Recognition and Assessment of Pain in Lambs

Graham N. Wood

Thesis presented for the degree of
Doctor of Philosophy
University of Edinburgh
March 1991

Recognition and Assessment of Pain in Lambs

Graham Notley Wood, Royal (Dick) Veterinary College and Moredun Research Institute, Edinburgh.

ABSTRACT

Local anaesthetic administered epidurally at the base of the tail, and infiltrated into the testes, spermatic cords and neck of the scrotum eliminated the behavioural and plasma cortisol responses associated with castration and tail-docking, confirming that the pain observed was dependent on afferent activity from the affected areas.

Having established the neural basis of the responses seen, attempts were made to modify the intensity of pain experienced so that measurement of the changes in behaviour and plasma cortisol concentration could be used to determine the usefulness of the behavioural and physiological indices adopted for the recognition and assessment of pain.

Pretreatment with intravenous naloxone (0.2mg/kg I/V), which was anticipated to antagonise an endogenous opioid antinociceptive system activated following castration and tail-docking, provided limited evidence for an increase in pain intensity. 6 procedures which were proposed to represent a range of intensities and duration of noxious stimulation based on the amount and type of tissue involved were ranked in the expected order using the physiological and behavioural indices selected.

A study was undertaken to determine if morphine (16mg) etorphine (10nmol) or xylazine (50μg) by either the epidural or intrathecal route could provide a method by which pain intensity could be reduced in a dose dependant manner, to further improve the indices used for recognising and assessing pain. The results of these studies suggested that the pain produced by castration or castration and tail-docking cannot be eliminated by the doses of the drugs tested and the routes used. To confirm these results preliminary experiments were performed, morphine or xylazine was administered intrathecally to demonstrate active drug gained access to, and had the expected effect on the Sural-semitendinosus reflex in the lamb. Morphine failed to suppress the semitendinosus reflex which is consistent with the results obtained. The suppression of the semitendinosus reflex by xylazine is not consistent with the absence of analgesia in lambs to which either epidural or intrathecal xylazine (50μg) had been administered prior to castration or castration and tail-docking, as the general view is that reflex suppression is highly correlated with the level of analgesia.

The results of this study present data which show physiological and behavioural indices can be used to recognise and assess a range of pain intensities in this age of lamb originating from the scrotum, testes and tail.

DECLARATION

The work presented in this thesis was part of a larger project concerned with the recognition and assessment of pain in both lambs and calves. Except where indicated, a full role has been played in the initiation, design and execution of the experiments described herein and in the interpretation of the results.

GRAHAM.N.WOOD
February 1991

ACKNOWLEDGEMENTS

My sincere thanks are due to the following:

The Agriculture and Food Research council for providing me with a Veterinary Schools Fellowship for the three years of this project;

Dr Ian D. Aitken (Director, Moredun Research Institute), for providing the research facilities;

Dr Chris Hodgson for being my supervisor at Moredun and for help with numerous aspects of the work including reading the thesis;

Dr Susan Fleetwood-Walker for being my assistant university supervisor and for help with electrophysiological methods;

Professor David Mellor for initiating this project and providing continuing support despite being based in New Zealand;

Dr Geoff Beckitt for providing facilities and equipment at the clinical chemistry department of the Edinburgh Royal Infirmary and advice on cortisol assay techniques;

Mr Colin Warwick (photography), for guidance in producing the diagrams presented in posters, papers and this thesis;

Mr Jim Brown (mechanical workshop) for constructing the specialised equipment used in this project;

Mr Mike McLauchlan and Mr Frank Wright (Scottish Agricultural Statistics Services) for advice on statistical analysis;

Mr Benny Sim and Mr Norrie Jones for help with handling animals;

All the staff at the Moredun Research Instititute and the Royal "Dick" Veterinary College for their advice and cheerful assistance;

Dr Rob Clarke for allowing me to visit him at the School of Agriculture (Sutton Bonnington) to learn electrophysiological techniques;

I thank my family for their concern and support, and my girlfriend Fiona who hasn't seen me this year.

I am deeply indebted to Dr Vince Molony, my supervisor, for accepting me as a pre-doctoral student for this project, for guiding me from the beginning of this project to the completion of this thesis, without whose advice, constant encouragement, stimulating and thought-provoking discussions, this work could not be possible.

CONTENTS

CHAPTER CONTENTS

GENERAL INTRODUCTION

Review of legislation to improve animal welfare and the importance of the concept of pain

There has been an active interest in animal welfare in the UK for over 150 years. The Royal Society for the Prevention of cruelty to animals was founded in 1824, and the Cruelty to Animals Act, designed to protect experimental animals from unnecessary suffering, was introduced in 1876. In order that this and related legislation such as the Protection of Animals Act 1911 and 1912 may be applied appropriately, methods of recognising and assessing suffering in animals are required. That interpretation of animal behaviours could provide a useful approach to this problem was indicated in the Report of the technical committee to inquire into the welfare of animals kept under Intensive Livestock Husbandry Systems (Brambell, 1965).

The Farm Livestock Advisory Committee, at a symposium on stress in farm animals (F.L.A.C. 1973), concluded that to interpret the meaning of "unnecessary suffering", the key phrase in the 1911 Protection of Animals Act and all subsequent legislation, objective assessment of suffering was necessary and that this could possibly be achieved using ethological evidence. Despite these recommendations little apparent progress in solving the primary problem of how to recognise and assess pain in animals was made before the Cruelty to Animal act 1876 was updated in a 1983 white paper (command 8883, HMSO. London). This indicated that the concept of "pain" in the 1876 act should be broadened to include disease, other disturbances of normal health, adverse changes in physiology, discomfort and distress. A supplementary white paper (command 9521, HMSO. London) was produced in 1985 concerning scientific procedures on living animals. This white paper proposed not only the control of pain but of all the factors which may compromise the welfare of animals used in experiments, such as the impairment of health or well-being, morbidity and mortality. In 1986 further guidance on the operation of the new Animals (Scientific Procedures) act 1986 (Home office, London) was provided in which the onus was placed on those conducting experiments on animals to alleviate pain, though no formal guidance on pain recognition was included.

Existing veterinary and farm husbandry practices where pain recognition is required to improve animal welfare

The importance of pain recognition was addressed at a symposium organised by the British Veterinary Association Animal Welfare Foundation (1985) on "The detection and relief of pain in animals". At this meeting it was proposed that pain occurred in many of the procedures undertaken by vets, but that this pain was poorly recognised due to inadequate training (Flecknell, 1985). Concern was expressed that until methods become available, pain in animals will remain undetected, particularly following surgery, including surgery undertaken without any form of anaesthesia for husbandry purposes. Such husbandry procedures include partial amputation of the chicken beak using a heated blade. This mutilation has been suggested to be painful because Breward (1983), while recording from cutaneous afferents in the chicken beak, elicited responses in C- fibres with thermal stimuli in the temperature range known to be noxious in man.

Groups investigating procedures such as castration and tail-docking in lambs (Shutt *et al*, 1988), castration in calves (Fell *et al*, 1986), muelsing in sheep (Shutt *et al*, 1987) and dehorning in cattle (Carter *et al*, 1983), were primarily concerned with increases in plasma cortisol concentrations as an indication of the magnitude of the stress imposed on the animal. It has been assumed in the present study that the underlying stressor producing these responses was pain. As stated by Carter *et al* (1983), measurement of increases in plasma cortisol concentrations alone cannot be regarded as a satisfactory method for assessment of pain. Unwillingness to address the problem of pain recognition directly, may derive from the contention that the characteristics of the response to pain differ considerably between animals of the same or different species (Tyers, 1985). The variable characteristics of the response to pain was incorporated in a recent definition of pain in animals by Uvarov (1989); "Pain is an aversive sensory experience caused by actual or potential injury which is accompanied by protective somatic and visceral reactions and induces changes in behaviour including social behaviour which can be specific for an individual animal". Although reactions to pain may appear to be different between animals and between animals and man it is likely that there are common features due to the similarity of the nervous system, particularly in vertebrate mammals (Brain, 1963).

Recognition and assessment of pain in man including children and how it helps in the recognition and assessment of pain in animals

An extensive literature on pain perception and alleviation in humans (Beecher, 1957; Chapman *et al*, 1985; Wall and Melzack, 1984) should be relevant to pain perception in animals because of the similarity in the structure and functioning of nociceptors and afferent pathways found in man and animals (Zayan, 1986). For example with regard to structure, the spinothalamic tract which has been regarded as the 'pain pathway' on the basis that transection may relieve intractable pain in humans (Spiller and Martin, 1912), is well developed in animals such as the rat and monkey (Kevetter and Willis, 1984). If there is sufficient similarity between the mechanisms in animals and man then there is no reason to suppose that they do not feel pain (Brain, 1963). These anatomical and physiological similarities support the observation by Thorpe (1965), that the reaction of animals to kinds of stimuli which cause pain in ourselves are very often (though not always) very similar to our own, including escape reactions, contortions of the body and intense vocalisation. Although there are exceptions, not only are the types of reaction to pain similar but so are the thresholds for these reactions (Kitchell and Johnson, 1985). Ethically, therefore, it is safest to assume that what is painful in man is painful in animals.

(a) Recognition and assessment of pain in adult humans

People in pain, convey this to others by a variety of expressive cues such as language, paralinguistic vocalisations (screaming etc), facial distortion, withdrawal reflexes, diffuse locomotor activity, posture and autonomic activity (Craig and Prkachin, 1983), however, the assessment has typically depended on self-report in humans over about 7 years old (Jeans, 1983). Once over 12 years of age, sensitive assessment of pain intensity, location, and quality can be made using a standardised examination such as the McGill Pain Questionaire -MPQ (Melzack, 1975). The MPQ and techniques where patients describe pain in terms of pictures, or as a deflection on a scale such as the visual analogue scale (VAS) depend on verbal communication and are inapplicable in animals. Monitoring human behaviour suffers ethical and logistical disadvantages (Fordyce, 1983), such as invasion of privacy which even if acceptable to the subject may alter the behaviour observed. An approach which might be adopted for use in expressive animals, however, is a system developed by Ekman and Friesen (1978), that uses highly trained observers to recognise and identify discrete facial expressions (Facial Action Coding System- FACS).

(b) Recognition and assessment of pain in children

Until recently there has been virtually no systematic study of pain in children, and in the absence of verbal communication, more emphasis has to be placed on observation of behaviour

(Jeans, 1983). A behavioural observation model was developed for use in children, the Childrens Hospital of Eastern Ontario Pain Scale (CHEOPS) by McGrath *et al* (1985), to measure pain intensity. In older children, however, little relationship was found between the self-reports and the behavioural measure (CHEOPS) of pain (Beyer *et al*, 1990). McGrath (1990) emphasised the importance of the type of pain to which the method is applied, for short sharp pain behavioural measures were reliable. For example Dixon *et al* (1984) found that infants undergoing circumcision exhibited marked behavioural disturbances which could be avoided if local anaesthetic was used. For medium term pain, behaviour was found to be an unreliable measure but physiological responses were helpful (McGrath, 1990).

The problem of nonverbal pain recognition in humans appears similar to that encountered in animals. The findings above indicate that the approach requires that a number of readily available behavioural and physiological measures need to be measured and developed as reliable indices of pain.

New approaches to recognition and assessment of pain in animals

Interest in the recognition and assessment of pain in animals was more widespread in the 1980's. A workshop was held in 1984 concerned specifically with the problem of assessing pain in farm animals (Molony and Duncan, 1986). It was proposed at this workshop that although direct measurement of pain was not yet feasible, an examination of both an animals behavioural and physiological responses to procedures or noxious stimulation likely to produce pain were anticipated to provide a fruitful way forward. An experimental scheme for the quantitative assessment of pain, distress and discomfort in animals was devised by Morton and Griffiths (1985). This scheme required the grading of 5 independent variables: bodyweight, appearance, clinical signs, unprovoked behaviour and behavioural responses to stimuli. Each variable was given a score of between 0-3, and the combined score was used to make an assessment of the degree of suffering. Reservations were expressed over the applicability of this scheme as it was thought inappropriate to assign each variable with the same weighting before their relative significance had been fully evaluated for each species (Sanford *et al*, 1985).

Guidelines for the recognition and assessment of pain in animals were produced by Sanford *et al* (1986). These were developed from the results of a questionaire distributed to members of the Association of Veterinary Teachers and Research Workers (A.V.T.R.W.) on assessment of pain in animals. Sanford *et al*, (1986) concluded that although desirable, it was not yet possible to develop a pain scoring system and pain severity would have to be judged by observation using a range of parameters including behavioural, physiological and biochemical signs. Blackshaw (1986) supported the use of behaviour as a monitor of welfare status and proposed that it can be an extremely sensitive measure. The prime physiological indicator of stress ie plasma cortisol (Munck *et al*, 1984), although now frequently used as an indicator of stress in a variety of husbandry procedures (Broom, 1986), remains to be validated as a suitable indicator of pain. The AVTRW guidelines (Sanford *et al*, 1986) were partly revised (Sanford *et al*, 1989) and were published by U.F.A.W. These guidelines provided a framework for recognition and assessment of pain in animals but lacked the detail necessary for the recognition and quantitative assessment of pain of various kinds in different ages and species of animals.

Recent studies on the response of a particular species to specific types of pain some of which provide useful models with which to develop methods for recognition and assessment of pain

The intimation by Sanford *et al* (1989), that research on recognition and assessment of pain in animals should focus on particular pains in particular species determined the strategy for current work. Castration and tail-docking of lambs provide two such examples, though others for this species include muelsing, disbudding and common natural diseases such as broken mouth, footrot and some acute infectious diseases though some of these are much less reproducible.

Several authors have already studied the responses of lambs to castration and/or tail-docking including Shutt *et al* (1988) and Mellor and Murray (1989a,b). Shutt *et al* (1988), found that following castration and tail-docking with tight rubber rings, lambs initially showed no reaction but by 2 minutes they were lifting and stamping their hind legs. Additional behaviour included bleating, looking around, shaking their hind limbs and tail, and a progressive increase in running back and forth. By 5 minutes most lambs were rolling and vocalising, this erratic behaviour reaching a peak at 15 minutes. By 60 minutes most lambs were judged to be behaving normally. No attempt was made, however, to analyse the behavioural data in a quantitative manner. Plasma cortisol concentrations were estimated at 15 minutes and 24 hours post-treatment and were found to be elevated at both times from a basal level of 43 ± 5 nmol/l to 128 ± 9nmol/l and 99 ± 11 nmol/l respectively.

Mellor and Murray 1989a, provided a quantitative assessment of 5 categories of lamb behaviour (lateral recumbency, ventral recumbency, standing, teat-seeking and sucking) following castration and tail-docking with tight rubber rings, and characterised the plasma cortisol response in greater detail. They noted similar behaviour to Shutt *et al* (1988) in the first 15 minutes, in addition, however, they noted that as lambs became less active after 15 minutes there was an increase (40% of observations at 30 minutes) in the abnormal posture of lateral recumbency. Lambs in both lateral and ventral recumbency lay with their necks extended, eyes closed and were hyperventilating with deep, frequent breaths. Plasma cortisol concentrations were found to increase from basal levels (8ng/ml) to a peak of 40 ng/ml at 30 minutes before returning to basal levels by 90 minutes, indicating the sampling regime adopted by Shutt *et al* (1988), provided insufficient data to characterise the plasma cortisol response, as the first sample at 15 minutes is taken before peak levels are obtained, while the acute plasma cortisol response has ceased long before the sample is taken at 24 hours. Although Mellor and Murray (1989a,b), made quantitative measurements of the 5 categories of lamb posture, no attempt was made to make statistical comparisons between the behaviours seen following four different treatments (castration and tail-docking, tail-docking, handling plus ACTH or control handling).

In the studies described above, methods for pain recognition were emerging because comparisons were being made between specific treatments proposed to cause different levels of "disturbance". To direct this type of work exclusively at the problem of pain assessment, the plasma cortisol response needed to be validated as a sensitive indicator of pain, while observation and measurement of lamb behaviour required greater emphasis and more than a subjective analysis if a sensitive, reliable and practical method of pain recognition and assessment were to be developed.

Why must physiological and behavioural indices be validated for a specific pain in a particular species?

At one time it was thought that the body reacted in a uniform way to a number of forms of adversity (Selye, 1936) and therefore a biochemical index such as plasma cortisol concentration, would indicate the severity of a stressor such as pain. Now, however, the correlation between biochemical parameters such as plasma cortisol and pain are regarded to be unclear (Sanford *et al*, 1989). This view was not new, as

Terman *et al* (1984), had demonstrated that changing the timing of a fixed intensity of noxious stimulus (foot shock) in the rat, could produce different changes in the plasma corticosterone levels. The selection of a particular painful procedure such as that produced by castration and tail-docking, the intensity of which changes with time, allowed correlation of the the changes in behaviour with the plasma cortisol concentration. Such a correlation only validates the use of plasma cortisol concentration as an indicator of pain intensity for pain originating from the affected tissues in this model. Similar plasma cortisol concentrations may be found in the same animal under different circumstances which may not be indicative of pain (Rushen, 1986; Dantzer and Mormede, 1983).

Although small changes in behaviour may be a sensitive monitor of changes in welfare status (Blackshaw, 1986), each behavioural index selected requires validation for the particular situation in which it is to be used. Fraser *et al* (1975) when discussing behaviour in animals resulting from the stressful nature of a husbandry system stated that " no single physiological or behavioural feature will ever be a useful indicator of stress in all situations", This is also relevant to behaviour and physiological changes resulting from pain. For example the lateral recumbency found to be an abnormal posture adopted by lambs following castration and tail-docking (Mellor and Murray, 1989a) may not indicate pain under all conditions. Lambs in warm environments may frequently adopt such a posture (Duncan, personal communication), and it would be completely misleading if used as an index of pain in other species (such as the dog), where lateral recumbency is a normal and commonly adopted posture. Thus both behavioural and physiological indices should be validated for a particular pain in a specific species, and then tested for applicability to other types/origin of pain in the same and different species.

What was required initially was access to a well defined reproducible painful experience in a particular species to enable development of physiological and behavioural indices for its assessment. Castration and tail-docking using tight rubber rings produces a painful experience in lambs. Some studies of the physiological and behavioural responses to these procedures have been described above (Shutt *et al*, 1988; Mellor and Murray, 1989a).

Use of castration and tail-docking as models for the investigation of somatic, visceral, acute and chronic pain in lambs

Castration and tail-docking are not natural stimuli but are performed as routine husbandry procedures and while these practices are allowed to continue they provide useful models of clinical pain. The investigation of these procedures was licenced under the Protection of Animals Act 1986 in order that future regulation of these procedures could be based on adequate evidence. So two purposes were being served, first methods for quantitative assessment of pain were being developed and second, they were being applied to the welfare problems posed by the practice of these procedures.

Castration or castration and tail-docking is a stimulus which, in contrast to most fleeting experimentally induced pains such as the thermal stimulus used in the tail flick test or hot plate tests in rats, produce lasting pain that is both visceral and somatic in origin. Unlike most stimuli now accepted for experimental use in conscious animals castration and tail-docking causes tissue damage from which the lamb cannot escape and a response which does not dissipate quickly. The alternative of investigating pain produced by natural disease or injury i.e. clinical cases as suggested by Zimmerman (1984), is not as useful since there is no clear control of the stimulus. The use of a stimulus which causes substantial tissue trauma partly avoids the criticism that contrived acute pain models may not be the best method of assessing behaviour associated with natural clinical pain or the efficacy of analgesics (Flecknell, 1986).

Approach adopted in an attempt to establish some physiological and behavioural measurements as indices for the recognition and assessment of pain

In order to develop methods for quantifying pain experienced by lambs, it was decided that one possible approach would be to expose lambs to graded pain intensities which could then be correlated with changes in behaviour and plasma cortisol concentrations, thus providing supporting evidence for these indices as measures for pain. Two possible methods of varying pain intensity are (a) to select a variety of stimuli which represent a range of intensities and durations of noxious stimulation, and (b) to select a suitable noxious stimulus and increase or decrease the intensity of pain experienced by the use of analgesics or hyperalgesics. Both approaches were tried, but the the latter approach where a single noxious stimulus is selected was preferred, as any changes in the plasma cortisol response or behaviour following administration of analgesics or hyperalgesics could be attributed directly to the change in pain intensity, and not to a change in the nature or origin of the pain.

Pain intensity as judged by the amount and type of tissue injured

Mellor and Murray (1989a), showed that castration and tail-docking in lambs produced a greater plasma cortisol response and a greater incidence of abnormal behaviour when compared with the less severe stimulus of tail-docking only. This approach was extended by exposing the lamb to a broader range of procedures. A range of intensities was accomplished by the use of castration with tight rubber rings and with the "short scrotum" technique, in which pain from the testes was avoided by pushing the testes and associated tissues into the inguinal region. The term "scrotal castration" is used throughout to indicate when lambs have been castrated by the "short scrotum" technique. Each of these procedures were further modified using selective local anaesthetic to block afferent activity from a proportion of the affected tissues. Complete anaesthetic blocks were performed in initial experiments to evaluate the practicability of alleviating all the acute pain following castration or castration and tail-docking and to confirm that neural activity originating from the affected tissues was responsible for the behavioural and plasma cortisol responses recorded.

Modification of the pain experienced following a selected noxious stimulus

(a) Using hyperalgesics

The noxious stimulus selected initially was castration and tail-docking. The hypothesis to be tested was that pain produced by these procedures could be modified by hypo or hyperalgesic drugs. Naloxone, an opioid antagonist, was selected as a potential hyperalgesic. It has long been recognised that there are central nervous system mechanisms that can alter the transmission of activity evoked by noxious stimulation (Mayer and Price, 1976). Akil *et al* (1975), found that the analgesia produced by by focal electrical stimulation of the brain could be partially reversed by naloxone. This opioid based system can also be initiated by stress (Bodnar *et al*, 1979; Amit and Galina,1986) and pain (Basbaum and Fields, 1984; Terman *et al* 1984). There is evidence that in sheep, stress such as electroimmobilisation (Jephcott *et al*, 1986) and pain such as from muelsing (Shutt *et al*, 1987), result in activation of endogenous opioid systems. It was proposed therefore, that lambs might utilise an endogenous opioid antinociceptive system following castration and tail-docking and that this could be antagonised by naloxone.

(b) Using analgesics

The stimulus selected was castration only. The proposal tested was that the pain intensity following castration could be reduced in a dose dependent manner by epidural or intrathecal injection of established analgesics. Spinal administration of analgesics is now accepted as an effective method of producing regional analgesia, even in cases where systemic drug administration has failed (Cousins and Mather, 1984). When morphine is administered by the intrathecal route to rats, 15μg produces profound analgesia as defined by the hot plate and tail flick tests and higher doses do not increase analgesia. Up to 10 times this dose administered intravenously has no detectable analgesic effect (Yaksh and Rudy, 1976). The regional extent of intrathecal analgesia in the sheep was demonstrated by Waterman *et al* (1988), who showed that 50μg of xylazine injected intrathecally at C5 produced analgesia restricted to the forelimbs and shoulders. The importance of these findings to the present study was that it indicated analgesia of the hind end of the lamb might be obtained following intrathecal injection of analgesics into the Thoraco-Lumbar subarachnoid space and that central effects which might modify behavioural indices could be avoided.

In the experiments designed to demonstrate the effectiveness of established analgesics (chapter 4 and 5) no analgesia was detected. In order to investigate this result and to determine if these drugs were gaining access to the spinal cord they were administered intrathecally in a range of doses to the same locations and their effects on the sural- semitendinosus reflex in anaesthetised spinalised lambs were studied. These pilot studies indicated that morphine did not reduce reflex responses, but conversely was responsible for hyperreflexia at doses similar to those used in attempts to alleviate the pain of castration and tail-docking. Xylazine markedly reduced reflex responses but no evidence for alleviation of the pain of castration and tail-docking was obtained when similar doses were administered intrathecally.

Physiological and behavioural measurements for the recognition and assessment of pain

Pain causes a range of physiological effects, some of which are due to hormonal responses. For example, injury results in sodium and water retention due to secretion of antidiuretic hormone and aldosterone, and hyperglycaemia due to increased cortisol and adrenalin secretion (Kehlet, 1978). Increased sympathetic activity as well increasing circulating adrenaline levels increases heart rate and peripheral resistance causing hypertension and increased cardiac work (Atkinson *et al*, 1989). The least intrusive but still quantitative measurement available was analysis of hormonal changes such as catecolamines or cortisol. Catecholamines were not chosen as analysis by HPLC was not readily available and is both time consuming and expensive. Furthermore preliminary studies by Ley *et al* (1989), suggest that plasma concentrations of adrenalin and noradrenalin are poor indicators of pain in lambs naive to the sensation of severe pain. The pattern of cortisol release, however, following administration of ACTH or imposition of various stressor agents has been well characterised in the sheep (Purchas, 1973; Fulkerson and Jamieson, 1982), and used subsequently by a number of groups, including Mellor and Murray (1989a), whose model was used as a starting point for these studies.

Zimmerman (1986) described some acute responses to pain in animals, which are also present in lambs during castration and tail-docking. Medium and long term pain is yet to be described and these studies are designed to find indices with which to determine if medium or long term pain is caused by castration and tail-docking, and the intensity of this pain. The types of behaviour anticipated following medium to long term pain (Chapman *et al*, 1985) include;

(a) Guarding behaviour and avoidance of pain aggravation, where a posture is adopted which minimises the pain sensation while protecting the injured tissue from further damage.

(b) Seeking pain relieving factors or environments, which might include self administration of analgesics in trained animals and selection of a comfortable surface on which to lie to minimise stimulation of the injured tissue.

(c) Self care of painful region such as cleaning by licking, or possibly self mutilation if prolonged severe pain is present.

(d) Changes in the incidence of normal behaviours such as sleep, exploration, feeding and social interaction.

As the pain becomes overwhelming it demands immediate attention and disrupts ongoing behaviour in some of the ways described above, directing the organism into activity aimed at stopping the pain as quickly as possible (Melzack, 1983). In order to describe some of these changes in a group of animals an instantaneous sampling technique may be adopted where the observer records the individual's current activity at preselected moments in time (scan sampling). This method measures states (eg posture), not events and thus appropriate categories of instantaneous behaviour require selection (Altmann, 1973). The initial behaviours chosen, were as described by Mellor and Murray (1989a,b), the intention being to develop new categories once experience had been gained observing the responses of lambs to castration and tail-docking.

MATERIALS AND METHODS

Animals/Husbandry

Breeds

Three breeds of lamb were used: Finnish Landrace x Dorsets, Grey face x Suffolk and Scottish Blackface. The use of Dorset ewes allowed lambing to be brought forward to late November, continuing through to June with the other breeds.

Management

All lambs were supplied by the Dept. Clinical Studies, Moredun Research Institute. Ewes and lambs were transported to the Institute two days after lambing and penned individually on solid concrete floors covered with a deep layer of straw. Ewes were fed 0.5kg ESCA nuts (9-10 MJ/kg dry matter, 16% protein) twice daily with ryegrass hay ad libitum. In most circumstances animals were penned for no more than 5 days before return to their flocks and farm of origin. All animals were inspected at regular intervals by the named veterinary officer (Hay, L.A.) to comply with the Animal (Scientific procedures) Act, 1986.
NB Lambs had unlimited access to the dam (unrestrained).

General anaesthesia prior to epidural or intrathecal injection of opioid and alpha 2 agonists

All lambs were anaesthetised with a mixture of halothane (4%), nitrous oxide (1 litre/min), and oxygen (1 litre/min), gradually reducing the halothane to a maintenance level of 1.0-1.5%. Anaesthesia was maintained prior to and during the epidural, intrathecal or sham intrathecal procedures, except for the control group in experiment 1 (Chapter 3). This routine was adopted to minimise pain and distress, and the risk of spinal trauma, that could have resulted from attempting these procedures in conscious, restrained animals.

General anaesthesia was normally maintained for less than 10 minutes. Recovery from the effects of the general anaesthesia was rapid: induction, drug administration and return to consciousness were usually complete in 4-5 minutes. The lambs were able to walk by the time they were placed back in the pen with their respective dams after a further 2-3 minutes and, thereafter, their behaviour was not notably different to lambs which had not been anaesthetised. After recovery from the anaesthetic, 40 minutes were allowed before the start of an experiment to enable the drug under investigation to gain access to the spinal cord.

Selection of epidural dose of morphine, xylazine and etorphine

The dose of either xylazine, morphine or etorphine was selected on the basis that it was approximately 75% of a dose which would produce unacceptable side effects. Slight unsteadiness was considered to be an acceptable side effect, if intermittent. Slight unsteadiness which was a continual feature (S2) following drug administration was also considered to be an acceptable side effect if of short duration ie < 15 minutes. Pilot studies were carried out, the results of which are shown overleaf. Those doses selected are marked with an *. Note that the dose of xylazine selected for epidural administration was also used for intrathecal administration.

Pilot study of the effects of epidural xylazine and morphine

Table 2.1. Xylazine

Lamb	Dose	Route	Behavioural effects
24.03.88	2 x 20μg	E/D T10-T11 L6-L7	Swaying and unsteady (S2) 0-15 minutes
24.03.88 (3.5kg)	2 x 20μg	E/D T10-T11 L6-L7	CSF seen, not included
24.03.88	*2 x 25μg	E/D T10-T11 L6-L7	Slightly unsteady (S1-S2)
24.03.88	2 x 50μg	E/D T10-T11 L6-L7	Swaying (S3), almost unable to walk. Lying peacefully (V2) by 17 minutes

Table 2.2. Morphine

Lamb	Dose	Route	Behavioural effects
No tag (3.8kg)	10mg (0 minutes)	I/V	Some circling to the right and left
	10mg (+35 minutes)	I/V	Compulsive circling, head shaking and swaying (S2)
	10mg (+65 minutes)	I/V	Compulsive activity, turning towards flank and chewing itself
	10mg (+130 minutes)	I/V	Walking continually, some circling
346 (5.0kg)	2 x 16mg	E/D T10-T11 L6-L7	Weak hind legs (S2), shivering and vocalising
104 (4.8kg)	2 x 17mg	E/D T10-T11 L6-L7	Weak hind legs (S2), rubbing, licking flank, circling and shaking
299 (5.9kg)	2 x 12.5mg	E/D T10-T11 L6-L7	Shivering, trembling and crouching (S2). Unsteady, increased licking and chewing
109 (6.5kg)	*2 x 8mg	E/D T10-T11 L6-L7	Only occasional ataxia (S1-2). Some chewing
105	*2 x 8mg	E/D T10-T11 L6-L7	Only occasional ataxia (S1-S2). Some chewing and persistent bleating

Selection of intrathecal dose of etorphine and xylazine

Pilot studies of the effects of intrathecal etorphine

Table 2.3. Etorphine

lamb	Dose	Route	Behaviour
260 (3.6kg)	50 nmol	I/T T10-T11	Lateral recumbency (L2), and abnormal standing
266 (1.9kg)	20 nmol	I/T T10-T11	Lateral recumbency (L2), Moving head from side to side
262 (2.9kg)	20 nmol	I/T L6-L7	Compulsive wandering, weak hind legs (S2)
261 (4.5kg)	10 nmol	I/T L7-S1	Abnormal standing (S2)
263 (2.9kg)	10 nmol	I/T L7-S1	Abnormal standing (S2)
254 (3.8kg)	10 nmol	I/T L7-S1	Some abnormal ventral recumbency (V3) and abnormal standing (S3)
264 (3.4)	10 nmol	I/T L7-S1	Some lateral recumbency (L1-L2) and abnormal standing (S2)
250 (1.8kg)	10 nmol	I/T L7-S1	Weak hind legs (S1-S2)
271 (3.0kg)	7.5 nmol	I/T L7-S1	Normal ventral recumbency
13.02.90 (3.5kg)	5 nmol	I/T L7-S1	Weak hind legs (S1-S2)
276 (4.6Kg)	5 nmol	I/T L7-S1	Abnormal standing (S2), shaking and nibbling
282 (4.4kg)	*2x5 nmol	I/T T10-T11 L7-S1	Weak hind legs (S1-S2)
274 (4.6kg)	*2x5 nmol	I/T T10-T11 L7-S1	Weak hind legs (S2)
273 (4.3kg)	*2x5 nmol	I/T T10-T11 L7-S1	Weak hind legs (S2), licking flank
272 (4.6kg)	*2x5 nmol	I/T T10-T11 L7-S1	Unsteady (S2)

Pilot studies of the effect of intrathecal xylazine.

Table 2.4. Xylazine

Lamb	Dose	Route	Behaviour
1271 (5.0kg)	2x33μg	I/T T10-T11 L7-S1	Sedated, unsteady (S1-2)
1272 (5.4kg)	2x10μg	I/T T10-T11 L7-S1	Only occasionally unsteady
1282 (5.1kg)	2x50μg	I/T T10-T11 L7-S1	Unsteady, falling over (S2-S3)
1284 (4.7kg)	2x50μg	I/T T10-T11 L7-S1	Falling over (S3), immobile (L1)
1395	100μg	I/V	Sedation, some abnormal standing (S2)

Blood Sampling

A rectangular area of wool was clipped the day before an experiment from the ventral part of the neck exposing both jugular veins. Blood samples (2ml) were obtained from alternate veins to minimise vascular damage, using 21 gauge 1" needles and a Steriseal 2ml syringe. Samples were taken prior to anaesthesia (where this was part of the experimental procedure), immediately before the start of the experiment and then at 15, 30, 60, 90, 120, 150, 180 and 240 minutes after the start of the experiment. On some occasions sampling was terminated after three hours. It was found that experienced operators could obtain jugular blood samples in less than 15 seconds with little apparent disturbance to the animal. Samples were then transferred to glass tubes containing lithium heparin and mixed briefly on a Matburn blood cell suspension mixer. Blood samples were centrifuged at 3000RPM (1800 xg) for ten minutes and cooled to < 4°C within 10 minutes of being taken. Aliquots of plasma were removed, avoiding any lipid layer, using a pasteur pipette and stored at -20°C. Cortisol measurement by radioimmunoassay (RIA) was completed within 4 months of sampling.

Plasma cortisol estimation using the I.D.S and Amersham R.I.A. kits.

Samples from the local anaesthetic/naloxone and epidural xylazine/morphine series of experiments were analysed by radioimmunoassay (Read *et al*, 1977) using a IDS-Gamma-BCT cortisol kit (IDS, Tyne and Wear). This kit enables direct measurement of total cortisol concentrations in unextracted serum, plasma or urine using an antibody to cortisol covalently bound to the inner surface of a polypropylene tube. After the addition of sample and 125-I cortisol, the tubes were incubated at 37ρC for 45 minutes. The cortisol in the sample was free to compete with 125-I labeled cortisol derivative for binding sites on the antibody coated tubes. The amount of 125-I labeled cortisol derivative which is bound by the antibody is inversely proportional to the concentration of unlabeled cortisol in the sample. After decanting the solution from the tubes, tubes were counted in a Packard auto-gamma 5650.

In a second series of experiments, plasma samples were again assayed by radioimmunoassay (Read *et al*, 1977) using an Amerlex RIA kit (Amersham International plc, Amersham UK). This kit is for the direct measurement of total cortisol in human serum, plasma or urine covering the same approximate range as the previous kit (0-1700 nmol/l). The assay employs a specific antibody having a very low cross reactivity with other naturally occurring steroids and which has been immobilised on the surface of polymer particles of uniform diameter. Particles were mixed thoroughly to ensure a homogeneous antibody suspension, but behaved as a solid phase separation system on centrifugation, yielding a highly stable precipitate containing the 'bound' fraction of the tracer. Cortisol in the sample was free to compete with the 125-I labeled cortisol as above, following release from transcortin by a chemical blocking agent. The antibody bound 125-I labeled cortisol derivative was separated by centrifugation for 30 minutes at 1800xg, with the polymer particles to which it was attached forming a readily observable precipitate in the assay tube. After removal of the supernatant, the radioactivity present in the pellet was measured on a gamma counter. Cortisol concentrations obtained by both methods were determined by reference to a standard curve derived using known concentrations of cortisol in lamb plasma.

Accuracy of the IDS kit was determined from analysis of lamb plasma samples to which known amounts of cortisol have been added. A cortisol stock solution was made by dissolving 38.06 mg cortisol (molecular weight 362.5) in 5mls of ethanol and making up to 100 ml with distilled water. 100μl of this solution was then diluted 1:1000, 1:500 and 1:250. A 25μl sample from each dilution was added to one of three 500μl plasma samples (containing negligible cortisol) to produce final cortisol concentrations of 50, 100 and 200 nmol/l, respectively. The recovery rate of added cortisol assayed in triplicate was 94, 97 and 100% respectively.

The precision of both assays was checked by including duplicate low and high concentration plasma cortisol samples at the beginning and end of every assay (the two pools of low and high concentration plasma cortisol being obtained from 5 day old lambs before or 30 minutes after castration and tail-docking respectively). The intra- and inter- assay coefficients of variation were 4.5 and 9.0%, respectively, for the IDS kit and 11.7 and 8.8%, respectively, for the Amersham kit.

In the present work the magnitude of the cortisol rise with time after treatment was more important than an accurate assessment of low basal levels. Nevertheless, in the second series of assays (using the Amersham kit), certain modifications were made to the manufacturer's instructions, to improve the kit sensitivity at low plasma cortisol concentrations. The lowest concentration of standard in each kit was diluted 1:2 with buffer control standard (B0; contains no hormone). The highest standard was discarded. The volume of lamb plasma assayed was doubled as this was found to have no effect on the performance of the kit, while increasing the sensitivity of the assay. Sensitivity (or limit of detection), was obtained for each assay by determining the intercept of the standard curves with the lower 95% confidence limit of percentage of binding by the B0 standards (Cekan, 1975) ie two standard deviations from zero. The minimal detectable concentration (MDC) for the IDS kit was 7.8 ± 1.1 nmol/l and the MDC of the Amersham kit using the modifications described above was 3.0 ± 0.9 nmol/l.

Statistical analysis of the plasma cortisol data

Plasma cortisol estimations for each of the 6 lambs in any of the treatment groups consisted of 9 or 10 consecutive measurements. Statistical analysis should take into account the non-independence of consecutive measurements and methods are available for certain classes of data (Rowell and Walters, 1976). There were, however, problems in applying such an analysis to the data from these experiments. Each of the treatments showed a peak followed by a slow return to the base level. Variability in the timing of this peak presented problems for comparing treatments, thus it was decided to compare the changes in plasma cortisol within the peaks independent of time.

Analysis 1 (all time periods studied)

An initial analysis of variance was carried out using a 2 by 2 or 2 by 3 treatment structures (factorial analysis) for 4 and 6 group experiments respectively, using the Genstat statistical program developed at Rothamstead. The reason for carrying out this type of analysis was to give an indication of the variation in cortisol levels with time in order to allow a decision on which time periods to use in the analysis. In each experiment the duration of the cortisol peak was determined from a plot of the mean cortisol values for the various treatments against time.

Analysis 1 (Restricted time periods)

This analysis only uses data from the consecutive time periods included in the peak, and therefore the effect of non-independence of measurements is not a problem. Regardless of the treatment considered, the plasma cortisol concentration rises from 0 to 30 minutes. The most interesting part of the output is either a 2 by 2 or 2 by 3 table. The plasma cortisol results for all experiments were presented in the following manner:

Plasma cortisol concentrations
Experiment n. Table of mean plasma cortisol concentrations (nmol/l) for the x groups

	DRUG	No Drug	Drug A
PROCEDURE			
Treatment 1		a	b
Treatment 2		c	d

These cortisol means (a,b,c and d) could be compared with a two way T-test using the standard error of differences (SED) and the appropriate number of degrees of freedom. It was found that similar results were obtained when the plasma cortisol concentrations found in the 15 and 30 minutes samples were compared with those obtained at 15, 30 and 60 minutes thus increasing the confidence in this approach.

Analysis 2 (Taking account of variation within individual lambs)

Analysis 1 concentrated on the cortisol measurements around the peak, disregarding the other information. The plasma cortisol concentrations outwith the peak can, however, be used to assess the base level of cortisol for each lamb and this can be used as a covariate. An analysis of variance (adjusted for this covariate) was then carried out. The covariate was calculated using cortisol levels from time periods 0, 120, 150, 180 and 240 and a mean base level cortisol was calculated for each lamb. This covariate was then used in the analysis of values at 15, 30 and 60 minutes. The output again is a 2 by 2 or 2 by 3 table, as shown above.

Analysis 2 makes the most of the available information, and was preferred. This method was used for analysis of plasma cortisol throughout these experiments.

Behavioural monitoring. Statistical analysis of the behavioural data

The behavioural monitoring technique adopted, was that of scan sampling (Altmann, 1973), which required that information was recorded as to the posture of the lamb at prescribed times, and did not necessitate interference with the lamb except at times when venous blood samples were collected. Initial behavioural recording (method 1) was based on the four behaviour patterns/postures used by Mellor and Murray (1989a). In the initial experiments behaviour was recorded using method 1. The measurement scale used in this method is the "nominal scale", which does not involve any quantification, but is concerned with description and identification of behaviour patterns. The behaviours chosen were distinct, easily recognised and were such that there was no doubt as to the posture or activity of the lamb at any recording time. The four behaviour patterns/postures measured by method 1 are outlined below;

Method 1

Lateral recumbency. An abnormal posture in young lambs under the conditions of these experiments, refers to the lambs lying on their sides with at least three limbs extended.

Standing or Walking. Refers to lambs engaged in these activities unless teat seeking. No attempt was made to distinguish normal from abnormal standing/walking.

Ventral recumbency. Refers to lambs lying with their forelimbs flexed under their chests. No attempt was made to distinguish between lambs that were awake or asleep, or lying in an abnormal form of ventral recumbency.

Teat seeking. Refers to lambs making active attempts to find and suck at the udder. No attempt was made to distinguish between attempts to suckle or suckling itself.

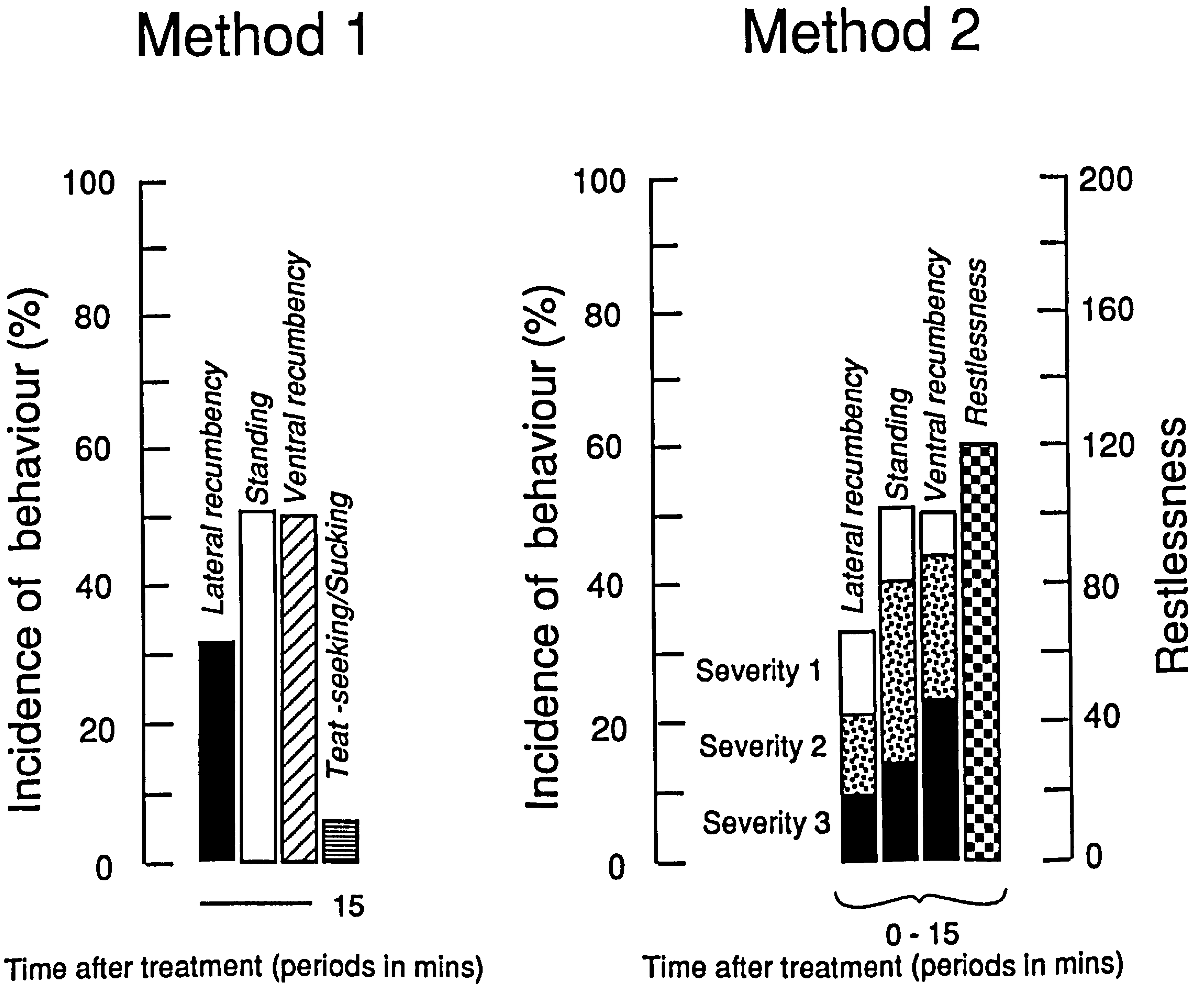
Observations were made at 1 minute intervals from the start of each treatment to 15 minutes (14 observations), at 2 minute intervals from 15 to 30 minutes (7 observations), at 5 minute intervals from 30 to 60 minutes (6 observations), at 10 minute intervals from 60 to 120 minutes (6 observations), and at 15 minute intervals from 120 to 240 minutes (8 observations). This produced a total of 41 observations of behaviour for each lamb during the four hour period. The results were normalised as percentages (%) and presented in bar chart form for each time period as shown in fig (2.1).

In subsequent experiments, (ie those investigating the effects of intrathecal etorphine, intrathecal xylazine and different local anaesthetic nerve blocks on the changes in behaviour and plasma concentration of cortisol produced by castration only, or other castration techniques in young lambs), a modified behavioural recording technique was used (method 2). The category of teat seeking and sucking was eliminated as it occurred infrequently with a low incidence when compared with the other behaviours, making statistical analysis difficult. One

FIGURE 2.1

Presentation of behavioural data

Behavioural data was presented in bar chart format using either method 1 or method 2. Note that the restlessness column which is only present in method 2 has a separate axis (0-200).


Method 1
Method 2
Incidence of behaviour (%)
100
80
60
40
20
0
Lateral recumbency
Standing
Ventral recumbency
Teat -seeking/Sucking
15
Time after treatment (periods in mins)
Restlessness
200
160
120
80
40
0
Severity 1
Severity 2
Severity 3
0 - 15

aim of this study was to develop reliable behavioural indices which could be used by inexperienced persons to assess pain in lambs within a practical period of time (5-10 minutes). The use of an intermittent behaviour requiring prolonged periods of observation to characterise fully, such as teat-seeking or sucking, was therefore considered to be unsuitable. The three remaining categories, however, were subdivided on an "ordinal" scale. This scale concerns the ranking of behaviour patterns into some sort of inter-relationship, for example, lateral lying has been divided into three categories. It is not known, however, what quantitative differences in the intensity of pain each category represents. The three categories of behaviour and their associated ranks are shown below. Lambs in borderline postures were observed for an extended period to allow subjective assessment of which category was most appropriate.

Method 2

Lateral recumbency

Lateral 1. Refers to lambs lying in an upright position and heads up, with one forelimb extended and one or both hind limbs extended.

Lateral 2. Refers to lambs in lateral recumbency with their heads raised or on the ground with all four limbs extended.

Lateral 3. Refers to lambs in lateral recumbency with their heads extended along the ground and all four limbs extended this posture being interrupted by rolling and kicking.

Standing/Walking

Standing 1. Refers to lambs standing or walking in a normal manner.

Standing 2. Lambs at first may appear to be normal but on prolonged observation, however, one or more kinds of abnormality can be detected e.g. lambs may weave and walk with a stiff unsteady gait. They may stand with the hind legs slightly splayed or positioned further back than normal.

Standing 3. Refers to lambs with an obviously abnormal gait. One or more kinds of abnormality occur e.g. the lamb kicks and stamps, and may fall over, occasionally the lamb may "walk" on the knees or walk backwards.

Ventral recumbency

Ventral 1. Refers to lambs in ventral recumbency, with an upright posture. All four limbs are tucked under the body, the head is curled around into the flank and the eyes are closed.

Ventral 2. Refers to lambs lying as for ventral 1, except the head is up and the eyes are open. Occasionaly the lamb lies with the head tucked into the flank but with the eyes open, these lambs are still recorded as ventral 2.

Ventral 3. Refers to lambs lying in ventral recumbency with both forelimbs tucked under body. One or both hind limbs are extended. On the rare occasions that the lambs adopted this posture, but had their head down and eyes closed, they were recorded as ventral 1.

In method 2 observations were made at 1 minute intervals from the start of each treatment to 15 minutes (14 observations), at 2 minute intervals from 15 to 30 minutes (7 observations), and then at 5 minute intervals from 30 to 180 minutes (30 observations). This produced a total of 51 observations for the 3 hour period. The results were normalised in percentages (%) and presented in bar chart format. The method of presentation with the 3 graduations of each behaviour is illustrated in fig (2.1).

Restlessness. This was a quantitative measure, requiring continuous observation, of how often a lamb got up and lay down in the first 15 or 30 minutes after the start of the experiment. This was presented as the combined score for all six lambs in each experimental group.

Statistical analysis of the behavioural data

The behavioural data could not be assumed to come from a population with a normal distribution, due to the small number of observations. Thus a distribution-free test, or non-parametric test was chosen (The Wilcoxon rank test) as an appropriate measure under these circumstances. The incidence of the 3 or 4 behavioural categories were compared between groups for similar time periods. This approach allowed differences to be measured between the incidence of a particular behaviour in one group when compared with another during the same time period. It does not, however, indicate whether differences are due to either a change in the mean or the standard deviation or both measures of the incidence of a particular behaviour.

EFFECTS OF REGIONAL LOCAL ANAESTHESIA OR INTRAVENOUS NALOXONE ON INDICES USED FOR ASSESSMENT OF PAIN FROM CASTRATION, SCROTAL CASTRATION OR CASTRATION AND TAIL-DOCKING

Origin and use of the opioid antagonists

The action and clinical pharmacology of certain opioid antagonists have been known in reasonable detail since early reviews (Lasagna, 1954). An opioid antagonist was first used in 1915 by Pohl who demonstrated that he could stimulate respiration using the allyl compound N-allylnorcodeine synthesised by Von Braun (1916). Clinically, nalorphine was not used until 1951 for the antagonism of morphine poisoning (Ekenhoff *et al*, 1951). There has, however, been a substantial increase in the number of opioid antagonists available most of which are not only used clinically, but also for the study of the mode of action of opioids (Duggan *et al*, 1985) and for the investigation of receptors by competitive binding studies (Heyman *et al*, 1986). These uses are extensively reviewed by Martin (1967) and more specifically use of naloxone has been reviewed by Sawynok *et al* (1979), and a comparative study of naloxone and naltrexone by Takemori and Portoghese (1984).

Choice of naloxone as an opioid antagonist

Naloxone was selected as the antagonist for a number of reasons. It has a well documented action both at the spinal and central levels (Duggan and North, 1984), and its selectivity ensures significant antagonism of the endogenous opioid ligands (Sawynok *et al*, 1979). It does not produce respiratory depression at the doses used (Foldes *et al*, 1964).

Evidence for both opioid and non opioid antinociceptive systems which may operate in the lamb

The complexity of the intrinsic inhibitory systems have been discussed by Terman *et al*, (1984). This group have suggested that pain/stress is the physiological trigger for an intrinsic opioid mediated analgesia system. The close association between the stress response and analgesia is illustrated by the discovery that hypophysectomy, adrenal demedulation or denervation attenuates opiate based stress analgesia (Lewis *et al*, 1981). The opioid proposed to be responsible for this analgesia was an enkephalin like peptide (Lewis *et al*, 1982). There appears to be another opioid antinociceptive system which is blocked by pentobarbital anaesthesia and which requires the animal to perceive the stressor as inescapable (Maier *et al*, 1982). Activity of this system can be conditioned, and is not dependent on the H.P.A-axis (Watkins *et al*, 1982). Thus there are at least two opioid systems. In the rat a non opiate mechanism also exists which is not be antagonised by naloxone (Terman *et al*, 1984). It was found that cross tolerance does not occur between these two forms of opioid and non opioid stress analgesia, confirming the separateness of their neurochemical substrates (Terman *et al*, 1983). Lesions of the spinal dorsolateral funiculus were found to disrupt both the opioid (Barton *et al*, 1980), and nonopioid forms of stress analgesia (Lewis *et al*, 1983). If any or all of these antinociceptive mechanisms are operative in the lamb before and/or after castration and tail docking, the use of the opioid antagonist naloxone, although unable to antagonise non-opioid forms of stress analgesia, could be expected to provide evidence for their operation.

Selection of a suitable naloxone dose to antagonise endogenous opioid activity in the lamb

No studies of the distribution and metabolism of naloxone have been found for sheep. In mice pharmacokinetic data shows that the half life of naloxone when administered intrathecally is very short ranging from 9-17 minutes (Heyman *et al*, 1986). Subcutaneous injection of naloxone in the mouse (0.2mg/kg) reduced the antinociceptive effect of morphine in the acetic acid writhing test for up to 1 hour, with maximum antagonist activity at 20 minutes (Smits and Takemori, 1970). This dose of naloxone in adult sheep (0.2mg/kg I/V) was found to eliminate the analgesia produced by pethidine (5mg/kg I/V) or buprenorphine (6μg/kg I/V) in thermal pain tests (Nolan, 1986). The intravenous route was chosen for this present study in preference to the subcutaneous route to reduce the time from injection to peak antagonist activity. The dose of naloxone selected (0.2 mg/kg), administered immediately before and one hour after the start of the experiment, was expected to exceed that required to antagonise endogenous opioid activity in the lamb during the two hours following castration and tail-docking. In a variety of species intravenous injection of naloxone at doses as low as 0.05 mg/kg have been demonstrated to increase the firing of multireceptive dorsal horn neurones to noxious input, indicating a reversal of the inhibition of these spinal neurones produced by endogenous opioids (for review see Duggan and North, 1984). Larger doses of naloxone although improving the likelihood that an effective antagonist concentration is maintained, could give agonist effects. Undesirable agonist and antagonist actions of naloxone are discussed below.

Agonistic actions of naloxone

Work by Woolf (1980), has demonstrated a dose dependent bi-phasic change in nociceptive threshold in the rat following intrathecal injection of naloxone. The initial antinociception obtained with low doses of naloxone was associated with the delta receptor as it could be blocked by pretreatment 24 hours earlier with a large intrathecal dose of naloxone or 20 minutes previously with the delta specific antagonist ICI-174,864 (Taiwo *et al*, 1989). The antagonistic effect at higher doses was due to an effect at mu receptors. In studies by Duggan *et al* (1985) and Clarke *et al* (1989), very small doses of naloxone (5 μg) administered intravenously increase the magnitude of a spinal nociceptive flexor reflex indicating that even at doses as low as these it can still act as an opioid antagonist.

Effects of naloxone on behaviour

In man, naloxone treatment can produce feelings of euphoria or unpleasant illusions and hallucinations. Although human subjects may feel drunk, there are no signs of gross ataxia or dysarthia even with large doses (Martin, 1984). There is evidence that naloxone inhibits deprivation induced food intake (Arjune and Bodnar, 1990). In rats administered naloxone that were not deprived of food there was a reduction in weight gain (Brands *et al*, 1979). In lambs this should be seen as a reduction in frequency or duration of suckling and/or teat seeking. Narcotic antagonists can produce convulsions but this is normally only at high dose rates (Martin, 1967). No signs of convulsions were expected in lambs given up to 1mg/kg intravenously, as much higher doses have been used experimentally (Duggan and North, 1984), without producing adverse effects.

Effects of naloxone on plasma glucocorticoid concentrations

It is now well known that plasma glucocorticoids rise in response to the opiate antagonist naloxone. Intraperitoneal injection of naloxone (50 mg/kg) to mice elevated plasma corticosteroid concentrations above control levels for 30 minutes, but inhibited the stress induced elevation of plasma corticosterone (Gibson *et al*, 1979). Intraperitoneal injection of naloxone (1mg/kg) in the rat produced a small, though significant increase in plasma corticosterone concentration 30 minutes after injection (Jezoua, 1985). Intravenous injection of naloxone (10mg/kg) to the rat also increased plasma corticosterone concentrations, however, as the dose was reduced so was this effect, until no significant change in plasma corticosterone concentration was seen at 2.0 mg/kg naloxone (Eisenberg, 1980). The dose of naloxone used in this study (0.2 mg/kg I/V) was not expected therefore, to have a significant effect on plasma cortisol concentrations in the lamb.

Effects of naloxone on thermoregulation

Changes in body temperature could produce behaviours adopted for thermoregulation and complicate interpretation of changes in behaviour as an index of pain. Subcutaneous injection of naloxone (10mg/kg) in the rat produced only a small (< 0.5°C) reduction in body temperature (Goldstein and Lowery, 1975), which was confirmed by Cox *et al* (1976), with a lower dose (0.5mg/kg). Similarly intravenous injection of naloxone (8mg I/V) did not effect the body temperature of the chronic spinal dog (Martin *et al*, 1976).

All the unwanted agonist and antagonist effects mentioned in this introduction can be minimised by using a dose of naloxone of less than 0.2 mg/kg. The naloxone does however, act on many different central and peripheral sub-populations of opioid receptors many of which may not be involved in antinociception etc. In addition, this dose of naloxone is only effective in antagonising some classes of opiate receptors, mu in particular.

SELECTION OF A LOCAL ANAESTHETIC

Properties of local anaesthetics

Local anaesthetics provide the single most effective approach to eliminating pain. Infiltration of local anaesthetic into appropriate tissues was used in attempts to modify the severity of pain experienced following application of tight rubber rings to the scrotum, scrotum and testes or tail. Such local anaesthetic techniques are used routinely for surgical castration of lambs 3 months after birth <Protection of Animals Act (Anaesthetics), 1964>. The various types of local anaesthetic have different properties and initial testing was undertaken in a similar manner to that described by Burn (1946), to obtain an agent with an appropriate duration of action. Selection of tissues to be infiltrated, and type of local anaesthetic to be used, allowed manipulation of the nociceptive input and the time course of the effects.

Recent studies (Wall, 1988; Codere and Melzack, 1987) have emphasised the importance of infiltrating local anaesthetic prior to any painful procedure, as evidence suggests that blocking noxious input from injured tissue entering the CNS, stops production and maintenance of secondary hyperalgesia (Hardy *et al*, 1952). If local anaesthetic is administered after the noxious stimulus although it eliminates all input and produces analgesia (Hardy *et al*, 1950), secondary hyperalgesia may appear when the effect of the local anaesthetic wears off (Beecher, 1957). It was thus anticipated that injection of local anaesthetic prior to application of the rubber rings would ensure adequate and complete anaesthesia, and prohibit secondary hyperalgesia.

Selection of lignocaine as a suitable local anaesthetic

Lignocaine was first described by Lofgren in 1943, and was selected mainly because of its duration of action, being longer lasting than procaine and shorter than bupivicaine. It penetrates nerves effectively, a property conferred by its lipophylicity, ensuring a rapid onset of action and it is non toxic and stable in tissues (Green, 1979). Once lignocaine enters the circulation it is rapidly metabolised by the liver though some lignocaine (10-20%) is excreted unchanged through the kidneys (Westhues and Fritsch, 1964). It was anticipated from pilot studies and work by Soma (1971), that lignocaine without added adrenalin would have a duration of action in the testes, scrotum and tail of about 40-60 minutes. This would permit nociceptive activity which continued for longer than this to gain access to the CNS, an alternative means by which the effects of gradually increasing pain intensity on the behavioural and physiological responses of the lamb could be investigated.

Secondary effects may be seen with epidural administration of local anaesthetic, such as systemic hypotension (Hall and Clarke, 1988), due to sympathetic blockade. Some respiratory depression may be seen due to central effects, but no evidence was obtained for this in the lambs of this age used here. It was concluded that if accidental intravenous administration of these doses was avoided, cardiovascular and respiratory side effects were unlikely. Local irritation at the injection site is rare (Green, 1979), however, systemic effects may occur. After subcutaneous or intramuscular injection peak blood levels occur at 30 minutes, when CNS depression, drowsiness, twitching, respiratory depression, convulsions and hypotension are most likely if sufficient lignocaine is absorbed (Hall and Clarke, 1988).

MATERIALS AND METHODS

Studies of the effects of varying the intensity of noxious stimulus experienced by the lamb were based on the results obtained from two separate experiments. The first in March 1988 and the second in March 1989. In total 72 lambs (all male) were used, weighing between 2.1-9.7 kg, and ranging from 5-7 days old. In the first group of 36 lambs the effect of blocking transmission of nervous activity in tail, testes and scrotum with local anaesthetic on behaviour patterns and cortisol responses produced by castration and tail-docking were examined. The effect of intravenous naloxone (opioid antagonist) was similarly examined. Behavioural and plasma cortisol data were recorded as described by Mellor and Murray (1989a), though the presentation and analysis of results was modified, see method 1, (general methods).

In the second group of 36 lambs, attempts were made to investigate the spectrum of behavioural responses elicited in the lamb by 5 procedures, which were designed to represent a range of intensitites and durations of noxious stimulation. Changes were made in the behavioural monitoring technique. The 3 main behavioural postures, lateral recumbency, standing and ventral recumbency were graded into 1 of 3 severities. Presentation of teat seeking/sucking data was discontinued. A restlessness score was introduced, which quantified how often a lamb got up or lay down, see method 2, (general methods). The drugs used in these studies were, Lignocaine (May and Baker) 2% solution; Naloxone hydrochloride (Sigma Chemical Company), in 0.9% NaCl solution diluted to 3mg/ml.

Experiment 1

The null hypothese were that blocking transmission of nervous activity in the scrotum, testes and tail with the local anaesthetic lignocaine, or intravenous administration of naloxone (0.2mg/kg) prior to castration and tail-docking, would not change the behaviour patterns or plasma cortisol concentrations in response to castration and tail-docking.

Animals

A total of 36 lambs (Dorset x Finnish Landrace) from 5-7 days old and weighing 3.2 ± 0.1 kg were used for this experiment. No lambs showing clinical signs of ill health were included in the investigation. Housing, feeding and bedding, followed routine husbandry practices, which were as described in the general methods.

Treatments

A day before the experiment, wool was shaved from the full length of the neck to expose both jugular veins. All lambs were bled (2ml samples) by venepuncture immediately before and at 15, 30, 60, 90, 120, 180 and 240 minutes after treatment. This procedure usually took less than 15 seconds.

There were six treatments.

Group 1. Control handling. Lambs (n=6) were handled as if they were being castrated and tail docked but without application of rubber rings, the testes were squeezed down into the scrotum and the tail was held, in each case for the time normally taken to apply the rubber ring (approximately 5 sec).

Group 2. Castration and tail-docking. Lambs (n=6) were castrated and tail-docked by application of tight rubber rings with an outside diameter of 14.8mm and inside diameter of 5.0mm both with SEM ±0.1mm (Paragon Rubber Co Ltd., Skegness, England). Rings were applied in accordance with the recommendations in the M.A.F.F. codes of practice for the welfare of sheep and as described by Eales and Small (1986).

This procedure is commonly used for castration and tail-docking in the United Kingdom.

Group 3. Local anaesthetic only. Lambs (n=6) had anaesthesia of the tail induced by epidural administration of 0.3ml of 2% lignocaine without adrenalin through the intervertebral space between coccygeal vertebrae 1 and 2. Local anaesthesia of the scrotum and its contents was produced by infiltrating 0.5 ml of 2% lignocaine into each spermatic cord, 1.0 ml of 2% lignocaine in a ring around the neck of the scrotum and 0.5 ml of 2% lignocaine into each testis 15-20 minutes before being treated as lambs in group 1. These multiple injections were designed to block the various nerves involved. In no case was more than 3.5 ml of lignocaine administered as higher doses produced behavioural changes including ataxia and sedation in lambs of this age and size.

Group 4. Local anaesthesia followed by castration and tail-docking. Lambs (n=6) were treated with local anaesthetic as in group 3, 15-20 minutes before being castrated and tail-docked, as in group 2.

Group 5. Intravenous naloxone only. Lambs (n=6) were slowly injected with 0.2 mg/kg naloxone (0.2mg/ml) into a jugular vein 10-12 minutes prior to the start of the experimental recording period (when they were treated as lambs in group 1) and again after 1 hour.

Group 6. Intravenous naloxone followed by castration and tail-docking. Lambs (n=6) were treated with naloxone 10-12 minutes before and 48-50 minutes after being castrated and tail-docked as in group 2.

Experiment 2

The null hypothesis was that a range of intensities and durations of noxious stimuli from the same area of the body will produce similar changes in the behaviour patterns and plasma cortisol responses.

Animals

A total of 36 lambs (Scottish Blackface) from 5-7 days old and weighing 5.7 ± 0.2 kg were used in this experiment.

Treatments

A day before the experiment, wool was clipped from the full length of the neck to expose both jugular veins. All animals were sampled from a jugular vein immediately before treatment, and at 15, 30, 60, 90, 120, and 180 minutes after treatment.
There were six treatments.

Group 1. Control handling (C). Lambs (n=6) were handled as if being castrated but without application of the rubber ring.

Group 2. Castration only (CN). Lambs (n=6) were castrated, by the application of tight rubber rings.

Group 3 Scrotal castration (SC). Lambs (n=6) had a tight rubber ring applied around the neck of the scrotum, after the testes have been pushed up into the inguinal canals.

Group 4. Castration with local anaesthetic in the testes (CNLAT). Lambs (n=6) were treated as for group 2, and 0.3 ml of 2% lignocaine without adrenalin was injected into the parenchyma of each testicle immediately after application of the tight rubber ring.

Group 5. Scrotal castration plus local anaesthetic distal to rubber ring (SCLADR). Lambs (n=6) were treated as for group 3, and 1.0 ml of 2% lignocaine without adrenalin was infiltrated subcutaneously in a ring around the scrotal neck, just distal to the tight rubber ring immediately after it had been applied.

Group 6. Castration with local anaesthetic distal to ring (CNLADR). Lambs (n=6) were treated as for group 2, and 1.0 ml of 2% lignocaine without adrenalin was infiltrated subcutaneously in a ring around the scrotal neck and into the spermatic cords just distal to the tight rubber ring immediately after it had been applied.

Ranking of the six treatments (Exp2)

In order to arrive at a ranking of the six treatments described above, the following principles were adopted.

(1) When the same procedure was carried out with and without local anaesthetic, it was accepted that the intensity and duration of the nociceptive activity would be less with local anaesthetic present.

(2) When separate procedures were carried out together on the same animal it was assumed that the intensity and duration of the nociceptive activity would be more than when each was carried out separately.

(3) It was not assumed that there is a direct relationship between the amount of tissue involved and the amount of nociceptive activity produced (Beecher, 1957).

(4) It was accepted that damage to different tissues may give rise to different amounts and types (e.g. somatic and visceral) of nociceptive activity.

(5) It was assumed that local anaesthetic would not always block all the activity from the tissues immediately, and that in some situations the action would persist for longer than one hour due to the occlusion of the blood supply by the rubber ring.

Using these principles it is clear that the most noxious procedure should be castration without local anaesthetic (CN). Then in descending order, castration plus local anaesthetic in the testes (CNLAT), this does however make the assumption that local anaesthetic will rapidly eliminate activity from the testes, this assumption may not be valid if the local anaesthetic has to diffuse long distances to reach the afferent axons or nociceptors in the testis. If all the activity from the testis is eliminated and all other activity remains this procedure should produce similar amounts of nociceptive activity to that produced by scrotal castration without local anaesthetic. If some testicular afferent activity remains in addition to nociceptive activity which could arise from the epididymis, vas deferens and peritoneum of the vaginal sac this procedure should be ranked higher than scrotal castration (SC). In both castration plus local anaesthetic distal to the ring (CNLADR), and scrotal castration plus local anaesthetic distal to the ring (SCLADR) the remaining sources of nociceptive activity should be the receptors in the skin and underlying tissues damaged by the rubber rings and the intact afferents to the CNS i.e. the skin proximal to the ring. Since following CNLADR there may be additional activity from the vaginal sac, epididymis and spermatic cords, it should be ranked above SCLADR but not above SC without local anaesthetic though this again assumes that the local anaesthetic penetrates to all tissues rapidly. The least noxious procedure is clearly control handling (C).

Plasma cortisol assays and presentation of results (experiment 1 and 2)

Cortisol concentrations in experiment 1 were measured using an IDS kit (IDS Ltd. Tyne and Wear) with lowest detectable concentration 7.8 ± 1.1 nmol/l and intra and inter assay coefficients of variation of 4.5 and 9.0% respectively. Cortisol concentrations in experiment 2 were measured using an Amerlex kit (Amersham, Amersham International plc, Amersham UK) with lowest detectable concentration 3.0 ± 0.9 nmol/l and intra and inter assay coefficients of variation of 11.7 and 8.8% respectively. Detailed methods are as described in general methods.

Where applicable the plasma cortisol concentrations were expressed as the mean ± the standard error of the mean (SEM) for the six lambs in each group. The analysis of variance was carried out covering time periods 15, 30 and 60 minutes, using the base cortisol level for each lamb as a covariate. A two by three and one by six treatment structure was used for experiment 1 and 2 respectively.

Experiment 1. (castration plus tailing (C/T) v control handling (C); control (Nil) v Local anaesthetic (LA) v Naloxone (Nx)).

Experiment 2. (Control (C) v Castration (CN) v Ring scrotum (RS) v Castration plus local anaesthetic in testes (CNLAT) v Ring scrotum plus local anaesthetic distal to ring (RSLADR) v Castration plus local anaesthetic distal to ring (CNLADR))

The main output of this analysis was the two by three and one by six tables of cortisol means and the appropriate standard error of difference (SED) to allow comparison of the means (obtained from the residual).

Behavioural monitoring and presentation of results (experiment 1 and 2)

Detailed description of the behavioural monitoring techniques and analysis of data using non parametric statistical tests is described in the general materials and methods chapter. In experiment 1 behavioural monitoring extended over a 4 hour period and the presence or absence of particular behaviours was noted for each lamb at specific times after treatment. The different behaviours were: lateral recumbency, ventral recumbency, standing and teat seeking or sucking. Behaviour was observed once every minute between one and 14 minutes, every two minutes between 17 and 29 minutes, every 5 minutes between 35 and 60 minutes, every ten minutes between 70 and 120 minutes, and every fifteen minutes between 135 and 240 minutes. Behavioural data was presented in bar chart form, the incidence of each behaviour pattern expressed as a percentage of the total number of observations during each period. In experiment 2 this time period was reduced to 3 hours. Most lambs return to near normal by this time as assessed by initial monitoring techniques and this allows more lambs to be investigated. Three severities of lateral recumbency, ventral recumbency and standing were were recorded as was restlessness, a quantitative measure of how often all lambs got up and lay down. Restlessness was reported between 1 and 15 minutes. Behaviour was observed once every minute between one and 14 minutes, every two minutes between 17 and 29 minutes, and at five minute intervals thereafter to the end of the experiment (180 minutes). Behaviour was presented as for experiment 1. A full description is given in the general methods.

RESULTS

EXPERIMENT 1 Effects of intravenous naloxone and complete local anaesthesia on behavioural and physiological indices following castration and tail-docking

Expt 1 Group 1- Behaviour of control lambs

The results are presented in Fig 3.1 and Tables 1 and 2. The behaviour of control lambs was consistent throughout the duration of the experiment (0-240 minutes), most lambs remaining in ventral recumbency (50-83% of observations). There was little evidence of teat seeking during the experiment (0-4% of observations) and only one time period (60-120 minutes) when a lamb lay in lateral recumbency.

Plasma cortisol concentrations

The results are presented in Fig 3.1 and Tables 1 and 2. The plasma cortisol concentration of control lambs increased from a basal level (22.4 ± 12.6 nmol/l) to a peak of 51.6 ± 13.2 nmol/l at 30 minutes, returning to basal levels by 60 minutes.

Expt 1 Group 2- Behaviour of lambs to which local anaesthetic had been administered

The results are presented in Fig 3.1 and Tables 1 and 2. Lambs following local anaesthesia lay in ventral recumbency more than control lambs from 0-240 minutes (74-88% of observations). This was significant from 120-240 minutes ($P<0.05$). One lamb lay in lateral recumbency from 60-120 minutes (3% of observations).

Plasma cortisol concentrations

The results are presented in Fig 3.1 and Tables 1 and 2. The plasma cortisol concentration of lambs to which local anaesthetic had been administered increased from a basal level of 35.3 ± 13.6 to a peak of 61.1 ± 19.8 nmol/l at 30 minutes, returning and falling to below pretreatment basal levels by 120 minutes (24.3 ± 5.3 nmol/l). Two further peaks were evident at 150 minutes (57.7 ± 16.8 nmol/l) and 240 minutes (53.2 ± 11.9 nmol/l). The plasma cortisol response in group 2 was not significantly different from control lambs when compared from 15-60 minutes ($P<0.05$).

Expt 1 Group 3- Behaviour of lambs to which local anaesthetic had been administered prior to castration and tail-docking

The results are presented in Fig 3.1 and Tables 1 and 2. Administration of local anaesthetic prior to castration eliminated the lateral recumbency associated with this procedure and restored the incidence of ventral recumbency to near control levels from 0-120 minutes, see group 4. Lateral recumbency was only recorded twice in this group from 120-180 minutes (8% of observations). These lambs lay more than control lambs from 0-240 minutes (72-88% of observations). This was significant from 120-180 minutes ($P<0.05$).

Plasma cortisol concentrations

The results are presented in Fig 3.1 and Tables 1 and 2. The plasma cortisol response of lambs to which local anaesthetic had been administered prior to castration and tail-docking increased from a basal level (29.1 ± 10.6) to a peak of 53.0 ± 8.5 nmol/l at 30 minutes, returning to basal levels by 60 minutes. The plasma cortisol response of group 3 lambs was not significantly different from control lambs when compared from 15-60 minutes ($P<0.05$).

Expt 1 Group 4- Behaviour of lambs castrated and tail-docked

The results are presented in Fig 3.1 and Tables 1 and 2. Lambs castrated and tail-docked lay in lateral recumbency from 0-120 minutes (22-58% of observations) and 180-240 minutes (4% of observations). This was significant when compared with control lambs from 0-60 minutes ($P<0.05$). As a consequence of the lateral lying, lambs in this group lay less in ventral recumbency from 0-60 minutes than control lambs ($P<0.05$). From 30 minutes these lambs showed progressively more ventral recumbeny until 180 minutes. From 120 to 240 minutes they also lay more in ventral recumbency (71-79% of observations) than control lambs. This was significant from 120-180 minutes ($P<0.05$). Group 4 lambs stood less (14-35% of observations) than control lambs throughout the duration of the experiment (0-240 minutes). This was significant from 120-180 minutes ($P<0.05$).

Plasma cortisol concentrations

The results are presented in Fig 3.1 and Tables 1 and 2. The plasma cortisol concentration of castrated and tail-docked lambs increased rapidly from basal levels (17.3 ± 8.4 nmol/l) to a peak of 142.5 ± 15.9 nmol/l at 30 minutes, returning to basal levels by 90 minutes. From 90 to 180 minutes the plasma cortisol concentration continued to fall, to a new basal level of 8.3 ± 2.4 nmol/l. The increase in plasma cortisol was significantly greater than that in control lambs and that in lambs to which local anaesthetic had been administered either with or without castration and tail-docking, when compared from 15-60 minutes ($P<0.05$).

Expt 1 Group 5- Behaviour of lambs to which naloxone had been administered (0.2mg/kg I/V)

The results are presented in Fig 3.2 and Tables 1 and 2.

Lambs to which naloxone had been administered, minutes lay predominantly in ventral recumbency from 30-240 minutes (58-83% of observations). These lambs lay more in ventral recumbency and stood less than control lambs from 120-240 minutes ($P<0.05$). Throughout the experiment (0-240 minutes) occasional teat seeking (0-15% of observations) and lateral recumbency (2-11% of observations) occurred.

Plasma cortisol concentrations

The results are presented in Fig 3.2 and Tables 1 and 2. The plasma cortisol concentration of lambs to which naloxone had been administered increased from basal levels (7.9 ± 6.7 nmol/l) to a peak of 65.2 ± 15.6 nmol/l at 30 minutes, returning to basal levels by 60 minutes. The plasma cortisol concentration increased from 60 minutes to 31.8 ± 16.5 nmol/l at 90 minutes, followed by a slow return to basal levels by 240 minutes. The plasma cortisol response of group 5 lambs was not significantly different from control lambs when compared from 15-60 minutes ($P<0.05$).

Expt 1 Group 6- Behaviour of lambs to which naloxone had been administered (0.2mg/kg i.v.) prior to castration & tail-docking

The results are presented in Fig 3.2 and Tables 1 and 2. Lambs to which naloxone had been administered prior to castration and tail-docking lay more in lateral recumbency (64-86% of observations) than lambs castrated and tail-docked without pretreatment with naloxone from 0-60 minutes. This was significant from 30-60 minutes ($P<0.05$). Lambs in group 6 lay less in ventral recumbency than control lambs from 0-60 minutes ($P<0.05$), after which the incidence increased. From 120-240 minutes these lambs lay in ventral recumbency (79-88% of observations) more than control lambs. This was significant from 120-180 minutes ($P<0.05$).

Plasma cortisol concentrations

The results are presented in Fig 3.2 and Tables 1 and 2. The plasma cortisol concentration of lambs to which naloxone had been administered prior to castration and tail-docking increased rapidly from basal levels (7.6 ± 7.8 nmol/l) to a peak of 151.1 ± 11.1 nmol/l at 30 minutes, returning to basal levels by 150 minutes. The plasma cortisol response of group 6 lambs although greater than, was not significantly different from that in in lambs castrated and tail-docked without naloxone pretreatment when compared from 15-60 minutes ($P<0.05$). The plasma cortisol response of these lambs was, however, significantly greater than that in control lambs, and those to which naloxone had been administered when compared over the same time ($P<0.05$).

Plasma cortisol concentrations

Experiment 1. Mean plasma cortisol concentrations nmol/l for the six groups.

	DRUG	NIL	LA	Nx
Procedure				
C		34.8	30.9	43.1
C/T		97.0	34.8	105.5

Thus for an SED of 14.51, and using a two way T-test, 5% significance is obtained with any difference greater than 64.2 nmol/l between the mean treatment cortisol concentrations tabulated above. Note that the plasma cortisol response of lambs to which local anaesthetic had been administered prior to castration and tail-docking (C/T-LA) was not significantly different ($P>0.05$) from control lambs (C-Nil). Note also that although the plasma cortisol response of lambs to which naloxone (0.2mg/kg) had been administered prior to castration and tail-docking (C/T-Nx) was greater than that recorded for castrated and tail-docked lambs (C/T-Nil), this difference was not significant ($P>0.05$).

EXPERIMENT 2 Effects of partial local anaesthesia on behavioural and physiological indices following castration and scrotal castration

Expt 2 Group 1- Behaviour of control lambs

The results are presented in Fig 3.3 and Tables 3 and 4. Control lambs lay predominantly in ventral recumbency throughout the duration of the experiment (55-83% of observations), furthermore most remained with their eyes open (50-72% of observations). Uncharacteristically some lambs lay in abnormal ventral recumbency (V3) from 30-60 minutes and from 90-180 minutes, this represented 8-12% of observations. There was little evidence of restlessness from 0-15 minutes (8 posture changes).

Plasma cortisol concentrations

The results are presented in Fig 3.3 and Tables 3 and 4. The plasma cortisol concentration in control lambs remained within 22 nmol/l of the basal level (45.2 ± 25.8 nmol/l) throughout the duration of the experiment (0-180 minutes).

Expt 2 Group 2- Behaviour of lambs castrated with tight rubber rings

The results are presented in Fig 3.3 and Tables 3 and 4. Castrated lambs lay in lateral recumbency (L1-L2) from 0-90 minutes (8-56% of observations), a behaviour which was not observed in control lambs or lambs subjected to scrotal castration with or without local anaesthetic. This difference was significant from 0-60 minutes ($P<0.05$). There was an associated decrease in ventral recumbency when compared with control lambs from 0-60 minutes (30-45% of observations). This approached significance from 0-15 minutes ($P<0.1$). Lambs in group 2 also stood less (10-25% of observations) when compared with control lambs in all time periods except 90-120 minutes. This was significant from 15-30 minutes and 60-90 minutes ($P<0.05$). Abnormal standing (S2-S3, 10-25% of observations) and abnormal ventral recumbency (V3, 18-46% of observations) was present throughout the experiment (0-180 minutes). Castrated lambs stood less than lambs subjected to scrotal castration from 0-60 minutes. This was significant from 15-30 minutes ($P<0.05$). When the main behavioural categories of the castration group are compared with the castration and local anaesthetic in testes group, there are no significant differences except for the increased incidence of ventral recumbency (94% of observations) in the latter group from 60-90 minutes, which approaches significance at $P<0.1$. Castrated lambs stood less than lambs administered local anaesthetic distal to the ring, from 0-180 minutes. This was significant from 15-30 and 60-90 minutes ($P<0.05$). There was evidence of substantial restlessness from 0-15 minutes (208 posture changes) which was significantly greater ($P<0.05$) than recorded in the control, castration and local anaesthetic in the testes and scrotal castration and local anaesthetic distal to the ring groups.

Plasma cortisol concentrations

The results are presented in Fig 3.3 and Tables 3 and 4. The plasma cortisol concentration of castrated lambs increased rapidly from a basal level of 42.2 ± 10.4 nmol/l to a peak of 231.3 ± 31.5 nmol/l at 60 minutes, returning to basal levels by 120 minutes. The plasma cortisol response of group 2 was not significantly different from that of castrated lambs with local anaesthetic injected into either the testes or distal to the ring when compared from 15-60 minutes. The plasma cortisol response of this group was, however, significantly greater

(P<0.05) when compared with the remaining groups over the same time.

Expt 2 Group 3- Behaviour of lambs following scrotal castration

The results are presented in Fig 3.3 and Tables 3 and 4. Following scrotal castration, lambs adopt progressively more ventral recumbency, and by 60-90 minutes the incidence (94% of observations) is significantly greater than control lambs (P<0.05). From 0-180 minutes lambs in group 3 stand in an abnormal manner (S2-S3, 3-48% of observations), and from 0-30 minutes almost all standing is abnormal. From 0-180 minutes lambs in group 3 also lie in abnormal ventral recumbency (V3, 3-14% of observations), the incidence decreases throughout the course of the experiment. There was evidence of substantial restlessness from 0-15 minutes (200 posture changes) which was significantly greater (P<0.05) than that recorded in the control and scrotal castration and local anaesthetic distal to the ring groups.

Plasma cortisol concentrations

The results are presented in Fig 3.3 and Tables 3 and 4. The plasma cortisol concentrations of lambs subjected to scrotal castration rose from a basal level of 15.7 ± 5.6 nmol/l to a peak value of 107.2 ± 14.5 at 30 minutes, returning to basal levels by 90 minutes. The plasma cortisol response of group 3 was significantly lower than that produced in lambs castrated with or without local anaesthetic administration (P<0.05) when compared from 15-60 minutes. The plasma cortisol response of this group was, however, greater than that produced in control lambs over the same time (approached significance at P<0.05).

Expt 2 Group 4- Behaviour of lambs following castration with local anaesthetic in the testes

The results are presented in Fig 3.4 and Tables 3 and 4. Lambs castrated with local anaesthetic in the testes lie in lateral recumbency from 0-60 minutes (12-45% of observations), a behaviour which was not seen in control lambs. When compared with control lambs this difference was significant from 15 to 30 minutes ($P<0.05$). The incidence of ventral recumbency in group 4 lambs increased from the 15-30 minute period remaining the predominant behaviour until 180 minutes (59-94% of observations). When compared with control lambs there was more ventral recumbency from 60-180 minutes. This was significant from 60-90 minutes ($P<0.05$). Group 4 lambs lay in abnormal ventral recumbency (V3) from 0-180 minutes, with the highest incidence from 0-90 minutes (25-55% of observations). These lambs also stood abnormally (S1-S2) from 0-180 minutes (6-30% of observations). The lambs were restless from 0-15 minutes (120 posture changes) which was significantly greater than that recorded for control lambs ($P<0.05$).

Plasma cortisol concentrations

The results are presented in Fig 3.4 and Tables 3 and 4. The plasma cortisol concentrations of lambs castrated with local anaesthetic in the testes rises rapidly from a basal level of 39.6 ± 10.8 nmol/l to a peak of 254.2 ± 18.4 nmol/l at 30 minutes, returning to basal levels by 150 minutes. When compared to the plasma cortisol response in lambs castrated with or without local anaesthetic distal to the ring, there are no significant differences in the period from 15-60 minutes ($P>0.05$). The plasma cortisol response in group 4 lambs was, significantly greater than that in control lambs, and those subjected to scrotal castration with or without local anaesthetic when compared from 15-60 minutes ($P<0.05$).

Expt 2 Group 5- Behaviour of lambs subjected to scrotal castration with local anaesthetic distal to the rubber ring

The results are presented in Fig 3.4 and Tables 3 and 4. Lambs subjected to scrotal castration plus local anaesthetic distal to the ring lay predominantly in ventral recumbency from 0-180 minutes (57-83% of observations). There was evidence of abnormal ventral recumbency (8-30% of observations) which declines from the start of the experiment to 120 minutes. There was also evidence of abnormal standing (S2-S3) from 0-120 minutes. The majority of the abnormal standing occurred in the first 30 minutes (25-31% of observations). These lambs stood less and lay more from 15-30 minutes than lambs subjected to scrotal castration without local anaesthetic distal to the rubber ring, the incidence was reversed by 60-90 minutes. These differences approach significance at $P<0.1$. There was some evidence of restlessness in these lambs from 0-15 minutes (65 posture changes), which was not, however, significantly different ($P<0.05$) from that recorded for control lambs.

Plasma cortisol concentrations

The results are presented in Fig 3.4 and Tables 3 and 4. The plasma cortisol concentrations of lambs subjected to scrotal castration and local anaesthetic distal to the ring increased from a basal level of 37.2 ± 11.7 nmol/l to a peak of 238.1 ± 25.2 nmol/l by 30 minutes, returning to basal levels by 60 minutes. The plasma cortisol response of group 5 lambs when compared with the response of lambs castrated with or without local anaesthetic from 15-60 minutes was significantly smaller ($P<0.05$). When the plasma cortisol response of group 5 lambs was compared with that of control lambs, and of those subjected to scrotal castration without local anaesthetic from 15-60 minutes there was no significant difference ($P>0.05$).

Expt 2 Group 6- Behaviour of lambs castrated with local anaesthetic distal to the rubber ring

The results are presented in Fig 3.4 and Tables 3 and 4. Lambs castrated with local anaesthetic distal to the ring, spent most time in ventral recumbency (64-69% of observations) after the first 30 minutes. Abnormal ventral recumbency (V3) occured from 0-180 minutes, though most was confined to the first 30 minutes (22-28% of observations). There was also abnormal standing from 0-120 minutes (8-48% of observations). When the incidence of the main categories of behaviour were compared with control lambs, however, there were no statistical differences (P>0.05). Occasional lateral recumbency was observed from 1-15 minutes and 30-60 minutes, which was significantly less than was recorded in lambs following castration from 0-60 minutes (P<0.05). There was also more standing from 15-30 minutes (55% of observations) and 60-90 minutes (36% of observations), when compared with both the castration group, and those castrated with local anaesthetic in the testes (P<0.05). These lambs lay less from 60-90 minutes when compared to lambs castrated with local anaesthetic in the testes (P<0.05). The lambs were restless from 0-15 minutes (145 posture changes) which was significantly greater (P<0.05) than that recorded for control lambs, and the difference approached significance when compared with lambs subjected to scrotal castration with local anaesthetic distal to the ring.

Plasma cortisol concentrations

Results are presented in Fig 3.4 and Tables 3 and 4. The plasma cortisol concentrations of lambs castrated with local anaesthetic distal to the ring increased rapidly from a basal level of 37.2 ± 11.7 nmol/l to a peak of 238.1 ± 25.2 nmol/l, returning to basal levels by 120 minutes. The plasma cortisol response in group 6 lambs was not significantly different from those of lambs castrated with or without local anaesthetic in the testes when compared from 15-60 minutes ($P>0.05$). The plasma cortisol response of these lambs was, however, greater than that of control lambs or those of lambs subjected to scrotal castration either with or without local anaesthetic when compared over the same time ($P<0.05$).

Experiment 2. Mean plasma cortisol concentrations nmol/l for the six groups.

Procedure	C	CN	RS	CNLAT	RSLADR	CNLADR
	31.5	186.6	90.1	197.1	67.8	193.7

Thus for a SED of 31.5, and using a two-way T-test, 5%, 10% and 20% significance is obtained with any difference greater than 64.2, 53.6 and 40.9 nmol/l respectively between the mean treatment cortisol concentrations tabulated above. Note that the plasma cortisol responses of lambs castrated with (CNLAT, CNLADR) or without (CN) local anaesthetic treatments following application of the ring were not significantly different ($P>0.05$). Note also that the cortisol responses of lambs subjected to scrotal castration with (RSLADR) or without (RS) local anaesthetic treatments following application of the ring were significantly ($P<0.05$) lower than the plasma cortisol responses observed in the three castration groups. Although these two groups (RS and RSLADR) were not statistically different when compared ($P>0.05$), the plasma cortisol response of lambs subjected to scrotal castration (RS) was, however, significantly greater ($P<0.05$) than that in control lambs (C).

Expt 2 Further comparison of behaviour of the castration with local anaesthetic distal to the ring (CNLADR) and scrotal castration (SC) groups based on the total time (minutes) spent by each lamb in each of the nine postures (L1-L3, S1-S3, V1-V3)

The approach adopted thus far to analyse behaviour, has been to compare the incidence of a particular category of behaviour (lateral recumbency, ventral recumbency and standing) between two groups for a given time period (e.g. 0-15 minutes). An alternative approach is to compare between groups the total time spent in each of the nine postures (L1, L2, L3, V1, V2, V3, S1, S2 and S3) or the three categories of behaviour over the entire course of the experiment. Results are presented using this alternative approach in Table 3.5 and 3.6. Lambs subjected to scrotal castration spend more time in ventral recumbency (788 minutes) than standing (280 minutes). There was no incidence of lateral recumbency. 19% of the time was spent in abnormal postures (201 minutes), of which 11%, 6% and 2% was spent in S2, S3 and V3 respectively. Lambs subjected to castration with local anaesthetic distal to the ring also spent more time in ventral recumbency (680 minutes) than either standing (377 minutes) or lateral recumbency (11 minutes). 25% of the time was spent in abnormal postures (257 minutes), of which 14%, 10%, and 1% was spent in S2, V3 and L1 respectively. When the incidence of the three main postures (lateral recumbency, ventral recumbency and standing) and the nine categories (L1-L3, S1-S3, V1-V3) were compared for the CNLADR and SC groups no significant differences were found ($P>0.05$). The largest differences between these groups were the greater incidence of normal standing (S1, 228 minutes) and abnormal ventral recumbency (V3, 108 minutes) in the CNLADR which were significant at $P<0.2$ and $P<0.3$ respectively.

FIGURE 3.1

The effect of regional local anaesthetic on the indices used for assessment of pain from castration and tail-docking

The results shown were obtained from 4 groups (2 groups are common to Fig 3.1 and 3.2) of 6 lambs, each group was subjected to one of the following treatments 1) control handling 2) local anaesthesia 3) local anaesthesia and castration and tail-docking 4) castration and tail-docking

Behaviour

The incidence (%) of four behaviours (L= Lateral recumbency, S= Standing, V= Ventral recumbency, T/S= Teat seeking or Sucking) were recorded for each group and presented in bar chart form in 6 time periods; 0-15, 15-30, 30-60, 60-120, 120-180 and 180-240 minutes, see materials and methods for numbers of observations in each period.

Plasma cortisol

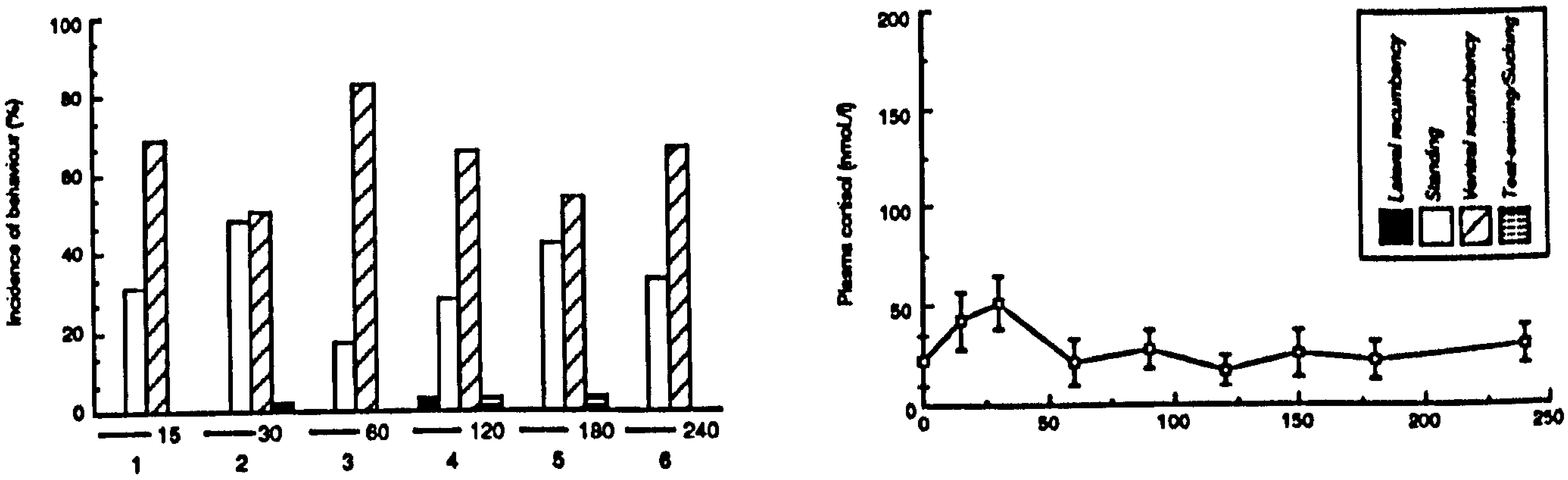
Plasma cortisol concentrations were presented in graph form (nmol/l ± S.E,(n=6)) at 9 times; 0, 15, 30, 60, 90, 120, 150, 180 and 240 minutes.

Statistical analysis

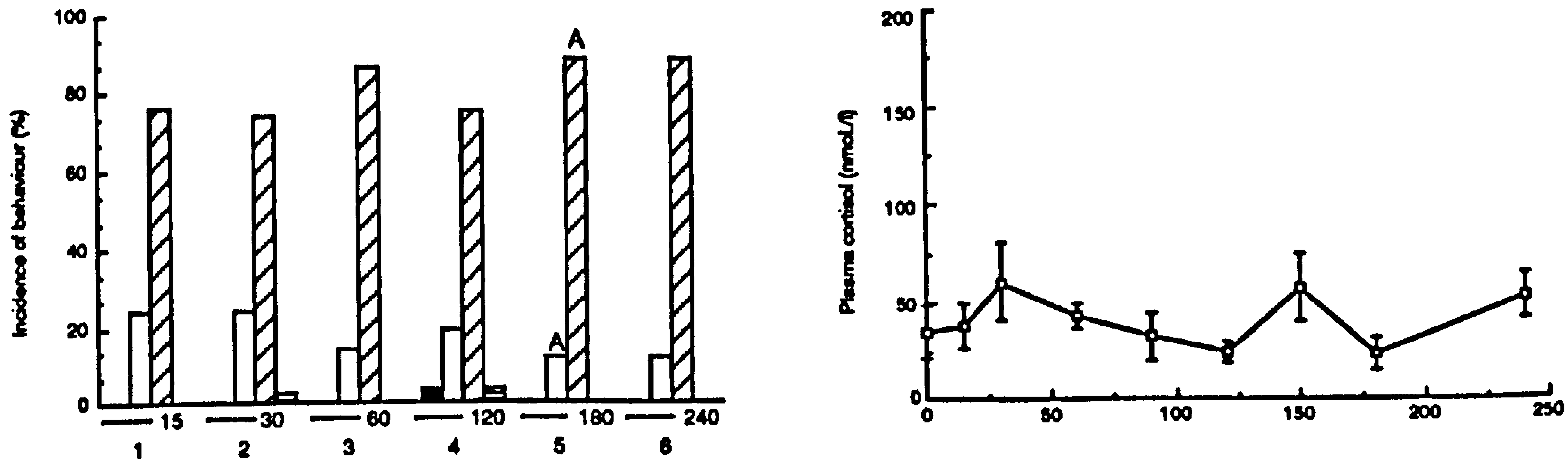
A Indicates that the difference between the behaviour of the treatment group and the control handling group was significant at ($P<0.05$).

Notes 1) Administration of local anaesthetic eliminates the lateral recumbency shown by the (castration and tail-docking) group and also eliminates the increase in plasma cortisol concentration ($P<0.05$), when compared with lambs castrated and tail-docked without local anaesthetic.

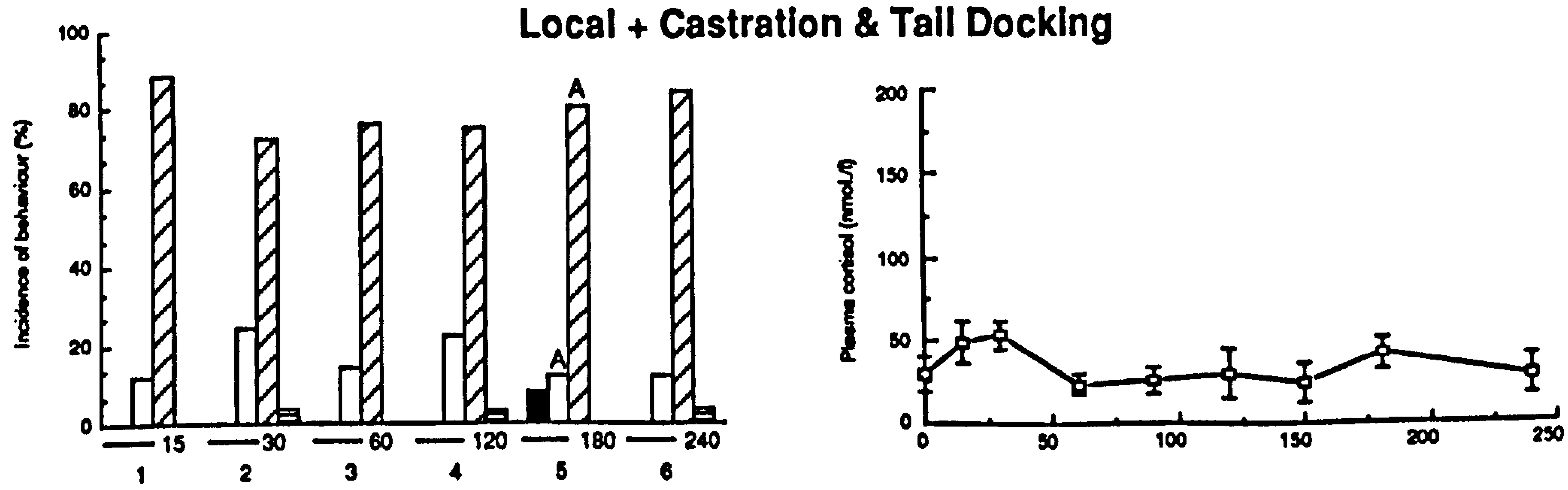
Control Handling



Local Anaesthesia



Local + Castration & Tail Docking



Castration & Tail Docking

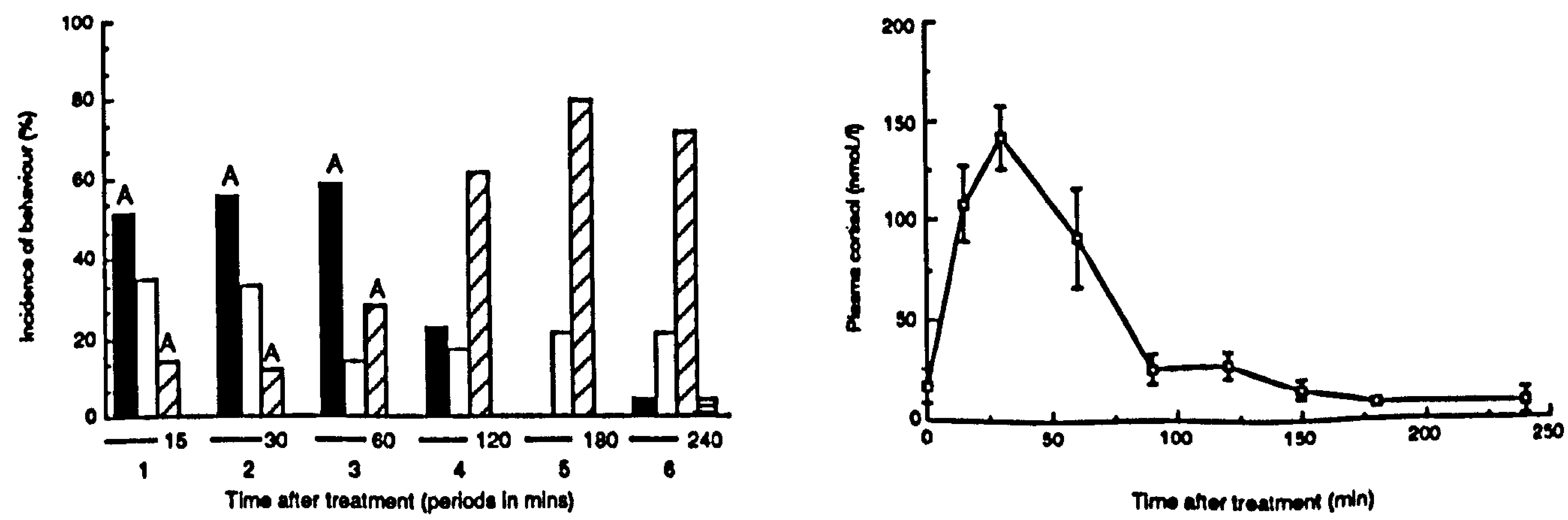


FIGURE 3.2

Effect of intravenous naloxone on the indices used for assessment of pain from castration and tail-docking

The results shown were obtained from 4 groups of 6 lambs, each group was subjected to a different treatment a) control handling b) intravenous naloxone c) naloxone + castration and tail-docking d) castration and tail-docking

Behaviour

The incidence (%) of four behaviours (L- Lateral recumbency, S- Standing, V-Ventral recumbency, T/S- Teat seeking and Sucking) were recorded for each group and presented in bar chart form in 6 time periods; 0-15, 15-30, 30-60, 60-120, 120-180 and 180-240 minutes, see materials and methods for numbers of observations in each period.

Plasma cortisol

Plasma cortisol concentrations were presented in graph form (nmol/l ± S.E, (n=6)) at 9 times; 0, 15, 30, 60, 90, 120, 150, 180 and 240 minutes.

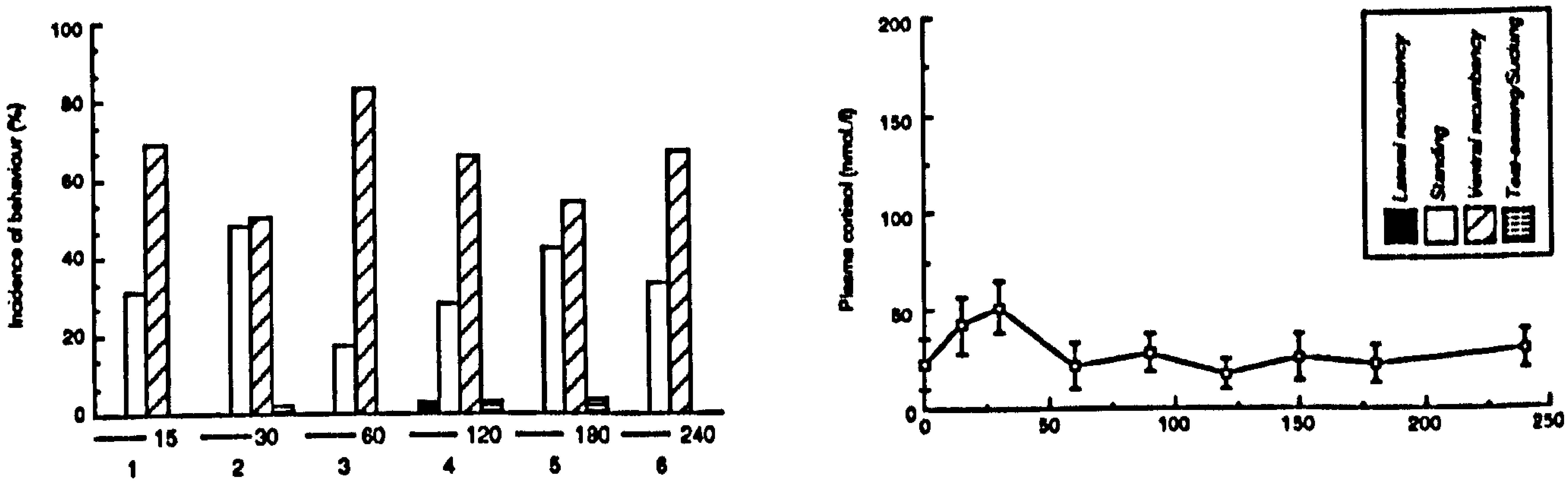
Statistical analysis

A Indicates that the difference between the behaviour of the treatment group and the (control handling) group was significant at ($P<0.05$).

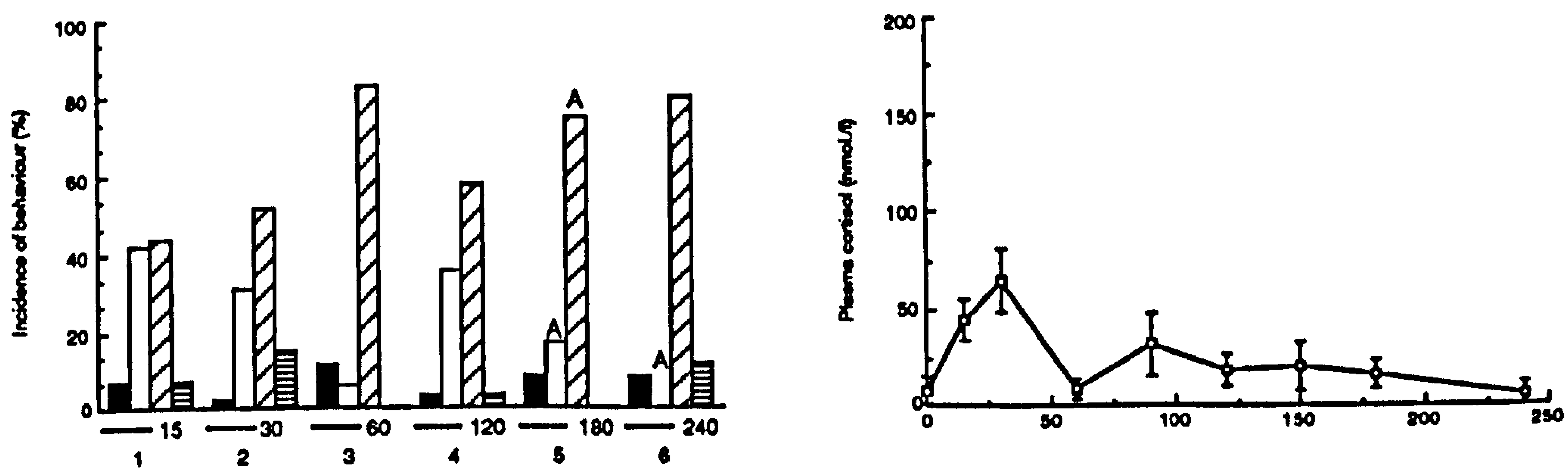
C Indicates that the difference between the behaviour of the (naloxone + castration and tail-docking) group and the (castration and tail-docking) group was significant at ($P<0.05$)

Notes 1) Intravenous naloxone increases the incidence of lateral recumbency after castration and tail-docking, this was significant ($P<0.05$) for the period 30-60 minutes. No significant difference ($P<0.05$) was found in the cortisol responses

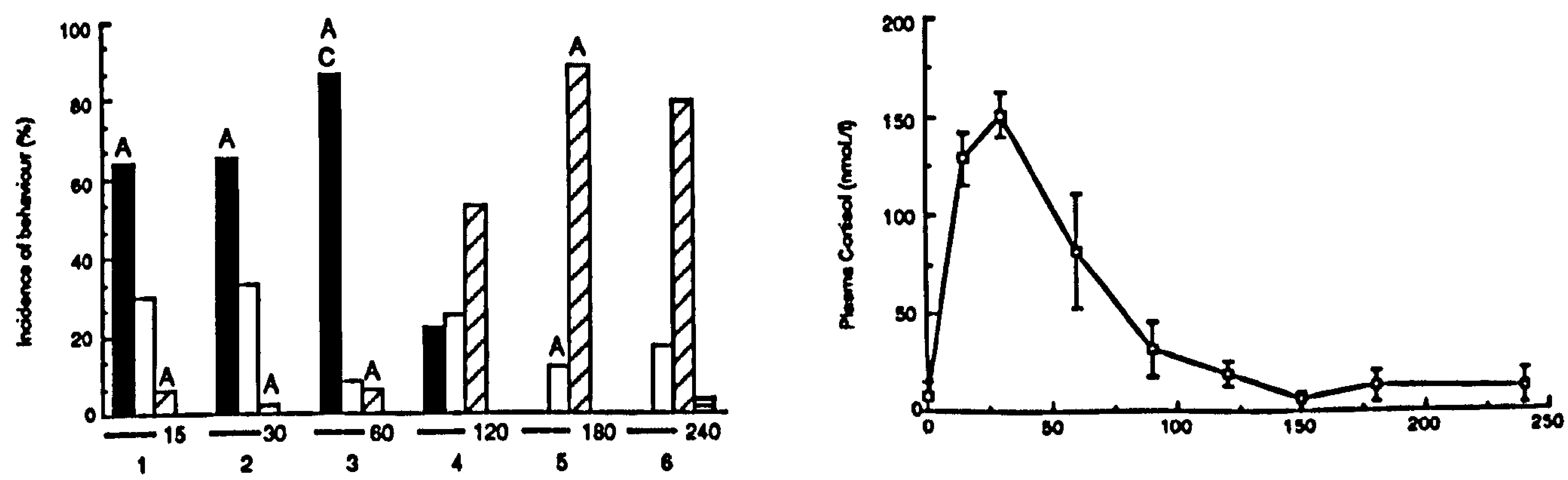
Control Handling



Intravenous Naloxone



Naloxone + Castration & Tail Docking



Castration & Tail Docking

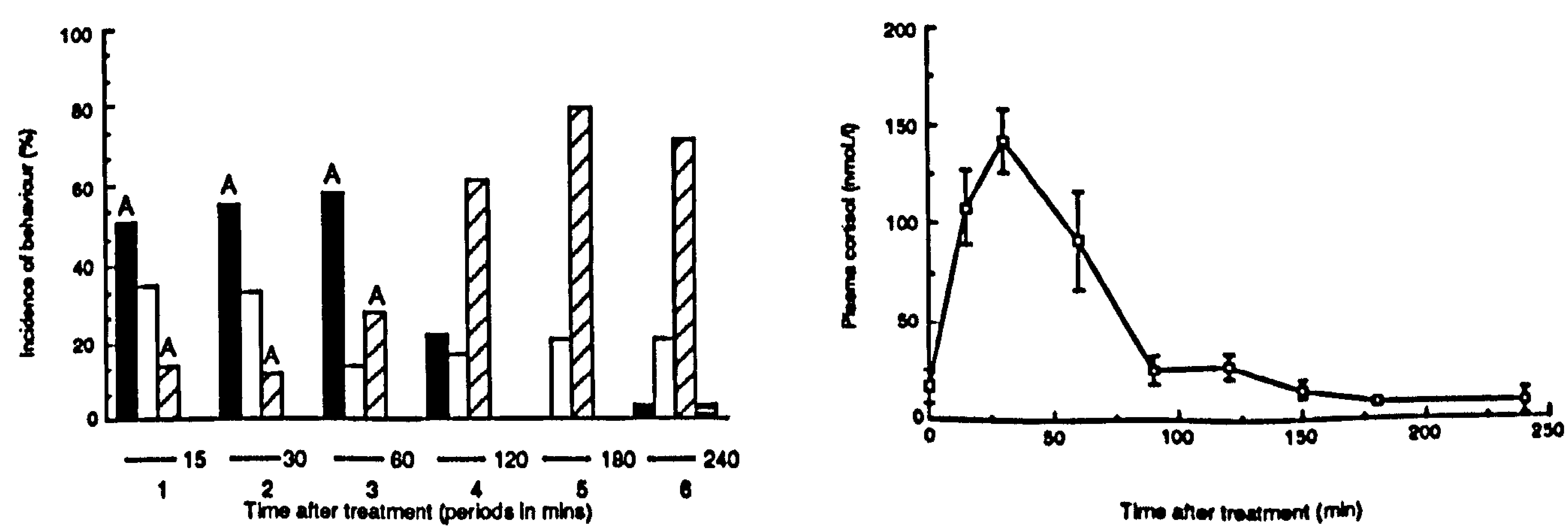


TABLE 3.1

THE EFFECTS OF SIX TREATMENTS ON LAMB BEHAVIOUR IN SIX TIME PERIODS

In each period after treatment the incidence of the four postures (i.e. Lateral Recumbency, Standing, Ventral Recumbency and Teat Seeking or Sucking) are presented as the number of times that behaviour is observed, and as a percentage of the total number of observations made in that period.

TABLE 3.1 Incidence of four lamb behaviours in six periods after six treatments.

PERIOD AFTER TREATMENT	0 - 15 mins				15 - 30 mins				30 - 60 mins				50 - 120 mins				120 - 180 mins				180 - 240 mins			
NUMBER OF OBSERVATIONS MADE IN PERIOD	n = 84				n = 42				n = 36				n = 36				n = 24				n = 24			
BEHAVIOUR OBSERVED	LR	St	VR	TS/S	LR	St	VR	TS/S	LR	St	VR	TS/S	LR	St	VR	TS/S	LR	St	VR	TS/S	LR	St	VR	TS/S
CONTROL HANDLING (6 lambs)	0 0%	26 31%	58 59%	0 0%	0 0%	20 48%	21 50%	1 2%	0 0%	6 17%	30 83%	0 0%	1 3%	10 28%	24 66%	1 3%	0 0%	10 42%	13 54%	1 4%	0 0%	8 33%	16 67%	0 0%
CASTRATION AND TAIL DOCKING (6 lambs)	43 51%	29 35%	12 14%	0 0%	23 55%	14 33%	5 12%	0 0%	21 58%	5 14%	10 28%	0 0%	8 22%	6 17%	22 61%	0 0%	0 0%	5 21%	19 79%	0 0%	1 4%	5 21%	17 71%	1 4%
LOCAL ANAESTHESIA (6 lambs)	0 0%	20 24%	64 76%	0 0%	0 0%	10 24%	31 74%	1 2%	0 0%	5 14%	31 86%	0 0%	1 3%	7 19%	27 75%	1 3%	0 0%	3 12%	21 88%	0 0%	0 0%	3 12%	21 88%	0 0%
LOCAL ANAESTHESIA AND CASTRATION AND TAIL DOCKING (6 lambs)	0 0%	10 12%	74 88%	0 0%	0 0%	10 24%	30 72%	2 4%	0 0%	5 14%	31 86%	0 0%	0 0%	8 22%	27 75%	1 3%	2 8%	3 12%	19 80%	0 0%	0 0%	3 12%	20 84%	1 4%
NALOXONE 0.2mg/Kg (INTRAVENOUS) (6 lambs)	6 7%	35 42%	37 44%	6 7%	1 2%	13 31%	22 52%	6 15%	4 11%	2 6%	30 83%	0 0%	1 3%	13 36%	21 58%	1 3%	2 8%	4 17%	18 75%	0 0%	2 8%	0 0%	19 80%	3 12%
NALOXONE 0.2mg/Kg AND CASTRATION AND TAIL DOCKING (6 lambs)	54 64%	25 30%	5 6%	0 0%	27 65%	14 33%	1 2%	0 0%	31 86%	3 8%	2 6%	0 0%	8 22%	9 25%	19 53%	0 0%	0 0%	3 12%	21 88%	0 0%	0 0%	4 17%	19 79%	1 4%

LR Lateral Recumbency
St Standing or Walking
VR Ventral Recumbency
TS/S Teat Seeking or Sucking

TABLE 3.2 Plasma cortisol measurements taken at 9 times after treatment for 6 different treatment groups

TIME AFTER TREATMENT (mins)	0	15	30	60	90	120	150	180	240
NUMBER OF MEASUREMENTS (No. of lambs)	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
CONTROL HANDLING Plasma cortisol (n.mol/1) Mean ± S.E.	22.4 ± 12.6	42.5 ± 14.6	51.6 ± 13.2	21.3 ± 11.6	27.6 ± 9.7	16.8 ± 7.5	25.6 ± 11.8	22.4 ± 9.7	30.0 ± 9.9
CASTRATION AND TAIL DOCKING Plasma cortisol (n.mol/1) Mean ± S.E.	17.3 ± 8.4	108.3 ± 18.9	142.5 ± 15.9	92.0 ± 24.6	25.9 ± 7.3	27.7 ± 6.7	15.5 ± 4.5	8.3 ± 2.4	8.7 ± 6.2
LOCAL ANAESTHESIA ONLY Plasma cortisol (n.mol/1) Mean ± S.E.	35.3 ± 13.6	38.3 ± 11.7	61.1 ± 19.8	43.6 ± 6.6	32.7 ± 12.0	24.3 ± 5.3	57.7 ± 16.8	23.9 ± 8.6	53.2 ± 11.9
LOCAL ANAESTHESIA AND CASTRATION AND TAILING Plasma cortisol (n.mol/1) Mean ± S.E.	29.1 ± 10.6	48.3 ± 12.5 (n = 5)	53.0 ± 8.5 (n = 5)	23.3 ± 5.7	25.1 ± 7.6	29.7 ± 14.4	24.2 ± 11.4	41.7 ± 9.6	28.5 ± 11.8
INTRAVENOUS NALOXONE 0.2mg/Kg^{-1} Plasma cortisol (n.mol/1) Mean ± S.E.	7.9 ± 6.7	45.1 ± 10.8	65.2 ± 15.6	8.7 ± 5.3	31.8 ± 16.5	18.3 ± 8.6	20.2 ± 13.5	16.1 ± 7.8	5.4 ± 6.1
INTRAVENOUS NALOXONE AND CASTRATION AND TAIL DOCKING Plasma cortisol (n.mol/1) Mean ± S.E.	7.6 ± 7.8	129.0 ± 13.0	151.1 ± 11.1	82.1 ± 28.7	32.0 ± 14.4	19.0 ± 6.5	6.9 ± 2.9	12.6 ± 8.2	11.8 ± 9.1

FIGURE 3.3 and 3.4

The effect of 6 treatments, proposed to represent 6 different levels of noxious stimulus on the indices used for the assessment of pain

The results shown were obtained from 6 groups of 6 lambs, each group was subjected to a different treatment 1) control handling 2) castration 3) scrotal castration 4) castration + local anaesthetic in the testes 5) scrotal castration + local anaesthetic distal to the ring 6) castration + local anaesthetic distal to the ring

Behaviour

The incidence (%) of three behaviours (L-Lateral recumbency (1-3) , S- Standing (1-3) , V- Ventral recumbency (1-3)) were recorded for each group and are presented in bar chart form in 6 time periods; 0-15, 15-30, 30-60, 60-90, 90-120 and 120-180 minutes, see materials and methods for numbers in each period.

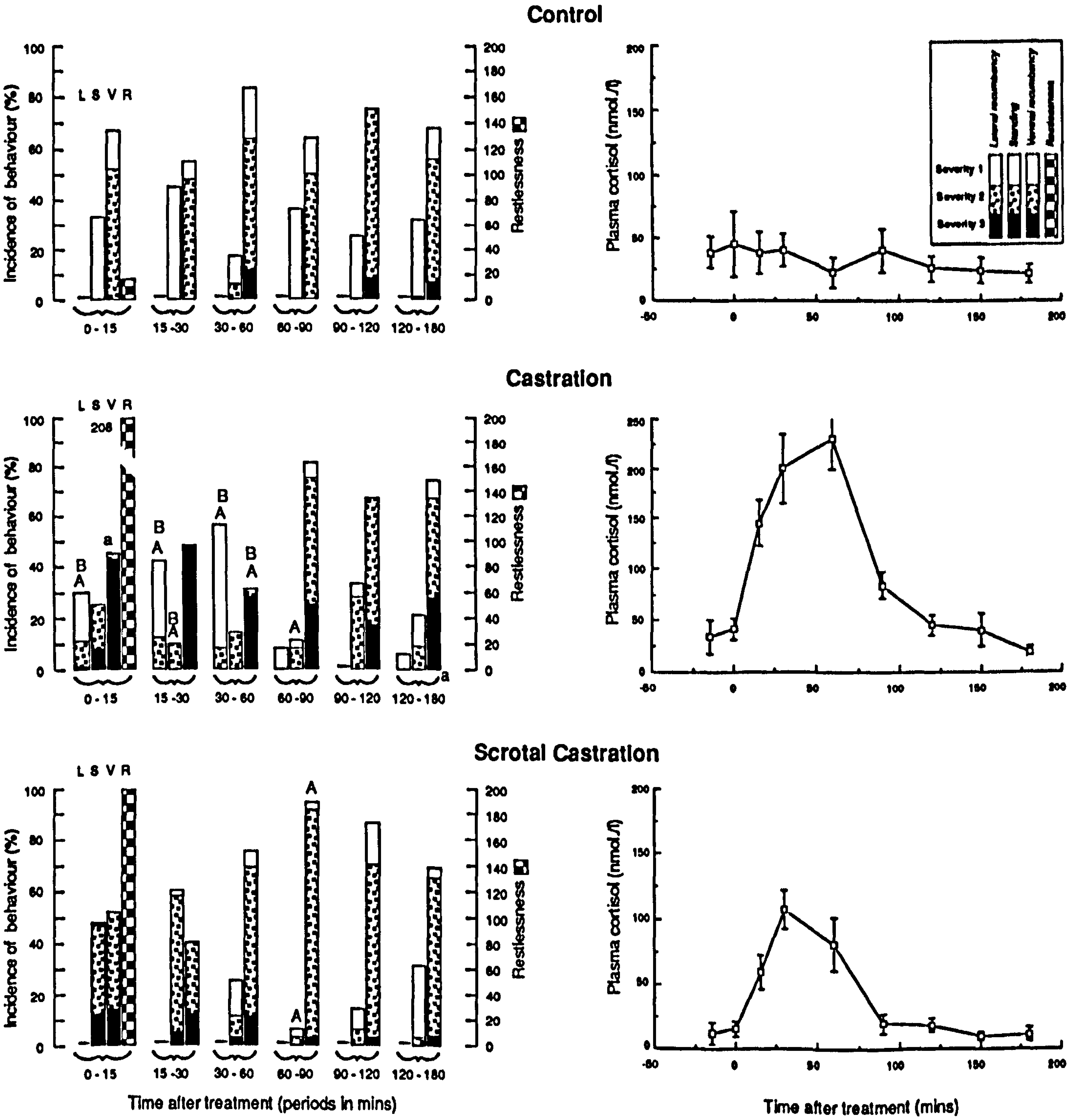
Plasma cortisol

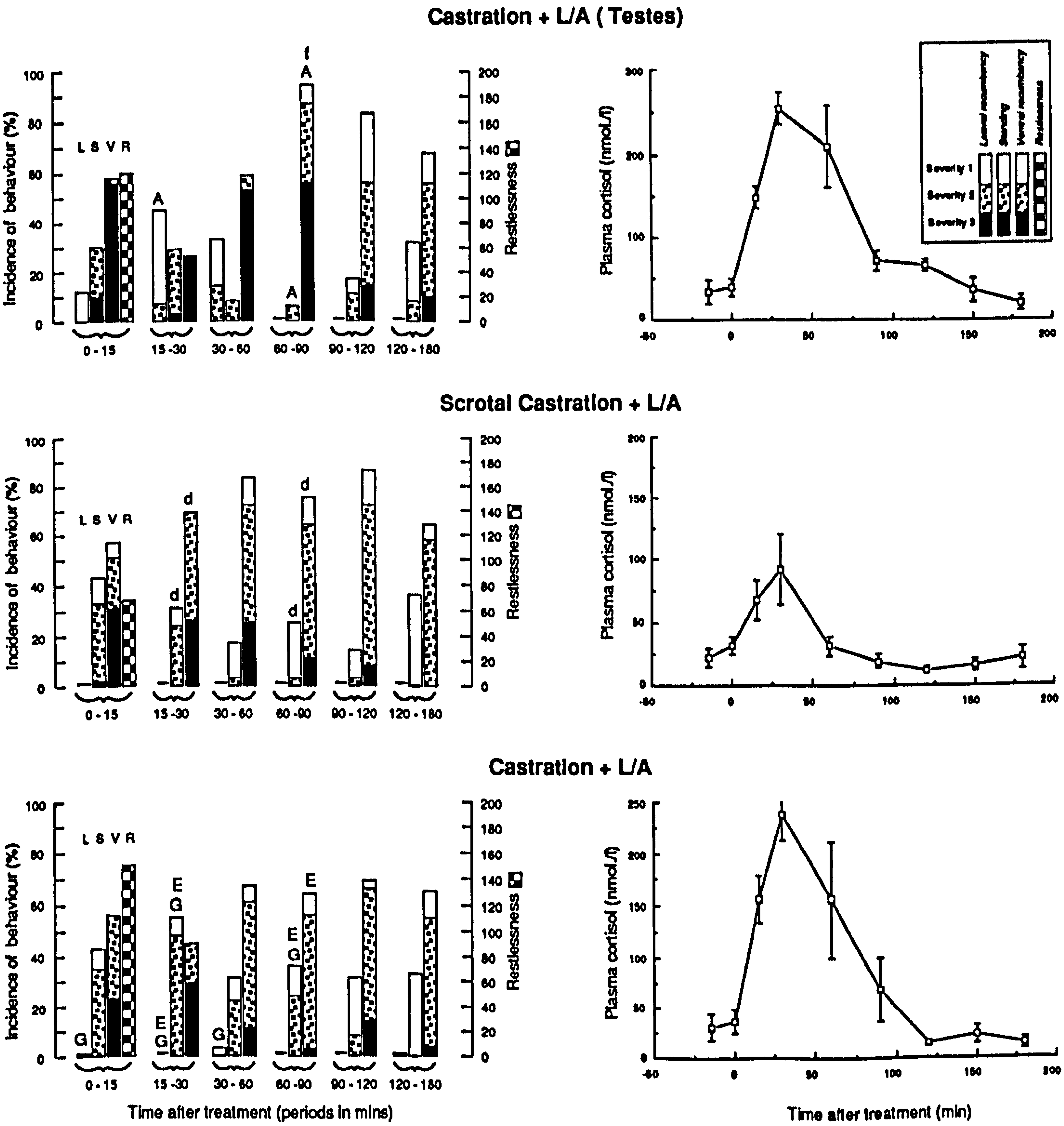
Plasma cortisol concentrations are presented in graph form (nmol/l ± S.E, (n-6)) at 9 times; -10, 0, 15, 30, 60, 90, 120, 150 and 180 minutes.

Statistical analysis

A Indicates that the difference between the behaviour of the treatment group and the (control handling) group was significant at ($P<0.05$)
a As above but ($P<0.1$)
B Indicates that the difference between the behaviour of the (castration) group and the (scrotal castration) group was significant at ($P<0.05$)
d Indicates that the difference between the behaviour of the (scrotal castration) group and the (scrotal castration + local anaesthetic distal to the ring) group was significant at ($P<0.05$)
E Indicates that the difference between the behaviour of the (castration + local anaesthetic in the testes) group and the (castration + local anaesthetic distal to the ring) group was significant at ($P<0.05$)
f Indicates that the difference between the behaviour of the (castration + local anaesthetic in the testes group) and the (castration) group was significant at ($P<0.05$)
G Indicates that the difference between the behaviour of the (castration + local anaesthetic distal to the ring) group and the (castration) group was significant at ($P<0.05$)

N.B. The statistical comparisons made between behaviours of different groups are restricted to the total incidence (%) of lateral recumbency, ventral recumbency and standing and not their respective subdevisions (i.e. L1-L3, S1-S3, V1-V3).


Control
Castration
Scrotal Castration
L S V R
208
Incidence of behaviour (%)
Restlessness
Plasma cortisol (nmol/l)
Severity 1
Severity 2
Severity 3
Lateral recumbency
Standing
Ventral recumbency
Restlessness
0 - 15
15 - 30
30 - 60
60 - 90
90 - 120
120 - 180
Time after treatment (periods in mins)
Time after treatment (mins)



Castration + L/A (Testes)
Scrotal Castration + L/A
Castration + L/A
Incidence of behaviour (%)
Restlessness
Plasma cortisol (nmol/l)
L S V R
0 - 15
15 -30
30 - 60
60 -90
90 - 120
120 - 180
Severity 1
Severity 2
Severity 3
Lateral recumbency
Standing
Ventral recumbency
Restlessness
Time after treatment (periods in mins)
Time after treatment (min)

TABLE 3.3

THE EFFECTS OF SIX TREATMENTS ON LAMB BEHAVIOUR IN SIX TIME PERIODS

In each period after treatment the incidence of three postures (i.e. Lateral Recumbency, Standing and Ventral Recumbency) are presented as the number of times that behaviour is observed, and as a percentage of the total number of observations made in that period.

Text bound into the spine

TABLE 3.3 Incidence of four lamb behaviours in six periods after six treatments.

PERIOD AFTER TREATMENT	0 - 15 mins				15 - 30 mins			30 - 60 mins			60 - 90 mins			90 - 120 mins			120 - 180 mins		
NUMBER OF OBSERVATIONS MADE IN PERIOD	n = 84				n = 42			n = 36			n = 36			n = 36			n = 72		
BEHAVIOUR OBSERVED	LR	St	VR	R	LR	St	VR	LR	St	VR	LR	St	VR	LR	St	VR	LR	St	VR
CONTROL HANDLING (6 lambs)	0 15%	28 33%	56 67%	15	0 0%	19 45%	23 55%	0 0%	6 17%	30 83%	0 0%	13 36%	23 64%	0 0%	9 25%	27 75%	0 0%	23 32%	49 68%
CASTRATION (6 lambs)	25 30%	21 25%	38 45%	208	18 42%	4 10%	20 48%	20 56%	5 14%	11 31%	3 8%	4 11%	29 81%	0 0%	12 33%	24 67%	4 6%	15 21%	53 74%
SCROTAL CASTRATION (6 lambs)	0 0%	40 48%	44 52%	187	0 0%	25 60%	17 40%	0 0%	9 25%	27 75%	0 0%	2 6%	34 94%	0 0%	5 14%	31 86%	0 0%	22 31%	50 69%
CASTRATION PLUS LOCAL ANAESTHETIC IN TESTES (6 lambs)	10 12%	25 30%	49 58%	120	19 45%	12 29%	11 26%	12 33%	3 8%	21 59%	0 0%	2 6%	34 94%	0 0%	6 17%	30 83%	0 0%	23 32%	49 68%
SCROTAL CASTRATION PLUS LOCAL ANAESTHETIC IN DISTAL TO RING (6 lambs)	0 0%	36 43%	48 57%	68	0 0%	13 31%	29 69%	0 0%	6 17%	30 83%	0 0%	9 25%	27 75%	0 0%	5 14%	31 86%	0 0%	26 36%	46 64%
CASTRATION PLUS LOCAL ANAESTHETIC DISTAL TO RING (6 lambs)	1 1%	36 43%	47 56%	151	0 0%	23 55%	19 45%	1 3%	11 30%	24 67%	0 0%	13 36%	23 64%	0 0%	11 31%	25 69%	1 1%	24 33%	47 66%

LR Lateral Recumbency
VR Ventral Recumbency
St Standing/walking
R Restlessness

TABLE 3.4 Plasma cortisol measurements taken at 9 times for 6 different treatment groups

TIME AFTER TREATMENT (mins)	-10.0	0	15	30	60	90	120	150	180
NUMBER OF MEASUREMENTS (No. of lambs)	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
CONTROL HANDLING Plasma cortisol (n.mol/l) Mean ± S.E.	38.6 ± 12.2	45.2 ± 25.8	38.1 ± 16.8	40.3 ± 13.1	23.2 ± 11.9	39.9 ± 17.0	25.7 ± 10.3	24.4 ± 10.2	21.5 ± 7.5
CASTRATION Plasma cortisol (n.mol/l) Mean ± S.E.	34.0 ± 16.4	42.2 ± 10.4	144.9 ± 22.7	200.2 ± 35.2	231.3 ± 31.5	84.0 ± 12.5	45.9 ± 9.8	40.7 ± 15.4	19.9 ± 5.0
SCROTAL CASTRATION Plasma cortisol (n.mol/l) Mean ± S.E.	11.7 ± 8.2	15.7 ± 5.6	59.8 ± 12.8	107.2 ± 14.5	81.2 ± 20.3	20.0 ± 7.9	18.3 ± 5.4	9.4 ± 3.7	11.0 ± 5.6
CASTRATION PLUS LOCAL ANAESTHETIC IN TESTES Plasma cortisol (n.mol/l) Mean ± S.E.	34.4 ± 14.7	39.6 ± 10.8	149.3 ± 14.0	254.2 ± 18.4	209.1 ± 48.8	71.5 ± 12.8	65.7 ± 7.6	35.5 ± 13.7	19.4 ± 8.8
CASTRATION PLUS LOCAL ANAESTHETIC DISTAL TO RING Plasma cortisol (n.mol/l) Mean ± S.E.	31.4 ± 13.3	37.2 ± 11.7	156.8 ± 22.4	238.1 ± 25.2	155.7 ± 55.3	68.5 ± 30.7	15.4 ± 3.1	23.4 ± 8.8	15.2 ± 5.7
SCROTAL CASTRATION PLUS LOCAL ANAESTHETIC DISTAL TO RING Plasma cortisol (n.mol/l) Mean ± S.E.	22.5 ± 6.8	32.4 ± 7.0	67.8 ± 15.8	92.1 ± 28.2	31.0 ± 7.7	19.1 ± 5.4	12.5 ± 3.5	16.4 ± 5.0	22.2 ± 8.8

TABLE 3.5

Total time (minutes) spent by each lamb subjected to scrotal castration in one of nine postures

LAMB	L1	L2	L3	TOTAL	S1	S2	S3	TOTAL	V1	V2	V3	TOTAL
436	0	0	0	0	20	25	8	53	20	98	7	125
444	0	0	0	0	15	39	9	63	15	81	19	115
463	0	0	0	0	25	21	2	48	5	120	5	130
460	0	0	0	0	15	8	0	23	5	138	12	155
666	0	0	0	0	15	9	0	24	10	123	21	154
472	0	0	0	0	58	11	0	69	5	101	3	109
TOTAL				0	148	113	19	280	60	661	69	788

TABLE 3.6

Total time (minutes) spent by each lamb subjected to castration + local anaesthetic distal to the ring in one of nine postures

LAMB	L1	L2	L3	TOTAL	S1	S2	S3	TOTAL	V1	V2	V3	TOTAL
395	0	0	0	0	30	66	0	96	5	73	4	82
433	0	0	0	0	56	10	0	66	0	97	15	112
458	5	0	0	5	36	38	0	74	5	62	32	99
481	0	0	0	0	10	21	0	31	10	114	23	147
501	6	0	0	6	40	12	0	52	30	61	29	120
1001	0	0	0	0	56	2	0	58	15	100	5	120
TOTAL	11			11	228	149	0	377	65	507	108	680

DISCUSSION

The three aims of the first experiment were achieved. First it was confirmed that the changes in plasma cortisol concentrations and behavioural responses obtained by Mellor and Murray (1989a) could be reproduced using the same stimulus (castration and tail-docking -C/T) after limited instruction by D.J. Mellor and by using a smaller group of animals. Second it was confirmed that the pain following castration and tail-docking is dependent on afferent neural activity from the affected areas and that by prohibiting access of all this activity to the CNS the behavioural indices and changes in plasma cortisol were eliminated. Third it was shown that lambs appear to utilise endogenous opioids to exert a limited control over the pain of C/T as judged by the use of these indices.

It was concluded that it should be possible to produce a range of pain intensities by using different doses of naloxone and that these could be used to further test the sensitivity of the behavioural and physiological indices selected. It would, however, be necessary to use large numbers of animals and preferably reduce experimental variance in order to achieve this. A preliminary report of some of this work has appeared elsewhere (Wood *et al*, 1988), see appendix.

The main aim of the second experiment was to compare a proposed sequence of increasingly noxious treatments with the sequence obtained by using physiological and behavioural changes to assess the pain produced. There were some discrepancies found and attempts have been made to explain these.

The changes in behaviour and plasma cortisol concentrations observed here in the control and castrated and tail-docked lambs (Fig 3.1 and Table 3.1) were similar in all major respects for the same two treatments reported by Mellor and Murray (1989a). The peak cortisol concentrations following castration and tail-docking or control handling were 142 ± 16 nmol/l and 22 ± 13 nmol/l respectively for this study, compared with 114 ± 5 nmol/l and 22 ± 2 nmol/l in the study of Mellor and Murray (1989a). The lower standard error in the latter study reflects the greater number of animals used. In both studies the time course of the response was similar with peak plasma cortisol concentrations occurring between 15 and 60 minutes after treatment. The time course of the cortisol changes, and the variability between lambs emphasised the importance of frequent blood sampling under strictly controlled conditions and using each animal as its own control (Fulkerson and Jamieson, 1982). In the present study lambs spent less time lying (54-83% of observations) in the later periods (35-240 minutes) than did those in the study by Mellor and Murray, 1989a (84-98% of observations). The difference may be due to postnatal age, breed difference, or both. Alternatively the behavioural monitoring methods adopted, inhibited the lambs from lying due to an increased level of disturbance.

The present study helps to demonstrate that the indices used by Mellor and Murray (1989a) to measure pain and distress in groups of 52 to 57 lambs can be applied successfully to smaller groups (eg n=6), first because of the similar mean results in the two studies, and secondly because each of the castrated and tail-docked lambs in the present study showed similar patterns of change in behaviour and plasma cortisol concentrations.

In this study it has been shown that local anaesthesia abolishes the behavioural and cortisol changes associated with castration and tail-docking, throughout the four hours of observation. This was expected during the period of local anaesthetic nerve block since similar techniques are used routinely in clinical veterinary practice to permit humane castration of older lambs as required by codes of practice for the welfare of sheep (Ministry of Agriculture, Fisheries and Food, 1988).

The duration of anaesthesia with lignocaine without added adrenalin is unlikely to exceed one hour (Soma, 1971). It was of interest therefore, to note that no changes occured in either behaviour or plasma cortisol concentration after the action of the local anaesthetic could have been expected to have ceased. The abnormal behaviour and elevated cortisol response in lambs castrated and tail-docked without local anaesthetic continues for up to two hours. This apparent discrepancy can be accounted for, by failure of redistribution of the local anaesthetic from the testes and scrotum following castration, due to occlusion of the blood supply. The afferent activity from the testes rather than the scrotum is likely to be responsible for the prolonged response observed in lambs castrated without local anaesthetic as studies on the effect of of the occlusion of the blood supply on the afferent activity from the testes in the rat showed that there was a gradual increase in afferent activity over the recording period (150 minutes) to more than 300% of the control (Grubb *et al*, 1990). These results provide evidence that changes in the indices used to assess pain are dependent on the afferent activity from the affected areas and support their use for recognition and assessment of pain and distress originating in this way in this age and species of animal.

Some evidence was found for endogenous opioid activity in the lamb, which may provide a mechanism for pain inhibition (Terman *et al*, 1984). A proportion of lambs treated with naloxone alone lay in lateral recumbency on several occasions and some showed increased teat seeking and sucking. The number of lambs showing such changes was small but these results may indicate that at least some of these lambs were using endogenous opioids to reduce pain originating from an unknown source. That greater effects would be seen with a larger dose of naloxone was unlikely, as 0.2 mg/kg has been found to effectively antagonise endogenous opioid activity in a variety of species (Duggan and North, 1984). Increases in teat seeking and sucking could not be explained by the action of naloxone on appetite, since it has been shown that naloxone generally reduces appetite (Brands *et al*, 1979; Nizielski *et al*, 1986; Arjune and Bodnar, 1990) and indicates that teat seeking may be part of an additional mechanism for alleviating acute pain and distress in lambs.

Lambs castrated and tail-docked after treatment with naloxone showed greater increases in the incidence of lateral lying and in plasma cortisol than without naloxone pretreatment. The differences were small, only being significant for behaviour during the period 30 to 60 minutes. This indicates that although some lambs were able to reduce the signs of pain and distress which follow castration and tail-docking, their capacity to do so was limited. The slight antinociceptive effect obtained with intrathecal morphine (500μg-3000μg) in sheep (Ley *et al*, 1989a) provides some further evidence that this species may have limited ability to use endogenous opioids to reduce pain and distress.

As naloxone had a limited effect, further investigation of the reliability and sensitivity of such indices require that an alternative approach must be adopted to show that consistent relationships exist between the indices used here, and increasing or decreasing amounts of noxious stimulation. One alternative approach formed the basis of experiment 2.

Experiment 2

For this experiment the proposition was made that six different treatments produced six intensities and durations of pain as judged by the amount and type of tissue damaged, and the time for which a functional afferent nerve supply from the damaged tissue was present (see Fig 4.3, chapter 4 for innervation of the scrotum and testes). The proposed ranking (see methods) of the 6 treatments from most to least severe was:

(1) Castration (CN)
(2) Castration plus local anaesthetic in the testes (CNLAT)
(3) Scrotal castration (SC)
(4) Castration + local anaesthetic distal to the ring (CNLADR)
(5) Scrotal castration + local anaesthetic distal to the ring (SCLADR)
(6) Control handling (C)

Comparison of the proposed order of severities of the six procedures with the changes in the behaviour and plasma cortisol responses

The magnitude and significance ($P<0.05$) of the changes in the behavioural and plasma cortisol responses associated with the 5 treatments were determined by comparing the behaviour over the 5 time periods and plasma cortisol response from 15-60 minutes of each group with the control handling group.

(a)Ranking of the six groups using behavioural indices

Castration (CN) was the most severe stimulus based primarily on the incidence of lateral recumbency, the most informative behavioural measure in lambs subjected to substantial pain. Lambs castrated without local anaesthetic spent more time in lateral recumbency over the first 90 minutes (8-56% of observations) when compared with all other groups. This increase in lateral recumbency was significantly greater than that seen in all groups, except CNLAT during the first hour ($P<0.05$). The incidence of lateral recumbency in the CNLAT group was only significantly different from the control group in the period from 15-30 minutes (45% of observations, $P<0.05$), and was therefore ranked 2nd to the CN group. Lateral recumbency was observed during all periods in the first hour for the CNLADR group, the incidence was low (1-3 % of observations) and was not statistically different ($P<0.05$) when compared with the control group during any time period. The ranking of SC against

CNLADR is difficult. Lambs in the SC group were more restless (187 v 151, not significant P>0.05) and stood in abnormal postures (S3) from 0-60 minutes (3-12% of observations) none of which were recorded in the CNLADR group. There was, however, no incidence of lateral recumbency in the SC group, and less abnormal ventral recumbency (V3, 3-14% of observations) was observed when compared with the CNLADR group (3-30% of observations) over the duration of the experiment (0-180 minutes). Furthermore the duration over which abnormal behaviour (L1-L3,S2-S3,V3) occured was shorter (<1 hour) in the SC group when compared with the CNLADR group (up to 2 hours). It was of interest to note that lambs in the SC group lay in ventral recumbency (60-180 minutes) more than control lambs (significant from 60-90 minutes, P<0.05) indicating they were either truly undisturbed, or alternatively were unprepared to stand due to the nature of the noxious stimulus from the scrotum. In the absence of statistically significant differences between the main behavioural categories of the CNLADR and SC groups, the CNLADR group was ranked third and the SC group was ranked fourth primarily on the basis that the shorter duration of marked abnormal activity (>5% of observations) in the SC group was the most significant feature. The SCLADR group was ranked 5th because there was significantly less restlessness in the first 15 minutes when compared with the SC group (187 v 68, P<0.05), the time spent in abnormal standing behaviour (S2) was shorter, and the incidence of abnormal standing (S2) was less than that recorded in the SC group.

The category S2 as recorded here appears to be a sensitive measure of disturbance. This category was recorded when there was any detectable difference in the standing behaviour when compared with that of the control lambs (S1), though this judgement was not made by "blind" observers and is a subjective judgement. In the comparison between SCLADR, SC and C, it has been assumed that at pain intensities where lateral recumbency does not occur, the restlessness score and incidence of standing, particularly if abnormal (S2-3) assume more importance. This assumption is still to be confirmed by subjecting individual animals to a range of gradually increasing pain intensities, which would reveal at what relative pain intensities the different behaviours appear. The control group was ranked 6th, as this group was used as the standard for comparison. Treatment of control lambs was as similar to that of the other groups as could be reasonably achieved and it was assumed that they were not subjected to any imposed noxious stimulation except that necessary to take blood samples.

(b) Ranking of the six groups using plasma cortisol concentrations

The plasma cortisol response in the CN, CNLAT and CNLADR groups appeared very similar (Figs 3.3 and 3.4) and were not significantly different when compared statistically for the periods from 15-60 minutes ($P>0.05$). They were ranked as equally severe treatments, despite the cortisol response in the CN group reaching a later peak at 60 minutes. The fourth most severe treatment was scrotal castration (SC), with a significantly smaller cortisol response than CN, CNLAT and CNLADR ($P<0.05$), and a greater response than that of the SCLADR group and the control groups. The difference between SC and the C groups was only significant at $P<0.1$, while the difference between SC and SCLADR groups was not significant ($P>0.2$). The SCLADR group was ranked fifth, as the cortisol response was less than the SC group and greater than that in control lambs though the differences were again not significantly different ($P>0.2$). The control group was ranked last with the lowest plasma cortisol response.

(c) Ranking of the six groups using plasma cortisol concentrations and the behaviour

The results of ranking the severity of the six treatments using both the behaviour and the plasma cortisol responses is summarised below.

Rank	Behaviour	Plasma cortisol
1	CN	
2	CNLAT	CN/CNLAT/CNLADR
3	CNLADR	
4	SC	SC
5	SCLADR	SCLADR
6	C	C

When the two sets of results are combined, the rank remains the same as that obtained using the behavioural indices only. These results suggest that plasma cortisol responses of groups of six lambs can only be used to discriminate between substantial differences in intensity of noxious stimulus and that plasma cortisol changes in individual lambs provide a relatively insensitive index for assessment. The similarity of the rank orders obtained increases the confidence in the use of behavioural indices for assessment of pain and suggests that measurement of plasma cortisol does not increase the sensitivity of the measurement technique.

(d) Comparison of the ranking obtained experimentally with that predicted by the amount and type of tissue injured (see methods)

A comparison of the ranking predicted by the amount and type of tissue injured with that obtained using changes in the behaviour and plasma cortisol response are summarised below;

Rank	Predicted	Experimental
1	CN	CN
2	CNLAT	CNLAT
3	SC	CNLADR
4	CNLADR	SC
5	SCLADR	SCLADR
6	C	C

The only discrepancy found was in the ranking of SC in relation to CNLADR. The ranking of these two groups experimentally, was difficult using the behavioural indices, though there was a much larger cortisol response associated with CNLADR (Significant when compared from 15-60 minutes $P<0.05$) which was used as the main evidence for ranking CNLADR above SC. This discrepancy can be explained if the basis for the predicted ranking of pain intensity was incorrect in some respect. For example if the diffusion of the local anaesthetic was inadequate to stop afferent activity from nociceptors distal to the ring the stimulus produced by CNLADR would be greater than expected and more akin to that produced by CN or CNLAT. Achievement of some local anaesthesia in the CNLADR distal to the rubber ring can explain why the incidence of abnormal behaviour such as lateral recumbency was significantly lower in this group when compared with CN and CNLAT, but does not explain why there was no reduction in plasma cortisol response. A possible explanation could be that above a certain intensity of pain, the lamb has a restricted capacity to increase plasma cortisol release, implying the cortisol response may not relate directly to pain intensity in agreement with Rushen (1986). This is not due to adrenal exhaustion as injection of ACTH was found to produce a cortisol response substantially greater than that seen following castration and tail-docking (Mellor and Murray, 1989b). Further discrepancies may occur between behaviour and plasma cortisol if β-endorphin (an endogenous opioid), which is released concomitantly with ACTH (Guillemin *et al*, 1977) during the cortisol response can provide some analgesia (Shutt *et al*, 1988) for moderately intense pain in the CNLADR group, but insufficient to attenuate intense pain as is proposed to occur in the CN and CNLAT groups. Insufficient is known, however, of the physiological role of β-endorphin (Dantzer, 1986) to predict if the latter proposal is likely. This possibility could be tested by undertaking analgesic tests in the lamb following administration of β-endorphin in doses that would reproduce the plasma concentration found in lambs following castration and tail-docking.

Care must also be taken when interpreting the restlessness score, as it does not appear to be a reliable index of stimulus intensity. A lamb that is lying in the most severe form of lateral recumbency (L3), although possibly rolling and kicking, does not stand up and lie down regularly and so under these circumstances restlessness would underestimate the pain intensity. There is such a discrepancy between the score seen in castration with L/A in testes (60) and stimuli proposed to be less severe using other indices, namely, castration with local anaesthetic distal to the ring (75) and scrotal castration (100). It may be invalid to assume as suggested by Shutt *et al*, (1988) that such agitated behaviour represents more severe pain than immobile standing or abnormal recumbency. Restlessness is more likely to indicate unsuccessful attempts to escape/relieve pain/discomfort, whereas lateral recumbency is adopted when the intensity is higher and escape is not possible. This is also likely to apply to immobile standing postures. The delayed appearance of immobile standing or recumbency after castration and tail-docking with tight rubber rings may be related to a slow increase in visceral pain following occlusion of the testicular blood supply as suggested by Grubb *et al*, (1990). Mellor and Murray (1989a) found restlessness decreased following castration and tail-docking, and that after 30 minutes most lambs were lying in lateral recumbency. Despite the reduction in restlessness, however, pain was estimated to be more intense at this time (30 minutes) as in these studies lateral recumbency was usually only associated with noxious stimulation, the incidence increasing with the severity of the stimulus. The restlessness score appears to be a better index for less intense pain which the animal appears to be able to accomodate by changes in posture. For this reason, the restlessness score was only used in the assessment of the rank order in those groups where no lateral recumbency was present, i.e SC, SCLADR and C.

Extended analysis of the behavioural data of the CNLADR and SC groups (see table 3.5)

Behavioural recording method 1, if applied to this data would show that the behaviour in the CNLADR and SC groups was largely similar to control lambs with only a small incidence of abnormal behaviour (lateral recumbency) in the CNLADR group. Examination of the behaviour using method 2 where the total time (minutes) spent in each of the nine postures (L1-L3, S1-S3, V1-V3) is used (see Table 3.5), shows that lambs in the SC and CNLADR groups spend 19% and 25% of the time respectively in some form of abnormal behaviour (L1-L3, S2-S3, V3). Can an approach where the total time spent by lambs in these abnormal behaviours is quantified, resolve the difficulties encountered ranking these two groups?

Lambs in the CNLADR group spend 56% and 14% more time in abnormal ventral recumbency (V3) and abnormal standing (S2-S3) respectively when compared with the SC group, which in addition to the small incidence of lateral recumbency (1% of total time) suggests that CNLADR is the most severe treatment of the two. There are no significant differences ($P>0.05$), however, when the total time spent in abnormal standing or ventral recumbency is compared between the two groups using a students T-test, because of the variation between lambs. Of the abnormal behaviour in the CNLADR group 69% is associated with only three lambs, two of which lay in lateral recumbency (458 and 501), while 67% of the abnormal behaviour in the SC group was associated with the only three lambs that stood in the most abnormal form of standing (436,444,463). These results suggest that although the behavioural indices selected are sensitive to changes in stimulus intensity in the individual lamb, time consuming comparisons as outlined above, are not warranted unless the stimulus intensity is equivalent in all animals in the group. An approach is required whereby pain intensity can be reduced with greater certainty, so that the subtle behavioural changes can be interpreted correctly.

Pain recognition and assessment in the individual lamb

In the practical clinical situation, where pain is assessed in one animal, and long observation periods are impracticable, recognition of behaviours which only occur at certain pain intensities would improve the sensitivity of the technique. This may already be possible using the classification in method 2. For example L3 and S3 behaviour suggests the presence of extreme and severe pain respectively. Such an approach can be extended by increasing the number of behavioural categories measured e.g. ventral 3 could be sub-divided into V3 (lamb lies with front legs tucked under body, one hind limb extended) and V4 which is a posture seen in transition from V3 to L1 (lamb lies with front legs tucked under body, one or both hind limbs actively extended).

Is it possible to use these behavioural and physiological indices to measure the intensity of pain produced by a particular treatment?

It has been possible to use the indices selected to rank several different treatments in order of severity. Lateral recumbency was found to be the most useful behavioural index for the highest pain intensities. Abnormal ventral recumbency (V3) and abnormal standing (S3) were indicative of high intensity but probably occurred more at moderate to high intensities. Restlessness and abnormal standing (S2) were more useful at lower pain intensities. The plasma cortisol response was useful when comparing the most and least severe stimuli. The sensitivity of this index could be improved by using more lambs to compensate for variation between lambs, though such an approach ignores the problem of accurate pain assessment in individual animals. The indices studied so far clearly produced a ranking of intensity which is close to that predicted by the alternative approach. Use of these indices may, however, be considered more accurate than the alternative method since it is based on quantitative experimental results as opposed to theoretical estimates of likely intensities based on knowledge of the anatomy and physiology of the tissues and the possible effects of local anaesthetic. No final conclusion can be drawn until more quantitative ways are used to manipulate the pain intensity e.g. by the use of increasingly effective doses of an analgesic. As results such as these are repeated and extended, however, it should be possible to establish an agreed scale for the assessment of pain from castration and tail-docking.

The approach used here to produce a range of noxious stimuli could be extended and additional intermediate procedures could be added such as castration following injection of local anaesthetic in one testicle which might be expected to reduce the pain by approximately 50%. This approach is, however, limited by the inability to accurately rank these procedures by an independent and quantitative method. Such considerations may explain why, although changes in behaviour have been proposed as the most important signs of pain or stress (Brambell, 1965), recent studies emphasise quantitative differences in the plasma cortisol concentration as the main indicator of pain/stress intensity (Fulkerson and Jamieson, 1982; Fell *et al*, 1985, 1986; Shutt *et al*, 1987, 1988; Mitchell *et al*, 1988; Mellor and Murray, 1989a,b). It must be considered when using different noxious stimuli, such as castration or scrotal castration, that the differences in behaviour observed were due to the different types of pain from different tissues of origin and not just differences in the overall intensity of the pain. Changes in plasma cortisol concentration are likely to be less sensitive to the source of pain than are changes in behaviour.

It is concluded that the behavioural indices used so far are sensitive to changes in pain intensity, but the quantitative relationships between different behaviours which may result from the nature or origin of the pain remains to be determined. This problem could be overcome by investigating the behaviours associated with different intensities of pain resulting from a standard noxious stimulus. Such an approach requires the use of an effective analgesic with which the pain intensity of the standard noxious stimulus can be manipulated.

EFFECTS OF EPIDURAL MORPHINE OR INTRATHECAL ETORPHINE ON INDICES USED FOR ASSESSMENT OF PAIN FROM CASTRATION OR CASTRATION AND TAIL-DOCKING IN YOUNG LAMBS

Opioids as antinociceptive drugs

The pain relieving qualities of opioids have been known for many centuries and their pharmacology in particular has been extensively researched with the first general review in a wide spectrum of species being completed by Krueger *et al*, in 1943. Due to the amount of detail now available in this field, more recent reviews are restricted in their coverage with some concentrating on the pharmacology (Martin, 1984; Veda , 1990; Kosterlitz, 1985; Kosterlitz and Walt, 1968), development of the opioid system (McDowell and Kitcher, 1987), the endogenous ligands (Weber *et al*, 1983; Woolf and Wall, 1983) or the electrophysiology (Duggan and North, 1984). As techniques have become available molecular aspects of the action (McFadzean, 1988) or structure of the opioid system (North, 1986), have also been examined.

The aims of the current work dictate that the main interest is in the analgesic effects of these drugs, which are generally accepted to result from actions on the central nervous system, see for example Basbaum and Fields (1978), and only to a limited extent a peripheral action (Levine and Taiwo, 1989).

The central opioid effects involve activation of (a) intrinsic pain suppression systems originating in the brainstem, which via descending pathways in the spinal cord dorsolateral funiculus inhibits the spinal cord and trigeminal pain transmission neurones (Barton *et al*, 1980) and (b) the opioid receptors in the spinal cord. This spinal antinociceptive action of opioids (Yaksh, 1987) was not unexpected given the dense concentration of the opioid peptide enkephalin (Sar *et al*, 1978) and opioid receptors (Atweh and Kuhar, 1977) in the superficial layers of the dorsal horn associated with small diameter fibres which are inextricably linked with pain mechanisms. Following spinal transection, however, 2-3 times the dose of intravenous morphine was required to suppress withdrawal reflexes initiated by noxious stimuli when compared with the dose needed for similar suppression in intact rats (Irwin, 1951). This indicates that following systemic administration, morphine produces analgesia predominantly through a supra spinal mechanism.

The components of supraspinal analgesia have been investigated by Barton *et al* (1980), by examining the effects on suppression of spinal withdrawal reflexes by opioids following lesions of several spinal funiculi. Lesions of the DLF consistently interfered with suppression by morphine of the tail flick response across the dose range studied (5-15mg/kg IP), as did lesions in the VQ though to a lesser extent. They concluded that both spinal and supraspinal sites contributed significantly to the analgesia produced by systemic administration of opioids. An important additional observation was that lesions in the DLF, and systemic administration of naloxone did not significantly alter baseline latencies indicating the descending inhibitory system activated by morphine is not tonically active.

Opioid receptors and their endogenous ligands

Binding sites for opioids were demonstrated in the early 1970's (Goldstein *et al*, 1971) and notably by Terenius (1973), who showed that dihydromorphine was bound by synaptic plasma membranes. Once these opioid binding sites had been demonstrated endogenous ligands were sought to confirm that these binding sites were likely to be receptors, and of physiological importance.

Li *et al* (1965), while investigating a procedure for isolating ACTH from sheep pituitary gland, discovered a lipotrophic peptide, distinct from ACTH, which they called β-Lipotrophin. This peptide could be degraded to give 5 smaller peptide fragments. Ten years later came the significant discovery that a number of these fragments possess opioid activity (Li and Chung, 1976). This was also observed by other groups and it was then suggested that the active peptide isolated from the same (Tescemacher *et al*, 1975; Cox *et al*, 1975) or other tissues such as brain (Hughes *et al*, 1975; Pasternack *et al*, 1975) was a fragment derived from breakdown of β-Lipotropin.

The identification of opioid binding sites in nervous tissue (Atweh and Kuhar, 1977; Traynor and Woods, 1987), the discovery of their endogenous ligands and the subsequent discovery of the variety of receptor subtypes at which they may act, have been reviewed by amongst others (Mansour *et al*, 1988; Kosterlitz, 1985; McDowell and Kitcher, 1987; North, 1986; Weber *et al*, 1983). These reflect a greatly improved understanding of the mechanism of action for compounds that had been in use therapeutically for hundreds of years.

Factors affecting opioid antinociception

The antinociceptive activity of opioids is determined mainly by route of administration, the receptors at which they are active and the nature and intensity of the noxious stimulus being applied.

(a) Route of administration

Analgesia is routinely obtained clinically by oral, intravenous or intramuscular administration of opioids. Sometimes, however, these methods of administration produce insufficient analgesia, or analgesia is only obtained at doses associated with unacceptable effects (Cousins and Mather, 1984).

Spinal administration of drugs, was first tried by Tsou and Jang in 1964, but became widely used as an experimental method following the report by Yaksh and Rudy (1976a), that introduction of a chronic indwelling cannula into the spinal subarachnoid space made possible the investigation of the spinal action of opioids. Opioid drugs administered epidurally or intrathecally, in this manner, gain access to mu, delta and kappa receptors which are seen at significant levels in the spinal cord, with the highest binding evident appropriately, in the dorsal horn of the gray matter (Yaksh, 1984; Wamsley, 1983). Microgram amounts of opioids administered by this route can produce profound analgesia (Yaksh and Rudy, 1976; Yaksh and Rudy, 1977), even in some cases when systemic drug administration has failed (Cousins and Mather, 1984). The high local concentrations obtained by this method, produce spinal analgesia which cannot be obtained following systemic administration without producing central sedation (Barton *et al*, 1980). These central sedative effects were considered unacceptable in experiments where subtle changes in the behaviour of the animal were being investigated as potential indices of pain.

(b) Opioid receptor affinity

Opioid drugs are classified as agonists, partial agonists and antagonists at one or more of three main receptor types, mu, delta or kappa, though there is evidence that more may exist (Mansour *et al*, 1988; Bhargava, 1989). There is also evidence for a number of subtypes of those already described eg $\mu 1$, $\mu 2$ (Paul *et al*, 1989) and $\kappa 1$, $\kappa 1a$, $\kappa 1b$, $\kappa 3$ (Clark *et al*, 1989).

During classification, however, few opioids can be described as pure agonists or antagonists. For example antagonists such as naloxone do exhibit agonist activity at low doses. Work by Woolf (1980), demonstrated a dose dependent bi-phasic change in nociceptive threshold in the rat following intrathecal injection of naloxone. This has been shown to be associated with agonist action at the delta receptor (Taiwo *et al*, 1989).

Clinically useful opioid drugs are not likely to be active at one receptor subtype only, due to a lack of specificity and also because there is evidence for allosteric coupling between mu and delta receptors (Porecca *et al*, 1987), in support of earlier work (Vaught *et al*, 1982; Rothman and Westfall, 1982). Drugs may be classified by their receptor affinity, with these receptors in turn being classified as to whether or not they mediate antinociception in a variety of experimental pain models in animals (Tyers, 1980). Not only can an opioid be active at more than one receptor, but also it can potentially act as either an agonist, partial agonist or antagonist at any of these. Furthermore agonists acting on mu, delta and kappa receptors are shown to have varying antinociceptive actions on visceral, chemical and thermal stimuli (Schmauss and Yaksh, 1984). These variables when combined result in a wide range of analgesic activity. For example delta agonists when given intrathecally in the rat, show greater potency than morphine on the cutaneous thermal tests, but little or no efficacy in visceral chemical writhing (Yaksh and Noueihed, 1985). Species differences also exist due to the different predomination of receptors (Gouarderes *et al*, 1982). Clearly it is important to have a knowledge of the potency and receptor affinity of the drug, its suitability for the type of noxious stimulus being used, and the species of animal.

(c) Stimulus intensity

Stimulus strength or pain intensity is also thought to be of importance in the effect produced by the opioids. It has been shown that the weaker of the stimuli, whether noxious heat or pinch (as measured by quantifying afferent volleys in the nerves subserving the region under stimulation), is reduced proportionately more (Parsons and Headley, 1989). The somatic or visceral nature of the stimulus may have an effect, as the writhing response to I/P injected irritants was more readily eliminated than the response to tail pinch, which in turn was more readily eliminated than the response to the hotplate test by mice and the tail flick response to radiant heat by rats (Martin, 1984). This supports findings by Gray *et al* (1970), that a very intense noxious stimulus, such as is used in the flick response to radiant heat, is not readily modified by analgesics and thus is not an appropriate way of testing these drugs.

Synaptic location of opioid receptors within the spinal cord

At present, the pre and/or post synaptic action is probably not the best way of thinking about the effect of morphine in the spinal cord (Lombard and Besson, 1989). What is known is that analgesic doses of I/T morphine (Yaksh and Rudy, 1977), result in a dose responsive inhibition of ascending activity evoked by A-delta and C but not Aβ afferent stimulation (Doi and Jurna, 1982). Other drugs have demonstrated similar effects and importantly the rate of onset of their effect relates to times for the block of the skin twitch reflex in cats (Noueihed *et al*, 1984) and their lipid partition coefficients (Herz and Teschemacher, 1971).

It is not known if this suppression by morphine is by presynaptic inhibition at primary afferents, though the ability of opioids to produce preferential suppression of small diameter fibres suggested that this was a possibility (Doi and Jurna, 1982; Yaksh and Noueihed, 1985). Furthermore a reduction in receptor binding is seen after dorsal root section (La Motte *et al*, 1976) or if small diameter primary afferents are destroyed with the neurotoxin capsaicin (Gamse *et al*, 1979). Evidence also exists for inhibition of primary afferent transmitters by encephalinergic neurones (Jessel and Iversen, 1977), demonstrated experimentally as a reduction of substance P release following the superfusion of the spinal cord with opioids in vivo (Yaksh *et al* 1980; Kuraishi *et al*, 1983).

The current view of the mechanism of presynaptic inhibition by morphine dictates that a slow depolarisation in the primary afferent nerve terinal is a causal link in reducing the release of neurotransmitter from the terminal (For review see Schmitt, 1971). Detection of depolarisation in the primary afferent nerve terminal, however, has proved to be technically very difficult and is not a consistent finding (Carstens *et al*, 1979). This view of presynaptic inhibition may be an oversimplification as etorphine has been found to reduce synaptic transmission without associated changes in spike threshold or configuration following stimulation of dorsal root ganglion cells (MacDonald and Nelson, 1978).

Evidence for a post-synaptic action was provided by Zieglgansberger and Bayerl (1976), while recording intracellularily in the nucleus proprius. They demonstrated that iontophoretic application of opioids in the vicinity of the cell bodies, reduced the rate of rise of EPSP. This action could, however, be accounted for by selective changes in sensitivity of membrane conductance (Duggan and North, 1984).

The nature of the drug may determine the pre or post synaptic action. It was demonstrated while recording extracellularly in the nucleus proprius of the spinal cord that morphine depressed the responses evoked by noxious heat, only when applied in the substantia gelatinosa near the dendritic terminals of cells found in lamina IV and V and not when applied near the cell bodies themselves (Duggan *et al*, 1977a). Opioid peptides, however, depressed the same responses evoked by noxious heat when applied near the dendrites or cell body (Duggan *et al*, 1977b; Duggan *et al*, 1981). This latter finding is supported by Yoshimura and North (1983), who demonstrated while recording intracellularly in an in-vitro preparation that extracellular enkephalin administration produced hyperpolarisation.

Choice of morphine and Etorphine

Morphine sulphate was used as the first choice because it has been shown to be active in adult sheep (Ley *et al*, 1989a) and was established as the standard for comparison, and is commonly assigned the potency of 1 (Martin, 1984). It is normally a potent analgesic, due to it being an almost pure mu ligand. Magnan *et al* (1982), used receptor binding studies in guinea pig brain and bioassays in guinea pig ileum, mouse and rat vas deferens, to characterise the relative receptor affinities of a series of opioids for mu, delta and kappa sites. Morphine was classified as a mu agonist due to the high selectivity observed for the mu site, with only 2% cross-reactivity with delta sites and 0.6% with kappa sites.

Etorphine was used as a second choice due to its high lipid solubility and hence rapid absorbtion from the CSF like other more routinely used lipophylic drugs such as pethidine (Gourlay *et al*, 1987). Although morphine is 1.8 times more potent than etorphine at the mu site, etorphine is 90 times more potent at the delta site and 700 times more potent at the kappa site than morphine (Magnan *et al* ,1982). Etorphine is one of the most potent narcotics known, and has been reported to be 1000-80000 times more potent than morphine depending on the parameter measured (Blane *et al*, 1967). Following subcutaneous injection in the mouse it has a rapid onset of action with a peak effect at 15 minutes, and duration of only 50 minutes (Takemori *et al*, 1972). Use of the intrathecal route in the lamb was expected to prolong the duration of action. Once etorphine has been made up in solution it should be stored in a sealed container and protected from light.

Absorbtion and distribution of epidural morphine

No studies of the absorbtion and distribution of morphine in lambs have been found, so at this stage estimates are based on work in humans. In adult humans there is a rapid vascular uptake of morphine following epidural administration, with peak concentrations being obtained systemically in the plasma at 2-17 minutes. Peak concentrations in the CSF are obtained much later (120 minutes), due to its polarity and hence hydrophylic nature. These results were obtained following the injection of 10mg morphine epidurally in human cancer patients, with subsequent CSF and blood sampling (Gourlay *et al*, 1987). The volume of injectate (either 1mg/5ml or 1mg/ml), again in humans was found to produce no difference in terms of analgesia, $PaCO_2$ or side effects (Fitzgibbon, 1989). When radiolabelled ligands in the same volume (100μl) as used in the intrathecal studies being reported here in the lamb, was administered intrathecally to adult sheep, it was found to be distributed over 5 vertebral spaces (Waterman *et al*, 1988; Bouchenafa *et al*, 1988).

Development of tolerance and dependence on opioids

Little is known of dependence or addiction in animals with opioid agonist-antagonists (Martin, 1984). Throughout the experimental series each lamb only ever received one dose of opioid and thus tolerance was not a problem. No lamb having received morphine was then used in etorphine experiments, so cross tolerance was not seen. What is known is that opioid receptors are specific for certain ligands, and their topography is determined by; species, the region of tissue, antagonist specificity and pharmacological actions (Martin, 1984).

Ontogenesis of opioid receptors in the CNS of lambs

At birth the brain of the lamb has reached 50% of the adult size, the cerebral hemispheres 52%, cerebellum 40% and the spinal cord 40%. The relative maturity of the brain at birth compared with that in the adult sheep justifies a classification of prenatal brain development (McIntosh *et al*, 1979). Despite this classification, results obtained by research on the CNS of adult sheep may not be applicable to the lamb. A study of the ontogenesis of opioid receptors by binding studies in homogeneates prepared from different regions of the sheep brain using radiolabled 3H naloxone and 3H Ala Met 5 enkephalinamide (Villiger *et al*, 1982), indicates that this process is not complete in lambs at birth. Binding increases during prenatal development but has not reached adult levels by birth. Opioid and alpha 2 binding sites have been demonstrated in adult sheep spinal cord (Brandt and Livingston, 1989; Bouchenafa and Livingston, 1987), but studies of such sites in the spinal cord of lambs have not been found. It is clear that it will be necessary to carry out such studies to help explain some of the results obtained.

Unwanted effects of opioids in this study

(a) Temperature regulation

The lambs body temperature may influence behaviour by initiating postures which are adopted for thermoregulation. Although little is known of the temperature regulatory effects of morphine in lambs it has been shown to produce an increase in body temperature in the rat unless, the environmental temperature is low, where the response is attenuated and may even be one of hypothermia (Herman, 1942). Hyperthermia was observed with as little as 2μg of morphine injected intrathecally in the rat (Rudy and Yaksh, 1977), indicating this is a spinal action, as such low doses of morphine do not gain access to supraspinal structures in significant concentrations (Yaksh and Rudy, 1976b). As observed by Herman (1942), this hyperthermic effect was dependent on environmental temperature, suggesting that morphine was disrupting the thermoregulatory mechanism. Morphine produces hyperthermia in the cat while in the dog it produces a dose related hypothermia. It is clear that opioid analgesics alter body temperature by acting at several sites, including the spinal cord, medullary raphe system and the anterior/ventromedial hypothalamus some of which effects may not be naloxone reversible (Martin, 1984). In conclusion the effect of opioids on temperature regulation are complex, dependant on species, dose, ambient temperature and time after injection. Where possible, therefore, the ambient temperature of the sheep housing should be kept constant, so any thermoregulatory effects of morphine will be the same for all animals.

(b) Effects of opioids on plasma corticosteroid concentrations

In unanaesthetised rats, morphine sulphate (10mg/kg S/C) significantly reduces ascorbic acid content in the adrenal cortex, indicating a increase in ACTH release (Nasmyth, 1954). The stimulatory effect of morphine on ACTH release was supported by Burdette *et al* (1961), paradoxically however, they found that morphine (20mg/kg I/P) inhibited the secretion of ACTH in stressed rats. Similarly Gibson *et al* (1979) found that although I/P administration of morphine (24mg/kg) to mice doubled basal levels of corticosterone in 15 minutes, the same dose of morphine administered to mice exposed to ethor vapour eliminated the stress induced elevation of plasma corticosteroids. Etorphine (2mg/kg I/P), however, was found to have no effect om plasma corticosteroid concentrations (Gibson *et al*, 1979). Nikolarakis *et al* (1989), have recently provided evidence showing that exogenously applied opioids increase ACTH release by both activating CRF secretion, and by activating peripheral sympathetic neuronal pathways. The dose of morphine administered to lambs (16mg) was anticipated to increase cortisol release in control lambs, but decrease the cortisol release associated with the different husbandry procedures.

(c) Effects of opioids on the cardiovascular system

Opioids have a complex action on the cardiovascular system which is dependent upon species, tolerance, anaesthetic state, drug, dose etc (Martin, 1984). In dogs and cats morphine causes a reduction in blood pressure, mainly due to peripheral vasodilation (Cohen and Coffman, 1979), but there is probably also a central component to this effect (Evans *et al*, 1952). When morphine (11-250nmol) is administered intracisternally in the chloralose anaesthetised rat there is a vasopressor response and bradycardia, which is followed by a hypotensive phase (Bolme *et al*, 1978). This apparent paradox can be explained by the opposing effects morphine has on different parts of the cardiovascular system. A stimulatory effect, via an increase in sympathetic tone (Borison *et al*, 1964), and a depressant effect whereby brainstem neurones are depressed, decreasing sympathetic tone and increasing vagal tone (Martin, 1984). Such opposing effects of morphine may explain the bell shaped dose-response curve of heart rate when morphine (>0.15 mg/hr) is administered intravenously in lambs (Zhu and Szeto, 1989).

(d) Effects of opioids on behaviour

Behavioural effects of the opioids in humans have been comprehensively reviewed by Lal (1977). Morphine and related drugs have mood altering and sedative properties, (Martin, 1984), which are dependent on the disposition of the subject prior to administration. The mu antagonist naloxone has been shown to inhibit food intake (Brands *et al*, 1979), and so conversely the mu agonist morphine could be expected to increase food consumption. In lambs this would be seen as an increase in suckling frequency and duration.

During initial pilot studies (see chapter 2), 3 lambs administered morphine (>32mg) showed hyperactivity. This is in agreement with Yaksh (1985), who suggests detectable motor dysfunction occured with mu agonists at doses at or above those classified as analgesic. Recent work by Nolan (1986), demonstrated that in sheep treated intravenously with buprenorphine, pethidine or fentanyl, there was initiation, to a greater or lesser degree of increased locomotor activity, a rigid base-wide stance, jerky head movements, bleating, chewing, sniffing and excitement such that physiological recording was made difficult. There is evidence to suggest that high doses of morphine produce hyperaesthesia mediated by a non opiate receptor. In rats to which morphine had been administered via epidural catheters this has been described as a pain syndrome, it involves intermittent bouts of biting and scratching at the dermatomes innervated by segments proximal to the catheter tip (Yaksh *et al*, 1986) and was not reversible with naloxone. They suggested that high doses of morphine might be having a strychnine like effect, antagonising glycinergic effects at spinal neurones. Glycine normally producing postsynaptic inhibition in dorsal horn neurones, thus limiting their excitability (Game and Lodge, 1975). It has been previously shown by Duggan and Curtis (1972) that high doses of opioids can effectively block glycine receptors in the (cat) spinal cord.

Epidural and intrathecal morphine and etorphine were expected to have significant analgesic activity in lambs. These routes of administration were chosen to minimise side effects, in particular behavioural changes, since such effects could not be completely eliminated, control groups were included to account for these effects. Hypoalgesia could not be consistently demonstrated in pilot studies involving reflex analgesic tests/ foot withdrawal to thermal stimuli in restrained lambs. Doses were therefore selected on the basis that they were 75% of a dose which produced noticeable locomotor effects in 100% of lambs tested in pilot studies, (see general methods). This was because any treatment causing such side effects would interfere with the measurement and interpretation of the changes in behaviour which were being investigated as indices for assessment of pain.

MATERIALS AND METHODS

Studies of the effects of the opioid agonists morphine and etorphine on responses to castration, or castration and tail-docking were based on the results obtained from two separate experiments, the first in March 1988 and the second in March 1989. In total 48 Finnish x Dorset lambs (all male) weighing between 3.0-7.0kg, and ranging from 5-7 days old. In the first group of 24 lambs, the effect of epidural morphine (16mg) on behaviour patterns and cortisol responses produced by castration and tail-docking were examined. Behavioural and plasma cortisol data were recorded as described by Mellor and Murray (1989a), though presentation and analysis of results was modified, (see general methods). In the second group of 24 lambs, the effect of intrathecal etorphine (10 nmol) on behaviour patterns and cortisol responses produced by castration alone, were examined. For this study changes were made in the behavioural monitoring technique. Behavioural monitoring was restricted to the main 3 postures; lateral recumbency, standing and ventral recumbency, which were graded into 1 of 3 severities. A restlessness score was introduced, which quantified how often a lamb got up or lay down. The drugs used in these studies were, morphine sulphate (Boots the Chemists), dissolved in 0.9% NaCl solution to 80mg/ml; etorphine hydrochloride (Reckitt and Coleman), dissolved in 0.9% NaCl solution to 50nmol/ml.

Experiment 1

The null hypothesis was that 2 x 8mg of morphine administered to young lambs via the epidural route would not change the behaviour patterns or plasma cortisol responses following castration and tail-docking.

Animals

A total of 24 lambs (Finnish x Dorset) from 5-7 days old and weighing 5.5 ± 0.3kg were investigated. No lambs showing clinical signs of ill health were included in the investigation. Housing, feeding and bedding, followed routine husbandry practices as described in the general methods.

Treatments

A day before the experiment, wool was shaved from the full length of the neck to expose both jugular veins. A 10cm square of wool was also shaved from the skin overlying the lumbosacral junction. All lambs were anaesthetised with a mixture of halothane, nitrous oxide and oxygen, prior to and during the epidural or sham epidural procedures. General anaesthesia was maintained for no more than 10 minutes, 40 minutes were allowed after drug administration before the start of the experiment, see general methods for greater detail.

While anaesthetised, the shaved area of skin on the dorsum of the lamb was cleaned with an antiseptic solution of Hibiscrub (Pittman Moore, Europe Ltd) followed by application of 70% alcohol. The lumbar spinal cord was flexed to locate the lumbosacral space and a small incision made in the skin directly above this space with a stylet. A Tuohy needle angled at 45° from the vertical and directed from left to right (as seen from the rear of the lamb), with the chamfer facing uppermost was inserted through the lumbosacral junction into the epidural space of the spinal canal. A catheter was introduced into the epidural space via the Tuohy needle and advanced 80mm as indicated by marks previously made on the catheter. Drug or vehicle could then be injected in the region of thoracic segment 10. The catheter was then withdrawn to the 10mm mark, so an injection could be made in the region of the lumbar segments 6-7. Where lambs were tail-docked, a tight rubber ring

(Paragon Rubber Co Ltd., Skegness, England) was applied to the tail so that the remnant would cover the anus in ram lambs. When castrated a tight ring was applied to the neck of the scrotum so that both testicles were below and the two teats above the ring (Eales and Small, 1986b). All lambs were bled (2ml samples) by venepuncture from a jugular vein immediately before and at 15, 30, 60, 90, 120, 180 and 240 minutes. This procedure usually took less than 15 seconds. There were four treatment groups.

Group 1 Control handling. Lambs were handled as if they were being castrated and tail-docked but without application of the rubber rings.

Group 2 Epidural morphine only. Lambs (n-6) were anaesthetised and 2 x 8mg of morphine in 100μl of saline was introduced into the epidural space at two separate locations (see above), 40 minutes before the start of the experiment.

Group 3 Epidural morphine followed by castration and tail-docking. Lambs (n-6) were treated as in group 2, and were castrated and tail docked at the start of the experiment.

Group 4 Epidural saline followed by castration and tail-docking. Lambs (n-6) were treated as lambs in group 3 except that saline (2 x 100μl) alone was administered into the epidural space.

Experiment 2

The null hypothesis was that 2 x 5nmol of etorphine administered to the young lamb via the intrathecal route would not change the behaviour patterns or plasma cortisol responses following castration.

Animals

A total of 24 lambs (Finnish Landrace x Dorsets) from 5-7 days old and weighing 5.5 ± 0.2 kg were investigated.

Treatments

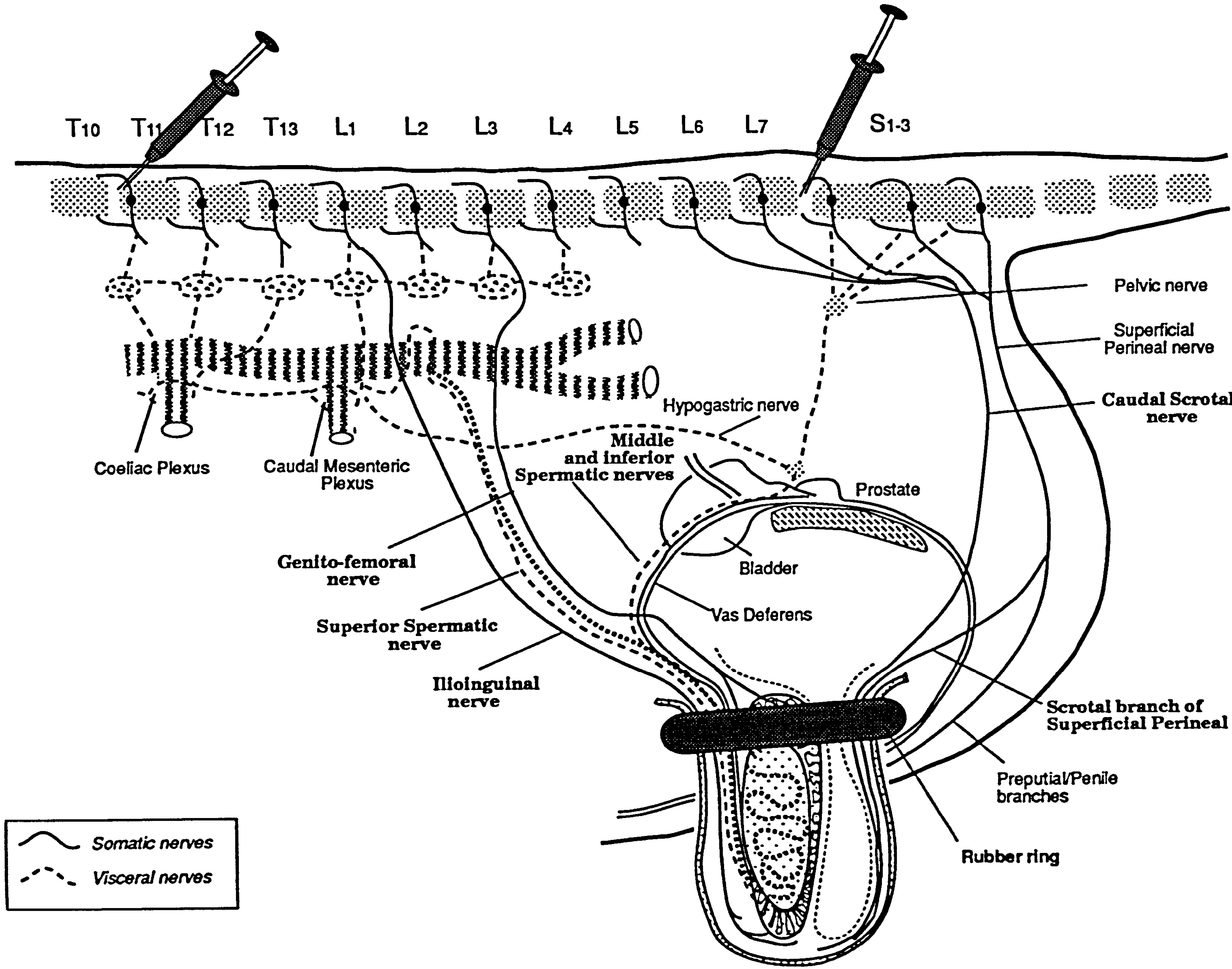
A day before the experiment wool was clipped from the full length of the neck to expose both jugular veins. Wool was also removed in a 10cm wide strip along the dorsum from mid-sacrum to thoracic vertebra 8. Preparation of the skin surface and anaesthesia were as described for experiment 1.

The T10-T11 intervertebral space, and the lumbosacral junction were located by palpation of vertebrae and other bony landmarks and marked on the skin surface with a pen. A 1" 23 guage needle was located centrally on the mark made at the lumbosacral space. While held vertically the needle was advanced through the skin and intervertebral space into the subarachnoid space until CSF was seen to flow from the hub (see fig 4.1). A new needle was located 5-10mm lateral to the mark previously made in the midline indicating the T10-T11 intervertebral space. The needle was then slowly advanced through the intervertebral space with the hub angled towards the rear of the lamb. Penetration was usually at a depth of about 15 mm for this size of lamb. If the needle hit the vertebral body it was retracted slightly and the angulation increased. The needle was then advanced again until until CSF was seen to flow from the hub. Due to the dead space of the needle, 120μl of solution was injected at both sites, after which the needle was removed with the syringe attached to stop the loss of CSF and drug. Despite the need for several attempts in early tests no neurological deficits were found that lasted for more than a few minutes and such deficits were only seen on 2 occasions. All animals were bled from a jugular vein before the animals were subjected to anaesthetic etc, then immediately before treatment, and at 15,

FIGURE 4.1

SCHEMATIC DIAGRAM ILLUSTRATING THE INTRATHECAL INJECTION SITES, LOCATION OF THE TIGHT RUBBER RING AND INNERVATION OF THE SCROTUM AND TESTES

See key opposite for somatic and visceral innervation


T10
T11
T12
T13
L1
L2
L3
L4
L5
L6
L7
S1-3
Pelvic nerve
Superficial Perineal nerve
Caudal Scrotal nerve
Hypogastric nerve
Middle and inferior Spermatic nerves
Prostate
Coeliac Plexus
Caudal Mesenteric Plexus
Bladder
Genito-femoral nerve
Vas Deferens
Superior Spermatic nerve
Ilioinguinal nerve
Scrotal branch of Superficial Perineal
Preputial/Penile branches
Rubber ring
Somatic nerves
Visceral nerves

30, 60, 90, 120, and 180 minutes after treatment. There were four treatment groups.

Group 1 Control handling. Lambs (n=6) received a general anaesthetic, and 1" 23 gauge needles were inserted in both injection sites (as described above) but no drug or vehicle was injected.

Group 2 Intrathecal etorphine only. Lambs (n=6) were anaesthetised and 2 x 5nmol etorphine in 100μl of saline was administered into the intrathecal space at two separate locations (see above), 40 minutes before the start of the experiment.

Group 3 Intrathecal etorphine followed by castration. Lambs (n=6), were treated as in group 2, and were castrated by application of tight rubber rings.

Group 4 Intrathecal saline followed by castration. Lambs (n=6), were treated as lambs in group 3 except that saline (2 x 100μl) was administered into the intrathecal space.

Plasma cortisol assays and presentation of results (Experiment 1 and 2)

Plasma cortisol concentrations in experiment 1, were measured by radioimmunoassay using an IDS kit (IDS Ltd. Tyne and Wear) with lowest detectable concentration of 7.8 ± 1.1 nmol/l and intra and inter assay coefficients of variation of 4.5 and 9.0% respectively. In experiment 2 cortisol concentrations were also measured by radioimmunoassay using an Amerlex kit (Amersham) with intra and inter assay coefficients of variation of 11.7 and 8.8% respectively and lowest detectable concentration 3.0 ± 0.9 nmol/l. Further details of the plasma cortisol estimation are discussed in the general methods chapter.

Where applicable the plasma cortisol results are expressed as the mean ± the standard error of the mean (SED). An analysis of variance was carried out covering the periods 15, 30 and 60 minutes for both experiments, using the base cortisol level for each lamb as a covariate. A 2 by 2 treatment structure was used as shown below (see general methods for further explanation).

Experiment 1. (Castration (CN) v Control handling (C); Control v Epidural morphine and castration (EDM)).
Experiment 2. (Castration (CN) v Control handling (C); Control v Intrathecal etorphine and castration (ITE)).

The main output of these analyses were 2 by 2 tables of cortisol means (nmol/l) and a standard error of differences (SED), which allowed comparison of the means. Any difference between the mean plasma cortisol concentrations which exceeds the figure accompanying the table (Derived from the SED) is statistically significant at the 5% level.

Behavioural monitoring and presentation of results (Experiment 1 and 2)

Detailed description of the behavioural monitoring techniques and analysis of data using non parametric statistical tests is described in the general materials and methods chapter. In experiment 1 behavioural monitoring was extended over a four hour period and the presence or absence of particular behaviour was noted for each lamb at specific times after treatment. The different behaviours were: lateral recumbency, ventral recumbency, standing and teat seeking or sucking. Behaviour was observed once every minute between between one and 14 minutes, every two minutes between 17 and 29 minutes, every 5 minutes between 35 and 60 minutes, every ten minutes between 70 and 120 minutes, every 15 minutes between 135 and 240 minutes. Behavioural data is presented in bar chart form, the incidence of each behaviour pattern being expressed as a percentage of the total number of observations during each period. In experiment 2 the overall recording time was reduced to 3 hours. Most lambs return to normal by this time as assessed by initial monitoring techniques and this reduction allowed more lambs to be investigated each day. Three severities of lateral recumbency, ventral recumbency and standing were recorded as was restlessness, a quantitative measure of how often a lamb got up and lay down. Restlessness was recorded by continuous observation between 1 and 15 minutes, and between 15 and 30 minutes. Behaviour was recorded once every minute between one and 14 minutes, every two minutes between 17 and 29 minutes, and at 5 minute intervals to the end of the experiment (180 minutes). Behavioural data is presented as for experiment 1. A fuller description is given in the general materials and methods (chapter 2).

RESULTS

Effect of epidural morphine on the behavioural and physiological indices selected, following castration and tail-docking

EXPERIMENT 1

Expt 1 Group 1- Behaviour of contol lambs

The behaviour of each lamb was recorded regularly but intermittently. It was not therefore possible to determine the total time for which a lamb exhibited each behaviour. The results are presented in Fig 4.2 and Tables 1 and 2. The behaviour of control lambs was consistent from 15 minutes to the end of the experiment (240 minutes), most lambs remaining in ventral recumbency (67-94% of observations). In the first 15 minutes following blood sampling, lambs lay in lateral recumbency (15% of observations) and showed teat seeking (18% of observations). Note, however, only one lamb was responsible for the lateral recumbency recorded. Furthermore in the first 15 minutes lambs spent more time standing (44% of observations) than lying in ventral recumbency (23% of observations).

Plasma cortisol concentrations

The results are presented in Fig 4.2 and Tables 1 and 2. There was a rise in plasma cortisol concentration from a basal level (21.5 ± 16.1 nmol/l) at the start of the experiment, reaching a peak of 48.5 ± 16.7 nmol/l at 15 minutes and returning to below initial basal levels by 60 minutes. The plasma cortisol concentration remained below the initial basal level for the remainder of the experiment (60-240 minutes).

Expt 1 Group 2- Behaviour of lambs following epidural morphine (16mg)

The results are presented in Fig 4.2 and Tables 1 and 2. Behaviour in lambs treated with epidural morphine (16mg) was not significantly different from control lambs in the first 15 minutes. In subsequent time periods there was more standing than in control lambs which was significant (P<0.05) during the periods 15-30 and 60-120 minutes. Lateral lying was observed during the time periods between 0 and 180 minutes, which was not significant when compared period by period with the control group (P<0.05). The total time spent in lateral recumbency in this groupis, however, greater than that in the control group (P<0.05). These lambs lay less in ventral recumbency than control lambs throughout the experiment, and this was significant during the periods 15-30, 30-60 and 60-120 minutes (P<0.05). Teat seeking and sucking was evident in the first 60 minutes (14-27% of observations) in group 2, while almost absent from all time periods in the other 3 groups. The total time spent teat seeking approached significance at P<0.05 when compared with control lambs.

Plasma cortisol concentrations

The results are presented in Fig 4.2 and Tables 1 and 2. The plasma cortisol levels were reduced in lambs to which epidural morphine had been administered. When compared with control lambs from 15-60 minutes, this approached significance at the 5% level. Following administration of epidural morphine the plasma cortisol level fell from basal levels (40.0 ± 9.4 nmol/l) to below the minimum detectable concentration of the kit (7.8 ± 1.1 nmol/l) by 30 minutes. Thereafter concentrations increased to establish a plateau value between 70-78 nmol/l from 120-180 minutes.

Expt 1 Group 3- Behaviour of lambs to which epidural morphine had been administered (2x8mg) 40 minutes prior to castration and tail-docking

The results are presented in Fig 4.2 and Tables 1 and 2. Lambs to which epidural morphine (2x8mg) had been administered 40 minutes prior to castration, showed no reduction in the abnormal behaviour associated with this procedure. On the contrary in the first 60 minutes a greater increase in lateral recumbency occurred than that in lambs castrated and tail-docked without epidural morphine. This increase in lateral recumbency was significant from 0-15 minutes ($P<0.05$) and approached significance from 15-30 minutes ($P<0.1$). After 60 minutes most lambs stopped lying laterally, but spend more time standing (75-79% of observations) than those lambs castrated and tail-docked without morphine. The increase in standing, and associated decrease in ventral recumbency was significant ($P<0.05$) when compared with control lambs from 60-180 minutes.

Plasma cortisol concentrations

The results are presented in Fig 4.2 and Tables 1 and 2. Lambs castrated and tail-docked 40 minutes after epidural morphine administration (2x8mg) produced a significantly smaller plasma cortisol response than lambs castrated and tail-docked following saline administration when compared for the period 15-60 minutes ($P<0.05$). The plasma cortisol changes in group 3 were not statistically different from control lambs over the same time period. A distinct secondary rise in plasma cortisol concentration occurred from 90-180 minutes reaching a peak of 70.8 ± 19.7 nmol/l by 150 minutes.

Expt 1 Group 4- Behaviour of lambs to which epidural saline had been administered 40 minutes prior to castration and tail-docking

The results are presented in Fig 4.2 and Tables 1 and 2. Lambs to which epidural saline had been administered 40 minutes prior to castration and tail-docking lay more in lateral recumbency (6-69% of observations) from 0-120 minutes than control lambs. This marked difference was significant from 0-60 minutes ($P<0.05$). Conversely lambs this group lay less in ventral recumbency (2-78% of observations) when compared with control lambs from 0-240 minutes, which was only significant ($P<0.05$), from 15-30 minutes. This reduction in ventral recumbency, was associated with a progressive increase in standing (10-46% of observations) from 15-240 minutes. The incidence of standing in this group was significantly greater than for control lambs ($P<0.05$) from 120-240 minutes.

Plasma cortisol concentrations

The results are presented in Fig 4.2 and Tables 1 and 2. The plasma cortisol response in lambs to which epidural saline had been administered 40 minutes prior to castration and tail-docking rose rapidly from a pretreatment basal level (16.3 ± 8.8 nmol/l) to a peak value of 183.2 ± 45.7 nmol/l at 60 minutes. The plasma cortisol concentrations returned to near basal levels by 120 minutes (33.4 ± 18.9 nmol/l). The plasma cortisol response was significantly larger than the other 3 groups ($P<0.05$), for the periods between 15-60 minutes.

Plasma cortisol concentrations

Experiment 1. Mean plasma cortisol concentrations (nmol/l) for the 4 groups.

Procedure \ Drug	Nil	E/D Morphine
C	52.8	-4.9
CN	167.0	69.1

Thus for a SED of 33.8, and using a two way T-test, 5% significance is obtained with any difference greater than 70.6 nmol/l between the mean treatment cortisol concentrations tabulated above. Note that the castration and tail-docking (CN-Nil) group produces a cortisol response significantly greater than the other 3 groups ($P<0.05$). Lambs to which epidural morphine (16mg) had been administered prior to castration and tail-docking (CN-E/D Morphine) produced a cortisol response (69.1 nmol/l) which was not significantly different from control lambs (C-Nil). Note also, however, that lambs administered morphine only (C-E/D Morphine), had depressed plasma cortisol concentrations which approached significance at $P<0.05$. As a result of this depression in plasma cortisol concentration in the C-E/D Morphine group the response in the CN-E/D Morphine group significantly greater ($P<0.05$).

EXPERIMENT 2

Effect of intrathecal etorphine on the behavioural and physiological indices selected, following castration

Expt 2 Group 1- Behaviour of control lambs

The results are presented in Fig 4.3 and Tables 3 and 4. The incidence of normal standing (S1) in control lambs decreased progressively from 59% of the observations in the first 15 minutes to 22% of observations by 120 minutes. This was due to an increase in mainly awake ventral recumbency (V2) from 41% of observations in the first 15 minutes to 78% of observations by 120 minutes. The trend of increasing ventral recumbency and decreasing standing was reversed from 120-180 minutes. There was no evidence of restlessness (11 posture changes) from 0-15 minutes.

Plasma cortisol concentrations

The results are presented in Fig 4.3 and Tables 3 and 4. The plasma cortisol concentration in control lambs rises from a basal level prior to the first blood sample (17.3 ± 5.5 nmol/l) to a peak of 76.8 ± 17.5 nmol/l at 30 minutes. Plasma cortisol concentrations then returned to basal levels by 60 minutes.

Expt 2 Group 2- Behaviour of lambs to which intrathecal etorphine (2x5nmol) had been administered 40 minutes prior to castration

The results are presented in Fig 4.3 and Tables 3 and 4. A similar pattern of behaviour occurred in lambs following intrathecal etorphine when compared to control lambs, there being no statistical differences between the behaviours of these two groups. In this group, the incidence of standing decreased progressively from 83% of the observations in the first 15 minutes to 28% of the observations at the end of the experiment (180 minutes). This was due to an increase in mainly awake ventral recumbency (V2) from 17% of observations in the first 15 minutes to 72% of the observations by 180 minutes. Unlike the control group, the standing was slightly abnormal (S2) from 0-90 minutes (10-35% of observations) in group 2. There was no evidence of restlessness (7 posture changes) from 0-15 minutes.

Plasma cortisol concentrations

The results are presented in Fig 4.3 and Tables 3 and 4. The plasma cortisol concentration of lambs to which intrathecal etorphine (2x5nmol) had been administered 40 minutes prior to castration increased from a basal level (41.5 ± 13.0 nmol/l) to a peak of 82.9 ± 25.3 nmol/l at 30 minutes. After the peak there was a gradual return to basal levels by 90 minutes. From 120 minutes there was a secondary increase which may have continued after the last sample at 180 minutes. The plasma cortisol response of this group was not significantly different from that in control lambs when compared for the periods between 15-60 minutes ($P<0.05$).

Expt 2 Group 3- Behaviour of lambs to which intrathecal etorphine (10 nmol) had been administered 40 minutes prior to castration

The results are presented in Fig 4.3 and Tables 3 and 4. Lambs to which intrathecal etorphine (2x5nmol) had been administered 40 minutes prior to castration lie mainly in ventral recumbency throughout the whole experiment from 0-180 minutes (61-72% of observations). Unlike control lambs, however, most ventral recumbency was abnormal (V3) from 0-90 minutes (45-60% of observations), changing progressively to mainly normal awake ventral recumbency (V2) by 120-180 minutes. The incidence of standing was reduced from 0-180 minutes when compared with control lambs. This was significant ($P<0.05$) from 0-60 minutes. Lambs in this group lay in lateral recumbency (L1 and L2) throughout the experiment, with the peak incidence occurring from 30-60 minutes (33% of observations). Although no lateral recumbency occurred in control lambs, and only to a limited extent from 0-60 minutes in lambs castrated following administration of intrathecal saline (4-10 % of observations, all L1), no significant differences were found between these groups and group 3. There was also evidence of restlessness in this group from 0-15 minutes (79 posture changes), which was significantly greater than that in control lambs ($P<0.05$), but was not significantly different when compared with lambs to whom intrathecal saline had been administered prior to castration (120 posture changes, $P>0.05$).

Plasma cortisol concentrations

The results are presented in Fig 4.3 and Tables 3 and 4. The plasma cortisol concentrations of lambs to which intrathecal etorphine (2x5nmol) had been administered 40 minutes prior to castration, rose rapidly from a basal level of 51.5 ± 22.5 nmol/l to a peak value of 325.5 ± 57.9 nmol/l at 60 minutes. There was evidence of a plateau between 30 and 60 minutes, indicating that an even higher peak probably occurred between these two sample times. Although the plasma cortisol response was greater in this group than that in lambs castrated following administration of intrathecal saline when compared between 15-60 minutes, this was not found to be significant ($P>0.05$). The plasma cortisol response in this group was, however, significantly greater than the other two groups when compared in the periods 15-60 minutes ($P<0.05$).

Expt 2 Group 4- Behaviour of lambs to which intrathecal saline had been administered 40 minutes prior to castration

The results are presented in Fig 4.3 and Tables 3 and 4. Lambs to which intrathecal saline had been administered 40 minutes prior to castration spent most time in ventral recumbency from 0-180 minutes (61-75% of observations). Ventral recumbency was mostly abnormal (V3) from 0-90 minutes (35-60% of observations), returning to normal awake ventral recumbency (V2) by 90-120 minutes. All standing was abnormal (S2) from 0-120 minutes (25-35% Of observations) but began to return to normal by 120-180 minutes. Some lateral recumbency (L1) was evident from 0-60 minutes (4-10% of observations), however, none of the main behavioural categories in this group were statistically different from either control lambs or lambs to which etorphine had been administered prior to castration ($P<0.05$). Consistent with the lateral recumbency, and abnormal standing the restlessness score was elevated to the highest frequency found in all four groups (120 posture changes) from 0-15 minutes, which was statistically significant when compared with control lambs ($P<0.05$).

Plasma cortisol concentrations

Results are presented in Fig 4.3 and Tables 3 and 4. The plasma cortisol concentrations of lambs to which intrathecal saline had been administered 40 minutes prior to castration increased from a basal level (44.7 ± 17.0 nmol/l) to a plateau similar to that seen in lambs castrated following intrathecal etorphine administration. The peak concentration of the plateau at 60 minutes was lower (223.9 ± 28.0 nmol/l) though not statistically different from that latter group ($P>0.05$) when compared for the periods 15-60 minutes. The plasma cortisol response in this group was significantly greater, however, than control lambs, and those to which etorphine had been administered, when compared over the same time period ($P<0.05$).

Experiment 2. Mean plasma cortisol concentrations (nmol/l) for the 4 groups.

Procedure	Drug	Nil	I/T Etorphine
C		57.6	68.5
CN		191.9	249.0

Thus for a SED of 39.9, and using a two way T-test, 5% and 20% significance is obtained with any difference greater than 83.4 and 52.7 nmol/l respectively between the mean treatment cortisol concentrations tabulated above. Note that lambs to which intrathecal etorphine (10nmol) had been administered prior to castration (CN-I/T Etorphine) produced the largest cortisol response (249.0 nmol/l). This response was significantly greater when compared to control lambs (C-Nil) and lambs receiving etorphine only (C-I/T Etorphine) at $P<0.05$, and lambs castrated without prior drug treatment at $P<0.2$.

FIGURE 4.1

Effect of epidural morphine (16mg) on the indices used for assessment of pain from castration and tail-docking

The results shown were obtained from 4 groups of 6 lambs, each group was subjected to one of the following treatments 1) control handling 2) epidural morphine 3) epidural morphine + castration and tail-docking 4) epidural saline + castration and tail-docking

Behaviour

The incidence (%) of four behaviours (L= Lateral recumbency, S= Standing, V= Ventral recumbency, T/S= Teat seeking and Sucking) were recorded for each group and presented in bar chart form in 6 time periods; 0-15, 15-30, 30-60, 60-120, 120-180 and 180-240 minutes, see materials and methods for numbers of observations in each period

Plasma cortisol

Plasma cortisol concentrations are presented in graph form (nmol/l ± S.E, (n=6)) at 10 times; -10, 0, 15, 30, 60, 90, 120, 150, 180 and 240 minutes.

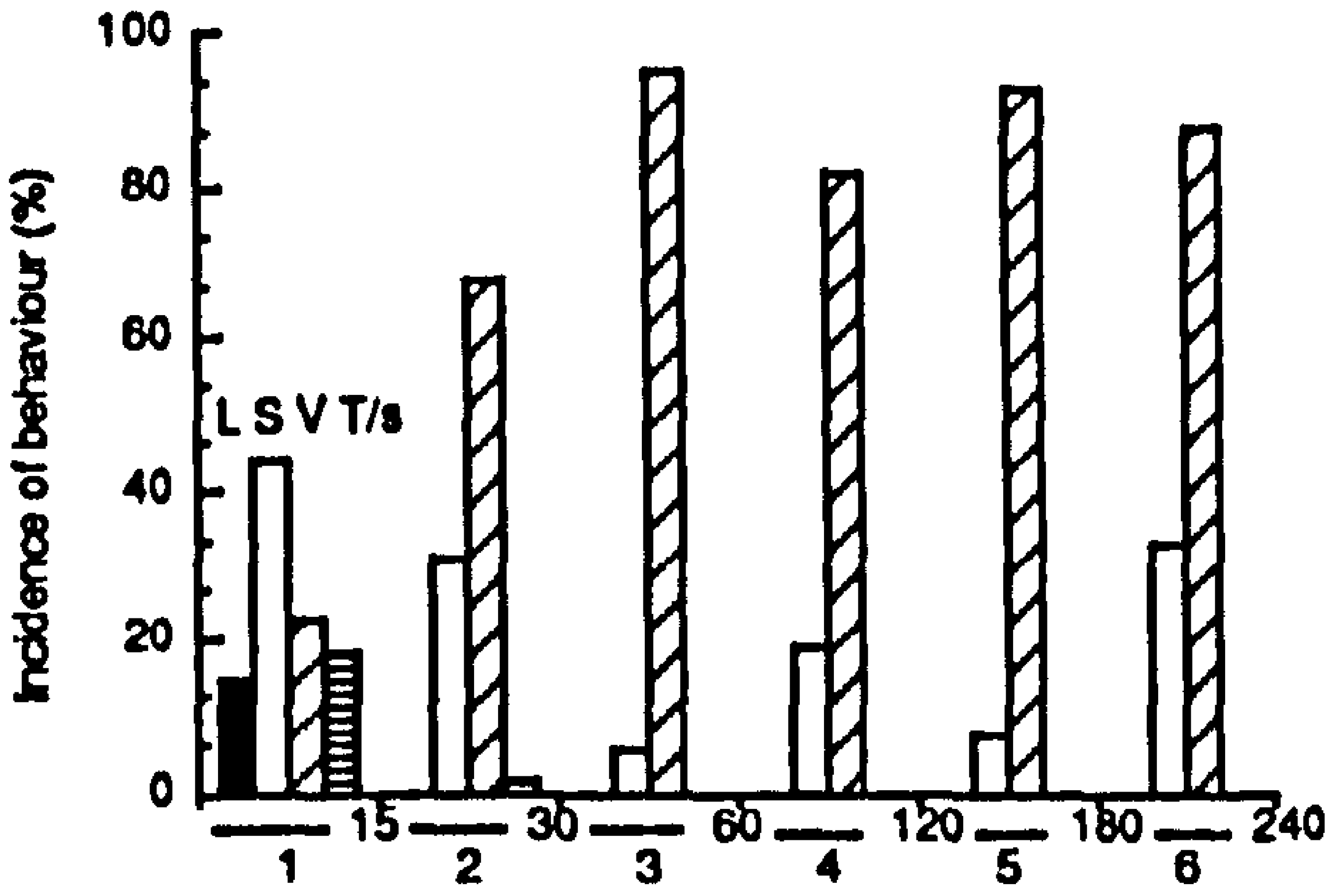
Statistical analysis

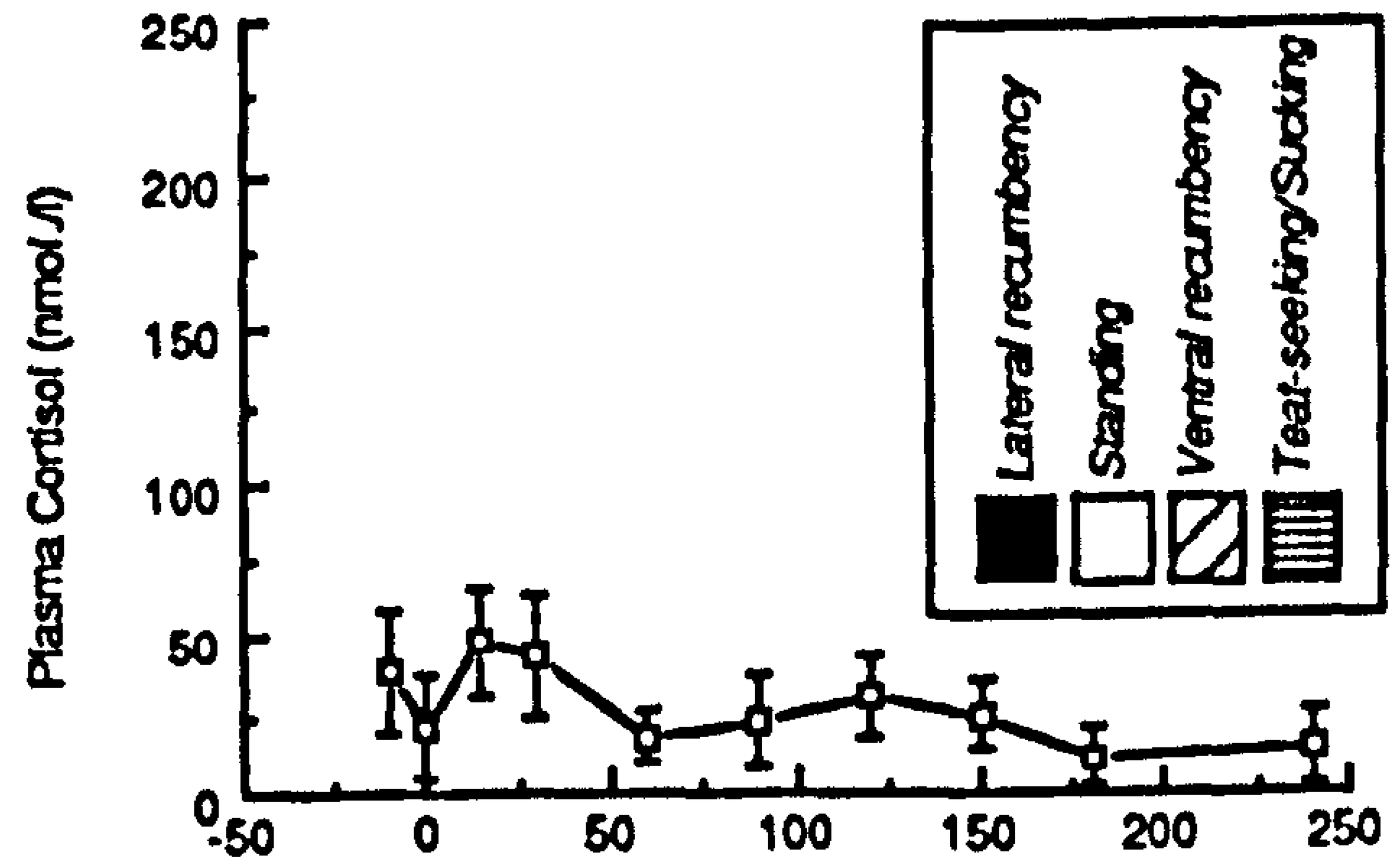
- A Indicates that the difference between the behaviour of the treatment group and the (control handling) group was significant at ($P<0.05$)
- a As above but ($P<0.1$)
- B Indicates that the difference between the behaviour of the (epidural morphine + castration and tail-docking) group and the (epidural saline + castration and tail-docking) group was significant at ($P<0.05$)
- b As above but ($P<0.1$)

Notes 1) Epidural morphine increases the incidence of lateral recumbency after castration and tail-docking, this was significant for the period 0-15 minutes ($P<0.05$) and approached significance for the period 15-30 minutes ($P<0.1$).

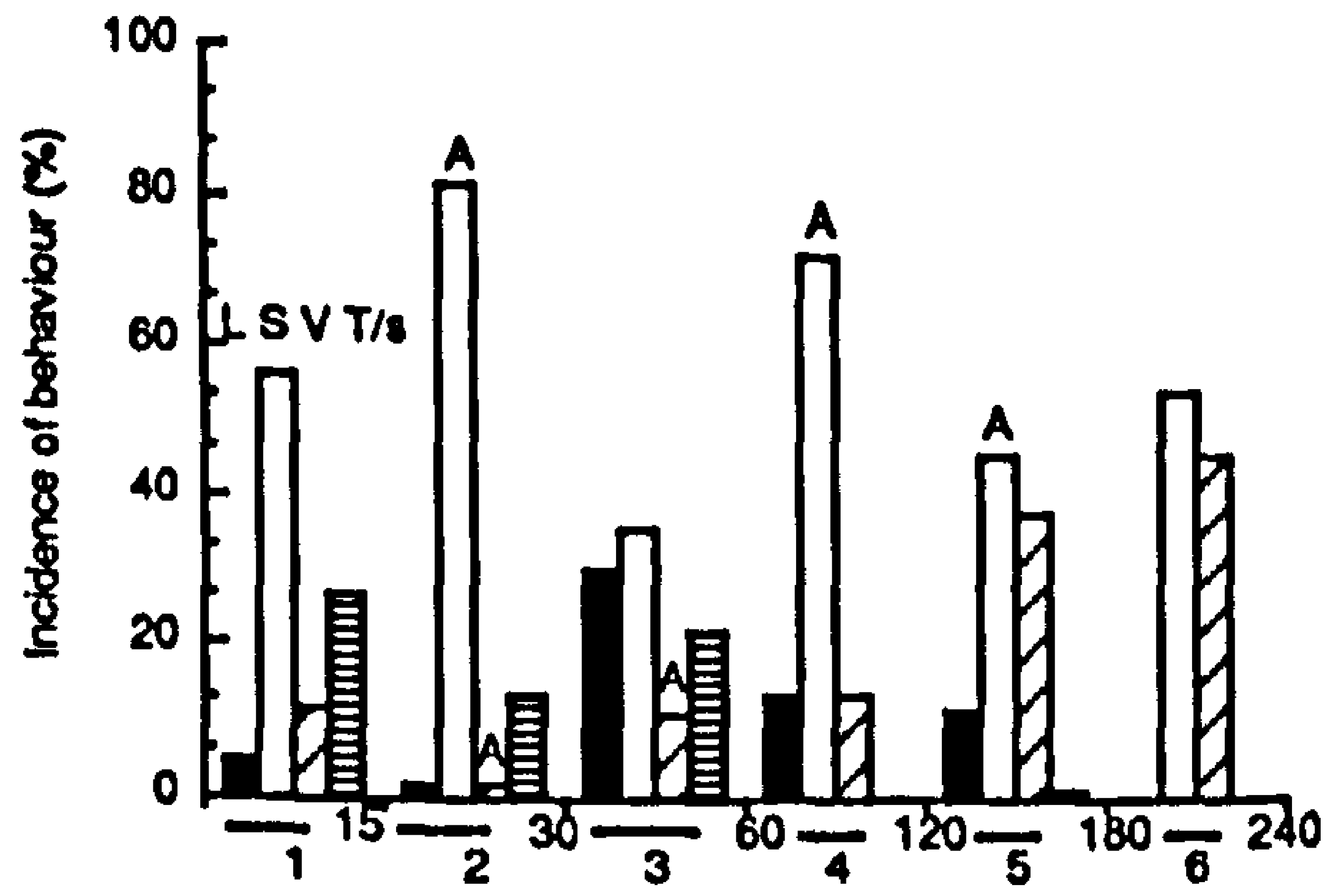
2) The plasma cortisol response of the (E/D Saline + C/T) group is significantly greater than the (E/D Morphine + C/T) group when compared between 15-60 minutes ($P<0.05$)

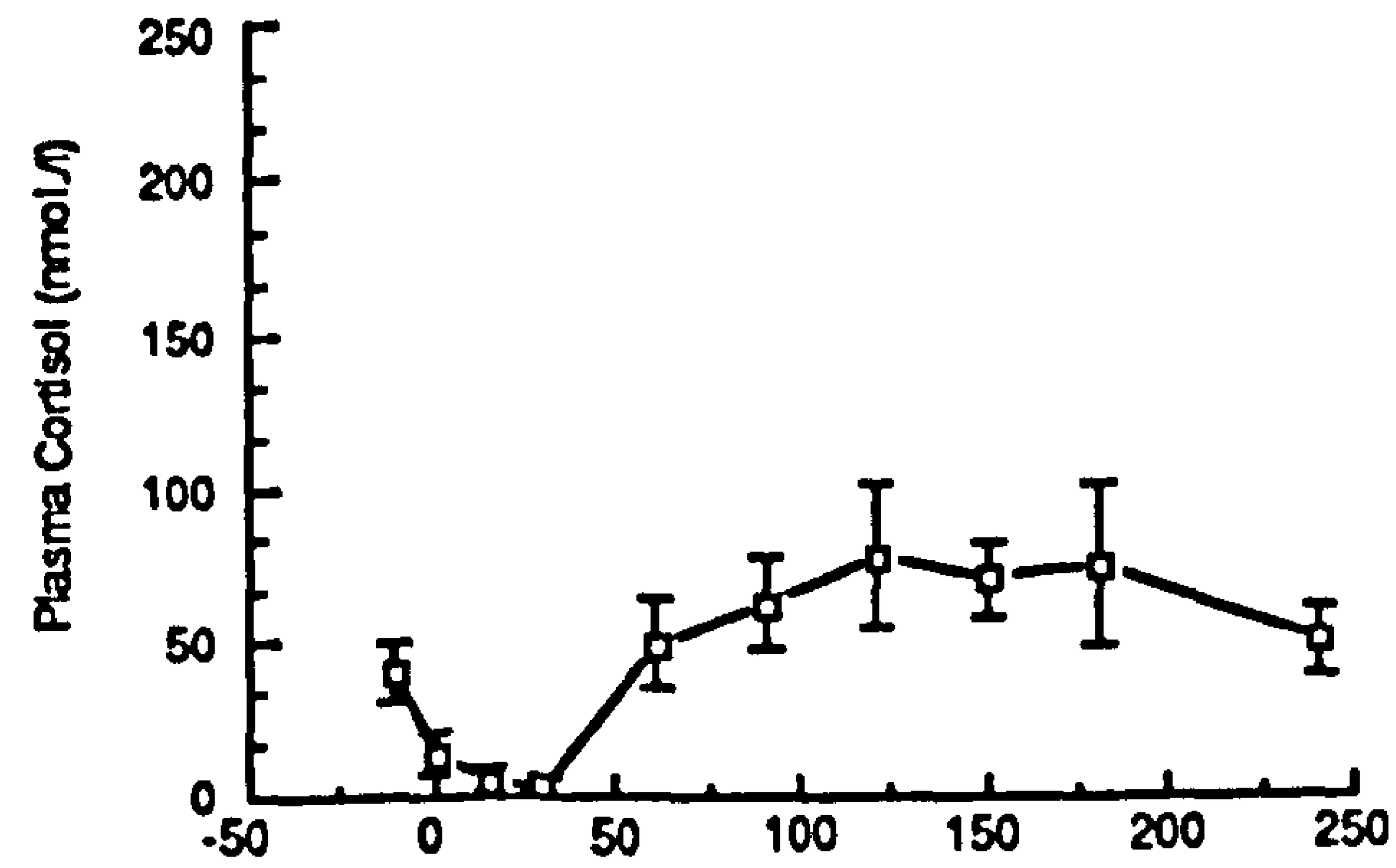
Control Handling



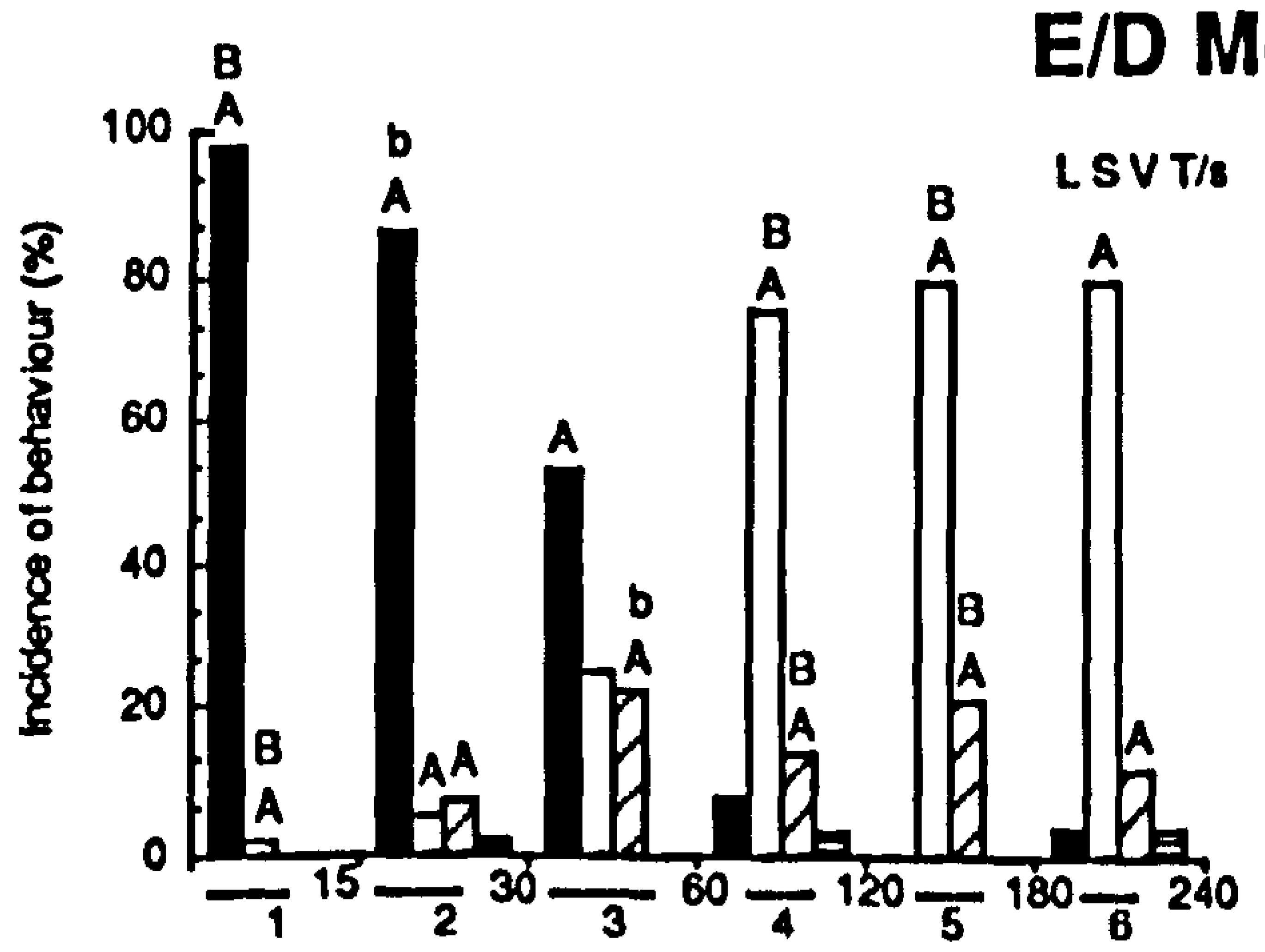


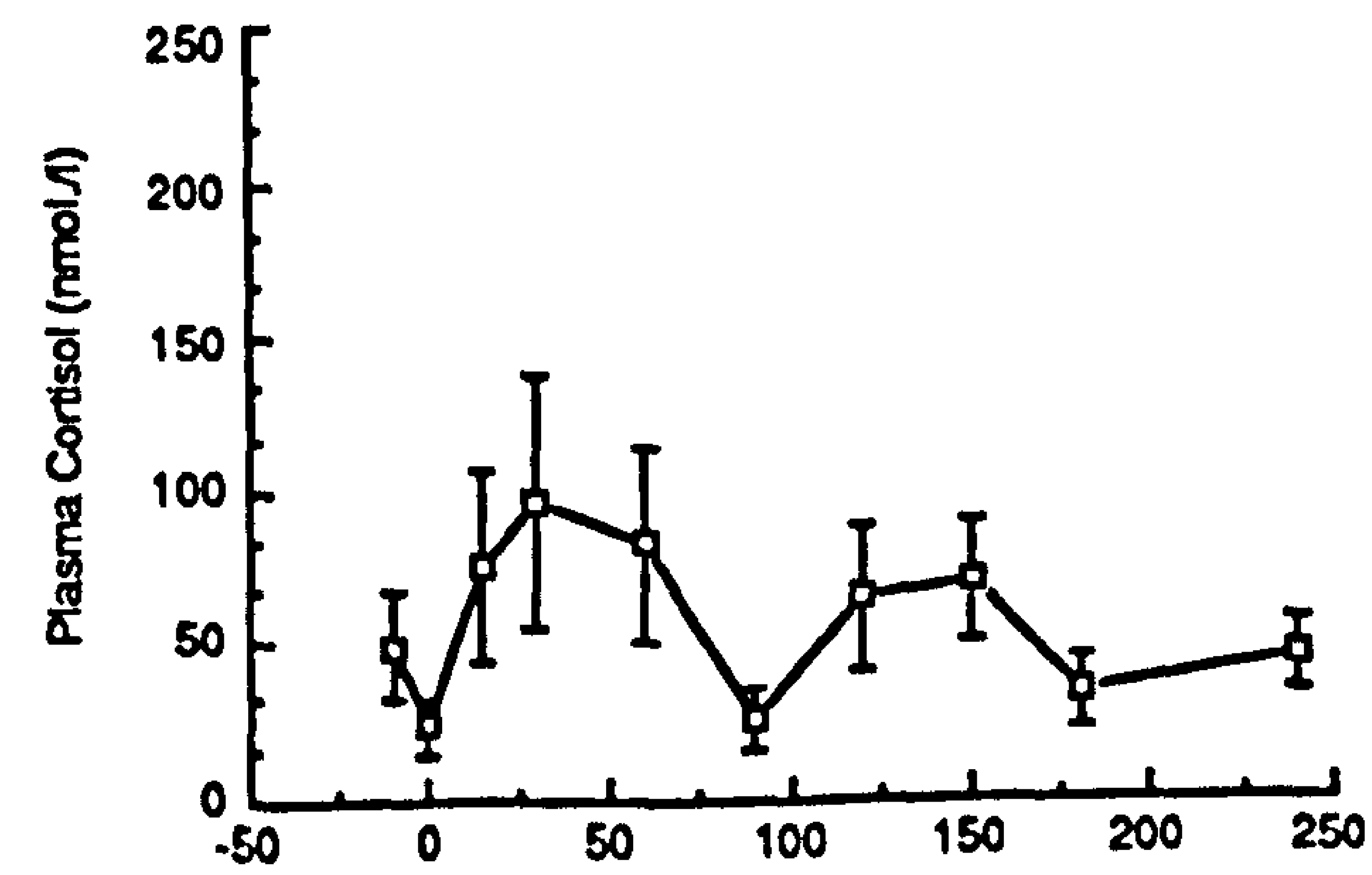
E/D Morphine



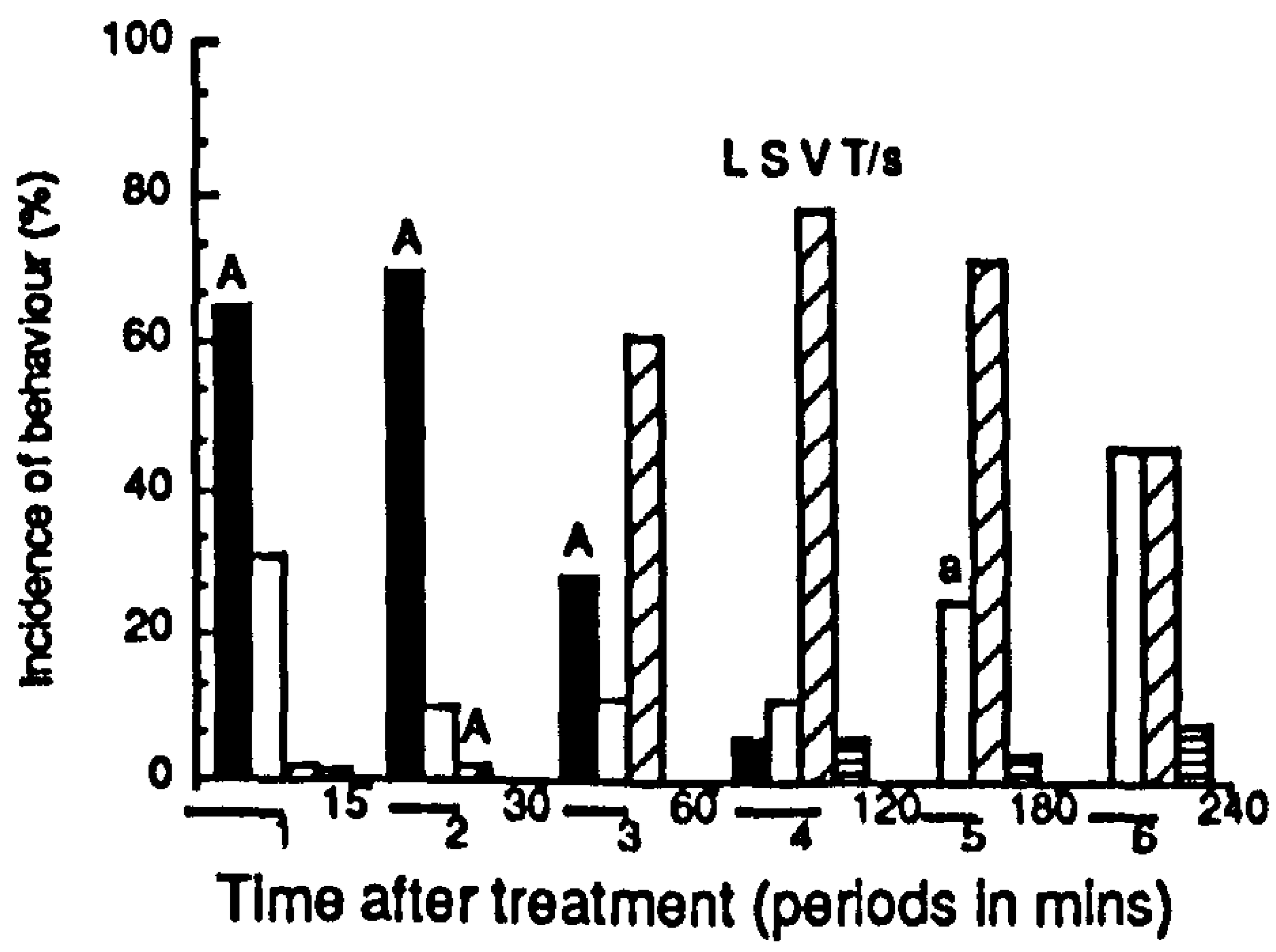


E/D Morphine+C/T





E/D Saline +C/T



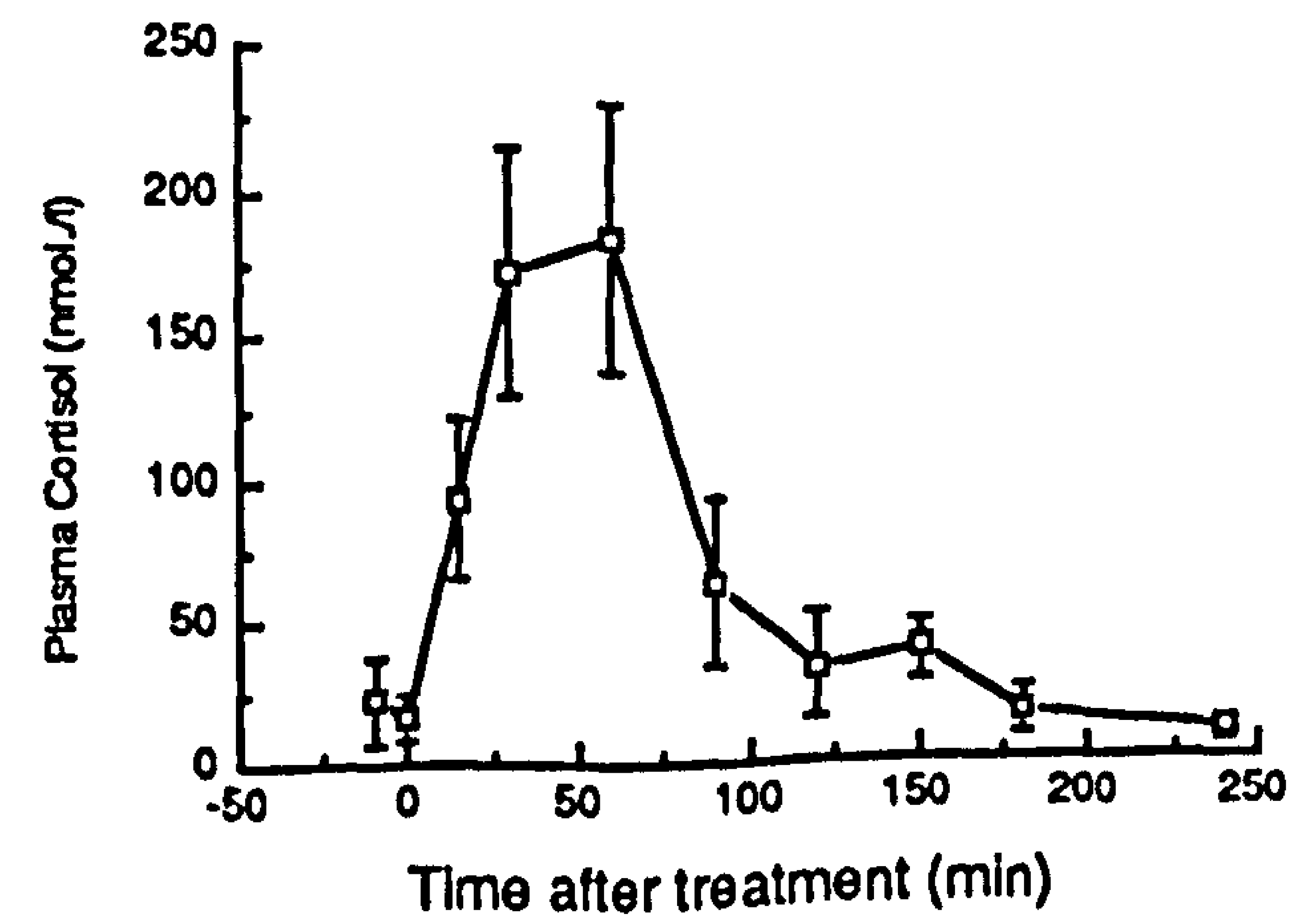


TABLE 4.1

THE EFFECTS OF FOUR TREATMENTS ON LAMB BEHAVIOUR IN SIX TIME PERIODS

In each period after treatment the incidence of four postures (i.e. Lateral recumbency, Standing, Ventral recumbency and Teat Seeking or Sucking) are presented as the number of times that behaviour is observed, and as a percentage of the total number of observations made in that period.

TABLE 4.1 Incidence of four lamb behaviours in six periods after four treatments.

PERIOD AFTER TREATMENT	0 - 15 mins				15 - 30 mins				30 - 60 mins				50 - 120 mins				120 - 180 mins				180 - 240 mins			
NUMBER OF OBSERVATIONS MADE IN PERIOD	n = 84				n = 42				n = 36				n = 36				n = 24				n = 24			
BEHAVIOUR OBSERVED	LR	VR	St	TS/S	LR	VR	St	TS/S	LR	VR	St	TS/S	LR	VR	St	TS/S	LR	VR	St	TS/S	LR	VR	St	TS/S
CONTROL HANDLING (6 lambs)	13 15%	19 23%	37 44%	15 18%	0 0%	28 67%	13 31%	1 2%	0 0%	34 94%	2 6%	0 0%	0 0%	34 94%	2 6%	0 0%	0 0%	22 92%	2 8%	0 0%	0 0%	16 67%	8 23%	0 0%
EPIDURAL MORPHINE (16mg) (6 lambs)	4 5%	10 12%	47 56%	23 27%	1 2%	1 2%	34 81%	6 14%	11 30%	4 11%	13 36%	8 22%	5 14%	5 14%	26 72%	0 0%	3 12%	9 38%	11 46%	1 4%	0 0%	11 46%	13 54%	0 0%
EPIDRUAL MORPHINE (16mg) PLUS CASTRATION AND TAIL DOCKING (6 lambs)	82 98%	0 0%	2 2%	0 0%	36 86%	2 5%	2 5%	2 5%	19 53%	8 22%	9 25%	0 0%	3 8%	5 14%	27 75%	1 3%	0 0%	5 21%	19 79%	0 0%	1 4%	3 12%	19 79%	1 4%
EPIDURAL SALINE PLUS CASTRATION AND TAIL DOCKING (6 lambs)	55 65%	2 2%	26 31%	1 1%	29 69%	9 21%	4 10%	0 0%	11 30%	22 61%	4 11%	0 0%	2 6%	28 78%	4 11%	2 6%	0 0%	17 71%	6 25%	1 4%	0 0%	11 46%	11 46%	2 8%

LR Lateral Recumbency
VR Ventral Recumbency
St Standing or Walking
TS/S Teat Seeking or Sucking

TABLE 4.2 Plasma cortisol measurements taken at 10 times for 4 different treatment groups

TIME AFTER TREATMENT (mins)	-10.0	0	15	30	60	90	120	150	180	240
NUMBER OF MEASUREMENTS (No. of lambs)	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
CONTROL HANDLING Plasma cortisol (n.mol/1) Mean ± S.E.	38.5 ± 19.6	21.5 ± 16.1	48.5 ± 16.7	43.4 ± 19.6	17.6 ± 8.6	22.9± 14.0	30.7 ± 13.3	25.2 ± 11.2	11.8 ± 8.9	15.0 ± 11.1
EPIDURAL MORPHINE Plasma cortisol (n.mol/1) Mean ± S.E.	40.0 ± 9.4	13.8 ± 7.1	6.0 ± 3.8	2.9 ± 2.8	48.9± 14.3	62.6 ± 15.0	78.0 ± 24.0	70.6 ± 12.0	75.3 ± 26.4	49.9.± 11.3
EPIDURAL MORPHINE PLUS CASTRATION Plasma cortisol (n.mol/1) Mean ± S.E.	49.5 ± 17.6	23.7 ± 8.2	74.8 ± 31.3	96.6 ± 40.9	82.8 ± 31.9	26.1 ± 10.2	64.6 ± 24.4	70.8 ± 19.7	34.4 ± 11.6	45.8 ± 11.2
EPIDURAL SALINE PLUS CASTRATION Plasma cortisol (n.mol/1) Mean ± S.E.	22.3 ± 15.6	16.3 ± 8.8	92.6 ± 28.0	171.9 ± 42.7	183.2 ± 45.7	64.0 ± 29.1	33.4 ± 18.9	38.7 ± 10.4	16.3 ± 8.6	9.1 ± 3.6

FIGURE 4.2

Effect of intrathecal etorphine (10nmol) on the indices used for assessment of pain from castration

The results shown were obtained from 4 groups of 6 lambs, each group was subjected to a different treatment 1) control handling 2) intrathecal etorphine 3) intrathecal etorphine + castration 4) intrathecal saline + castration

Behaviour

The incidence (%) of three behaviours (L- Lateral recumbency (1-3), S- Standing (1-3), V- Ventral recumbency (1-3)) were recorded for each group and presented in bar chart form in 6 time periods; 0-15, 15-30, 30-60, 60-90, 90-120 and 120-180 minutes, see materials and methods for numbers of observations in each period. A restlessness score was recorded from 0-15 minutes, and was presented as a quantitative measure of the total number of posture changes in all lambs.

Plasma cortisol

Plasma cortisol concentrations were presented in graph form (nmol/l +/- S.E, (n=6)) at 9 times; -10, 0, 15, 30, 60, 90, 120, 150 and 180 minutes.

Statistical analysis

A Indicates that the difference between the behaviour of the treatment group and the (control handling) group was significant at ($P<0.05$)

Notes 1) Intrathecal etorphine increases the incidence of lateral recumbency after castration during the time periods 0-15, 15-30, 30-60 and 60-90 minutes. This was not significant ($P<0.05$). 2) Intrathecal etorphine also increases the incidence and duration of abnormal ventral recumbency (V3) after castration 3) No abnormal ventral recumbency (V3) or lateral recumbency (L1-3) was seen in either the (control handling) group or the (I/T Etorphine) group 4) The cortisol response of the (I/T Etorphine + Castration) group was greater but not significantly different from ($P<0.05$) the (I/T Saline + Castration group).

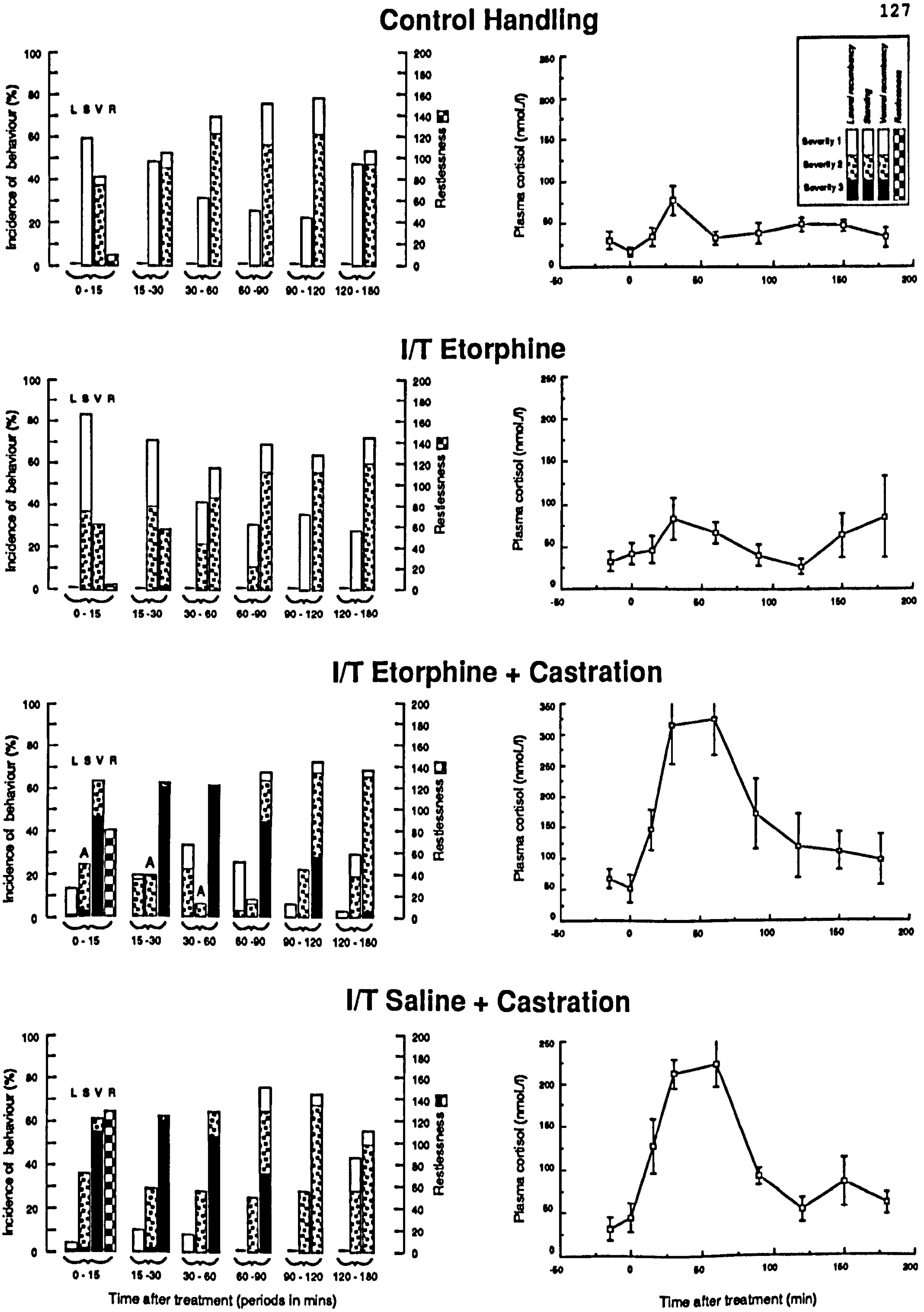

Control Handling
I/T Etorphine
I/T Etorphine + Castration
I/T Saline + Castration
L S V R
A
Incidence of behaviour (%)
Restlessness
Plasma cortisol (nmol/l)
Lateral recumbency
Standing
Ventral recumbency
Restlessness
Severity 1
Severity 2
Severity 3
0 - 15
15 -30
30 - 60
60 -90
90 - 120
120 - 180
Time after treatment (periods in mins)
Time after treatment (min)

TABLE 4.3

THE EFFECTS OF FOUR TREATMENTS ON LAMB BEHAVIOUR IN SIX TIME PERIODS

In each period after treatment the incidence of three postures (i.e. Lateral Recumbency, Standing and Ventral Recumbency) are presented as the number of times that behaviour is observed, and as a percentage of the total number of observations made in that period.

TABLE 4.3 Incidence of four lamb behaviours in six periods after four treatments.

PERIOD AFTER TREATMENT	0 - 15 mins				15 - 30 mins			30 - 60 mins			60 - 90 mins			90 - 120 mins			120 - 180 mins		
NUMBER OF OBSERVATIONS MADE IN PERIOD	n = 84				n = 42			n = 36			n = 36			n = 36			n = 72		
BEHAVIOUR OBSERVED	LR	St	VR	R	LR	St	VR	LR	St	VR	LR	St	VR	LR	St	VR	LR	St	VR
CONTROL HANDLING (6 lambs)	0 0%	50 59%	34 41%	11	0 0%	20 48%	22 52%	0 0%	11 31%	25 69%	0 0%	9 25%	27 75%	0 0%	8 22%	28 78%	0 0%	34 47%	38 53%
INTRATHECAL ETORPHINE (10nmol) ONLY (6 lambs)	0 0%	70 83%	14 17%	7	0 0%	30 71%	12 29%	0 0%	15 42%	21 58%	0 0%	11 31%	25 69%	0 0%	13 36%	23 64%	0 0%	20 28%	52 72%
INTRATHECAL ETORPHINE (10 nmol) PLUS CASTRATION (6 lambs)	11 13%	20 24%	53 63%	79	8 19%	8 19%	26 62%	12 33%	2 6%	22 61%	9 25%	3 8%	24 67%	2 6%	8 22%	26 72%	2 3%	21 29%	49 68%
INTRATHECAL SALINE PLUS CASTRATION (6 lambs)	3 4%	30 35%	51 61%	128	4 10%	12 29%	26 61%	3 8%	10 28%	23 64%	0 0%	9 25%	27 75%	0 0%	10 28%	26 72%	0 0%	31 43%	41 57%

LR Lateral Recumbency
St Standing or Walking
VR Ventral Recumbency
R Restlessness

TABLE 4.4 Plasma cortisol measurements taken at 9 times for 4 different treatment groups

TIME AFTER TREATMENT (mins)	-10.0	0	15	30	60	90	120	150	180
NUMBER OF MEASUREMENTS (No. of lambs)	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
CONTROL HANDLING Plasma cortisol (n.mol/l) Mean ± S.E.	30.5 ± 9.2	17.3 ± 5.5	33.9 ± 10.3	76.8 ± 17.5	33.1 ± 7.6	38.8± 11.6	48.7 ± 8.0	47.3 ± 6.8	34.0 ± 11.3
INTRATHECAL ETORPHINE Plasma cortisol (n.mol/l) Mean ± S.E.2	32.0 ± 11.5	41.5 ± 13.0	46.1 ± 16.4	82.9 ± 25.3	66.0 ± 12.5	40.0 ± 11.8	26.9 ± 9.3	63.0 ± 25.1	84.8 ± 47.2
INTRATHECAL ETORPHINE PLUS CASTRATION Plasma cortisol (n.mol/l) Mean ± S.E.	67.1 ± 15.3	51.5 ± 22.5	147.0 ± 32.2	315.3 ± 61.7	325.5 ± 57.9	172.0 ± 56.9	119.8 ± 51.1	111.7 ± 30.1	97.8 ± 40.1
INTRATHECAL SALINE PLUS CASTRATION Plasma cortisol (n.mol/l) Mean ± S.E.	31.6 ± 13.3	44.7 ± 17.0	127.9 ± 31.8	212.6 ± 17.1	223.9 ± 28.0	91.8 ± 9.5	52.9 ± 14.5	84.5 ± 28.6	60.9 ± 12.6

DISCUSSION

The aim of these experiments was to find an analgesic with which to reduce the pain experienced by lambs after castration and tail-docking or castration. Neither epidural or intrathecal morphine at the doses employed here produced recognisable analgesia in these experiments. This suggests that unless unknown factors prevented access of active opioids to the spinal cord, or the drugs used had an insufficient duration of action, the pain produced by castration and tail-docking or castration cannot be reduced by epidural morphine or intrathecal etorphine. Evidence was, however, obtained that suggests opioids, administered by this route and in these doses, produce hyperalgesia.

Interpretation of the results obtained in experiment 1

There was no evidence from the behavioural data of any analgesia following epidural administration of morphine. The incidence of lateral recumbency (98% of observations) was significantly higher ($P<0.05$) during the period 15 - 30 minutes in lambs castrated and tail-docked following epidural morphine administration than in lambs castrated and tail-docked after receiving epidural saline. This change in behaviour could not be attributed directly to morphine, as the lambs receiving just morphine showed increased standing throughout the duration of the experiment (36-81% of observations), with only a limited incidence of lateral recumbency which occurred later in the periods from 30-120 minutes (though the total time spent in lateral recumbency was significantly greater than that seen in control lambs ($P<0.05$)).

In contrast to the behavioural changes, the associated plasma cortisol response might be considered indicative of hypoalgesia, as this response was significantly smaller than that recorded in lambs castrated and tail-docked following epidural saline

($P<0.05$). Epidural morphine alone, however, reduced the plasma cortisol levels to below resting values over the same time period (0-60 minutes) i.e. at the time that peak concentrations would be expected in lambs castrated and tail-docked. The reduction in plasma cortisol concentration by morphine could therefore be responsible for the lower peak plasma cortisol response obtained in lambs castrated and tail-docked following morphine administration. Morphine has been associated with an inhibition of the plasma corticosteroid response in rats produced by stress (Burdette *et al*, 1961; Gibson *et al*, 1979). One possible explanation for the reduction in plasma cortisol levels in lambs administered morphine only, may be that there is a background level of stress in lambs because of human contact before the start of the experiment, which is revealed by the drug treatment.

Work by Carson and Challis, (1982) has demonstrated that administration of met- and leu-enkephalin increased plasma cortisol concentrations in lambs. Some evidence for such an effect in these experiments may be the long lasting increase in plasma cortisol which occurred after the initial suppression. This was seen as a gradual increase starting at about 30 minutes and rising to a peak at 120 minutes which was 95% higher than the basal level. The second peak which can be seen in the plasma cortisol response of lambs castrated and tail-docked following morphine from 90-180 minutes may also be explained by such an effect of morphine on plasma cortisol.

Interpretation of the results obtained in experiment 2

Intrathecal etorphine (2x 5nmol) unlike epidural morphine (2x8mg) was associated with behaviour which was not statistically different from the control lambs when the three main categories of behaviour were compared ($P>0.05$), though some abnormal standing (S2) was seen during the periods 0-15, 15-30 and 30-60 minutes. The total time spent in abnormal standing (S2) by lambs administered intrathecal etorphine was significantly greater than in control lambs ($P<0.05$). This abnormal standing is possibly attributable to the immobilising action of etorphine (Kania, 1985), which was seen in initial trials where larger doses were used (50nmol). An immobilising action could also explain why there was no increase in restlessness score following administration of etorphine. It was not possible to compare the effects of etorphine and morphine on restlessness because this index was was only developed for the etorphine experiment, though restlessness was observed after morphine administration.

Intrathecal etorphine did not eliminate or reduce the limited incidence of lateral recumbency associated with castration, conversely there was an increase in the incidence and duration of this behaviour when compared with lambs castrated following intrathecal administration of saline. Further comparison between these groups revealed that those lambs castrated following intrathecal etorphine administration lie with an increased incidence (2-60% of observations) of abnormal ventral recumbency (V3) throughout the duration of the experiment providing additional evidence for hyperalgesia rather than hypoalgesia.

Evidence for hyperalgesia included an increase in abnormal lying behaviour (lateral 1-2 and ventral 3) and a greater cortisol response than that recorded in lambs castrated following intrathecal saline administration but was only significant at $P<0.2$. The possibility cannot be excluded that the increase in abnormal lying behaviour is due to other physiological effects such as hyperreflexia or hyperaesthesia. It is likely that the association between abnormal activity and the plasma cortisol response demonstrated here, was maintained because I/T etorphine, unlike E/D morphine did not suppress cortisol release. On the basis of the behavioural indices the result obtained with morphine and etorphine is similar, in that both produced changes which can be interpreted as evidence for hyperalgesia.

Explanations for the absence of any analgesic effect following administration of either epidural morphine (16mg) or intrathecal etorphine (10nmol)

(a) Failure of active drug to gain access to the spinal cord

There were no obvious errors in drug administration which may be responsible for failed spinal analgesia (Munhall, 1988). Once in the epidural space morphine has been demonstrated to diffuse slowly into the spinal cord (Cousins and Mather, 1984), despite the necessity to cross the pia arachnoid, which may be a greater barrier to morphine than the dura mater (Bernards and Hill, 1990). Direct evidence that active morphine was administered was provided by behavioural changes following epidural administration of morphine. The increased incidence of teat seeking which approached significance, and lateral lying which was significant ($P<0.05$) (when the total time spent in these behaviours was compared to that seen in control lambs)was attributable to an action at higher levels of the CNS, as was the increase in standing, which was significantly increased during the periods 15-30, 60-120 and 120-180 minutes ($P<0.05$). The increase in teat seeking can be explained by the finding that opioid agonists can enhance intake of some nutrients, for review see Reid (1985). There was indirect evidence that active morphine gained access to the spinal cord due to the appearance of lateral recumbency from 0-180 minute in lambs administered just morphine (2x8mg), which could be produced by a hyper-reflexic action of morphine at the level of the spinal cord. Similar doses of morphine injected intrathecally (see chapter 5) were found to facilitate withdrawal reflexes in the hind limb, probably by depression of spinal inhibitory mechanisms at non opioid receptors (Curtis and Duggan, 1969). Such substantial changes were not seen following intrathecal etorphine administration, the appearance of abnormal standing from 0-90 minutes (10-35% of observations) could be due to central and/or spinal effects as etorphine can have immobilising actions in sheep (Kania, 1985). Direct evidence that morphine and etorphine gained access to the spinal cord is required. This could be obtained from studies of binding of radio labelled opioids to the spinal cord following epidural and intrathecal administration in-vivo.

(b) There is an inadequate population of functional opioid receptors in the spinal cord on which morphine and etorphine can act

It is possible that there are species and age differences in receptor distribution and numbers (Gouarderes *et al*, 1982) which account for the lack of analgesic activity found in these experiments with morphine and etorphine. Although lambs are physiologically well developed at birth, brain development is not complete (McIntosh *et al*, 1979), and at birth the adult complement of opiate binding sites has not been established (Villiger *et al*, 1982). In rats where there is comparable development of the opioid system after birth, morphine analgesia as measured by tail withdrawal from hot water, increases from a 40% increase in reaction time at 5 days to an 85% increase in reaction time by 15 days. These increases were not considered to be attributable to changes in the blood brain barrier, as this barrier did not develop fully until after 15 days (Auguy-Valette *et al*, 1978). It is possible that similar insensitivity to morphine analgesia may be occurring in the young lamb (5-7 days old) and since even adult sheep are also relatively insensitive to opioids (Krueger, 1941, 1943; Davies, 1985), a partially developed system could have little antinociceptive capability. Studies are necessary therefore to elucidate the functional state, location and density of opioid receptors in the lamb spinal cord over this period (5-7 days). Such work has been undertaken in adult sheep. Bouchenafa *et al*, 1989; Brandt and Livingston, 1989 demonstrated that opiate receptors are appropriately located in areas of the spinal cord associated with input from nociceptive primary afferent fibres. Autoradiographic studies using the mu selective agonist <3H> DAGO and the highly selective delta agonist <3H> DPDPE have demonstrated binding in lamina I(μ) and lamina I and II(δ) of the spinal cord in vitro. Intrathecal injection of <3H> DAGO in 100μl saline failed to reproduce the results seen in the in-vitro binding. They concluded that these receptors are not easily accessible to intrathecal opioids supporting the proposal that poor access of opioids to the lamb spinal cord is a possible explanation for failure to obtain analgesia.

(c) The distribution, receptor affinity and duration of activity of morphine and etorphine is not adequate

An alternative explanation for the absence of analgesia with morphine could be that a predominantly mu selective drug (Magnan *et al*, 1982) is less effective against a mixed cutaneous and visceral stimulus comprising both mechanical and chemical nociceptor activity than for example a purely cutaneous stimulus. Ness and Gebhart (1989) demonstrated morphine to be more potent in mechanical pain tests when compared with formalin tests in rats. Improved performance of morphine in one pain test does not imply, however, that it is ineffective in another, there being ample evidence that morphine is antinociceptive in a variety of thermal and non thermal, visceral and somatic pain tests (Tyers, 1980; Schmauss and Yaksh, 1984). In most human patients an epidural dose of 5mg of morphine has an onset of action within 20 minutes, produces complete pain relief by 40 minutes and has a minimum duration of action of 4 hours (Torda and Pybus, 1982). Reflex studies in the conscious animal and the anaesthetised spinalised preparation, which are generally used to identify the analgesic potency of drugs destined for human use, indicate that morphine is indeed a potent analgesic narcotic (Irwin *et al*, 1951).

Kappa agonists have also been shown to produce similar levels of analgesia in both thermal and non thermal nociceptive tests (Parsons and Headley, 1989) if the intensity of the stimului as judged by excitability levels of neurones in the dorsal horn are comparable. Since morphine has little effect on κ receptors, an opioid agonist with an action at κ receptors could be selected. Etorphine is such a drug with high potency (Reckitt and Coleman, 1982) and action at mu, delta and kappa receptor sites (Magnan *et al*, 1982). The finding that etorphine was no more effective at producing measurable analgesia than morphine was further evidence that young lambs are refactory to the action of the three main opioid receptor agonists administered in this way. Etorphine like other highly lipid soluble opioids such as fentanyl was expected to gain access to the spinal cord by a different mechanism than morphine (Gourlay *et al*, 1987) and this provided further evidence that access may not be the main explanation. The high receptor affinity of etorphine (Reckitt and Coleman, 1982) may, however, have resulted in a restricted spread of drug through the spinal cord producing antinociception, which did not include all the segments of the spinal cord receiving afferent activity from the testes and scrotum. A further explanation is that like fentanyl, there is a rapid onset of analgesia following etorphine administration but this is rapidly dissipated as the opioid is redistributed and analgesia does not last for long enough to counteract the effects of castration carried out 40 minutes after administration.

(d) The stimulus intensity was too great for any analgesia obtained with morphine or etorphine to be revealed by these assessment methods

The nociceptive stimuli of castration or castration and tail-docking cannot be directly compared quantitatively with stimuli such as just suprathreshold noxious mechanical pressure on the forelimb of the sheep, as described by Nolan *et al* (1987), but it is clear that they are much more intense and are maintained for much longer. Analgesic effects may thus be obtained using these less intense and short lived stimuli whereas with castration such effects are swamped by the intensity of the response. The intensity of the stimulus is also important because, in rats, it has been shown that the analgesia obtained with either mu or kappa selective opioids, was more evident with weaker stimuli (Parsons and Headley, 1989). Intrathecal morphine (3000μg) has only been shown to produce antinociception at limited noxious pressures produced by a 10N force applied by a metal pin to the skin of the sheep, this contrasts with the action of the alpha 2 agonist xylazine, intrathecal administration of (50μg) of which eliminated limb withdrawal responses when forces greater than 15 Newtons were applied to the skin (Waterman *et al*, 1988). It has also been shown that the response to certain extremely noxious stimuli such as intense noxious heat applied to the rats tail could not be readily reduced by analgesics (Gray *et al*, 1970).

The intensity of the noxious stimulus used for the assessment of etorphine was reduced, by changing from castration and tail-docking to castration on its own. This remains a complex stimulus with both visceral and somatic components and is still extremely noxious. A further refinement along these lines would have been achieved by using tail-docking on its own, which provides a less intense stimulus with only somatic components. Sufficient evidence is, however, available from these studies to consider it unlikely that the intensity of the stimulus is the explanation for the absence of any recognisable analgesic effect of epidural morphine or intrathecal etorphine. If morphine and etorphine produced analgesia at lower intensities of noxious stimulation, an earlier termination to the abnormal behaviour following castration and tail-docking, or castration would be expected, because as indicated by these responses the intensity of these stimuli reduces with time.

(e) The doses of morphine and etorphine selected were too high

Both morphine and etorphine, may have analgesic activity at doses below those selected for use in these experiments. Tyers (1980), demonstrated analgesia in the mouse using the hot plate test following 0.5-5.0 mg/kg of subcutaneous buprenorphine, whereas 5.0-20.0 mg was associated with a decrease in analgesia. This biphasic analgesic action of buprenorphine may account for the failure by Livingston *et al* (1986), to obtain analgesia in some sheep at doses above those which produced antinociception in all animals tested. The possibility of the existence of such a bell-shaped dose response curve in lambs was investigated in only a few preliminary experiments. No analgesia was found at morphine doses at or below 16mg in initial tests. However, the variability of the limb withdrawal and tail flick response to noxious heat or pinch when the lamb was restrained in a hammock, does not exclude the possibility that analgesia can be obtained. During the main experiment where lambs were castrated 40 minutes after administration of epidural morphine (16mg) or intrathecal etorphine (10nmol), if lower doses are more effective some evidence of analgesia might be expected to be found as the concentration of morphine or etorphine falls in the CSF and spinal cord. There was no evidence, however, that morphine or etorphine reduced the duration of the response to either castration and tail-docking or castration respectively. If this process produced analgesia later than 2 hours after castration or castration and tail-docking, it would be difficult to detect because plasma cortisol concentrations have returned to basal levels, and lamb behaviour is near normal after this time.

Furthermore, following 16mg of epidural morphine it might be a considerable time before concentrations fell to those found to be analgesic in other pain models, particularly as lower doses than those used here can have an analgesic action for in excess of 10 hours in humans (Yaksh and Rudy, 1978). A dose response study using castration or castration and tail-docking as pain models, might elucidate bi-phasic analgesic properties of morphine and etorphine, but such a study would require a large number of lambs. A higher dose, particularly of etorphine, might have produced analgesia, but because of the effects on behaviour it would not be possible to use the indices developed here. Since only one dose was used for each drug and since it is still not known if this dose lies on any analgesic dose-response curve for this age and weight of lamb it can only be concluded that the effectiveness of morphine and etorphine as analgesics capable of reducing the pain of castration and tail-docking in young lambs remains to be demonstrated.

Further approaches to finding a suitable analgesic with which to modify the pain of castration and tail-docking

Since these established analgesics have not been successfully applied new approaches must be found to attack this problem. One important requirement of such approaches is that an analgesic administration technique is used whereby a variety of drugs at a range of doses can be tested in the same lamb. This could be accomplished by chronically implanting catheters into the subarachnoid space of 5-7 day old lamb as described for the rat (Yaksh, 1976a), but modified so that one catheter tip lies at the level of thoracic segments T10-T11, and the other at Lumbo-sacral segments (L7-S1). Use of a short lived repeatable noxious stimulus in unrestrained lambs the intensity of which can be varied quantitatively for short periods of time (e.g. 10 seconds) could enable a suitable analgesic and range of stimuli to be found. Another approach using the anaesthetised and spinalised lamb is to investigate the effect of intrathecally administered potential analgesics on for example, activity in dorsal horn neurones evoked by noxious stimulation (Fleetwood-Walker *et al*, 1985; Dickenson and Sullivan, 1987). An effective analgesic would be expected to suppress activity initiated by noxious stimulation.

It was decided that it was first necessary to confirm that morphine and xylazine applied intrathecally to the spinal cord, in the doses chosen above, did not produce evidence of analgesia. As analgesic potency is routinely estimated by the ability of a drug to suppress reflex activity in spinalised animals (Irwin *et al*, 1951), the effect of both morphine and xylazine on the sural- semitendinosus reflex in the anaesthetised and spinalised lamb were studied.

EFFECTS OF EPIDURAL AND INTRATHECAL XYLAZINE ON INDICES USED FOR THE ASSESSMENT OF PAIN FROM CASTRATION AND TAIL-DOCKING IN YOUNG LAMBS

Adrenergic agonists as antinociceptive drugs

Initially interest in the adrenoceptor agonists was directed towards understanding their regulatory effects on catecholamine release and action on effector organs (Langer, 1974). This has expanded to include investigations of the sedative, hypertensive and hypotensive action (Solomon *et al*, 1989), and also the profound analgesia obtained in many species, including the sheep (Nolan *et al*, 1987). Selective adrenoceptor agonists could be expected to have an analgesic action because noradrenaline, one of the endogenous ligands, had been long established as having analgesic properties (Gross *et al*, 1948). The spinal action of such agonists was demonstrated by a number of techniques which are outlined below;

Analgesia produced by electrical stimulation of the brainstem and by intracerebroventricular (ICV) injection of opioid drugs.

The analgesia produced by electrical stimulation of the brainstem or by focal ICV injection of opioids may be by activation of the bulbospinal and pontospinal noradrenergic systems which exert an inhibitory modulation of the rostrad transmission of nociceptive information (Fields and Basbaum, 1978). The monoamine nature of these spinopetal pathways was demonstrated in the mid sixties by Dahlstrom and Fuxe (1964, 1965), using a histochemical fluorescence method, with immunochemical studies elucidating the finer anatomical detail of the descending noradrenergic projections and their spinal terminations (Westlund *et al*, 1982).

That these pathways can produce hypoalgesia is supported by electrical stimulation of noradrenergic nuclei from which they originate. Electrical stimulation in the region of the locus coeruleus of the rat resulted in a decrease in sensitivity to noxious stimuli (Segal and Sandberg, 1977). Hammond *et al* (1985), have shown electrical stimulation of noradrenergic nuclei to produce analgesia which is associated with an efflux of noradrenalin into spinal cord superfusates and can be attenuated by intrathecal injection of the noradrenergic antagonist phentolamine.

Intravenous injection of morphine in cats (7mg/kg) was shown to have an effect by a descending pathway, as the suppressive action on reflex activity was eliminated following spinal transection (Takagi *et al*, 1955). That this analgesia was in part mediated by spinal alpha adrenoreceptors is supported by evidence demonstrating that these effects can be reversed almost completely by intrathecal injection of noradrenergic antagonists such as phentolamine (Yaksh, 1979). This finding also demonstrated how an interaction could occur between alpha agonists and opioid agonists.

Release and turnover of spinal noradrenaline

Focal brain stem stimulation in the nucleus raphe magnus and the nucleus reticularis paragigantocellularis results in release of noradrenalin at the level of the spinal cord as measured in the CSF by high performance liquid chromatography (Hammond *et al*, 1985). Increased turnover of 3-methoxy- 4-hydroxy phenethene (MHPG), a metabolite of noradrenalin , has been demonstrated following electrical stimulation of the locus coeruleus. When rat spinal cord tissue was assayed by gas chromatography and mass spectrometry for MHPG, it was found to have doubled in concentration when compared with control animals (Crawley *et al*, 1979). Other groups found the levels of metabolites such as normetaphrine to be more reliable measures of adrenergic activity than the levels of monoamines themselves. Following opioid administration (Shiomi and Takagi, 1974) normetaphrine levels were seen to increase without associated changes in spinal noradrenalin concentrations.

Spinal application of adrenergic agonists produces antinociception

That the spinal cord is one site of action of alpha-adrenergic agents in producing antinociception, was shown by the intrathecal spinal application of adrenergic agonists. These agonists produced analgesia as measured by the tail flick test and mimicked central activation of the bulbospinal and pontospinal systems (Yaksh, 1985). It has also been shown that iontophoretic application of noradrenalin into the substantia gelatinosa directly above or near to the cell bodies reduces the activity of lamina 4 and 5 cells elicited by noxious heating (45°C) of the hind paw of the cat (Headley *et al*, 1978).

A spinal analgesic effect of alpha 2 agonists has been shown in in adult sheep (Waterman *et al*, 1988; Eisenach and Dewan, 1990) following intrathecal administration of xylazine or clonidine. The pharmacology of this spinal adrenergic agonist mediated antinociception has been described by Reddy *et al*, (1980). They showed that the increased thresholds in rats to the hot plate and tail flick test was dose dependent, reversed by alpha 2 antagonists such as tolazoline and yohimbine and was not due to an indirect action such as vasoconstriction (Reddy *et al*, 1980).

Classification of adrenoceptor sites

The classification of adrenoceptor binding sites into alpha and beta was based originally on whether they produced excitation or inhibition of particular effector cells (Alquist, 1948). Langer (1974) referred to the post-synaptic alpha-receptor that mediates the response of the effector organ as α_1 while he called the pre-synaptic alpha-receptor that regulated transmitter release α_2. Since then, however, post-synaptic receptors which behave like pre-synaptic receptors have been found and are referred to as α_2 (Langer and Hicks, 1984). More recently, Bylund (1985), demonstrated that yohimbine binding in the rat CNS could be inhibited by prazocin in a biphasic manner indicating there were at least two populations of α_2 receptors. Two subtypes of alpha 1 receptors have also been discovered (Gross *et al*, 1990). It has, however, been suggested by McGrath and Wilson (1988) that the proliferation of receptor subtypes, based on their affinities for different agonists or antagonists is no more helpful than the broad functional classification, where receptors were identified by their physiological action, as suggested by Berthelsen and Pettiger (1977).

Location of receptors in the spinal cord: binding and electrophysiological studies

(a) Binding studies

The localisation of alpha 1 and alpha 2 adrenoceptors in rat brain and parts of the spinal cord was demonstrated in 1980 by Young and Kuhar (1980) by a light microscopic autoradiographic technique. They found that α_1 and α_2 binding sites were present at a low density over the grey matter in the spinal cord, except in lamina 2 which had a moderate level of α_2 binding sites. Similar radioligand binding studies showed the presence of alpha 2 adrenoceptors in the dorsal horn of the rat (Unnerstall *et al*, 1984). The affinities and number of α_1 and 2 receptors was determined in homogenised sections of spinal cord using radio labelled ligands (Jones *et al*, 1982). It was thus possible to conclude that adrenergic receptors are present in the spinal cord. The location and density of α_2 receptors in sheep spinal cord has been investigated using <3H> clonidine as a ligand (Bouchenafa and Livingston, 1987; Brandt and Livingston, 1989). Substantial binding was found in the superficial laminae of the spinal cord, with less intense binding being evident around the central canal and the intermediolateral bundle. It appears therefore that the autoradiographic distribution of α_2 receptors matches the proposed analgesic function, because nociceptive primary afferents terminate predominantly in the superficial laminae.

Labelled clonidine which was used in these binding studies is not, however, a specific α_2 antagonist. Bouchenafa (1988), demonstrated an interaction of <3H> clonidine and <3H> prazosin binding in the sheep spinal cord, suggesting distinct functional α_1 and α_2 adrenergic binding sites are present in this species. Binding of <3H> clonidine was further reduced in the presence of the alpha 2 antagonist idazoxan, with a small residue of activity remaining. This residual activity was attributed to binding at another receptor type described by Ernsberger *et al* (1987), the imidazoline receptor.

(b) Electrophysiological studies (presynaptic and postsynaptic location of adrenoceptors)

The pre and post synaptic action of the adrenoceptor agonists outwith the CNS is unclear though initially the view was that such drugs as clonidine, xylazine and yohimbine acted more through a presynaptic mechanism controlling the release of noradrenaline from nerve terminals (Langer, 1974; Starke, 1977). In the spinal cord the alpha 2 receptors involved in antinociception are probably post synaptic to adrenergic terminals because clonidine is effective in the spinalised rabbit in suppressing the sural-gastrocnemius withdrawal reflex (Clarke *et al*, 1988). A location postsynaptic to the terminals of descending noradrenergic fibres is supported by the finding of Howe *et al* (1987a) who showed that cervical (C1) hemi-section or intrathecal administration of the noradrenergic neurotoxin 6-hydroxydopamine does not significantly alter the density of α_2 adrenoceptors in the spinal cord. Evidence for either a presynaptic effect on primary afferent terminals or by a postsynaptic action on cells in the dorsal horn was provided by Davies and Quinlan (1985). They inhibited lamina 5 neurones in the cat following noxious cutaneous stimuli by ionophoretic application of clonidine close to the cell bodies suggesting a postsynaptic action. The possibility of presynaptic action at the primary afferent terminal cannot be excluded as Davies and Quinlan (1985) also inhibited lamina 5 cells by iontophoretic application of clonidine in the substantia gelatinosa.

The presynaptic location of receptors on primary afferent terminals would also be supported by the effect of unilateral dorsal root ganglionectomy which produces a 20% reduction in dorsal horn α_2 binding (Howe and Yaksh 1984), and why more extensive unilateral dorsal root rhizotomy reduces the monoamine levels in the rat spinal cord, both ipsilateral and contralateral to the lesion as measured by HPLC (Colado *et al*, 1988).

The analgesic action of α_2 agonists appears to be by a direct action on the systems in which nociceptive activity is processed. α_2 agonists do not appear to act indirectly via other neurotransmitter systems known to modulate spinal function including the opioids, G.A.B.A. and glycine. Electrophoretic application of noradrenalin is not antagonised by naloxone or mimicked by G.A.B.A. (Headley *et al*, 1978). In hot plate tests, the action of intrathecal noradrenalin in the rat is not antagonised by methysergide (serotonin antagonist), phentolamine (alpha adrenergic antagonist), picrotoxin (Gamma aminobutyric acid antagonist) or strychnine (glycine antagonist) (Reddy *et al*, 1980). The antinociceptive action of noradrenaline is not reproduced by other, potent, vasoconstrictors such as angiotensin II, and hence is not due to reduction in the blood supply to the spinal cord (Reddy *et al*, 1980). Evidence by direct observation of the spinal cord that adrenalin does not cause blanching was reported 90 years ago (Webber, 1904).

Drugs acting on adrenoceptor sites, agonists and antagonists

Adrenoceptor drugs can be broadly classified as either agonists or antagonists at α_1, α_2, β_1, and β_2 receptor sites. Work by Kuraishi *et al* (1979) showed the antinociceptive properties of the adrenergic drugs to be via their action on the α-adrenoceptor drugs as intrathecal pretreatment with the α-antagonist phenoxybenzamine substantially reduced the analgesia obtained in the rat after intrathecal noradrenalin. For this reason the following review is restricted to the α-adrenoceptor agonists.

Choice of α_1 or α_2 agonist

Howe *et al* (1983), stated that alpha agonists are selective but not specific, and that any of them at high enough doses will activate both adrenoceptor subtypes. Their work was designed to determine which alpha adrenoceptor was activated at antinociceptive doses. The motor effects of clonidine were found to be due to α_1 agonist activity while the antinociceptive effect, seen at low doses was due to α_2 agonist activity. α_1 agonists were found to produce motor excitation and antinociception at similar doses (excitatory motor syndrome).

The pharmacological profile of adrenergic agonists provides strong evidence that spinal administration of α_2 drugs specifically produce antinociception. When the antinociceptive potency of alpha adrenergic drugs are ranked, the α_2 compounds are found to be more potent than the α_1, this is also supported by the order in which their actions are reversed by antagonists (Yaksh, 1985). This may be a slight oversimplification since Tasker and Melzack (1989) demonstrated that intraperitoneal clonidine was more potent as a analgesic agent when assessed by the rat formalin test than when compared to the hot water (50°C) tail flick test. The effect on the tail flick test was antagonised better by yohimbine (α_2 antagonist) and the effect on the formalin test was antagonised better by prazocin (α_1 antagonist). The possibility that this was a peripheral effect cannot, however, be excluded.

Howe *et al* (1983) concluded that because of the marked disturbance of motor and autonomic function observed following intrathecal administration of α_1 agonists, such agents were unlikely to be useful analgesics and that α_2 agonists were more promising candidates for therapeutic use. Electrophysiological evidence for specific α_2 antinociceptive activity has been reported by Fleetwood-walker *et al* (1985), who showed that ionophoretic application of the mixed agonist noradrenalin on multireceptive neurones of the spinocervical tract in the dorsal horn of the spinal cord produced an inhibition of the responses to noxious stimulation of the skin (heat/pinch) while it had no effect on the responses to non-noxious stimuli (brushing). This selective action was blocked by idasoxan indicating that an α_2 receptor was involved. Below is a table of some commonly used α_1 and α_2 agonists and antagonists. Those drugs marked by an (*), are not only potent at that receptor but also specific. For example clonidine is a potent but non specific α_2 agonist, with 4-15 times the affinity of phenylephrine at the α_1 receptor but only 0.1-1.0 x the potency. At the α_2 receptor it has 50-1000x the affinity and potency of phenylephrine (Starke, 1981). Those drugs without an * have, like clonidine, some degree of mixed activity.

α_1 agonists	α_1 antagonists	α_2 Agonists	α_2 Antagonists
methoxamine	prazosin	ST-91	yohimbine
phenylephrine	phenoxybenzamine	oxymetazoline	tolazoline
cirazoline	methysergide	clonidine	rauwolscine
	8-OH-DPAT	xylazine	idazoxan
	RU24969	detomidine	imiloxan
	WB40101	medetomidine	DG-5128*
	phentolamine	B-HT933*	
		phentolamine	

Noradrenalin and adrenalin, the endogenous ligands are mixed alpha and beta agonists. As for opioids it is likely that the discovery of different receptor types has preceded the discovery of the endogenous ligands (Terenius, 1973). It is therefore possible that endogenous ligands do exist for the multiplicity of adrenergic receptor sites.

Interaction of α-agonists with opioid systems

Since both opioids and α_2 -agonists can produce useful antinociception at the spinal level it is interesting to examine possible interactions between these effects. The antinociceptive action of α_2 drugs is only reversed by α_2 antagonists whereas the antinociceptive activity of morphine and other opioids is not only reversed by the opioid antagonist naloxone but also attenuated by the α_2 antagonist yohimbine (Browning *et al*, 1982). This effect could be explained if morphine has a supraspinal action on the descending noradrenergic pathways. Direct evidence was provided by Yaksh (1979) that spinal noradrenaline terminals mediate the antinociceptive effect of morphine administered into the peri-aqueductal gray by microinjection. Not all interaction is supraspinal since Fleetwood-Walker *et al* (1987) have reported that responses to noxious stimuli of spinocervical tract neurons are inhibited by ionophoretic application of kappa opioids in the dorsal horn and that this effect can be blocked by idazoxan.

Choice of xylazine as an α_2 agonist for these studies

Xylazine is the British Veterinary Codex-approved non-proprietrary name for 2- (2, 6-dimethylphenylamino) 5-6-dihydro-4H-1, 3-thiazine, a compound first synthesised in 1962, described as a sedative, analgesic and muscle relaxant. Xylazine is soluble in dilute acids but practically insoluble in water. Xylazine like clonidine was developed to meet the requirement for a direct acting alpha 2 agent which was able to cross the blood brain barrier (Schmitt *et al*, 1974). Following clinical trials in West Germany it was subjected to experimental and preliminary clinical trials in horses and cattle (Clarke and Hall, 1969), the results of which demonstrated that intravenous injection of 50-100μg/kg or the intramuscular injection of 200.0μg/kg produced good basal narcosis for 1-2 hours in cattle. It was concluded that xylazine might prove to be a valuable sedative in this species and subsequent work has shown this to be the case. Work with sheep provided similar findings. In the sheep, optimal anaesthesia can be obtained with intramuscular injection of 3.0mg/kg xylazine (Straub, 1972), however, analgesia and muscle relaxation is evident at lower doses (0.6 mg/kg i/m) which is associated with slowing of the heart and respiratory rate (Kosuch, 1973). At even lower doses of xylazine (0.1mg/kg i/m) sedation, analgesia and muscle relaxation could be obtained without respiratory and cardiovascular side effects (Shokry *et al*, 1976). Recent studies indicate that xylazine at doses as low as 50μg/kg does have some respiratory effects, as administration was shown to cause a significant degree of arterial hypoxia in conscious sheep (Waterman *et al*, 1986/87). Xylazine has now been in routine clinical use in sheep for over 20 years at anaesthetic doses up to 60 times greater than those found to produce the respiratory effects shown by Waterman *et al* (1986/87), therefore this did not preclude the use of xylazine in lambs. Xylazine is a cheap and readily available sedative/analgesic licensed for use in sheep. Xylazine is also a safe drug for use in sheep, as although intravenous administration of 1-4mg/kg is lethal, up to 15mg/kg may be administered without ill effect if the anaesthesia is reversed before respiration stops, by administration of an α_2 antagonist such as tolazoline (2.0mg/kg) (Zingoni *et al*, 1982). The dose of xylazine chosen for these experiments (50μg total dose) was in the range found to be subneuroleptic in healthy sheep and cattle (40-80μg/kg i/m) (Goranov et al, 1983), and was identical to that which was found when administered intrathecally to the adult sheep to produce clear antinociceptive effects when using mechanical pressure threshold detection tests (Waterman *et al*, 1988).

Choice of the epidural and intrathecal route of administration

Xylazine is routinely administered via the intravenous, intramuscular (Clarke and Hall, 1969) and subcutaneous routes (Goranov *et al*, 1983). One of the first objective evaluations of the analgesic action of intramuscular or intravenous xylazine was undertaken in the horse (Pippi and Lumb, 1979) using three kinds of pain (superficial and deep thermal, and visceral). An objective assessment of analgesic potency of intravenous xylazine (50.0μg/kg) has also been undertaken in the sheep by Nolan *et al* 1987 using noxious thermal and mechanical tests. In the sheep even low doses of systemic xylazine (50-150μg/kg) are associated with sedation (Nolan, 1986) which complicate the interpretation of the changes in behaviour based mainly on posture as an index of pain. The epidural route was selected because administration of opioids in humans via this route has been shown to produce pain relief without sedation or respiratory depression, showing the feasibility of selective blockade of pain at the spinal cord level and the safety of this method (Cousins and Mather, 1984). This route was expected to be successful in the lamb as α_2 receptors have been demonstrated in the spinal cord of sheep (Bouchenafa and Livingston, 1987), where they have been shown in a number of species to mediate antinociception. The intrathecal route was not used initially because, despite the advantage of obtaining higher concentrations of xylazine in the CSF, the risk of causing spinal damage was greater. The intrathecal route has been used in the sheep (Waterman *et al*, 1988), following surgical implantation of an indwelling cannula and spinal analgesia was produced after a dose of 50μg of xylazine, with little evidence of side effects.

Unwanted effects of α_2 agonists in this study

(a) Behavioural effects

Since α_2 agonists have effects on the CNS and change behaviour (Vanio and Vaha, 1990), administration of xylazine could complicate the interpretation of the changes in behaviour as an index of pain. Clonidine (10-1000μg/kg) produces a dose related increase in activity in rats less than 10 days old, following intraperitoneal injection (Smythe and Pappas, 1989) which may be associated with an effect at the α_1 receptors known to be responsible for the motor excitation syndrome (Howe *et al*, 1983). In adult rats 5-40 μg of xylazine given by intracerebroventricular injection caused sedation, and reduced the time they could remain on an accelerating rotarod (Drew *et al*, 1977). The dose used in the present studies was 50μg of xylazine or approximately 10μg/kg by epidural or intrathecal injection and is a low dose by comparison with those above. At antinociceptive doses of noradrenalin (1 nmol) injected by the intrathecal route in the rat, there is no change in non-nociceptive reflexes such as placing or stepping (Reddy *et al*, 1980; Reddy and Yaksh, 1980).

Shortly after intravenous injection of clonidine or xylazine in the sheep, behavioural changes were observed (Nolan, 1986).

Animals became sedated often adopting sternal recumbency (Xylazine, 50μg/kg) or a sitting position (Clonidine, 25μg/kg), and then commenced dribbling from the mouth after 15-20 minutes. Clonidine produced more profound and longer sedation, with increased respiratory effort and mouth breathing (Nolan, 1986), accompanied by hypoxaemia as measured by blood gas analysis (Waterman *et al*, 1986/87; Eisenach and Dewan, 1990).

Clonidine administration produces hyperphagia, and has been used clinically in the treatment of anorexia nervosa in humans, and has been shown at high dose levels (0.1 mg/kg) to increase food intake in rabbits (Katz *et al*, 1989). With the dose of xylazine (0.05mg total) used in this study, little increase in feeding behaviour such as teat seeking or sucking was expected in the lambs.

(b) Effects of α_2 agonists on the cardiovascular system

The amount of pain produced by rubber ring castration and tail-docking is likely to be related to blood pressure. When the blood vessels supplying these tissues are occluded the hydrostatic pressure in the tissues will be related to the blood pressure at the time of occlusion and mechano-sensitive noci-receptors would be expected to be stimulated more. Therapeutic doses of xylazine in sheep (0.5 mg/kg i/v) produces an initial hypertension followed by a prolonged fall in blood pressure (Aziz and Carlyle, 1978). This initial increase in blood pressure is due to adrenergic action at peripheral α_1 and α_2 receptors both of which may be found post-synaptically in blood vessels where they mediate vasoconstriction (Langer and Hicks, 1984). The prolonged fall in blood pressure (Aziz and Carlyle, 1978) and heart rate (Hsu *et al*, 1987) seen following xylazine administration in the sheep is likely to be due to a central (Unnerstall *et al*, 1985) and spinal action (Guyenet and Cabot, 1981) at α_2 receptors. It might be anticipated that both epidural and intrathecal xylazine (0.05mg) would have a hypotensive effect, due to a restricted access to the spinal α_2 receptors. Eisenach *et al* (1987), however, using the lowest maximally analgesic dose of epidural clonidine (300µg), did not find any marked hypotensive effect in sheep, and therefore no effects would be expected with a lower dose (50µg) of a less potent α_2 agonist (xylazine). Intrathecal clonidine produced a biphasic change in blood pressure, and a dose dependent decrease in heart rate, which is consistent with a central and peripheral action (Eisenach and Dewan, 1990). Eisenach *et al* (1989) concluded that the haemodynamic effect of clonidine may differ between epidural and intrathecal injection.

(c) Effects of α_2 agonists on plasma cortisol concentrations

Since catecholamines have an action on the CRF-ACTH system (Plotsky *et al*, 1989) administration of α_2 agonists could effect cortisol release and complicate the interpretation of changes in plasma cortisol as an index of pain. Nanomolar concentrations of noradrenalin have been shown to increase ACTH release from rat anterior pituitary cells in primary culture (Giguere *et al*, 1981), and there is now also evidence that endogenous noradrenalin release from catecolaminergic nerve terminals in the hypothalamic PVN can increase cortisol release (Richardson *et al*, 1990). This effect on cortisol release was anticipated because a close association had been shown between adrenergic nerves and the CRF neurones found in the PVN (Mezey *et al*, 1984). There is, however, no evidence that an increase in plasma cortisol as produced by administration of catecolamines in the vicinity of these noradrenergic neurones also occurs after intrathecal injection of an α_2 agonist such as clonidine up to a dose of 1500µg (Eisenach *et al* 1989; Eisenach and Dewan 1990). On the contrary any increase in plasma cortisol could be prohibited since some central α_2 receptors have been implicated in an inhibition of ACTH release (Al-Damluji *et al*, 1990).

MATERIALS AND METHODS

Studies of the effects of the alpha 2 analgesic xylazine on responses to castration, or castration and tail-docking were based on the results obtained from two separate experiments, the first in April 1988 and the second in April 1989. In total 48 lambs (all male) were used, weighing between 3.0 and 7.0 Kg, and ranging from 5 to 7 days old. In the first group of 24 lambs, the effect of epidural xylazine (50μg) on behaviour patterns and cortisol responses produced by castration and tail-docking were examined. Behavioural and plasma cortisol data were recorded as described by Mellor and Murray (1989a), though presentation and analysis of results were modified (see general methods). In the second group of 24 lambs, the effect of intrathecal xylazine (50μg) on behaviour patterns and cortisol responses produced by castration alone, were examined. For this study changes were made in the behavioural monitoring technique. Behavioural monitoring was restricted to the 3 main behavioural postures; lateral recumbency, standing and ventral recumbency and they were graded into 1 of 3 severities. A restlessness score was introduced, which quantified how often a lamb got up or lay down (see general methods). The drug used in this study was xylazine hydrochloride (Sigma Chemical Company), dissolved in 0.9% NaCl solution made slightly acidic with 2 drops of 0.1M HCl acid to 250μg/ml.

Experiment 1

The null hypothesis was that 2x 25μg of xylazine administered to young lambs via the epidural route would not change the behaviour patterns or plasma cortisol responses following castration and tail-docking.

Animals

A total of 24 lambs (Scottish Blackface) from 5-7 days old and with a mean weight of 4.9 ± 0.2 kg were investigated. No lambs showing clinical signs of ill health were included in the investigation. Housing, feeding and bedding, followed routine husbandry practices, which were as described in the general methods.

Treatments

A day before the experiment, wool was shaved from the full length of the neck to expose both jugular veins. A 10cm square of wool was also shaved from the skin overlying the lumbosacral junction.

All lambs were anaesthetised with a mixture of halothane, nitrous oxide and oxygen, prior to and during the epidural or sham epidural procedures. General anaesthesia was maintained for no more than 10 minutes, 40 minutes were allowed after drug administration before the start of the experiment. See general methods for greater detail.

While anaesthetised, the shaved area of skin on the dorsum of the lamb was cleaned with an antiseptic solution of Hibiscrub followed by application of 70% alcohol. The lumbar spinal cord was flexed to locate the lumbosacral space and a small incision made in the skin directly above this space with a stylet. A Tuohy needle angled at 45° from the vertical and directed from left to right (as seen from the rear of the lamb), with the chamfer facing of the Tuohy needle uppermost was inserted through the lumbosacral junction into the epidural space of the spinal canal. A catheter was introduced to the epidural space via the Tuohy needle and advanced 80mm as indicated by a mark previously made on the catheter. Drug could then be injected in the region of thoracic segment 10. The catheter was then withdrawn to the 10 mm mark, so an injection could be made in the region of the lumbar segments 6-7.

All lambs were bled (2ml samples) by venepuncture from a jugular vein immediately before and at 15, 30, 60, 90, 120, 180 and 240 minutes after treatment. This procedure usually took less than 15 seconds.

There were four treatment groups.

Group 1. Control handling. Lambs (n=6) were handled as if they were being castrated and tail-docked but without application of the rubber rings.

Group 2 Epidural xylazine only. Lambs (n=6) were anaesthetised and 2 x 25μg of xylazine in 100μl of saline was introduced into the epidural space at two separate locations (see above), 40 minutes before the start of the experiment.

Group 3 Epidural xylazine followed by castration and tail-docking. Lambs (n=6) were treated as in group 2, and were castrated and tail-docked at the start of the experiment.

Group 4 Epidural saline followed by castration and tail-docking. Lambs (n=6) were treated as lambs in group 3 except that saline (2 x 100μl) alone was administered into the epidural space.

Experiment 2

The null hypothesis was that 2 x 25μg of xylazine administered to the young lamb via the intrathecal route would not change the behaviour patterns or plasma cortisol responses following castration.

Animals

A total of 24 lambs (Scottish Blackface) from 5-7 days old and with a mean weight of 5.2 ± 0.2 kg were investigated.

Treatments

A day before the experiment wool was clipped from the full length of the neck to expose both jugular veins. Wool was also removed in a 10 cm wide strip along the dorsum from mid-sacrum to thoracic vertebra 8. Preparation of the skin surface and anaesthesia were as described for experiment 1.

The T10-T11 intervertebral space, and the lumbosacral junction were located by palpation of vertebrae and other bony landmarks and marked on the skin surface with an pen. A 1" 23 guage needle was located centrally on the mark made at the lumbosacral space. While held vertically the needle was advanced through the skin and intervertebral space into the subarachnoid space until CSF was seen to flow from the hub. A new needle was located 5-10 mm lateral to the mark previously made in the midline indicating the T10-T11 intervertebral space. The needle was then slowly advanced through the intervertebral space with the hub angled towards the rear of the lamb. Penetration was usually at a depth of about 15 mm for this size of lamb. If the needle hit the vertebral body it was retracted slightly and the angulation increased. The needle was then advanced again until CSF was seen to flow from the hub. Due to the dead space of the needle, 120μl of solution was injected at both sites, after which the needle was removed with the syringe attached to stop the loss of CSF and drug.

All animals were bled from a jugular vein before the animals were subjected to anaesthetic etc, immediately before the treatment, and at 15, 30, 60, 90, 120, and 180 minutes after treatment. There were four treatment groups.

Group 1 Control handling. Lambs (n=6) received a general anaesthetic, and a 1" 23 gauge needle inserted (as described above) but no drug or vehicle was injected.

Group 2 Intrathecal xylazine only. Lambs (n=6) were anaesthetised and 2 x 25μg of xylazine in 100μl of saline was administered into the intrathecal space at two separate locations (see above), 40 minutes before the start of the experiment.

Group 3 Intrathecal xylazine followed by castration. Lambs (n=6) were treated as in group 2, and were castrated by application of tight rubber rings.

Group 4 Intrathecal saline followed by castration. Lambs (n=6), were treated as lambs in group 3 except that saline (2x100μl) was administered into the intrathecal space.

Plasma cortisol assays and presentation of results (experiment 1 and 2)

Cortisol concentrations in experiment 1 were measured by radioimmunoassay (Read *et al*, 1977) using an IDS kit (IDS Ltd. Tyne and Wear) with lowest detectable concentration 7.8 ± 1.1 nmol/l and intra and inter assay coefficients of variation of 4.5 and 9.0% respectively. Cortisol concentrations in experiment 2 were measured using an Amersham kit with lowest detectable concentration 3.0 ± 0.9 nmol/l and intra and inter assay coefficients of variation of 11.7 and 8.8% respectively. Details of these methods are described in the general methods.

Where applicable the plasma cortisol concentrations were expressed as a mean ± the standard error of the mean (SED). An analysis of variance for both experiments was carried out covering the periods 15, 30 and 60 minutes, using the basal cortisol level for each lamb as a covariate. A two by two treatment structure was used as described in the general methods.

Experiment 1. (Castration (CN) v Control handling (C); Control v Epidural xylazine and castration (EDX)).
Experiment 2. (Castration (CN) v Control handling (C); Control v Intrathecal xylazine and castration (ITX)).

The main outputs of this analysis were two by two tables of cortisol means (nmol/l) and a standard error of differences (SED), which allowed comparison of the means. Any difference between the mean plasma cortisol concentrations which exceeds the figure accompanying the table (Derived from the SED) is statistically significant at the 5% level.

Behavioural monitoring and presentation of results (experiment 1 and 2)

Detailed description of the behavioural monitoring techniques and analysis of data using non parametric statistical tests is described in the general materials and methods chapter. In experiment 1 behavioural monitoring extended over a 4 hour period and the presence or absence of particular behaviours was noted for each lamb at specific times after treatment. The different behaviours were: lateral recumbency, ventral recumbency, standing and teat seeking or sucking. Behaviour was observed once every minute between one and 14 minutes, every two minutes between 17 and 29 minutes, every five minutes between 35 and 60 minutes, every ten minutes between 70 and 120 minutes, and every fifteen minutes between 135 and 240 minutes. Behavioural data was presented in bar chart form, the incidence of each behaviour pattern expressed as a percentage of the total number of observations during each period. In experiment 2 this time period was reduced to 3 hours. Most lambs return to near normal by this time as assessed by initial monitoring techniques and this allows more lambs to be investigated. Three severities of lateral recumbency, ventral recumbency and standing were recorded as was restlessness, a quantitative measure of how often all lamb got up and lay down. Restlessness was reported between 1 and 15 minutes, and between 15 and 30 minutes. Behaviour was observed once every minute between one and 14 minutes, every two minutes between 17 and 29 minutes, and at five minute intervals thereafter to the end of the experiment (180 minutes). Behavioural data was presented as for experiment 1. A fuller description is given in the general methods (Chapter 2).

RESULTS

Effect of epidural xylazine on the behavioural and physiological indices selected, following castration and tail-docking

Expt 1 Group 1- Behaviour of control lambs

The results are presented in Fig 5.1 and Tables 1 and 2. The behaviour of control lambs was consistent from 30 minutes to the end of the experiment (240 minutes), lambs remaining in ventral recumbency (69-89% of observations). In the first 30 minutes, although ventral recumbency was predominant, the incidence of standing was higher than in later times (28-38% of observations). The incidence of teat seeking was low only reaching 17% of observations from 15-30 minutes. This was the highest incidence of teat seeking recorded in any of the four groups.

Plasma cortisol concentrations

The results are presented in Fig 5.1 and Tables 1 and 2. There was a rise in plasma cortisol concentration following blood sampling from the basal level (15.7 ± 7.0 nmol/l) to 39.6 ± 19.0 nmol/l at 30 minutes. Plasma cortisol concentrations were, however, similar to basal levels throughout the duration of the experiment.

Expt 1 Group 2- Behaviour of lambs to which epidural xylazine (2x25μg) had been administered

The results are presented in Fig 5.1 and Tables 1 and 2. Lambs to which epidural xylazine (2x25μg) had been administered stood (29-64% of observations) more than control lambs throughout the duration of the experiment (0-240 minutes). This was significant for the period 30-60 minutes ($P<0.05$). As for the control group however, the incidence of ventral recumbency predominated overall. The behaviour in this group and control lambs was only significantly different for the period 30-60 minutes.

Plasma cortisol concentrations

The results are presented in Fig 5.1 and Tables 1 and 2. Lambs to whom epidural xylazine (2x25μg) had been administered produced a rise in plasma cortisol concentration from the basal level (16.5 ± 7.2 nmol/l) to a peak of 73.4 ± 12.9 at 30 minutes, returning to basal levels by 90 minutes. The plasma cortisol response seen in group 2 was not significantly different to that seen in control lambs when compared from 15-60 minutes (P>0.05).

Expt 1 Group 3- Behaviour of lambs to which epidural xylazine (2x25μg) had been administered 40 minutes prior to castration and tail-docking

The results are presented in Fig 5.1 and Tables 1 and 2. Lambs to which epidural xylazine (2x25μg) had been administered 40 minutes prior to castration and tail-docking lay in lateral recumbency for the periods between 0-60 minutes and 120-180 minutes, which does not occur in control lambs. This was significant from 15-60 minutes (P<0.05), and approached significance from 0-15 minutes (P<0.1). The behaviour in this group is similar to control lambs after 60 minutes, with most lambs lying in ventral recumbency (69-75% of observations). There was more lateral recumbency in the periods from 0-60 minutes in this group (31-50% of observations) than recorded in lambs administered epidural saline prior to castration (23-36% of observations). There were, however, no significant differences between any behaviours in these two groups (P>0.05).

Plasma cortisol concentrations

The results are presented in Fig 5.1 and Tables 1 and 2. Lambs to which epidural xylazine (2x25μg) had been administered 40 minutes prior to castration and tail-docking produced a rapid rise in plasma cortisol concentration from a basal level of 13.7 ± 3.4 nmol/l to a plateau from 30-60 minutes. The peak concentration of this plateau occured at 60 minutes (182.0 ± 40.4 nmol/l), before returning to basal levels by 120 minutes. The plasma cortisol response of this group when compared with the control and epidural xylazine groups between 15-60 minutes, was significantly greater ($P<0.05$). Plasma cortisol concentrations in this group reach a lower peak which occurred later than that in lambs castrated and tail-docked following epidural saline administration. When compared between 15-60 minutes there were no significant differences ($P>0.05$).

Expt 1 Group 4- Behaviour of lambs to which epidural saline had been administered 40 minutes prior to castration and tail-docking

The result are presented in Fig 5.1 and Tables 1 and 2. Lambs to which epidural saline had been administered 40 minutes prior to castration and tail-docking lay in lateral recumbency for the periods between 0-60 minutes (23-36% of observations), which did not occur in control lambs. This was significant from 0-30 minutes ($P<0.05$). The trend in this group, as in the other 3 groups was towards an increase in ventral recumbency. For the periods between 30-240 minutes the behaviour in this group was not significantly different from the control lambs ($P>0.05$).

Plasma cortisol concentrations

The results are presented in Fig 5.1 and Tables 1 and 2. Lambs to which epidural saline had been administered 40 minutes prior to castration and tail-docking produced a rapid rise in plasma cortisol from a basal level of 41.0 ± 13.8 nmol/l to a peak of 226.4 ± 35.6 at 30 minutes, returning to basal levels by 120-150 minutes. The plasma cortisol response of this group was significantly greater ($P<0.05$) than that seen in the control group or the epidural xylazine group when compared from 15-60 minutes.

Plasma cortisol concentrations

Experiment 1. Table of mean plasma cortisol concentrations (nmol/l) for the four groups.

Procedure	Drug	Nil	E/D Xylazine
C		41.0	45.9
CN		186.6	177.1

Thus for a SED of 35.7, and using a two way T-test, 5% significance is obtained with any difference greater than 74.6 nmol/l between the mean treatment cortisol concentrations tabulated above. Note that the cortisol responses of lambs to which no drug (CN-Nil), or epidural xylazine (CN-E/D Xylazine) had been administered prior to castration and tail-docking are statistically greater ($P<0.05$) than that recorded in control lambs (C-Nil). There is, however, no statistical difference ($P>0.05$) in the plasma cortisol responses when these two groups are compared.

Effects of intrathecal xylazine on the behavioural and physiological indices selected, following castration

Expt 2 Group 1- Behaviour of control lambs

The results are presented in Fig 5.2 and Tables 3 and 4. The behaviour of control lambs changed from a high incidence of standing (79% of observations) from 0-15 minutes to predominantly awake ventral recumbency (V2) from 15-180 minutes (47-69% of observations). Abnormal behaviour was limited to standing (S2) in the first 15 minutes (20% of observations). There was no evidence of restlessness from 0-30 minutes (15 posture changes in total).

Plasma cortisol concentrations

The results are presented in Fig 5.2 and Tables 3 and 4. The plasma cortisol concentrations of control lambs remain within 10nmol/l of the basal level (10.3 ± 3.3 nmol/l) throughout the duration of the experiment (0-180 minutes).

Expt 2 Group 2- Behaviour of lambs to which intrathecal xylazine (2x25μg) had been administered

The results are presented in Fig 5.2 and Tables 3 and 4. Lambs to which intrathecal xylazine (2x25μg) had been administered lay more in ventral recumbency than control lambs throughout the duration of the experiment (0-180 minutes). This approached significance ($P<0.1$) from 60-90 minutes. The incidence (12-35 % of observations) and duration (0-60 minutes) of abnormal standing (S2) was greater in this group than in control lambs. There was no evidence of increased restlessness from 0-30 minutes (30 posture changes in total).

Plasma cortisol concentrations

The results are presented in Fig 5.2 and Tables 3 and 4. The plasma cortisol concentrations of lambs to whom intrathecal xylazine had been administered, remain within 10 nmol/l of the basal level (14.8 ± 7.9 nmol/l) throughout the duration of the experiment (0-180 minutes). There was no significant difference ($P>0.05$) in plasma cortisol responses when this group was compared with control lambs from 15-60 minutes.

Expt 2 Group 3- Behaviour of lambs to which intrathecal xylazine (2x25μg) had been administered 40 minutes prior to castration

The results are presented in Fig 5.2 and Tables 3 and 4. Lambs to which intrathecal xylazine (2x25μg) had been administered 40 minutes prior to castration stand less and lie more than control lambs from 0-15 minutes. This approaches significance for both behaviours ($P<0.1$). Lambs in this group lie in lateral recumbency (L1-L2) for the periods between 15-60 minutes, a behaviour which was not recorded in control lambs. This was significant from 15-60 minutes ($P<0.05$). In this group abnormal ventral recumbency (V3) was recorded from 0-90 minutes (22-60% of observations) and abnormal standing (S2-3) was recorded throughout the duration of the experiment (10-50% of observations). The incidence of abnormal standing was higher in this group for the periods between 0-90 and 120-180 minutes than that seen in lambs to which intrathecal saline had been administered prior to castration, while the reverse was true for abnormal ventral recumbency (V3) from 0-60 minutes. When the main behavioural categories were compared for each time period between group 3 and lambs to which intrathecal saline had been administered prior to castration, no significant differences were found ($P>0.05$). Restlessness in this group of lambs was evident from 0-30 minutes, increasing from 172 posture changes in the first 15 minutes to over 208 posture changes from 15-30 minutes, in contrast to group 4 lambs where the restlessness decreased over this time. The restlessness seen from 0-30 minutes in group 3 lambs was significantly greater than control lambs ($P<0.05$), but was not significantly different from the restlessness seen in group 4 lambs ($P>0.05$).

Plasma cortisol concentrations

The results are presented in Fig 5.2 and Tables 3 and 4. The plasma cortisol concentrations of lambs to which intrathecal xylazine (2x25µg) had been administered 40 minutes prior to castration increased rapidly from a basal level of 38.1 ± 6.7 nmol/l to a peak of 365.8 ± 69.3 nmol/l at 60 minutes, returning to basal levels by 120 minutes. The plasma cortisol response when compared from 15-60 minutes with the other 3 groups, was found to be significantly greater ($P<0.05$) in all cases.

Expt 2 Group 4- Behaviour of lambs to which intrathecal saline had been administered 40 minutes prior to castration

The results are presented in Fig 5.2 and Tables 3 and 4. Lambs to which intrathecal saline had been administered 40 minutes prior to castration stand less than control lambs (3-39% of observations) for the periods between 0-120 minutes. This was significant for the periods 0-30 and 60-90 minutes ($P<0.05$). Lambs in this group lay more in ventral recumbency (60-85% of observations) when compared with control lambs from 0-120 minutes. This was significant from 0-15 minutes ($P<0.05$). The majority of cases of ventral recumbency in these lambs was abnormal (V3) from 0-90 minutes (42-58% of observations). These lambs lay in lateral recumbency (L1-L2) from 0-90 minutes (2-33% of observations). This behaviour did not occur in control lambs, and when compared approached significance ($P<0.1$) from 15-30 minutes. Restlessness was evident from 0-30 minutes, decreasing from 192 posture changes in the first 15 minutes to 137 posture changes from 15-30 minutes. There was significantly more restlessness in these lambs than control lambs when compared from 0-30 minutes ($P<0.05$).

Plasma cortisol concentrations

The results are presented in Fig 5.2 and Tables 3 and 4. The plasma cortisol concentrations of lambs to which intrathecal saline had been administered 40 minutes prior to castration increased from a basal level of 9.2 ± 3.3 nmol/l to a peak of

190.4 ± 47.0 nmol/l at 60 minutes, before reducing to basal levels at 120 minutes. When the plasma cortisol response was compared from 15-60 minutes with the other groups it was found to be significantly greater than the control group and the intrathecal xylazine group ($P<0.05$), but significantly lower than that recorded in lambs administered intrathecal xylazine prior to castration ($P<0.05$).

Plasma cortisol concentrations

Experiment 2. Table of mean plasma cortisol concentrations (nmol/l) for the four groups.

	Drug	Nil	I/T Xylazine
Procedure			
C		36.4	24.4
CN		160.6	224.3

Thus for a SED of 26.3, and using a two way T-test, 5% significance is obtained with any difference greater than 55.0 nmol/l between the mean treatment cortisol concentrations tabulated above. Note the cortisol responses of lambs to which no drug (CN-Nil), or intrathecal xylazine (CN-I/T Xylazine) had been administered prior to castration are statistically greater ($P<0.05$) than that recorded in control lambs (C-Nil). The plasma cortisol response in the latter group (CN-I/T Xylazine) is, however, statistically greater ($P<0.05$) than the former group (CN-Nil).

Figure 5.1

Effect of epidural xylazine on the indices used for assessment of pain from castration and tail-docking

The results shown were obtained from 4 groups of 6 lambs, each group subjected to one of the following treatments 1) control handling 2) epidural xylazine 3) epidural xylazine + castration and tail-docking 4) epidural saline + castration and tail-docking

Behaviour

The incidence (%) of four behaviours (L=Lateral recumbency, S= Standing, V= Ventral recumbency, T/S= Teat Seeking and Sucking) were recorded for each group and presented in bar chart form in 6 time periods; 0-15, 15-30, 30-60, 60-120, 120-180 and 180-240 minutes, see the materials and methods for number of observations in each period.

Plasma cortisol

Plasma cortisol concentrations were presented in graph form (nmol/l ± S.E, (n=6)) at 10 times; -10, 0, 15, 30, 60, 90, 120, 150, 180 and 240 minutes.

Statistical analysis

A Indicates that the difference between the behaviour of the treatment group and the (control handling) group was significant at ($P<0.05$)

a As above but ($P<0.1$)

Notes 1) Epidural xylazine increases the incidence of lateral recumbency after castration and tail-docking during the time periods 0-15, 15-30 and 30-60 minutes. This was not significant ($P>0.05$)

2) No significant differences were found ($P<0.05$) in the cortisol responses.

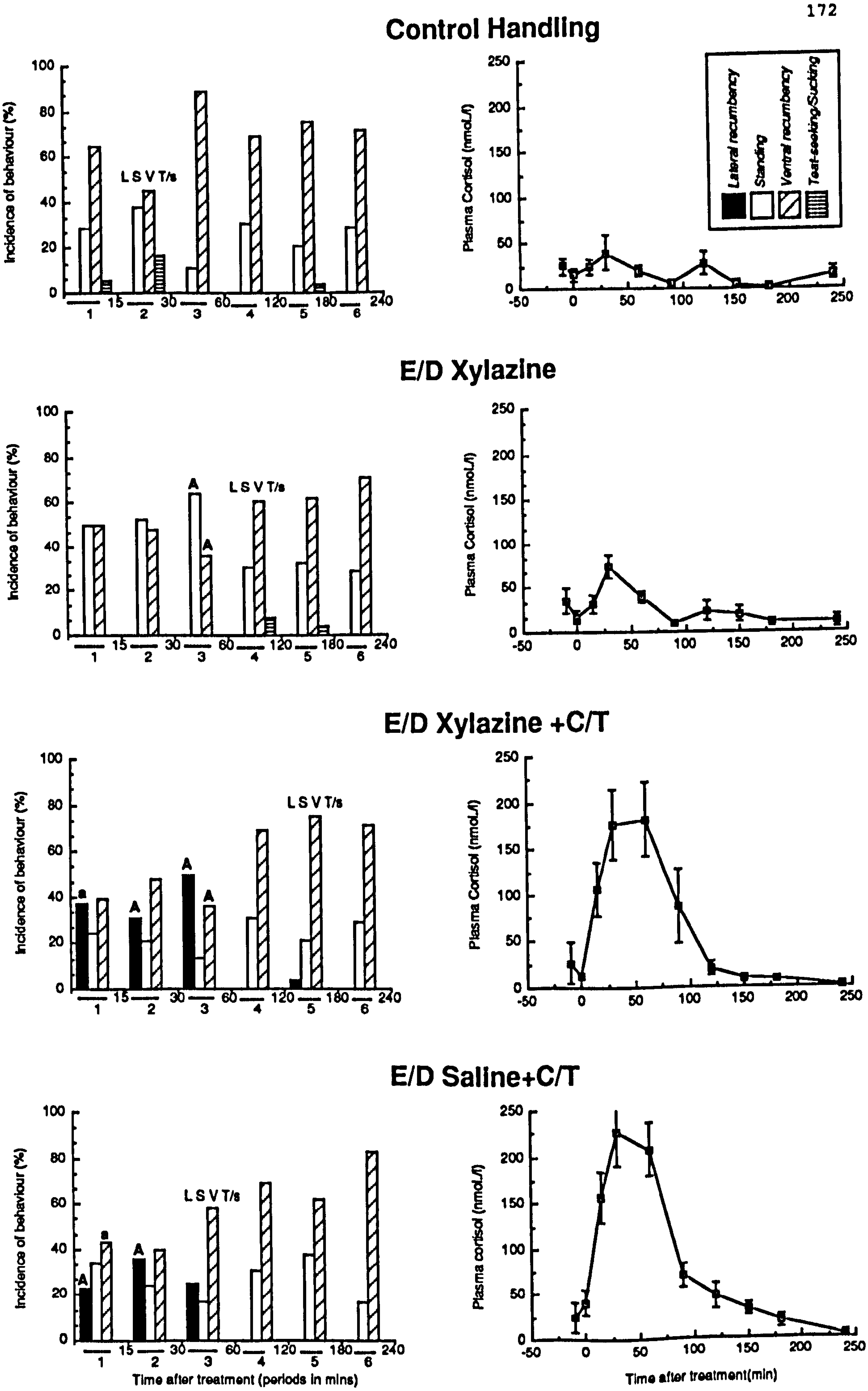

Control Handling
E/D Xylazine
E/D Xylazine +C/T
E/D Saline+C/T
Incidence of behaviour (%)
Plasma Cortisol (nmol./l)
Plasma cortisol (nmol./l)
L S V T/s
Lateral recumbency
Standing
Ventral recumbency
Teat-seeking/Sucking
Time after treatment (periods in mins)
Time after treatment(min)

TABLE 5.1

THE EFFECTS OF FOUR TREATMENTS ON LAMB BEHAVIOUR IN SIX TIME PERIODS

In each period after treatment the incidence of four postures (i.e. Lateral Recumbency, Standing, Ventral Recumbency and Teat Seeking or Sucking) are presented as the number of times that behaviour is observed, and as a percentage of the total number of observations made in that period.

TABLE 5.1 Incidence of four lamb behaviours in six periods after four treatments.

PERIOD AFTER TREATMENT	0 - 15 mins				15 - 30 mins				30 - 60 mins				50 - 120 mins				120 - 180 mins				180 - 240 mins			
NUMBER OF OBSERVATIONS MADE IN PERIOD	n = 84				n = 42				n = 36				n = 36				n = 24				n = 24			
BEHAVIOUR OBSERVED	LR	VR	St	TS/S	LR	VR	St	TS/S	LR	VR	St	TS/S	LR	VR	St	TS/S	LR	VR	St	TS/S	LR	VR	St	TS/S
CONTROL HANDLING (6 lambs)	0 0%	55 65%	24 28%	5 6%	0 0%	19 45%	16 38%	7 17%	0 0%	32 89%	4 11%	0 0%	0 0%	25 69%	11 31%	0 0%	0 0%	18 75%	5 21%	1 4%	0 0%	17 71%	7 29%	0 0%
EPIDURAL XYLAZINE (50ug) ONLY (6 lambs)	0 0%	42 50%	42 50%	0 0%	0 0%	20 48%	22 52%	0 0%	0 0%	13 36%	23 64%	0 0%	0 0%	22 61%	11 31%	3 8%	0 0%	15 62%	8 33%	1 4%	0 0%	17 71%	7 29%	0 0%
EPIDURAL XYLAZINE (50ug) PLUS CASTRATION AND TAIL DOCKING (6 lambs)	31 37%	33 39%	20 24%	0 0%	13 31%	20 48%	9 21%	0 0%	18 50%	13 36%	5 14%	0 0%	0 0%	25 69%	11 31%	0 0%	1 4%	18 75%	5 21%	0 0%	0 0%	17 71%	7 29%	0 0%
EPIDURAL SALINE PLUS CASTRATION AND TAIL DOCKING (6 lambs)	19 23%	36 43%	29 34%	0 0%	15 36%	17 40%	10 24%	2 0%	9 25%	21 58%	6 17%	0 0%	0 0%	25 69%	11 31%	0 0%	0 0%	15 62%	9 38%	0 0%	0 0%	20 83%	4 17%	0 0%

LR Lateral Recumbency VR Ventral Recumbency
St Standing or Walking TS/S Teat Seeking or Sucking

TABLE 5.2 Plasma cortisol measurements taken at 10 times after treatment for 4 different treatment groups

TIME AFTER TREATMENT (mins)	-10.0	0	15	30	60	90	120	150	180	240
NUMBER OF MEASUREMENTS (No. of lambs)	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
CONTROL HANDLING Plasma cortisol (n.mol/1) Mean ± S.E.	25.5 ± 8.7	15.7 ± 7.0	25.0 ± 8.3	39.6 ±19.0	19.8 ± 5.8	6.5 ± 3.2	28.4 ± 11.8	5.1 ± 4.0	2.1 ± 5.2	16.9 ± 6.8
EPIDURAL XYLAZINE Plasma cortisol (n.mol/1) Mean ± S.E.	35.1 ± 14.5	16.5 ± 7.2	30.7 ± 9.5	73.4 ± 12.9	39.2 ± 6.5	9.5 ± 2.9	23.4 ± 10.9	20.1 ± 8.0	10.8 ± 8.0	11.8 ± 6.9
EPIDURAL XYLAZINE: CASTRATION PLUS TAIL DOCKING Plasma cortisol (n.mol/1) Mean ± S.E.	26.7 ± 21.7	13.7 ± 3.4	106.9 ± 29.3	176.8 ± 37.6	182.0 ± 40.4	87.3 ± 40.6	20.0 ± 7.2	9.2 ± 3.7	8.1 ± 2.5	1.1 ± 2.9
EPIDURAL SALINE: CASTRATION PLUS TAIL DOCKING Plasma cortisol (n.mol/1) Mean ± S.E.	24.9 ± 16.0	41.0 ± 13.8	157.3 ± 28.1	226.4 ± 35.6	208.0 ± 28.5	70.3 ± 13.4	46.6 ± 13.4	32.6 ± 6.1	18.9 ± 8.0	3.0 ± 5.2

FIGURE 5.2

EFFECT OF INTRATHECAL XYLAZINE ON THE INDICES USED FOR ASSESSMENT OF PAIN FROM CASTRATION

The results shown were obtained from 4 groups of 6 lambs, each group was subjected to one of the following treatments 1) control handling 2) intrathecal xylazine 3) intrathecal xylazine + castration 4) intrathecal saline + castration and tail-docking

Behaviour

The incidence (%) of three behaviours (L- Lareral recumbency (L1-L3), S- Standing (S1-S3), V- Ventral recumbency (V1-V3)) were recorded for each group and presented in bar chart form in 6 time periods; 0-15, 15-30, 30-60, 60-90, 90-120 and 120-180 minutes, see materials and methods for numbers of observations in each period. A restlessness score was recorded from 0-30 minutes, and was presented as a quantitative measure of the total number of posture changes in all lambs.

Plasma cortisol

Plasma cortisol concentrations were presented in graph form (nmol/l ± S.E, (n=6)) at 9 times; -10, 0, 15, 30, 60, 90, 120, 150 and 180 minutes.

Statistical analysis

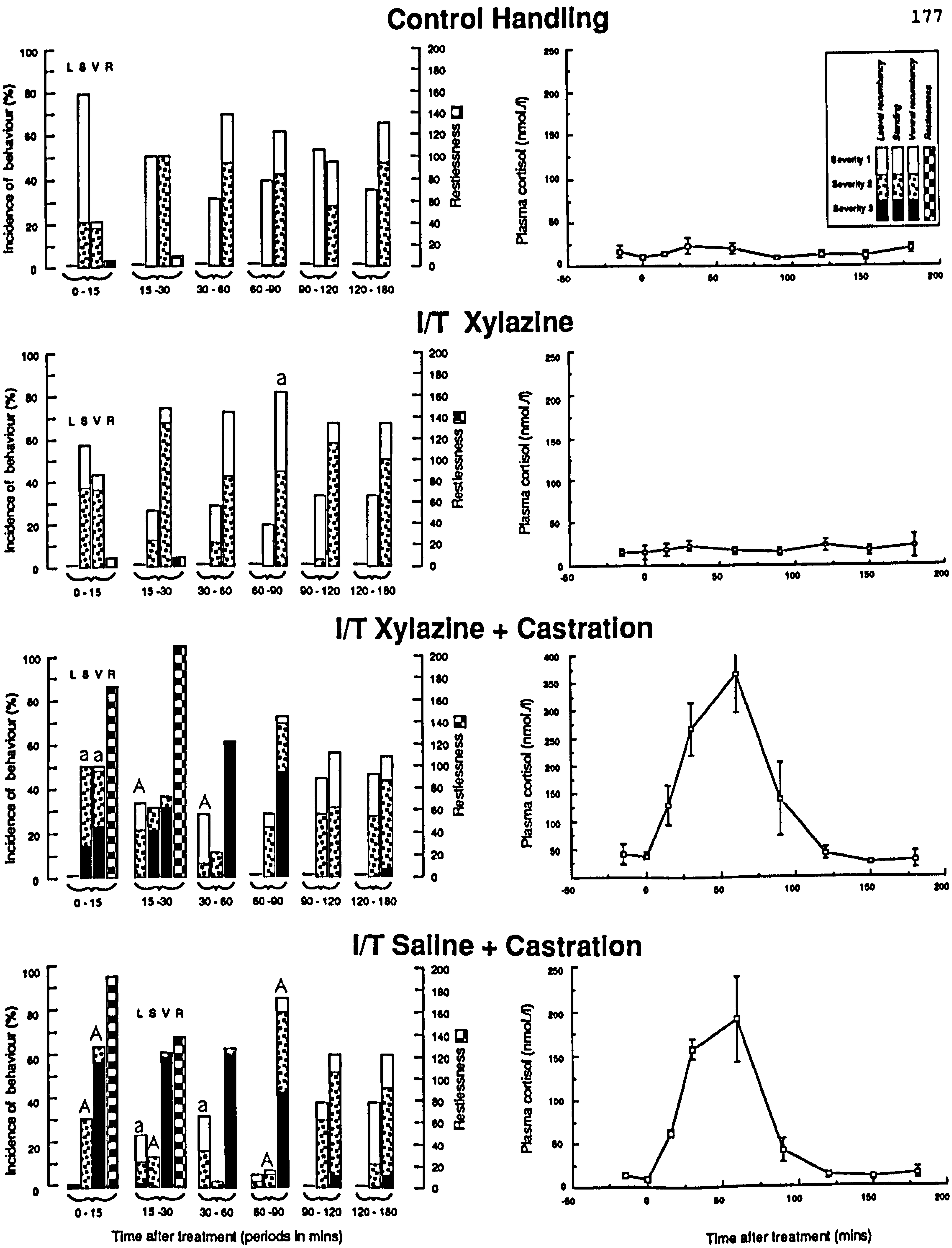
A Indicates that the difference between the behaviour of the treatment group and the (control handling) group was significant at ($P<0.05$)

a As above but ($P<0.1$)

Notes 1) Intrathecal xylazine increases the incidence of abnormal standing (S2-S3) after castration during the periods 1-15, 15-30 , 30-60 and 120-180 minutes

2) No S3 standing was seen in the (I/T Saline + Castration) group

3) The plasma cortisol response of the (I/T Xylazine + Castration) group was significantly greater ($P<0.05$) than the (I/T Saline + Castration) group when compared from 15-60 minutes.



Control Handling
I/T Xylazine
I/T Xylazine + Castration
I/T Saline + Castration
L S V R
Incidence of behaviour (%)
Restlessness
Plasma cortisol (nmol/l)
0 - 15
15 -30
30 - 60
60 -90
90 - 120
120 - 180
Lateral recumbency
Standing
Ventral recumbency
Restlessness
Severity 1
Severity 2
Severity 3
a
A
Time after treatment (periods in mins)
Time after treatment (mins)

TABLE 5.3

THE EFFECTS OF FOUR TREATMENTS ON LAMB BEHAVIOUR IN SIX TIME PERIODS

In each period after treatment the incidence of three postures (i.e. Lateral Recumbency, Standing, Ventral Recumbency and Teat Seeking or Sucking) are presented as the number of times that behaviour is observed, and as a percentage of the total number of observations made in that period.

TABLE 5.3 Incidence of four lamb behaviours in six periods after four treatments.

PERIOD AFTER TREATMENT	0 - 15 mins				15 - 30 mins				30 - 60 mins			60 - 90 mins			90 - 120 mins			120 - 180 mins		
NUMBER OF OBSERVATIONS MADE IN PERIOD	n = 84				n = 42				n = 36			n = 36			n = 36			n = 72		
BEHAVIOUR OBSERVED	LR	St	VR	R	LR	St	VR	R	LR	St	VR	LR	St	VR	LR	St	VR	LR	St	VR
CONTROL HANDLING (6 lambs)	0	66	18	6	0	21	21	9	0	11	25	0	14	22	0	19	17	0	25	47
	0%	79%	21%		0%	50%	50%		0%	31%	69%	0%	39%	61%	0%	53%	47%	0%	35%	65%
INTRATHECAL XYLAZINE (50ug) ONLY (6 lambs)	0	48	36	6	0	11	31	5	0	10	26	0	7	29	0	12	24	0	24	48
	0%	57%	43%		0%	26%	74%		0%	28%	72%	0%	19%	81%	0%	33%	67%	0%	33%	67%
INTRATHECAL XYLAZINE (50ug) PLUS CASTRATION (6 lambs)	0	42	42	172	14	13	15	208	10	4	22	0	10	26	0	16	20	0	33	39
	0%	50%	50%		33%	31%	36%		28%	11%	61%	0%	28%	72%	0%	44%	56%	0%	46%	54%
INTRATHECAL SALINE PLUS CASTRATION (6 lambs)	2	27	55	192	10	6	26	137	12	1	23	2	3	31	0	14	22	0	28	44
	2%	32%	65%		24%	14%	62%		33%	3%	64%	6%	8%	86%	0%	39%	61%	0%	39%	61%

LR Lateral Recumbency
St Standing or Walking
VR Ventral Recumbency
R Restlessness

TABLE 5.4 Plasma cortisol measurements taken at 9 times for 4 different treatment groups

TIME AFTER TREATMENT (mins)	-10.0	0	15	30	60	90	120	150	180
NUMBER OF MEASUREMENTS (No. of lambs)	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6	n = 6
CONTROL HANDLING Plasma cortisol (n.mol/l) Mean ± S.E.	17.3 ± 7.0	10.3 ± 3.3	15.0 ± 2.1	23.4 ± 9.0	20.0 ± 6.0	9.8± 2.4	13.5 ± 4.4	12.2 ± 4.7	20.0 ± 5.1
INTRATHECAL XYLAZINE Plasma cortisol (n.mol/l) Mean ± S.E.2	14.8 ± 3.7	14.8 ± 7.9	18.3 ± 7.2	22.5 ± 5.4	16.8 ± 4.4	15.3 ± 4.7	23.0 ± 7.3	17.0 ± 5.5	21.7 ± 13.3
INTRATHECAL XYLAZINE PLUS CASTRATION Plasma cortisol (n.mol/l) Mean ± S.E.	41.4 ± 18.1	38.1 ± 6.7	128.8 ± 35.3	267.9 ± 47.5	365.8 ± 69.3	139.4 ± 65.9	41.8 ± 10.7	25.2 ± 3.4	30.0 ± 15.1
INTRATHECAL SALINE PLUS CASTRATION Plasma cortisol (n.mol/l) Mean ± S.E.	13.0 ± 3.1	9.2 ± 3.3	62.1 ± 4.2	158.4 ± 11.4	190.4 ± 47.0	41.9 ± 13.5	12.8 ± 3.9	10.0 ± 3.8	14.9 ± 6.7

DISCUSSION

The aim of these experiments, as for the previous series (chapter 4) was to find an analgesic with which to reduce the pain experienced following castration, or castration and tail-docking. Neither epidural or intrathecal xylazine at the doses employed here produced recognisable analgesia. This suggests that unless unknown factors prevented access of active xylazine with a suitable duration of action, the pain produced by castration and tail-docking or castration cannot be reduced by epidural or intrathecal xylazine in lambs of less than one week of age.

Interpretation of the results obtained in experiment 1

There was no evidence from the behavioural measurements or the changes in plasma cortisol concentration to indicate that epidural xylazine produced analgesia. Epidural xylazine (2x25μg) did not reduce the incidence of lateral recumbency associated with castration and tail-docking, conversely the incidence of lateral recumbency was higher than that recorded in lambs to which only saline had been administered prior to castration and tail-docking, though the difference was not significant ($P>0.05$). This increase in lateral recumbency was unlikely to be due to general sedation as similar doses injected systemically did not produce central effects (Goranov *et al*, 1983). Furthermore lambs administered xylazine only, spent less time recumbent than control lambs, the decrease being significant ($P<0.05$) during the period 30-60 minutes. There were no differences in the plasma cortisol responses of those groups where either saline or xylazine were administered prior to castration and tail-docking when compared from 15-60 minutes ($P>0.05$). The similarity of the plasma cortisol responses in these two groups and the small increase in lateral recumbency (proposed to be the prime indicator of severe pain, see chapter

3) in the latter group suggests there is no analgesia following administration of xylazine.

Interpretation of the results obtained in experiment 2

Unlike epidural administration of xylazine, at these doses intrathecal xylazine results in lambs lying in ventral recumbency more than the control lambs throughout the duration of the experiment (0-180 minutes). This increase in ventral recumbency is significant at $P<0.1$ during the time period 60-90 minutes. This suggests that intrathecal administration of xylazine at these doses produces sedation, which may also account for the abnormal standing/walking (S2) seen during the time periods 15-30 and 30-60 minutes (12% of observations) as opposed to 0% in control lambs.

There was little evidence for analgesia following intrathecal administration of xylazine, but some weak evidence for hyperalgesia. Xylazine pretreatment did not eliminate or reduce the lateral recumbency associated with castration, and there was an increase in abnormal standing (10-50% of observations, S2-S3) during the time periods 0-15, 15-30 and 30-60 minutes when compared to lambs administered saline prior to castration. The limited evidence that intrathecal xylazine might have some analgesic action is the decrease in abnormal ventral recumbency (22-60% of observations - V3) during the time periods 0-15, 15-30 and 30-60 minutes and the absence of lateral recumbency from 60-90 minutes when compared with lambs administered just saline prior to castration. It is difficult to explain the effects on standing and ventral recumbency produced by prior treatment with intrathecal xylazine in castrated lambs in the absence of an independent measure of pain intensity. As they may reflect dysaesthesia or other modifications of the responses to the noxious stimulus by action of xylazine at higher levels of the CNS, they are not considered to be evidence of analgesia.

The increase in plasma cortisol, seen in lambs administered intrathecal xylazine prior to castration, was significantly greater ($P<0.05$) in the period from 15-60 minutes than in lambs administered just saline prior to castration supporting the conclusion that intrathecal xylazine did not produce analgesia. The increase in plasma cortisol concentration was not attributable to xylazine itself, as no change in plasma cortisol concentration was seen in lambs administered just xylazine.

Explanations for the absence of analgesic effects following administration of either epidural or intrathecal xylazine (2x25μg)

(a) Failure of active drug to gain access to the spinal cord

As described for morphine and etorphine administration, no obvious errors could be found in either the epidural or intrathecal administration technique which could occasionally be responsible for failed spinal analgesia (Munhall, 1988). There was good evidence that active drug was administered because there was an increase in standing (Significant during the period 30-60 minutes, $P<0.05$) in lambs administered epidural xylazine and an increase in ventral recumbency (significant during the period 60-90 minutes, $P<0.05$) in lambs administered intrathecal xylazine when compared with control lambs, which can only be explained by the presence of an agent which has effects on the central nervous system (Vainio and Vaha-Vahe, 1990; Kitzman *et al*, 1982). Whether the active drug gained access to the spinal cord or not is less clear though weak indirect evidence that it did is provided by the increase in standing throughout the duration of experiment 1. This could be attributed to action of xylazine at alpha 1 receptors in the ventral horn since increases in motor activity have been described following intraperitoneal injection of the the alpha 2 agonist clonidine (10-1000μg) to rats (Howe *et al*, 1983) and

Brandt and Livingston (1989) have demonstrated that the α_2 agonist <3H> clonidine binds to the ventral spinal cord of adult of adult sheep. As argued for the opioids, direct evidence that xylazine gained access to the spinal cord in this age of lamb is required. Such evidence could be obtained by demonstrating binding of radiolabelled α_2 agonists in the spinal cord following intrathecal injection as described in the next section. It is concluded that active xylazine was administered but only inconclusive evidence was obtained that xylazine gained access to the spinal cord.

(b) There is an inadequate population of functional α_2 receptors in the spinal cord on which xylazine can act

There is evidence that alpha 2 binding occurs in lamina II of the dorsal horn in the rat which is associated with nociceptive primary afferents (Young and Kuhar, 1980; Unnerstall *et al*, 1984), and a similar distribution has also been demonstrated in the adult sheep using <3H> clonidine (Bouchenafa and Livingston, 1987). Despite these findings, it cannot be assumed that a similar distribution will be found in the young lamb. At birth the CNS of the lamb is still developing, with the cerebellum and spinal cord less well developed than the cerebral hemispheres (McIntosh *et al*, 1979), furthermore it is likely that the adrenergic and opioid receptors are regulated in a specific manner which does not necessarily parallel brain growth (Villiger *et al*, 1982). Initially autoradiographic studies are required to locate adrenoceptors in the spinal cord of the young lamb. To quantify the density of binding sites, sections of spinal cord from the appropriate level (T10-T11 and L6-L7) could be dissected and saturation binding studies performed using the alpha 2 agonist <3H> clonidine (Brandt and Livingston, 1989). Although the presence of such binding implies that specific receptors are present and they are an important or integral component of the nervous system, it does not permit the conclusion that their development is directly related to any particular process, such as antinociception (Clendeninn *et al*, 1976). For example, although the sensitivity to morphine analgesia in young rats increases from day 5 to day 15 postnatally (Auguy-Valette *et al*, 1978), there is a paradoxical decrease in opioid binding by brain and spinal cord homogenates from day 6 (Kirby, 1981). Even if adrenoceptors can be demonstrated to be present in the lamb spinal cord it does not necessarily follow that intrathecal xylazine should have an analgesic action; however, if limited α_2 binding can be demonstrated it would help to confirm the results obtained here.

(c) The duration and distribution of activity of xylazine is not adequate

Kalpravidh *et al* (1984), showed that intramuscular xylazine (2.2mg/kg) could provide 3-4 hours of analgesia against cutaneous and visceral pain in experimental ponies, while Waterman *et al* (1988) obtained 2 hours of analgesia against mechanical pain following intrathecal administration of xylazine (50μg) in the adult sheep.

Waterman *et al* (1988), demonstrated that peak analgesic effects of intrathecal xylazine (50μg) in the sheep occur 40 minutes after administration and lasts for up to 120 minutes. Although some differences in duration of action of xylazine may occur in lambs it was expected that similar doses to those used by Waterman *et al* (1988) when given to lambs would have at least the same duration of action and it was not expected that any analgesic action would wear off between administration of xylazine and the treatment 40 minutes later. This possibility should be further investigated, however, by means of chronically implanted catheters in the intrathecal space (Yaksh and Rudy, 1976a; Waterman *et al*, 1988). This approach would allow administration of the drug to a group of lambs immediately prior to the treatment and would permit incremental dosing during the course of the noxious stimulus. In this way it would be possible to determine if xylazine has both a short duration of action and a narrow range of doses over which it is analgesic in the lamb.

Intrathecal injection was expected to produce a restricted distribution in the spinal cord since Solomon *et al* (1989), showed that although peak blood levels of clonidine following intrathecal injection in the rat, occurred after 5 minutes, this only represented 2% of the injected dose (3.2-32.0 µg). Autoradiographic studies by Castro and Eisenach (1989) in the sheep using <3H> clonidine, demonstrated that 36% of the drug remained in the lumbar spinal cord at the injection site, 5 minutes after administration. The concentration of clonidine in the cervical cord was 10 fold lower, suggesting that although rostral distribution does occur, a greater proportion remains present at the site of injection.

Similar results have been obtained following intrathecal injection of <3H> clonidine in 100µl of saline via a chronic indwelling cannula in the adult sheep (Bouchenafa *et al*, 1988; Waterman *et al*, 1988). The highest concentration of <3H> clonidine bound to the spinal cord was found at the intrathecal injection site, with a measurable distribution restricted to about 5 vertebral segments around the catheter tip. What may be assumed is that the distribution was at least as extensive in the lamb. Some evidence suggests that it was more extensive. The sedation seen in lambs administered intrathecal, but not epidural xylazine at the level of thoracic segment 10 suggests that a greater rostral distribution occurs with intrathecal administration. This could be attributable to the smaller spinal canal of the lamb, or a combination of differences in passive diffusion and active transport by bulk flow in the CSF of xylazine compared with clonidine (Sato *et al*, 1971). Although increased distribution increases the likelihood that all levels of the spinal cord receiving afferent input from the affected areas are exposed to xylazine, insufficient concentrations of drug may be present to provide useful analgesia. Studies such as those described above are therefore required to determine details of the pharmacokinetics of xylazine following intrathecal injection in the lamb.

(d) The origin and intensity of the pain was such that any analgesia obtained with xylazine was not apparent using these assessment methods

Abdominal visceral pain, which is produced by castration, is poorly understood and has often been found to be difficult to treat in humans (Procacci *et al*, 1986). Despite this, alpha 2 agonists such as clonidine have been shown to attenuate certain types of visceral pain (chemical) more effectively than somatic pain in the rat (Ness and Gebhart, 1989). In other species, such as the horse, it was found that the alpha 2 agonist xylazine did not consistently produce analgesia in either visceral or somatic (thermal) pain tests (Pippi and Lumb, 1979). Castration, or castration and tail-docking in the lamb combines visceral and somatic pain (chemical and mechanical) and may represent a type and intensity of stimulus which can only be attenuated by a combination of analgesics directed at the different origins of the pain. Visceral pain originating from the spermatic cords, testis, epididymis and vaginal sac as a result of mechanical effects of the tight rubber ring may not be attenuated by α_2 agonists to the same extent as visceral pain produced by chemical effects of the rubber rings (Colburn *et al*, 1989). Furthermore, nociception may be occurring during the complex stimulus of castration or castration and tail-docking via mechanisms with greater susceptibility to the analgesic action of α_1 than α_2 agonists (Tasker and Melzack, 1989).

Since xylazine produced no evidence of analgesia it is unlikely that a combination of agonists will be helpful, however, a possible approach to obtaining analgesia, would involve the combination of a potent α_1 and α_2 agonist or an opioid and an α_2 agonist. Combination of an opioid and α_2 agonist, when administered intrathecally have been shown to have a synergistic action (Stevens *et al*, 1988), showing only limited cross tolerance (Solomon and Gebhart, 1988). Such an approach relies on opioid and adrenergic drugs operating via separate mechanisms to produce a synergistic action when combined and administered intrathecally (Wilcox *et al*, 1987).

As for the opioids there is sufficient evidence from these studies to consider it unlikely that the intensity of the stimulus provides an explanation for the absence of any recognisable analgesic effect of epidural or intrathecal xylazine. If xylazine produced analgesia at lower intensities of noxious stimulation, an earlier termination to the abnormal behaviour following castration or castration and tail-docking would be expected, because as indicated by these responses the intensity of these stimuli reduces with time. The only evidence for an earlier termination of the abnormal behaviour was the absence of lateral recumbency from 60-90 minutes in lambs administered intrathecal xylazine prior to castration which was recorded in lambs administered only saline prior to castration (6% of observations). This incidence of lateral recumbency, however, was only attributable to 1 lamb and the incidence was not significant ($P>0.05$).

Further approaches to finding a suitable analgesic with which to modify the pain of castration and tail-docking

Several approaches to finding a suitable dose and administration technique for xylazine or alternative analgesics were described in the discussion of chapter 4 which are also applicable here.

THE WITHDRAWAL REFLEX IN THE CHLORALOSE ANAESTHETISED LAMB AND ITS USE FOR THE ASSESSMENT OF THE ANALGESIC POTENCY OF XYLAZINE AND MORPHINE WHEN ADMINISTERED INTRATHECALLY TO THE SPINAL CORD

Since Sherrington's classic studies (Creed *et al*, 1932), reflex responses to noxious and electrical stimuli have been extensively investigated. In many of the early experiments with electrical stimulation, mixed fibre types were excited. That it was not necessary to excite unmyelinated fibres to elicit flexor reflexes (Lundberg, 1964), indicates drug effects may be investigated with a stimulus that might not be painful in the conscious animal. Results obtained by this method should always therefore be complemented by clinical trials using pain originating from disease or trauma (Beecher, 1957). Opioids (Brodo and Brooks, 1937; Martin *et al*, 1964) and alpha agonists (Anden *et al*, 1966) have both been shown to inhibit the withdrawal reflex. Many of these studies were performed in spinal animals (Irwin *et al*, 1951), where it was found that both excitatory and inhibitory interneurones in the spinal cord are under powerful, tonic descending inhibition which arises from the brainstem (Lundberg, 1964; Engberg *et al*, 1968). Other workers have argued that the spinal cord must be intact for morphine to have an effect on spinal reflexes (Takagi *et al*, 1955). This latter view although not generally accepted could be explained by recent work indicating that much of the inhibition of reflex activity following systemic morphine administration was indirect via descending systems originating in the brainstem (Barton *et al*, 1980).

Descending inhibitory influences on the withdrawal reflex

Release from descending inhibition following spinalisation increases and stabilises reflexes and this makes it easier to investigate the effects of various drugs. This has been clearly demonstrated by several groups including Clarke *et al*, (1988) in decerebrate, low spinalised and intact rabbit preparations. In non spinalised decerebrate rabbits the threshold and latency of the sural- gastrocnemius reflex were significantly higher than spinalised preparations. Intravenous naloxone and idazoxan were found to enhance reflexes while clonidine and morphine depressed them in a dose dependent fashion. Naloxone was more effective in spinalised rabbits and idasoxan in non spinalised rabbits, which is evidence that endogenous opioids have a segmental action which can be increased by descending adrenergic pathways acting through alpha 2 receptors. That descending inhibition is primarily by non opioid pathways was demonstrated by producing a discrete lesion in the Dorsolateral Funiculus. This lesion eliminates most of the inhibitory effects obtained with systemic morphine on spinal reflexes, yet the baseline latency of these reflexes remained unaltered. This indicated that the descending inhibitory system had not been disrupted and was therefore non opioid in nature (Barton *et al*, 1980).

Evidence for the proposition that opioids will inhibit the sural-semitendinosus withdrawal reflex in the lamb

Morphine, related narcotics and certain adrenoceptor drugs depress withdrawal reflexes initiated in the lumbar spinal cord in response to impulses in small diameter primary afferent fibres of both cutaneous and muscular origin (Koll *et al*, 1963). An example being the inhibition by morphine of reflex contractions observed in the tibialis anterior muscle following stimulation of C-fibre afferents in the superficial peroneal (Bell and Martin, 1977) or sural nerves (Willer and Brussel, 1980). Morphine was also found to inhibit monosynaptic reflexes (primarily extensor reflexes) initiated by activity in large diameter fibres (Brodo and Brooks, 1937; Wikler, 1950). The inhibition of these reflexes was, however, dependent on the nerve stimulation frequency. Duggan and North (1984) proposed that at low stimulation frequencies the amount of excitation or inhibition of the interneurones, and any modification by morphine, was not sufficient to change the subsequent reflex.

Morphine can, however, enhance reflex activity at high doses (Kuschinsky *et al*, 1978; Jurna *et al*, 1978) which can be accounted for by its ability to reduce both direct and indirect recurrent inhibition in cats at doses of 20mg/kg (Curtis and Duggan, 1969) and 5-10 mg/kg respectively (Krugalov, 1964). This action was thought to be via an antiglycinergic effect at the spinal neurones (Curtis and Duggan, 1969). This was supported by Yaksh *et al* (1986), who demonstrated that these effects were not dependent on opioid receptors. This action of morphine was not antagonised, but was enhanced following systemic administration of naloxone or naltrexone.

The mechanism of inhibition of the withdrawal reflex described by Koll *et al* (1963), was illucidated by their finding that in spinal cats, morphine (0.3-0.4mg/kg) suppressed activity in motoneurones elicited by stimulation of Aδ and C-fibres in preference to Aα and Aβ-fibres. A similar effect was also found with activity produced by natural stimuli such as thermal > 42°C (Eisenphar and Piercey, 1980; Toyyoka *et al*, 1978) and cutaneous pinch and pressure (Yaksh, 1978) which in the conscious animal would evoke pain behaviour. In support of this inhibition of reflex withdrawal being an opioid receptor mediated effect, is the evidence that this reflex could be antagonised in a dose dependent fashion by naloxone (Yaksh, 1978). Evidence that the site of this action is in the dorsal horn gray matter, was obtained by Johnson and Duggan (1981) who antagonised this suppression of activity by local iontophoretic application of naloxone. Barton *et al* (1980) found little tonic descending opioid related inhibition but naloxone increased activity of dorsal horn neurones to more than the initial value when administered after systemic morphine (Duggan *et al*, 1980), which indicates that there is some tonic segmental opioid activity. Such tonic activity is made more obvious when the tonic descending inhibition is removed by spinalisation (Clarke *et al*, 1988). In the preparation used here i.e. the chloralose anaesthetised, spinalised lamb, it was expected that morphine at the lower doses (32μg-1600μg) selected would suppress the sural-semitendinosus reflex, but could cause facilitation of this reflex at the highest doses selected (16mg).

Evidence for the proposition that α_2 agonists will inhibit the sural-semitendinosus withdrawal reflex in the lamb

Anden *et al* in 1964, demonstrated that intravenous administration of L-Dopa (which increases noradrenalin release from nerve terminals) profoundly changed neuronal transmission in the spinal cord of the acute spinal cat by inhibiting transmission from the flexor reflex afferents to the flexor and extensor motoneurones. Kawasaki *et al* (1978), demonstrated that intravenous administration of the alpha 2 agonist clonidine could depress reflex activity. Kawasaki *et al* (1978), however, found inhibition of reflexes at low doses (0.03-0.06mg/kg), and facilitation at higher doses (0.5-1.0 mg/kg). The inhibitory effects were found to be antagonised by the α_2 antagonists yohimbine (3.0mg/kg) and tolazoline (20mg/kg) and the alpha antagonist phentolamine (10.0mg/kg), while the excitatory effects were eliminated by the $\alpha 1$ antagonist phenoxybenzamine (5mg/kg). These results suggest that clonidine can affect spinal reflexes in the acutely spinalised rat in two ways, one involving an inhibitory mechanism and the other an excitatory one, and that different alpha receptor antagonists interact with one or other or both adrenoceptive mechanisms. Bell and Matsumiya, (1981), also found facilitation of C-fibre reflexes following microinjection of noradrenalin (10μg) into the ventral horn resulting in what has been called the motor excitation syndrome (Howe *et al*, 1983).

The increase in noradrenalin release from nerve terminals in the spinal cord elicited by L-DOPA administration (Anden *et al*, 1964), reproduced activation of the descending noradrenergic endogenous inhibitory system (Basbaum and Fields, 1978). Yaksh

(1985), concluded from the available evidence, including the work by Fleetwood-Walker *et al* (1985) that local application of noradrenaline (mimicing activation of the descending noradrenergic system) to the spinal cord selectively inhibits Aδ and C-fibre evoked activity in wide dynamic range dorsal horn neurones. This being both by a presynaptic action on primary afferent excitability, and a post synaptic effect on the wide dynamic range cells as demonstrated by the suppression seen with noradrenaline on excitatory amino acid evoked discharge. There is also evidence that many other interneurones in the dorsal horn are inhibited by noradrenaline (Engberg and Ryall 1966). Ness and Gebhart (1989), however, did report that noradrenaline facilitated the discharges in dorsal horn neurones to innocuous stimulation. They concluded that inhibitory effects on dorsal horn neurones following noxious stimulation, which they also found, were due to an action of noradrenaline at α_2 receptors. In the preparation used here it was expected that intrathecal administration of the potent α_2 agonist xylazine would suppress the sural-semitendinosus withdrawal reflex.

Significance of suppression of a withdrawal reflex to pain sensation in the conscious lamb

Not all inhibition of reflex activity is associated with pain suppression. Etorphine like morphine, reduces dorsal horn neurone excitation to a natural stimulus such as pinching the skin but etorphine was found at doses of 1-10 μg/kg to also reduce responses to light touch (Yaksh, 1978; Eisenphar and Piercey 1980). In most instances, however, a strong correlation has been maintained between suppression of reflex activity and analgesia obtained with a drug when used to treat 'natural' pain, explaining why the use of reflex tests to determine the analgesic potency of drugs has been so successful. To relate inhibition of reflex activity by opioids and adrenergic agonists to the changes observed in pain experienced in a meaningful way it is important to demonstrate a connection between the spinal reflex and the central perception of pain. Depression of reflex activity in itself is not responsible for pain relief, as surgical manipulations, such as leukotomy can eliminate intractable pain while resulting in facilitation of reflex activity (Wikler, 1953).

Evidence for a central connection of spinal reflexes was provided by Zimmerman (1986), when eliciting the withdrawl reflex in cats using a thermal stimulus applied randomly to either one or other of the hind limbs. He found they sometimes lifted the wrong leg, which was evidence that there was some cognitive or learning mechanism in this reflex (Zimmerman, 1986). The presence of a supraspinal connection from these spinal reflex centres was also shown electrophysiologically by Jurna and Gossman (1976), following intravenous administration of morphine (0.5mg/kg). They demonstrated a reduction of firing in the anterolateral tract axons in the cat in spinal segments, cephalic to the site of entry of dorsal root fibres conveying impulses produced by electrical stimulation of a peripheral nerve. This reduction in firing in anterolateral tract axons was associated with a concurrent inhibition of activity produced by Aδ and C-fibre primary afferents, which was naloxone reversible.

Reduction in the response of dorsal horn neurones involved in these reflexes by opioids and adrenergic agents, is likely to be an important component of their analgesic effect (Zieglgansberger and Bayerl, 1976; Davies and Dray, 1978; Zieglgansberger and Tulloch, 1979; Satoh *et al*, 1979). This association between analgesic doses of opioids or adrenergic drugs and the suppression of reflex activity is fundamental to analgesic testing in anaesthetised animals as it depends on modifications in reflex activity being closely aligned to modulation of pain.

Further investigation of the apparent failure to obtain analgesia with epidural morphine and intrathecal xylazine in the conscious lamb

Administration of epidural morphine and intrathecal xylazine did not produce demonstrable hypoalgesia following castration or castration and tail-docking using the behavioural and physiological indices selected. In order to demonstrate that these drugs at the doses used were gaining access to spinal cord, the action of these agents on the sural-semitendinosus withdrawal reflex in the hindlimb of the chloralose anaesthetised, spinalised lamb were studied. On the basis of the review above, both intrathecal morphine and xylazine were expected to suppress this reflex in a dose dependent manner.

MATERIALS AND METHODS

Experiment 1 and 2

The null hypothesis was that a range of doses of morphine (32μg-16mg) or xylazine (0.2μg-200μg) administered via the intrathecal route to chloralose anaesthetised and spinalised lambs would not suppress the sural-semitendinosus withdrawal reflex.

Animals

Experiments were conducted on 9 male Scottish Blackface lambs weighing between 3.3 and 7.0 Kg and 5-14 days old. In one lamb no reflex could be elicited. The results presented were obtained from 5 of the lambs. Results from three lambs were incomplete and were not included. Lambs were randomly selected and assigned to either the Morphine or Xylazine group.

Anaesthesia and cannulation

Prior to experimentation the throat, dorsal lumbosacral region, lateral aspect of the left leg and head were clipped free of wool. Induction of anaesthesia was with 4% halothane (Fluothane, ICI) in 1L Oxygen and 2L N_2O/minute administered through a Fluotec III vapouriser via a rubberised face mask. After induction, lambs were maintained on 1.0-1.5% halothane. A jugular vein, carotid artery and the trachea were cannulated. Gaseous anaesthesia was replaced by α-Chloralose (Fluka Chemicals Ltd) administered intravenously by infusion pump at a rate of 840 mg/hr for 20 minutes, reducing to 80 mg/hr for 4.5 hours and then 60mg/hr to the end of the experiment. These infusion rates were adjusted where necessary to meet individual requirements for surgical anaesthesia as assessed by reference to the response of blood pressure to high intensity electrical stimulation and response to corneal stimulation. The initial infusion rates of α-chloralose outlined above was used for all lambs independent of weight, as individual requirements varied considerably. End tidal CO_2 was measured continuously via a catheter inserted into the tracheal cannula.

Maintenance of the anaesthetised and paralysed lamb

The lamb was paralysed with Gallamine Triethiodide once exposure of the sural and semitendinosus nerves was complete (Flaxedil, May and Baker; 8 mg total dose initially and supplemented as necessary with 4-6 mg). The effects of gallamine were allowed to wear off periodically (every 2-3 hours) in order to assess the depth of anaesthesia obtained with α-chloralose. The animal was artificially respired using a reciprocating pump (Harvard Instruments). The stroke volume and rate was adjusted to maintain end tidal CO_2 at 4%, which was monitored with a CO_2 analyser (901 Mk2 Analytical Dev. Company) calibrated using a 5% CO_2 mixture (Speciality gases Ltd). For most lambs a stroke volume of about 40 mls at 32/min was sufficient to maintain an end tidal CO_2 level of 4.0-4.5%. Carotid arterial blood pressure was monitored with a pressure transducer calibrated by mercury manometer. After spinalisation the lambs maintained a blood pressure of between 60 and 100 mmHg. An infusion of an isotonic glucose saline solution at approximately 20mls/hr was used to combat dehydration and maintain blood pressure. The core temperature was maintained at 37°C ± 1.0°C by a thermostatically controlled heating blanket.

Spinalisation and location of intrathecal cannulae

Spinal transection was performed under deep halothane and chloralose anaesthesia as soon as possible to permit the maximum time for recovery from spinal shock before recording reflex responses. Spinalisation was performed in the cervical region C1-C2 in the first experiment then subsequently at T8-T9 to limit the fall in blood pressure. After the laminectomy and exposure of the spinal cord the dura was removed and then 300μl of lignocaine (2%) without adrenalin was applied both to the surface and injected into the substance of the spinal cord while slowly advancing the needle (25 SWG). In order to permit direct application of drugs to the spinal cord from T10 to L7 a laminectomy was performed on lumbar vertebra 3-4. Two cannulae were then introduced into the subarachnoid space, via small holes made in the dura with a 1" needle (23 SWG). One cannula was directed up the cord to the level of T10, the other directed down to L6-L7, and in some cases they were sealed in position with superglue. The cannulae were fixed with ligatures to the dorsal spines of L2 and L5 and the incised tissues were sutured together.

Exposure of the sural and semitendinosus nerves

The left leg of the lamb was supported at the knee(femur) and ankle(distal tibia), using rubber covered adjustable clamps. The biceps femoris muscle was removed from the quadriceps group along the fascial attachment and then bisected exposing the Sciatic nerve and its branches in the popliteal fossa. Bleeding was controlled by cutting all tissues with a cautery unit. The major noxious stimulus imposed by this surgery does not change the reflexes under examination when compared with those obtained from animals that have been subjected to the above procedures after local anaesthesia, of all tissues being stimulated, using lignocaine (Clarke *et al*, 1988). The cut muscle was retracted and a pool was formed by suturing the skin to a fixed ring. The pool was then filled with warm paraffin oil (37°C), to prevent dehydration and permit insulation of the recording and stimulating electrodes.

The Sural nerve was dissected free from the sciatic nerve and cut peripherally in the popliteal fossa. The central end was then dissected free of connective tissue and placed over silver bipolar stimulating electrodes. The sural nerve was also disected free more proximally in the leg to permit recording electrodes to be placed underneath it. These were used to record the compound action potential produced by electrical stimulation. The nerve suppling the semitendinosus (ST) muscle was dissected free from the muscle and placed over bipolar silver recording electrodes. The nerve was crushed between the electrodes to give a monophasic signal. Visualisation of the semitendinosus nerve required removal of most of the biceps femoris muscle.

Stimulation of the sural nerve

After surgery was completed, with recording and stimulating electrodes in place a period of at least one hour was left before continuing the experiment, this permitted recovery from any supplementary halothane anaesthesia and the surgical interference.

The sural nerve (except in lamb 1.03.90, where the tibial nerve was used), was electrically stimulated in initial tests to determine the threshold of the lowest thresholds fibres at a stimulation duration of 0.2 msec. The compound action potential was measured as described. When the threshold had been determined a minimum of 30 control responses of the semitendinosus nerve to stimulation of the sural at 10 times threshold and 0.6 msec duration were performed to ensure that all Aδ and Aβ fibres were being stimulated. Tests at stimulus intensities of 30 times threshold and 1.2 msec duration were carried out but no changes in the early responses being recorded were found. During the experiment at predetermined times before and after drug treatment, 12 successive stimuli were applied, one every 2 seconds, at five minute intervals. The stimulator, computer and oscilloscope were all controlled by a Digitimer programmer to synchronise stimulation and recording.

Changes in the magnitude of the reflexes evoked by sural or tibial nerve stimulation were measured as changes in the size of the compound action potential in the semitendinosus nerve. The compound action potential was amplified 1000 times and filtered (0.16-250 Hz). 10 successive responses were recorded on an FM tape recorder at 1 $^{7}/_{8}$" /sec and single examples of responses were simultaneously recorded on a digital storage oscilloscope (Hewlett Packard) from which one example was recorded on a paper recorder. An average signal was obtained from these 10 successive responses using a Cromenco computer which sampled each signal (CAP) every 390µsec, see Fig 6.1. Responses were then quantified as the voltage/time integral (area) of the averaged neurograms. For presentation the responses were expressed as a percentage of the pre-treatment control levels Fig 6.2 and 6.3.

Drugs

The drugs used in this study were naloxone hydrochloride (Sigma Chemical Company), dissolved in 0.9% NaCl solution to 3mg/ml; idazoxan hydrochloride (Reckitt and Coleman), dissolved in 0.9% NaCl solution to 1mg/ml; morphine sulphate (Boots the Chemists), dissolved in 0.9% NaCl solution to 160mg/ml; xylazine hydrochloride (Sigma Chemical Company), dissolved in 0.9% NaCl solution made slightly acidic with 2 drops of 0.1M HCl acid to 1mg/ml. All subsequent dilutions were made in 0.9% NaCl solution.

Drug administration

The following regime was used: Xylazine was given in doses of 0.1, 1.0, 5.0, 10.0, 50.0, and 100.0 µg at T10-11 and L6-L7, except in cases where drug was only administered at L6-L7. The Intervals between drug doses (15-35 minutes) was dependent on the effects observed. Idazoxan (0.1mg/kg I/V) was administered from 10-20 minutes after the last dose of xylazine. Morphine was given in doses of 16.0, 160.0, 200.0, 400.0, 800.0, 1600.0, and 8000.0µg at T10-11 and L6-L7. The intervals between drug doses

FIGURE 6.1

Example of how an average signal was obtained from the 10 successive responses in the semitendinosus nerve

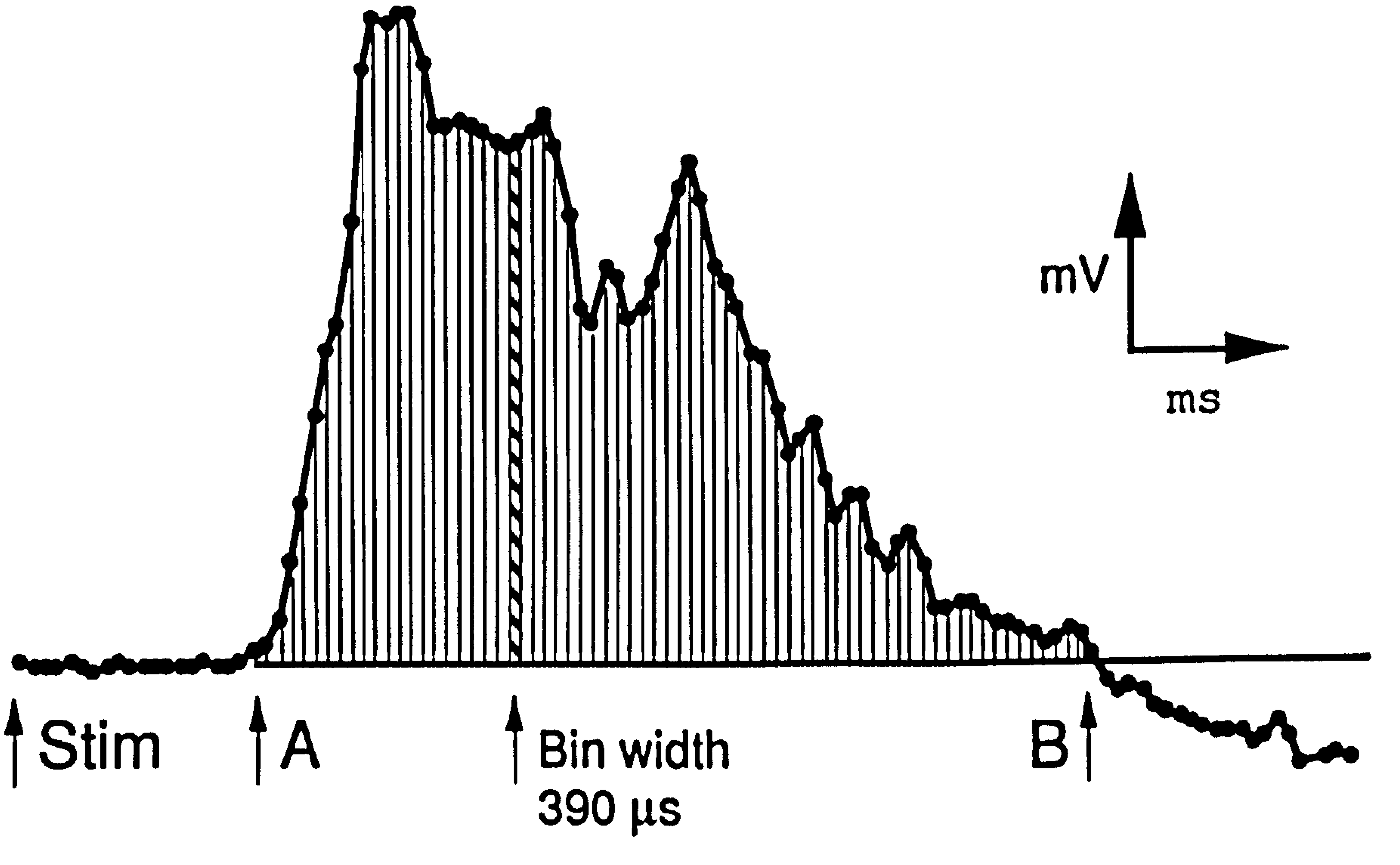
The diagram represents the data from one compound action potential in the semitendinosus nerve which has been adjusted to lie on a baseline determined by those values obtained after the stimulus to before the start of the response (see lamb 21.02.90, Fig 6.2: 3B). The voltage/time integral (area) of this response was determined for those values above the baseline between arrows A and B, by adding the area of each successive column shown by hatched marking (Bin width 390μsec). 10 responses were used for each averaged signal.

KEY

Stim - Stimulation of the sural nerve at 10x threshold.

A - Start of the compound action potential in the semitendinosus nerve (8msec post-stimulus)

B - End of recording


mV
ms
Stim
A
Bin width
390 µs
B

(15-45 minutes) was again, dependent on the effects observed. All drugs injections were flushed in with 140μl of isotonic saline (i.e. the catheter volume).

Presentation of results

The absolute sizes of the responses in the semitendinosus nerve were widely variable between experiments (6043-33058 Mvμsec), and the data were normalised for presentation and analysis, using the control responses prior to drug treatment as a 100% response. The time shown on each table (see Fig 6.2 and 6.3) was absolute, zero representing the start of the tests. Recordings were made at 5 minute intervals throughout the experiment. For clarity of presentation not all recordings are shown.

RESULTS

The effects of intrathecal morphine on the sural-semitendinosus reflex in the lamb

Morphine increased the reflex response in all 3 lambs tested. The dose at which this increase occured was between 320- 3200μg and the size of the increase was between 12-23%, see fig 6.2 and table 6.1.

Lamb 15.02.90. Initially the response fell to 94% of the control value 10 minutes after a total dose of 32μg of morphine. The response rose from 94% to 112%, 5 minutes after 320μg, reaching a maximum of 165%, 30 minutes after 3200μg of morphine.

Lamb 19.02.90. The lower doses of morphine used (32-1600μg), produced inconsistent increases in response when compared with the control value. Administration of 32μg, 320μg, 800μg, 1600μg and 3200μg of morphine, resulting in responses of 120%, 113%, 111%, 107% and 123% respectively. The highest dose of morphine (16000μg), resulted in a further increase to 143%, 50 minutes after administration.

Lamb 21.02.90. There was a progressive increase in response when compared with the control value following 800μg of morphine which reached 207%, 25 minutes after administration. This further increased following 400μg of morphine to a maximum of 668%, 15 minutes after administration.

These changes were not reversed by intravenous post-treatment with 0.2 mg/kg naloxone. The increase in activity noted in lambs 15.02.90 and 21.02.90 was due to increased amplitude and duration of the response. The increase noted in lamb 19.02.90 was confined to the early part of the response.

The effects of intrathecal xylazine on the sural/tibial-semitendinosus reflex in the lamb

Xylazine produced a dose dependent suppression of the sural-semitendinosus reflex, see figs 6.3 and table 6.2. There was some variability in the dose/response relationship in the 4 lambs in which it was tested. The most potent response being found in lamb 21.02.90 (dose 20.0 μg) and the least in lamb 23.02.90. (total dose 46.2 μg). In lamb (1.03.90) administration of xylazine (20μg) increased the latency of the response from 8-12 msec.

Lamb 19.02.90. There was an initial decrease in response to 58% of the control value, 5 minutes after administration of xylazine (100μg). This response continued to fall and by 15 minutes after xylazine administration was 19% of the control value. Intravenous idasoxan (0.1mg/kg) was administered 20 minutes after xylazine, restoring the response to 71% of the control value within 5 minutes.

Lamb 21.02.90. There was a complete elimination of reflex activity within 5 minutes of administration of xylazine (20μg). Administration of 0.1mg/kg idasoxan I/V restored the response to 67% of the control value within 5 minutes and to 71%, within 5 minutes of a second I/V dose of 0.1mg/kg idasoxan.

Lamb 23.02.90. Five minutes after administration of 0.1 μg xylazine the response was reduced to 69% of the control value. An additional 0.1μg 20 minutes later had little additional effect. The dose was increased, with 1.0, 5.0 and 20.0 μg of xylazine administered 55, 90 and 105 minutes after the first dose of xylazine. These doses resulted in a reduction to 47%, 29%, and 11% of the control value respectively. Administration of 0.1 mg/kg of idasoxan I/V restored the response to 17% of the control value within 5 minutes and 29%, 5 minutes after a second intravenous dose of 0.1mg/kg idasoxan.

Lamb 1.03.90. Five minutes after administration of 0.2 μg of xylazine the response increased to 102% of the control value. The dose was increased, with 2.0 and 10.0μg of xylazine administered 30 and 55 minutes after the first dose of xylazine. These doses resulted in in an increase of the response to 116% and 106% of the control value respectively. When the dose was increased further, with 20.0 and 200.0μg of xylazine administered 85 and 115 minutes after the first dose of xylazine, these doses resulted in an decrease in the response to 59% and 2% of the control value respectively. Administration of 0.1 mg/kg of idasoxan I/V restored the response to 102% of the control value within 5 minutes.

FIGURE 6.2

Facilitation of the sural-semitendinosus withdrawal reflex with intrathecal morphine and the effect of intravenous naloxone

Lamb 21.02.90.

A (1-4) Shows single recordings of the compound action potential recorded from the semitendinosus nerve, following stimulation of the sural nerve at 10 x threshold (volts) and 0.6 msec duration.

B (1-4) Shows the average of 10 recordings for each treatment.

Treatment

1 Control
2 25 minutes after intrathecal injection of 2x 400μg morphine
3 5 minutes after a further intrathecal injection of 2x 200μg morphine
4 5 minutes after intravenous injection of 0.2 mg/kg naloxone

Note that the sensitivity of the recording in 3A and 4A are 2.5 times less than in 1A and 2A. The sensitivity of the recording in 1B, 2B, 3B and 4B are the same.

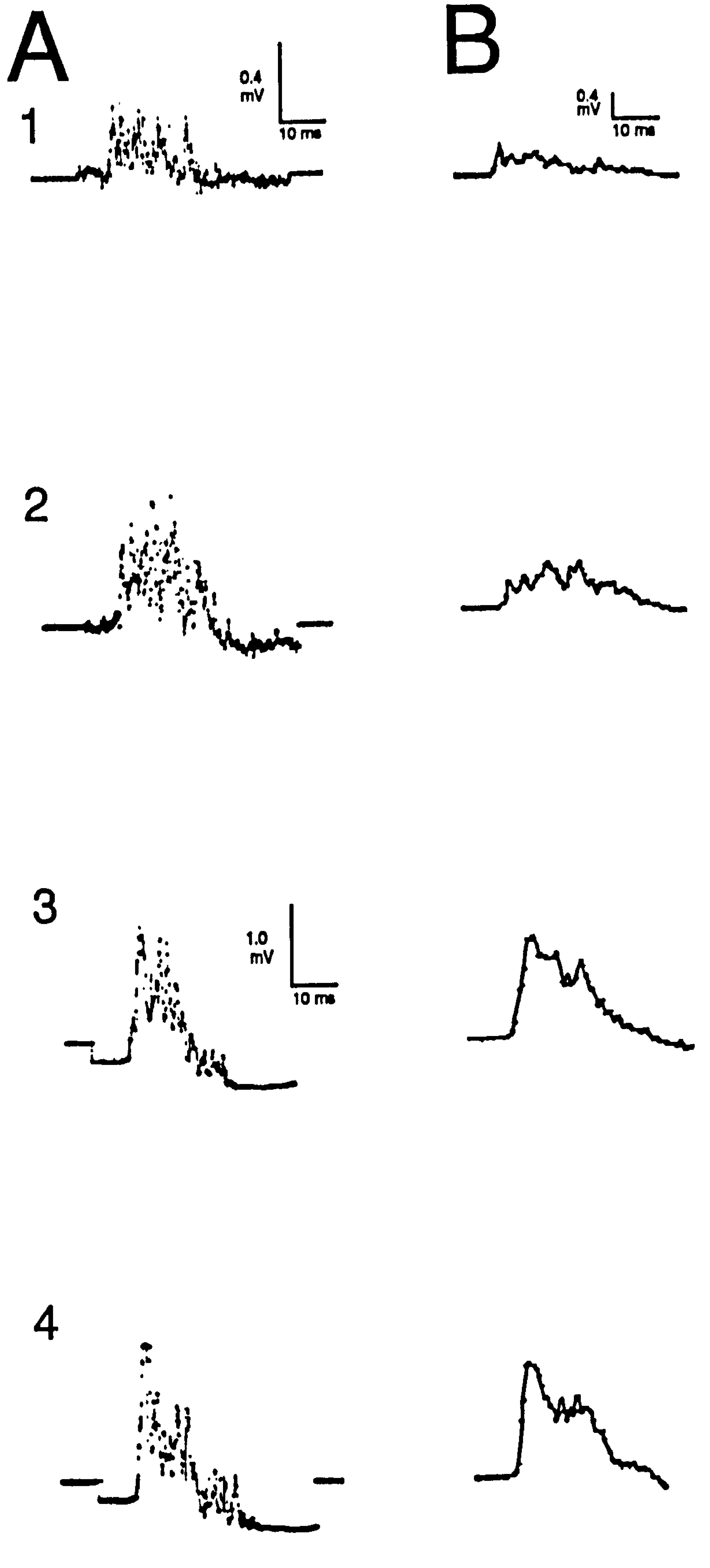

A
B
1
0.4 mV
10 ms
0.4 mV
10 ms
2
3
1.0 mV
10 ms
4

TABLE 6.1

EFFECT OF INTRATHECAL MORPHINE ON THE SURAL-SEMITENDINOSUS REFLEX IN THE LAMB

The results shown were obtained from three lambs

TIME Indicates the amount of time in minutes that has elapsed since the start of the experiment (00)

TREATMENT Indicates the dose of morphine in μg administered intrathecally at the level of T10 and L7

RESPONSE Indicates the average signal from 10 successive responses quantified as the voltage/time integral (area) of the averaged neurograms (See Fig 6.1 for example). For presentation, in the bottom row the responses are expressed as a percentage of the pre-treatment control levels

* Note this data is presented in another way in Fig 6.2

TABLE 6.1 RESPONSES TO MORPHINE SULPHATE

	TIME (mins)	00	05	10	15	25	30	35	65	85	90	95	100
	TREATMENT	Control	Control	Control	2x16ug MSA		2x160ug MSA		2x1600 ug MSA				
LAMB 15.02.90	**RESPONSE** (mV x time)	30696	29584	31163 ——(Av. 30481)——		28622		34002		38444	45086	50227	48731
	% of Control			100%		94		112		126	148	165	160

	TIME (mins)	00	05	06	40	45	65	75	100	105	130	135	175	180	210	220	230
	TREATMENT	Control	Control	2x16ug MSA		2x160ug MSA		2x400ug MSA		2x800ug MSA		2x1600ug MSA		2x8000ug MSA			
LAMB 19.02.90	**RESPONSE** (mV x time)	33058	30562 (Av. 31810)		38148		35978		35312		33978		39216		39768	43258	45566
	% of Control		100%		120		113		111		107		123		125	136	143

	TIME	00	05	*10	15	20	25	*35	40	45	*50	55	60	65	*75
	TREATMENT	Control	Control	Control	2x400ug MSA					2x200ug MSA					
LAMB 21.02.90	**RESPONSE** (mV x time)	6578	6043 (Av. 6157)	5851		9304	10361	11692	12766		24105	28277	41148	18652	24298
	% of Control			100%		151%	168%	190%	207%		392%	459%	668%	303%	395%

FIGURE 6.3

Suppression of the Tibial-semitendinosus withdrawal reflex with intrathecal xylazine and reversal by idasoxan

Lamb 1.03.90.

A (1-4) Shows single recordings of the compound action potential recorded from the semitendinosus nerve, following stimulation of the sural nerve at 10 x threshold (Volts) and 0.6 msec duration. Recorded as output from digital storage oscilloscope.

B (1-4) Shows the average of 10 recordings for each treatment.

Treatment

1 Control
2 25 minutes after a further intrathecal injection of 2x 10.0μg xylazine
3 5 minutes after a further intrathecal injection of 2x 100.0μg xylazine
4 5 minutes after intravenous injection of 0.1 mg/kg idasoxan

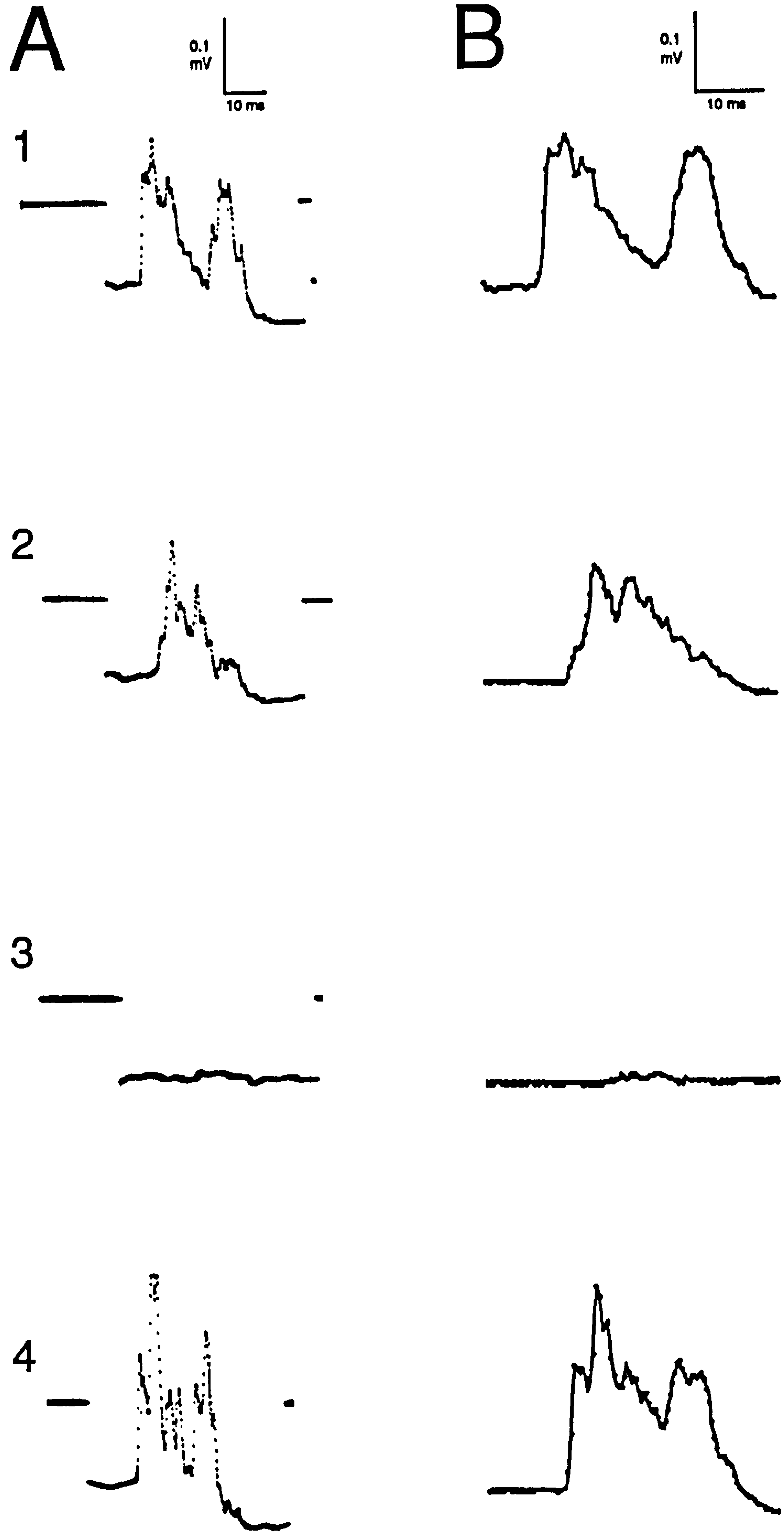

A
0.1
mV
10 ms
B
0.1
mV
10 ms
1
2
3
4

TABLE 6.2

EFFECT OF INTRATHECAL XYLAZINE ON THE SURAL OR TIBIAL -SEMITENDINOSUS REFLEX IN THE LAMB

The results shown were obtained from three lambs

TIME Indicates the amount of time in minutes that has elapsed since the start of the experiment (00)

TREATMENT Indicates the dose of xylazine in μg administered intrathecally at the level of T10 and L7. Where the symbols 2X are absent, xylazine was only administered at the level of L7

RESPONSE Indicates the average signal from 10 successive responses quantified as the voltage/time integral (area) of the averaged neurograms (see Fig 6.1 for example). For presentation, in the bottom row the responses are expressed as a percentage of the pre-treatment control levels

* Note, this data is presented in Figure 6.3

TABLE 6.2 RESPONSES TO XYLAZINE

	TIME (mins)	00	10	15	20	30	35	40	45
	TREATMENT	Control	Control	2x50ug xylaz.			0.1mg/kg idasox.		
LAMB 19.02.90	RESPONSE (mV x time)	49753	54254 (Av. 52004)		30429	10024		36946	30582
	% of Control		100%		58%	19%		71%	59%

	TIME (mins)	00	10	15	30	35	40	45
	TREATMENT	Control	2x10ug xylaz.		0.1mg/kg idasox.		0.1mg/kg idasox.	
LAMB 21.02.90	RESPONSE (mV x time)	12639		35.5		8444		8930
	% of Control	100%		0%		67%		71%

	TIME (mins)	00	20	30	45	50	80	85	115	120	130	135	140	150	155	160	165	170
	TREATMENT	Control	Control	0.1ug xylaz.		0.1ug xylaz.		1.0ug xylaz.		5.0ug xylaz.		2x10.0ug xylaz.		0.1mg/kg idasox.			0.1mg/kg idasox.	
LAMB 23.02.90	RESPONSE (mV x time)	13100	12110 (Av. 12605)		8734		8412		5905		3706		1385		2286	2145		3681
	% of Control		100%		69%		67%		47%		29%		11%		18%	17%		29%

	TIME (mins)	00	*15	20	45	50	70	75	100	105	120	125	*130	135	*140	145	*150	160
	TREATMENT	Control	Control	2x0.1ug xylaz.		2.1.0ug xylaz.		2x5.0ug xylaz.		2x10.0ug xylaz.				2x100.0ug xylaz.		0.1mg/kg idasox.		
LAMB 1.03.90	RESPONSE (mV x time)	22756	24816 (Av. 23786)		24374		27724		25224		13588	14031	14930		503		24389	21380
	% of Control		100%		102%		116%		106%		57%	59%	59%		2%		102%	90%

DISCUSSION

The main aim of this series of experiments was to determine if morphine or xylazine administered intrathecally to lambs at dose levels similar to those used in the castration and tail-docking, or castration experiments (see chapter 3 and 4) had any effect on the spinal cord and in this way to confirm that active agent was able to gain access to the spinal cord via this route and at this dose. Time permitted only a limited number of experiments to be performed.

The sural-semitendinosus reflex provided consistent results in most preparations but no response was obtained in two lambs. In one lamb this was the case for both limbs. No differences in anaesthesia, spinalisation or isolation of the nerves could be found to explain this. Distal tibial (plantar nerve) stimulation produced a consistent response in one of these lambs (1.03.90) and this nerve should be used subsequently. In the rabbit, the sural-semitendinosus reflex can also be unreliable (Clarke, personal communication), possibly because this "pairing" is not part of a withdrawal reflex in this species. There is evidence that afferents in the sural and other nerves from the region of the stifle can in the rabbit inhibit reflex activation of S.T motoneurones (Clarke *et al*, 1988). A similar problem had not been anticipated for the lamb because the sural -semitendinosus reflex was expected to be part of the limb withdrawal mechanism in the lamb and in 4 of 6 lambs this was the case. The limb was clamped at the stifle and the stimulation by the clamp might be responsible for inhibition of this reflex. In the absence of a response to sural nerve stimulation in 2 lambs a response was produced by tibial nerve stimulation. This response was larger than that obtained by sural nerve stimulation, and may be more suitable for such reflex studies in this species. It was used in preference to the sural nerve for the last lamb in the series (1.03.90). Thus data presented was from two different reflexes. The results for both were similar i.e. in both cases xylazine produced a dose dependent suppression of the reflex which was reversed by intravenous idazoxan (1.0 mg/kg). (see table 5.2, lamb 1.03.90 v lamb 21.02.90).

Possible effects of spinalisation on the results obtained

Lambs were spinalised to confirm that any effects seen following drug administration were due to a spinal rather than supraspinal action (Sinclair *et al*, 1988; Vigouret *et al*, 1973). Following spinalisation reflex activity below the level of the spinalisation is exaggerated due to the removal of more descending inhibition than excitation (Engberg *et al*, 1968; Foong and Duggan, 1986; Osburn *et al*, 1990; Dickenson and Le Bars, 1987), and because the reflex is enhanced it is easier to demonstrate the spinal action of drugs in such preparations (Clarke *et al* 1988). Damage to the spinal cord has been shown to produce a naloxone reversible decrease in blood flow to the cord (Faden *et al*, 1984), suggesting spinalisation itself may be a stimulus to release of endogenous opioids, which could obscure the results obtained following administration of morphine.

All drug doses were injected in 100μl vehicle volumes which in the adult sheep was found for <3H> clonidine to give a distribution of 5 spinal segments around the catheter tip (Waterman *et al*, 1988). Spinalisation, as carried out here, did, however, interrupt the normal flow of CSF and this could interfere with the distribution of drugs. Injection of dye did indicate that this volume of injectate distributed itself within the subarachnoid space from the level of spinalisation proximally, to beyond the sacrum distally. This gives no quantitative information about the drug concentrations that might be expected in the CSF around the spinal cord at the different levels, but this could be determined using <3H> clonidine. Morphine sulphate is hydrophylic and it has been shown to remain in the intrathecal space for many hours (Yaksh and Rudy, 1978) and with the high doses used (2x8mg) the distribution was expected to be adequate despite interruption of CSF flow.

The effect of morphine on the sural-semitendinosus reflex

In all 3 lambs, except in lamb 15.02.90 for one dose of morphine, intrathecal administration of morphine produced an increase in the sural-semitendinosus reflex. The difference in response between animals may be accounted for, by the different total injection volumes changing the CSF volume and hence the drug distribution and dilution. Alternatively they could be accounted for by individual differences in the development of the receptors at which opioids act, as the final complement of CNS opioid receptors has not been attained in this age of lamb (McIntosh *et al*, 1979). The possibility of procedural errors, such as incorrect weighing of drugs and subsequent dilution or differences in rate of uptake following administration cannot be entirely excluded.

The results for morphine were unexpected as opioid administration in most species results in dose dependent suppression of reflex withdrawal when elicited by activity in small diameter and unmyelinated fibres (Brodo and Brooks, 1937; Kivoy *et al*, 1973; Duggan and North, 1984; McClane and Martin, 1967; Clarke and Ford, 1987). Furthermore Clarke and Ford, 1987 have demonstrated supression by morphine of all classes of myelinated fibre reflex. The more general view for species other than the rabbit is that mu and kappa agonists selectively inhibit nociceptive reflexes (Parsons and Headley, 1989). Nociceptive reflexes would have been reproduced with greater certainty if electrical stimuli of the sural nerve likely to have stimulated all C- fibres had been used, however, no clear cut responses with latencies greater than 100msec were recognised when stimului of 1-2 msec and 30 x threshold were used. In this present model it was, however, expected that opioids would have an effect on the early responses attributable to Aβ and Aδ stimulation which occured within 8msec of stimulation (Clarke *et al*, 1988). These earlier responses were not changed by increasing the stimulus intensity to above C-fibre threshold.

These results were consistent with findings in experiments (see chapter 3) where E/D morphine failed to produce measurable analgesia following castration and tail-docking as determined by our indices.

Failure of intrathecal morphine to suppress the sural-Semitendinosus reflex

The possibility exists that spinalisation in the lamb disrupts the mechanism by which morphine suppresses reflex activity. It is unlikely, however, that the spinal cord must be intact to obtain an inhibitory effect of morphine on withdrawal reflexes as once suggested by Takagi *et al* (1955). Opioid inhibition has been shown to be largely segmental and the inhibitory effect on withdrawal reflexes remained after spinalisation (Duggan *et al*, 1980). It is possible that the intense noxious stimulation resulting from preparative surgery resulted in the maximum activation of opioidergic inhibition. Such inhibition has been reproduced by high intensity peripheral nerve stimulation (Clark *et al*, 1989), and by the application of "natural" noxious cutaneous stimuli to the limb being used (Pettit *et al*, 1989). It has also been shown that intense noxious stimuli can enhance some reflexes, possibly as a protective mechanism (Woolf and Wall, 1986). Such effects could obscure an insubstantial morphine action. To investigate the possibility that opioid systems are operative prior to the start of the testing procedure, systematic investigation of the effects of naloxone on the sural- semitendinosus reflex in this preparation are required. Some tonic segmental opioid inhibition of reflex activity in the spinal cord has been shown in species other than the sheep (Goldhart and Hugh, 1976; Clarke *et al*, 1988; Bell, 1989).

Facilitation of the sural-semitendinosus reflex with intrathecal morphine

The sural- semitendinosus reflex was increased by what may be considered high doses of morphine and was not reversed by naloxone (0.2mg/kg), evidence that it was acting at non opioid receptors. Similar responses have been described following intrathecal injection of morphine in the rat, and has been associated with hyperaesthesia (Yaksh *et al*, 1986). This hyperaesthesia may be mediated by a non opioid receptor, the response being compared with a strychnine like action. Such an action is dependent on an antiglycinergic effect at those spinal neurones which mediate postsynaptic inhibition in dorsal horn neurones (Duggan and Curtis, 1972; Yaksh *et al*, 1986). The antiglycinergic effect is seen in the conscious animal as excitation (Krueger, 1943) motor rigidity and hyperreflexia. Thus "low" doses of morphine in lambs do not affect the reflex and "high" doses produce effects similar to those described for high doses in other species. There is thus no evidence that opioids produce antinociceptive effects in the spinal cord of the lambs either in these experiments or in those described in chapter 3. The possibility cannot be excluded, however, that access of morphine to the glycine receptors is better than for opioid receptors. Under these circumstances analgesia though present, would be obscured. To show this morphine could be administered in small incremental doses from 16μg until the first indication of an increase in reflex response. If subsequent administration of naloxone (0.2 mg/kg I/V) increased the response further, this would provide evidence for an underlying inhibitory effect of morphine at opioid receptors. The results obtained here suggest that the increase in lateral lying observed in lambs castrated and tail-docked following epidural morphine administration, may be due at least in part to hyper-reflexia, and not necessarily hyperalgesia.

In order to determine if the results are due to absence or late development of opioid receptors, studies such as those of Gouarderes *et al* (1982), must be undertaken to determine if there are differences in the development and distribution of opioid receptors in the spinal cord of the lamb when compared with other species. The argument being that the final CNS complement of functional opioid receptors has not been attained in this age of lamb (McIntosh *et al*, 1979). In vivo binding studies in the lamb would also provide evidence with respect to the accessibility of opioid receptors in the sheep spinal cord to intrathecal opioids (Brandt and Livingston, 1989). It would be helpful to compare the effects of morphine on the sural semitendinosus- reflex in the spinalised and non spinalised lamb as has been done in the rat (Sinclair *et al*, 1988). The development of a non spinalised preparation, would allow CSF flow, which may be important for effective drug distribution when a small injection volume is used ($100\mu l$).

The possibility that, in young lambs, the action of morphine is confined to spinal motor effects (Duggan and Curtis, 1972) cannot be excluded. In attempts at analgesic testing carried out in these studies, lambs regularly remained refractory to noxious thermal and mechanical stimuli when restrained, with all four feet of the ground, in a rubber hammock. This observation provided some evidence for a "stress" induced analgesia. If this analgesia was not dependent on endogenous opioid or adrenergic ligands as suggested by the results obtained with morphine, etorphine and xylazine some other system(s) such as the spinal seretonin system acting on 5-HT_3 receptors must be responsible for such analgesia in the lamb (Glaum *et al*, 1990).

Effects of intrathecal xylazine on the sural/tibial-semitendinosus reflex

Intrathecal xylazine at doses of 0.1 to 20 μg produced inhibition of the sural/tibial- semitendinosus reflex in all 4 lambs. A dose dependent suppression in this reflex following xylazine administration was particularly evident in one lamb (23.02.90). Others have shown that spinal release or administration of adrenergic agonists decreased reflex withdrawal (Anden *et al*, 1966; Yaksh , 1985; Barasi and Clatworthy, 1987; Clarke *et al*, 1988). The variability in responses observed can be attributed partly to the different dosing regimes adopted and partly to variation between lambs. Where the first dose (10-50μg) produced a significant effect, such as in lambs (19.02.90 and 21.02.90) there was a substantial decreases in reflex activity within 5 minutes. Incremental dosing regimes, however, starting at 0.1μg or 0.2μg (lambs 23.02.90 and 1.03.90), delayed the marked decrease in reflex activity until higher doses were administered.

In lamb (1.03.90) administration of xylazine (20μg) not only suppressed reflex activity, but also increased the latency of the response from 8 msec to 12msec. This increase in time from stimulus to response could be explained if summation of afferent activity was necessary to elicit activity in neurons of the reflex arc. Any reduction of afferent activity by pre or post-synaptic inhibition would slow summation and delay excitation of the motoneurones.

Facilitation of reflex activity by alpha agonists

It has been shown that because noradrenergic drugs can have excitatory effects on motoneurones of the spinal cord (White and Neuman, 1980) focal administration in the dorsal horn gray matter could be used to avoid this problem (Bell and Matsumiya, 1981; Bras *et al*, 1988). Excitation of neurones in the ventral horn (White and Neuman, 1980), is primarily associated with α_1 agonist activity. Xylazine like clonidine would be anticipated to have a significant action on α_1 receptors only at high doses (Howe *et al*, 1983), a finding that was used to explain the observation by Kawasaki *et al* (1978) that clonidine, inhibits the tail flick reflex generated by pinch in the spinal transected animal at low doses (0.06mg/kg), whereas facilitation was seen at higher doses (0.5mg/kg), (Wiesenfeld-Hallin, 1987). An excitatory effect was only seen in lamb 1.03.90 at lower doses of xylazine (0.2μg to 10.0μg). This increase in reflex activity was limited to a 16% increase above control levels, indicating that if α_1 effects are present, they are either small or concealed by α_2 effects.

Explanation for the absence of analgesia following administration of intrathecal xylazine in castrated lambs, when similar doses inhibit the sural/tibial-semitendinosus withdrawal reflex

Xylazine inhibited the sural/tibial-semitendinosus reflex in all 4 lambs tested. The inhibition of such reflexes has been associated with an analgesic action in the conscious animal (Gamble and Milne, 1989). It cannot be assumed, however, that similar doses of intrathecal xylazine would also suppress the guarding reflex (rectus abdominus muscle) which is more likely to be elicited by stimulation of the spermatic nerve. This reflex would have been a more appropriate test of xylazine as a likely analgesic for castration, as occlusion of the testicular blood supply (which occurs following rubber ring castration) has been shown to substantially increase afferent activity in the spermatic nerve (Grubb *et al*, 1990). It was, however, considered appropriate to use the sural-semitendinosus reflex in initial tests to detect if two of the drugs used (morphine and xylazine), gained access to the spinal cord and produced an effect on a well characterised spinal mechanism, the sural-semitendinosus withdrawal reflex.

Gray *et al* (1970), have reported that although analgesics suppress reflex activity elicited by just suprathreshold noxious stimuli in the conscious rat, reflex activity elicited by severe noxious stimuli may remain refractory to their effects, lending support to the view that such reflex tests may be an unreliable way of estimating analgesic potency against severe clinical pain. Castration with tight rubber rings is such a stimulus with both visceral (testes) and somatic (scrotum) components and is presumed to be a more severe stimulus than that represented by intermittent, short duration (0.6ms) stimulation of a cutaneous nerve.

Drug distribution problems associated with the intrathecal injection method may contribute to the anomaly between reflex effects and analgesia. The concentration of xylazine at the catheter tip (located at L6-L7) may be sufficient to suppress reflex activity organised at this level but insufficient, even with injection of additional xylazine at T10-T11, to modify the noxious input to various levels of the cord produced by castration or castration plus tail docking (see Fig 4.1 chapter 4). Similar α_2 compounds such as clonidine have, however, been shown to readily gain access to the intact spinal cord in the sheep following intrathecal administration, and with an injection volume of 100μl should distribute to all levels of the cord (Waterman *et al*, 1988) receiving scrotal and testicular afferent innervation.

Conclusion

The main aim of this experiment was accomplished in that both morphine and xylazine at dose levels covering the range used in the castration and tail-docking, or castration experiments, were shown to have effects on spinal function. The failure of morphine sulphate to suppress the sural-semitendinosus reflex in these experiments is consistent with findings in the conscious lambs, where no evidence for analgesia was obtained.

Suppression of the sural/tibial- semitendinosus reflex by xylazine while apparently producing no analgesia in the conscious lamb is not, however, consistent with the general view that reflex suppression is highly correlated with the level of analgesia.

The implications of these results are that more extensive investigation is required into the effects of a range of xylazine doses on the response of the lamb to castration. Electrophysiological work could also be extended to examine the effect of xylazine on reflexes initiated by spermatic nerve stimulation which would more closely approximate to the stimulus produced by castration. The spermatic nerve has a mainly C-fibre population, which would require late responses to be recorded in nerves associated with muscles used in the guarding reflex. In addition to selecting a nerve more likely to reproduce the source of pain experienced by castration, some consideration should be given to increasing the stimulation frequency from once every 2 seconds, as this may not be representative of the more consistent barrage present in clinical pain (Duggan and North, 1984). Initially, however, doses of xylazine found to suppress the sural/tibial -semitendinosus reflex should be used to repeat experiment 2 in chapter 3 in order to confirm these results. Confirmation is also required that adequate levels of xylazine reaches the spinal cord receptors. One approach would be to undertake radioligand binding studies, using a labelled alpha 2 ligand injected via the same route and volume of vehicle to demonstrate the distribution of drug in the spinal cord. Alternatively CSF samples may be obtained, following intrathecal drug administration, from the conscious lamb at various levels of the spinal cord to confirm the distribution and concentrations of the drug. These samples should also be taken at frequent intervals to obtain a half life of the drug in the CSF. That possible explanations exist for the failure to obtain analgesia with morphine, etorphine and xylazine, indicate a single trial with one dose of drug, is insufficient to provide conclusive evidence that these drugs either do or do not provide analgesia in young lambs.

GENERAL DISCUSSION

Can pain be recognised in young lambs?

Pain could be described as a physiological phenomena which is hard to define in humans, and extremely difficult to recognise and interpret in animals. Pain cannot be measured directly and its assessment is always subjective in man and animals, though the experience is usually associated with actual or potential tissue damage (Kitchell and Johnson, 1985). In these studies, the assumption was made that activity in nociceptors would be induced by the tissue damage caused by castration, scrotal castration or castration and tail-docking with tight rubber rings, and by analogy with man, produce the sensation of pain. On the basis of this assumption, pain was recognised in the lamb, primarily by changes in the behaviour and also by the associated increases in plasma cortisol concentration (see chapters 3-5).

Can pain be assessed in young lambs?

Assessment of pain in lambs following a variety of painful procedures has been carried out using the indices described in the general methods. The noxious procedures were ranked on the basis of significant differences in the behaviours and plasma cortisol responses of the different groups and these rankings agreed well with the alternative method of ranking. How reliable were these indices and how can they be further improved?

(1) Plasma cortisol

The correlation between biochemical parameters and the experience of pain were described by Sanford et al (1989) as unclear. The current study has shown that a biochemical response such as the release of plasma cortisol can reflect the intensity of pain experienced, provided that comparisons are restricted to a particular pain in a specific species of uniform age. The plasma cortisol response was, however, shown to be susceptible to drugs, particularly following high doses of epidural morphine (16mg). This should be taken into consideration if pain intensity is to be manipulated using analgesics or hyperalgesics. As discussed in chapter 3, plasma cortisol concentrations could only be used to discriminate between high and low intensity stimuli. The sensitivity of this measure would be best improved by reducing the inter and intra assay variation, increasing the number of lambs in each group, and increasing the sampling frequency. There is a limit imposed, however, by availability of lambs, practicability and cost of the experiments which restrict the use of the latter two approaches. The usefulness of plasma cortisol concentration as an indicator of pain was such, that it was neither practical or reliable in the individual animal for diagnostic purposes but was helpful when investigating pain intensity in groups of animals experimentally.

(2) Behaviour

Behaviour resulting from pain, is likely to be species specific, designed to alert conspecifics to possible danger, and marshall aid for the individual in distress (Craig and Prkachin, 1983). Behaviours were found which appeared to reliably reflect the pain experienced by the lamb and which did alert the attention of the dam. As with plasma cortisol, behaviour was also shown to be influenced by drug effects, and is an important consideration when attempting to assess pain following administration of such drugs. Despite evidence that the drugs used in this study produced measurable changes in behaviour, these changes were small when compared with those elicited by the various noxious procedures and hence lamb behaviour remained a reliable indicator of pain.
The sensitivity of the behavioural monitoring technique was such that it discriminated between various intensities of noxious

stimulus whereas this could not always be determined by their plasma cortisol responses. Furthermore behavioural changes were present in some cases for more than 2 hours after plasma cortisol responses had returned to basal levels and when casual inspection indicated lambs had returned to "normal".

To improve the sensitivity of the behavioural recording method, the monitoring frequency could be increased, the number of categories of behavioural postures/events extended or more sensitive behavioural changes sought. Practical pain recognition in an individual animal, when time is limited would best be obtained using the latter two approaches. The ideal would be to associate particular changes in behaviour with a particular intensity of pain, which would allow quick pain assessment in either small groups or the individual animal.

Many of the subtle changes in behaviour noted with the current monitoring method, however, could not be interpreted easily. The intention had been to reduce pain intensity gradually using established analgesics to reveal the full spectrum of lamb behaviour and plasma cortisol responses. The appearance and disappearance of particular behaviours could then be closely correlated with a relative pain intensity. It is imperative therefore that a method of producing analgesia is found if this goal is to be achieved. To increase the likelyhood of finding such analgesics, the stimulus selected should produce somatic pain, as visceral pain is less well understood and can be difficult to treat clinically with analgesics (Procacci et al, 1986). Tail-docking provides such a somatic stimulus and could be used to assess the effects of various analgesics. Possible approaches to investigating explanations for the absence of analgesia following administration of morphine, xylazine and etorphine were discussed (see discussion Chapter 4 and 5). If it transpires that young lambs are indeed refractory to the action of these drugs, alternative approaches such as those described below should be investigated.

One option would be the use of spinal anaesthesia using epidural bupivicaine, since this is successfully used for abdominal surgery (Bengisson et al, 1983). Although such an approach is almost guaranteed to produce analgesia, any associated paralysis would interfere with the use of behavioural indices currently being investigated. Another option, would be to develop a reliable quantitative method for use in unrestrained lambs. Any such experimental design would require that the noxious stimulus was applied remotely and that the intensity and duration of the applied stimulus was acceptable under codes of practice endorsed by the IASP.

Are the findings here applicable to different ages and species of animal?

In the general introduction it was stated that the reaction of animals to stimuli which cause pain in ourselves are similar to our own (Thorpe, 1965). By inference, therefore, the reaction of different ages and species of animals to pain is considered likely to be similar. Although indices as used in this study can be further developed, the reliability of those presented is thought to be sufficient to warrant investigation of their validity for the the same pain in other species and different types of pain in the same and other species. The long term aim is to establish a library of the behavioural and physiological responses to various types of pain from various sources in domestic and companion animals. Such a library would enable (a) rapid detection of the condition so that analgesic therapy can be indicated where appropriate and (b) allow bodies such as the Farm Animal Welfare Council (F.A.W.C.) in Great Britain and the Animal Welfare Advisory Committee (A.W.A.C.) in New Zealand to assess the degree of pain experienced during a variety of husbandry practices. The F.A.W.C. is now committed to making recommendations as to how or when painful husbandry procedures should be undertaken (if they are to continue). If a reliable technique for pain assessment were available, these recommendations could be made with a scientific basis.

Welfare considerations

If these experiments do indicate the intensity of pain and distress experienced it must be concluded that young lambs subjected to castration and tail-docking, castration only or scrotal castration with tight rubber rings experience intense pain and distress for at least one hour. This contrasts with findings of Barrowman et al (1954), that lambs of less than one week old castrated with tight rubber rings showed few signs of pain and distress. The details of the techniques described by Barrowman et al (1954), do not permit an explanation of the differences found, but it is suspected that they may be due to differences in the rubber rings used. It can be concluded that young lambs cannot significantly reduce the pain and distress of castration and tail-docking by endogenous opioid activity but that it can be eliminated by pretreatment with local anaesthesia of the affected areas. Ideally the procedure should not be undertaken, particularly in fat lamb production where castration has been accepted as unnecessary (Robertson, 1966).

Akil, H., Mayer, D.J. and Liebeskind, J.C. (1975). Antagonism of stimulation-produced analgesia by naloxone, a narcotic antagonist. Science, 191, 961-962.

Al-Damluji, S., Bouloux, P., White, A. and Besser, M. (1990). The role of a_2-adrenoceptors in the control of ACTH secretion; interaction with the opioid system. Neuroendocrinology, 51(1), 76-82.

Alquist, R.P. (1948). A study of the adrenotropic receptors. Am. J. Physiol., 153, 586-600.

Altmann, J. (1973). Observational study of behaviour: Sampling methods. Behaviour, XLIX, 3-4, 227-267.

Amit, Z. and Galina, Z.H. (1986). Stress-induced analgesia; adaptive pain suppression Physiol. Rev., 66(4), 1091-1120.

Anden, N.E. Jukes, M.G.M. and Lundberg, A. (1964). Spinal reflexes and monoamine liberation. Nature, 202, 1222-1223.

Anden, N.E., Jukes, M.G.M. and Lundberg, A. (1966). The effect of DOPA on the spinal cord. 2. A pharmacological analysis. Acta Physiol. Scand., 67, 387-397.

Arjune, D. and Bodnar, R.J. (1990). Inhibition of deprivation-induced feeding by naloxone and cholecystokinin in rats: effects of central alloxan. Brain Res. Bull., 24(3), 375-381.

Atkinson, L., Bogduk, N., Cousins, M.J., Hodder, P., Kilham, H.A., Pilowsky, I., Pollard, B. and Quinn, R. (1989). Management of severe pain. (Report of the Working Party on Management of Severe Pain), Australian Government Publishing Service, Canberra, 29.

Atweh, S.F., and Kuhar, M.J. (1977). Autoradiographic localisation of opiate receptors in rat brain. I. spinal cord and lower medulla. Brain Res., 124, 53-67.

Auguy-Valette, A., Cros, J., Gouarderes, Ch., Gout, R., and Pontonnier, G. (1978). Morphine analgesia and cerebral opiate receptors: A developmental study Br. J. Pharmacol., 63, 303-308.

Aziz, M.A. and Carlyle, S.S. (1978). Cardiovascular and respiratory effects of xylazine in sheep. Zentralblatt fur Veterinarmedizin, 25A(3), 173-180.

Barton, C., Basbaum, A.I. and Fields, H.L. (1980). Dissociation of supraspinal and spinal actions of morphine: a quantitative evaluation. Brain Res., 188, 487-498.

Barrowman, J.R., Boaz, T.G. and Towers, K.G. (1954). Castration and docking of lambs : use of the rubber ring ligature technique at different ages. Emp. J. Exp. Agric., 22(87), 189-202.

Barasi, S. and Clatworthy, A. (1987). The effects of intrathecally applied noradrenaline and 5-hydroxytryptamine on spinal nocifensive reflexes on the rostral transmission of noxious information to the thalamus in the rat. Neurosci. Lett., 78(3), 328-332.

Basbaum, A.I. and Fields, H.L. (1978). Endogenous pain control mechanisms: Review and hypothesis. Ann, Neurol., 4, 451-462.

Basbaum, A.I. and Fields, H.L. (1984). Endogenous pain control systems: Brainstem spinal pathways and endorphin activity. Rev. neurosci., 7, 309-338.

Beecher, H.K. (1957). The measurement of pain. Pharmacol. Rev., 9, 59-209.

Bell, J.A. (1989). Naloxone induced facilitation of C-fibre reflexes is reduced by chronic morphine. Eur. J. Pharmacol., 168(1), 101-107.

Bell, J.A. and Martin, W.R. (1977). The effect of narcotic antagonists naloxone, naltrexone and nalorphine on spinal cord C-fibre reflexes evoked by electrical stimulation or radiant heat. Eur. J. Pharmacol., 42, 147-154.

Bell, J.A. and Matsumiya, T. (1981). Inhibitory effects of dorsal horn and excitant effects of ventral horn intraspinal microinjection of norephinephrine and seretonin in the cat. Life Sci., 29, 1507-1514.

Bengisson, M., Edstrom, H.H. and Lofstrom, J.B. (1983). Spinal analgesia with bupivicaine, mepivacaine and tetracaine. Acta. Anaesthesiol. Scand., 27, 278-283.

Bernards, C.M. and Hill, H.F. (1990). The pia-arachnoid mater not the dura mater is the major barrier to the diffusion of morphine from the epidural space to the spinal cord. Pain, Suppl 5, S1-S528, Abs 253.

Berthelsen, S. and Pettinger, W.A. (1977). A functional basis for classification of alpha adrenergic receptors. Life Sci., 21, 595-606.

Beyer, J.E., McGrath, P.J. and Berde, C.B. (1990). Behavioural responses to post-operative pain in 3-7 year olds. Pain Suppl., 5, S33.

Bhargava, H.N., Gulati, A. and Ramarao, P. (1989). Effect of chronic administration of U-50, 488H on tolerance to its pharmacological actions and on multiple opioid receptors in rat brain regions and spinal cord. J. Pharmacol. Exp. Ther., 251(1), 21-26.

Blackshaw, J.K. (1986). Objective measures of welfare in farming environments. Aust. Vet. J., 63(11), 361-364.

Blane, G.F., Boura, A.L.A., Fitzgerald, A.E. and Lister, R.E. (1967). Actions of etorphine hydrochloride (M99): a potent morphine like agent. Br. J. Pharmacol., 30, 11-22.

Bodnar, R.J., Kelly, D.D., Brutus, M. and Glusman, M. (1979). Stress-induced analgesia: neural and hormonal determinants. Neurosci. Biobehav. Rev., 4, 87-100.

Bolme, P., Fuxe, K., Agnati, L.F., Bradley, R. and Smythies, J. (1978). Cardiovascular effects of morphine and opioid peptides following intracisternal administration in chloralose- anaesthetised rats. Eur. J. Pharmacol., 48, 319-324.

Borison, H.L., Fishburn, B.R. and McCarthy, L.E. (1964). A possible receptor role of the subfornical organ in morphine-induced hyperglycaemia. Neurology, 14, 1049-1053.

Bouchenafa, O. (1988). Structural aspects of neurotransmitters in the sheep spinal cord. Thesis. University of Bristol.

Bouchenafa, O., Ley, S., Livingston, A. and Waterman, A. (1989). Intrathecal opioids in the sheep. British Opioid Colloquium, University of Reading, 21-23 March, P.22.

Bouchenafa, O. and Livingston, A. (1985). Autoradiographic localisation of <3H>-clonidine and <3H>-prazocin in the spinal cord of sheep. Br. J. Pharmacol., 86, 518P.

Bouchenafa, O. and Livingston, A. (1987). Autoradiographic localisation of a_2 adrenoceptor binding sites in the spinal cord of the sheep. Res. Vet. Sci., 42(3), 382-386.

Bouchenafa, O., Livingston, A. and Waterman, A. (1988). Autoradiographic localization of a_2-adrenoceptor agonists in sheep spinal cord following intrathecal administration. J. Physiol., 401, 54P.

Brain, W.R. (1963). Animals and pain. New Scientist, 19(353), 380-381.

Brambell, F.W. (1965). Report of the technical committee to enquire into the welfare of animals kept under the intensive livestock husbandry system. Command paper 2836. London HMSO.

Bras, H., Cavallas, P. and Janhowsha, E. (1988). An investigation of iontophoretically applied DOPA in the spinal cord. Exp. Brain Res., 71, 447-449.

Brandt, S.A. and Livingston, A. (1989). Receptor binding studies for a_2-adrenergic and opioid agonists in selected areas of the sheep spinal cord. British Opioid Colloquium. University of Reading 21-23 March, O.5.

Brands, B.J., Thornhill, J.A., Hirst, M. and Gowdey, C.W. (1979). Suppression of food intake and body weight gain by naloxone in rats. Life Sci., 24, 1773-1778.

Breward, J. (1983). Cutaneous nociceptors in the chicken beak. Proc. Phys. Soc. J. Physiol., 346, 56P.

Brodo, R.C. and Brooks, C.M. (1937). Effects of morphine on blood sugar and reflex activity in the chronic spinal cat. J. Pharmacol . Exp. Ther., 61, 82-88.

Broom, D.M. (1986). Indicators of poor welfare. Br. Vet. J., 142, 524-526.

Browning, S., Lawrence, D., Livingston, A. and Morris, B. (1982). Interactions of drugs active at opiate receptors and drugs active at a_2- receptors on various test systems. Br. J. Pharmacol., 77, 487-491.

Burdette, B.H., Leeman, S. and Munson, P.L. (1961). The reversal of the inhibitory effect of morphine on the secretion of adrenocorticotropic hormone in stress. J. Pharmacol. Exp. Ther., 132, 323-328.

Burn, J.H. (1946). The testing of local anaesthetics. Br. Med. Bull., 4, 82-85.

Bylund, D.B. (1985). Heterogeneity of $_2$ adrenergic receptors. Pharmacol. Biochem. Behav., 22(5), 835-843.

Carson, G.D. and Challis, J.R.G. (1982). Changes in plasma cortisol and progesterone after administration of enkephalins to newborn lambs. Can. J. Physiol. Pharmacol., 60, 1166-1170.

Carter, P.D., Johnston, N.E., Corner, L.A. and Jarrett, R.G. (1983). Observations on the effect of electro-immobilisation on the dehorning of cattle. Aust. Vet. J., 60, 17-19.

Carstens, E., Tulloch, I., Zieglgansberger, W. and Zimmermann, M. (1979). Presynaptic excitability changes induced by morphine in single cutaneous afferent C- and A-fibres. Pflugers Arch., 379, 143-147.

Castro, M.I. and Eisenach, J.C. (1989). Pharmacokinetics and dynamics of intravenous, intrathecal, and epidural clonidine in sheep. Anaesthesiology, 71(3), 418-425.

Cekan, Z. (1975). Assessment of reliability of steroid radioimmunoassays. J. Steroid Biochem., 6, 271-275.

Chapman, C.R., Casey, K.L., Dubner, R., Foley, K.M., Gracely, R.H. and Reading (1985). A.E. Pain measurement: an overview. Pain, 22(1), 1-31.

Clarke, R.W. and Ford, T.W. (1987). The contribution of m-, d- and k-opioid receptors to the actions of endogenous opioids on spinal reflexes in the rabbit. Br.J. Pharmacol., 91, 579-589.

Clarke, R.W., Ford, T.W. and Taylor, J.S. (1988). Adrenergic and opioidergic modulation of a spinal reflex in the decerebrated rabbit. J. Physiol., 404, 407-417.

Clarke, R., Galloway, F., Ford, T. and Taylor, J. (1989). Naloxone enhancement of flexor reflexes in the spinalised rabbit. British Opioid Colloquium, University of Reading 21-23 March, P.21.

Clarke, K.W. and Hall, L.W. (1969). "Xylazine"- A new sedative for horses and cattle. Vet. Rec., 85, 512-517.

Clark, J.A., Liu, L., Price, M., Hersh, B., Edelson, M. and Pasternak, G.W. (1989). Kappa-opiate receptor multiplicity: evidence for two U50,488- sensitive kappa 1 subtypes and a novel kappa 3 subtype. J. Pharmacol. Exp. Ther., 251(2), 461-469.

Clendeninn, N.J., Petraitis, M., and Simon, E.J. (1976). Ontological Development of opiate receptors in rodent brain. Brain Res., 118, 157-160.

Codere, T.J. and Melzack, R. (1987). Cutaneous hyperalgesia: contribution of the peripheral and central nervous systems to the increase in pain sensation after injury. Brain Res., 404, 95-106.

Cohen, R.A. and Coffman, J.D. (1979). The effects of morphine on cutaneous capacitance and resistance vessels. Circulation, 60, part 2, 80.

Colado, M.I., Arnedo,A., Peralta, E. and Del Rio, J. (1988). Unilateral dorsal rhizotomy decreases monoamine levels in the rat spinal cord. Neurosci. Lett., 87(3), 302-306.

Colburn, R.W., Coombs, D.W., Degnan, C.C. and Rogers, L.L. (1989). Mechanical Visceral Pain Model: Chronic Intermittent Intestinal Distension in the Rat. Physiol. Behav., 45, 191-197.

Cousins, M.J. and Mather, L.E. (1984). Intrathecal and epidural administration of opioids. Anesthesiology, 61, 276-310.

Cox, B., Ary, M., Chesarek, N. and Comax, P. (1976). Morphine hyperthermia in the rat: an action of the central thermostats. Eur. J. Pharmacol., 36, 33-39.

Cox, B.M., Opheim, K.E., Teschemacher, H. and Goldstein, A. (1975). A peptide-like substance from pituitary that acts like morphine. 2. Purification and properties Life Sci., 16, 1777-1782.

Craig, K.D. and Prkachin, K.M. (1983). Nonverbal measures of pain. In: Pain measurement and assessment. Ed. Melzack, R. Raven Press, New York, 173-179.

Crawley, J.N., Roth, R.H. and Maas, J.W. (1979). Locus coeruleus stimulation increases noradrenergic metabolite levels in rat spinal cord. Brain Res., 166, 180-184.

Creed, R.S., Denny-Brown, D., Eccles, J.C., Liddell, E.G.T. and Sherrington, C.S (1932). Reflex activity of the spinal cord. Oxford university press, London.

Curtis, D.R. and Duggan, A.W. (1969). The depression of spinal inhibition by morphine. Agents Actions, 1, 14-19.

Dahlstrom, A. and Fuxe, K. (1964). Evidence for the existence of monoamine-containing neurones in the central nervous system. 1. Demonstration of monoamines in the cell bodies of brain stem neurones. Acta. Physiol. Scand., 62, suppl 232, 1-55.

Dahlstrom, A. and Fuxe, K. (1965). Evidence for the existence of monoamine neurones in the central nervous system. 2. Experimentally induced changes in the intraneuronal amine levels of the bulbospinal neuron systems. Acta. Physiol. Scand., 64, suppl 247, 1-36.

Dantzer, R. (1986). Assessing pain by studying neuroendocrine responses. In: Assessing Pain in Farm Animals, edited by Duncan, J.H. and Molony, V., Commission of the European Communities, Luxembourg.

Davis, L.E. (1985). Species differences in disposition as factors in alleviation of pain. In: Animal pain, perception and alleviation. Eds. Kitchell, R.L. and Johnson, H.E. The Williams and Wilkins Company, Baltimore, 173.

Davies, J. and Dray, A. (1978). Pharmacological and electrophysiological studies on morphine and enkephalin on rat supraspinal neurones and cat spinal neurones. Br. J. Pharmacol., 63, 87-96.

Davies and Quinlan (1985). Selective inhibition of responses of feline dorsal horn neurons cutaneous stimuli by tizanide (DS103-282) and noradrenaline: involvement of a_2 adrenoceptors. Neurosci., 16, 673-682.

Dickenson, A.H. and Sullivan, A.F. (1987). Subcutaneous formalin-induced activity of dorsal horn neurones in the rat: differential response to an intrathecal opiate administered pre or post formalin. Pain, 30(3), 349-360.

Dixon, S., Snyder, J., Holve, R. and Bromberger, P. (1984). Behavioural effects of circumcision with and without anaesthesia. Dev. Behav. Pediat., 5, 246.

Doi, T. and Jurna, I. (1982). Analgesic affect of intrathecal morphine demonstrated in ascending nociceptive activity in the rat spinal cord and ineffectiveness of caerulein and cholecystokinin octapeptide. Brain Res., 234, 399-407.

Drew, G.W., Gower, A.J. and Marriot, A.S. (1977). Pharmacological characterisation of a-adrenoceptors which mediate clonidine- induced sedation. Br. J. Pharmacol., 61, 468P.

Duggan, A.W. and Curtis, D.R. (1972). Morphine and the synaptic activation of renshaw cells. Neuropharmacology, 11, 189-196.

Duggan, A.W., Griersmith, B.T. and North, R.A. (1980). Morphine and supraspinal inhibition of spinal neurones; evidence that morphine decreases tonic descending inhibition in the anaesthetised cat. Br. J. Pharmacol., 69, 461-466.

Duggan, A.W., Hall, J.G. and Headley, P.M. (1977b). Enkephalins and dorsal horn neurones of the cat: effects on responses to noxious and innocuous skin stimuli. Br. J. Pharmacol., 61, 399-408.

Duggan, A.W., Hall, J.G., Foong, F.W. and Zhao, Z.Q. (1985). A differential effect of naloxone on transmission of impulses in primary afferents to ventral roots and ascending spinal tracts. Brain Res., 334(2), 316-321.

Duggan, A.W., Hall, J.G. and Headley, P.M. (1977a). Suppression of transmission of nociceptive impulses by morphine: selective effects of morphine administered in the region of the substantia gelatinosa. Br. J. Pharmacol., 61, 65-76.

Duggan, A.W., Johnson, S.M. and Morton, C.R. (1981). Differing distributions of receptors for morphine and met 5 enkephalinamide in the dorsal horn of the cat. Brain Res., 229, 379-387.

Duggan, A.W. and North, R.A. (1984). Electrophysiology of the opioids. Pharmacol. Rev., 35(4), 219-281.

Eales, A. and Small, J. (1986). Practical lambing: a guide to veterinary care at lambing. Longman, Essex.

Eisenberg, R.M. (1980). Effects of naloxone on plasma cortisone in the opiate naive rat. Life Sci., 26, 935-943.

Eisenach, J.C., Castro, M.I., Dewan, D.M. and Rose, J.C. (1989). Epidural clonidine analgesia in obstetrics: sheep studies. Anaesthesiology, 70(1), 51-6.

Eisenach, J.C., Dewan, D.M., Rose, J.C. and Angelo, J.M. (1987). Epidural clonidine produces antinociception, but not hypotension, in sheep. Anaesthesiology, 66(4), 496-501.

Eisenach, J.C. and Dewan, D.M. (1990). Intrathecal clonidine in obstetrics: Sheep studies. Anaesthesiology, 72(4), 663-668.

Eisenphar, F.J. and Piercey, M.F. (1980). Morphine depresses dorsal horn neurone responses to controlled noxious and non-noxious cutaneous stimulation. J. Pharmacol. Exp. Ther., 213, 456-461.

Ekenhoff, J.E., Elder, J.D. and King, B.D. (1951). The effect of N-allyl normorphine in treatment of opioid overdose. Am. J. Med. Sci., 222, 115-117.

Ekman, P. and Friesen, W.V. (1978). Manual for the Facial Action Coding System. Consulting Psychologists Press, Paolo Alto.

Engberg, I., Lunberg, A. and Ryall, R.W. (1968). Is the tonic descending inhibition of reflex pathways mediated by monoaminergic pathways? Acta Physiol. Scand., 72, 123-133.

Engberg, I. and Ryall, R.W. (1966). The inhibitory action of noradrenaline and other monoamines on spinal neurones. J. Physiol., 185, 298-322.

Ernsberger, P. Meeley, M.P. Mann, J.J. Reis, D.J. (1987). Clonidine binds to imidazole binding sites as well as a_2-adrenoceptors in the ventrolateral medulla. Eur. J. Pharmacol., 134(1), 1-13.

Evans, A.G.J., Nasymth, P.A. and Stewart, H.C. (1952). The fall of blood pressure caused by intravenous morphine in the rat and the cat. Br. J. Pharmacol., 7, 542-552.

Faden, A.I. (1984). Opiate antagonists and thyrotropin releasing hormone. II. Potential role in the treatment of central nervous system injury. J. Am. Med. Assoc., 252, 1452-1454.

Fell, L.R., Shutt, D.A. and Bentley, C.J. (1985). Development of a salivary cortisol method for detecting changes in plasma "free" cortisol arising from acute stress in sheep. Aust. Vet. J., 62(12), 403-406.

Fell, L.R., Wells, R. and Shutt, D.A. (1986). Stress in calves castrated surgically or by the application of rubber rings. Aust. Vet. J., 63(1), 16-18.

Fields, H.L. and Basbaum, A.I. (1978). Brainstem control of spinal pain-transmission neurons. Annu. Rev. Physiol., 40, 217-248.

Fitzgibbon, D., Kelly, I., Fitzpatrick, G. and Moriarty, D.C. (1989). Intrathecal morphine- the influence of volume of injectate. British Opioid Colloquium, University of Reading 21-23 March, O.12.

F.L.A.C. (1973). Stress in farm animals. Society for Veterinary Ethology. Proceedings of joint symposium with the Royal Society for the Prevention of Cruelty to Animals. Br. Vet. J., 130, 85.

Flecknell, P. (1986). Discussion. In: Assessing pain in farm animals. Eds. Duncan, I.J.H. and Molony, V. (Proceedings of 1984 workshop), Commission of European Communities, Luxembourg, 35-38.

Flecknell, P. (1985). Detection and relief of pain. BVA Animal Welfare Association (symposium). Vet. Rec., 116(19), 509-511.

Fleetwood-Walker, S.M., Mitchell, R., Hope, P.J., Molony, V. and Iggo, A. (1985). An a_2 receptor mediates the selective inhibition by Noradrenaline of nociceptive responses of identified dorsal horn neurones. Brain Res., 334(2), 243-254.

Fleetwood-Walker, S.M., Mitchell, R., Hope, P.J. and Molony, V. (1987). Is there an interaction between opioid and adrenergic antinociceptive mechanisms in the dorsal horn? Neurosci. Lett. Suppl., 29, S41.

Foldes, F.F., Duncalf, D., Davidson, G.M., Yun, D.S. and Schapira, M. (1964). Comparison of the respiratory and circulatory effects of narcotic antagonists. Anaesthesiology, 25, 95-96.

Foong, F.W. and Duggan, A.W. (1986). Brainstem areas tonically inhibiting dorsal horn neurones: studies with microinjection of the GABA analogue piperidine-4-sulphonic acid. Pain, 27(3), 361-371.

Fordyce, W.E. (1983). The validity of pain behaviour measurement. In: Pain measurement and assessment. Ed. Melzack, R. Raven Press, New York, 145-153.

Fraser, D., Ritchie, J.S.D. and Fraser, A.F. (1975). The term "stress" in a veterinary context. Br. vet. J., 131, 653.

Fulkerson, W.J. and Jamieson, P.A. (1982). Pattern of cortisol release in sheep following administration of synthetic ACTH or imposition of various stressor agents. Aust. J. Biol. Sci., 35, 215-22.

Gamse, R., Holzer, P. and Lembeck, F. (1979). Indirect evidence for presynaptic location of opiate receptors in chemosensitive primary sensory neurones. Naunyn-Schmiedebergs Arch. Pharmacol., 308, 281-228.

Game, C.J.A. and Lodge, D. (1975). The pharmacology of the inhibition of dorsal horn neurones by impulses in myelinated cutaneous afferents in the cat. Exp. Brain Res., 23, 75-84.

Gamble, G.D. and Milne, R.J. (1989). Repeated exposure to sham testing procedures reduces reflex withdrawal and hot plate latencies: attenuation of tonic descending inhibition? Neurosci. Lett., 96(3), 312-317.

Gibson, A., Ginsburg, M., Hall, M. and Hart, S.L. (1979). The effect of opiate receptor agonists and antagonists on the stress induced secretion of corticosterone in mice. Br. J. Pharmacol., 65, 139-146.

Giguere, V., Cote, J. and Labrie, F. (1981). Characteristics of the a-adrenergic stimulation of adrenocorticotropin secretion in rat anterior pituitary cells. Endocrinology, 109, 757.

Glaum, S.R., Proudfit, H.K. and Anderson, E.G. (1990). 5-HT3 receptors modulate spinal nociceptive reflexes. Brain Res., 510(1), 12-17.

Goldhart, J. and Hugh, (1976). Enhances of reflexes by naloxone in the spinal cat. Neuropharmacology, 15, 585-792.

Goldstein, A., Lowney, L.I. and Pal, B.K. (1971). Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. Proc. Natl. Acad. Sci., USA 68, 1742-1747.

Goldstein, A. and Lowery, P.J. (1975). Effect of opiate antagonist naloxone on body temperature in rats. Life Sci., 17, 927-932.

Goranov, Kh., Zhelyazkov, S. and Popgeorgieva, M. (1983). Hyperglycaemic and therapeutic effect of sub-neuroleptic doses of xylazine in healthy sheep and cattle, and in cows with subclinical ketosis. Veterinarnomeditsinski Nauki, 20(3/4), 34-39.

Gouarderes, C., Audigier, Y. and Cros, J. (1982). Benzomorphan binding sites in rat lumbo-sacral spinal cord. Eur. J. Pharmacol., 78, 483-486.

Gourlay, G.K., Cherry, D.A., Plummer, J.L., Armstrong, P.J. and Cousins, M.J. (1987). The influence of drug polarity on the absorption of opioid drugs into CSF and subsequent cephalad migration following lumbar epidural administration: application to morphine and pethidine. Pain, 31(3), 297-305.

Gray, W.D., Osterberg, A.C. and Scuto, T.J. (1970). Measurement of the analgesic efficacy and potency of pentazocine by the D'Armour and Smith method. J. Pharmacol. Exp. Ther., 172, 154-162.

Green, C.J. (1979). Local and regional anaesthesia. In: Animal anaesthesia. London, UK; Laboratory animals Ltd., 45-48.

Gross, G., Hanft, G. and Mehdorn. (1990). Demonstration of alpha 1-A and alpha 1 B adrenoceptor binding sites in human brain tissue. J. Appl. Physiol., 68(3), 897-905.

Gross, E.G., Holland, H., Carter, H.R. and Christensen, E.M. (1948). The role of epinephrine in analgesia. Anaesthesiology, 9, 459-471.

Grubb, B.D., Molony, V. and Wood, G.N. (1990). Response of afferents in the superior spermatic nerve of rats to occlusion of the testicular artery and vein. Pain, Suppl 5, Abs 785.

Guillemin, R., Vargo, T., Rossier, J., Minick, S., Ling, N., Rivier, C., Vale, W. and Bloom.F. (1977). b-Endorphin and adrenocorticotropin are secreted concomitantly by the pituitary gland. Science, 197, 1367-1369.

Guyenet, P.G. and Cabot, J.B. (1981). Inhibition of sympathetic preganglionic neurones by catecolamines and clonidine: Mediation by an alpha-adrenergic receptor. J. Neurosci., 1, 908-917.

Hall and Clarke (1988). General principles of local analgesia Veterinary Anaesthesia, 8th edition, 183-188.

Hammond, D.L., Tyce, G.M. and Yaksh, T.L. (1985). Efflux of 5-hydroxytryptamine and noradrenaline into spinal cord superfusates during stimulation of the rat medulla. J. Physiol. London, 359, 151-162.

Hardy, J.D., Wolff, H.G. and Goodell, H. (1950). Experimental evidence on the nature of cutaneous hyperalgesia. J. Clin. Invest., 29, 115-140.

Hardy, J.D., Wolff, H.G. and Goodell, H. (1952). Pain sensations and reactions. Williams and Wilkins, Baltimore.

Headley, P.M., Duggan, A.W. and Griersmith, B.T. (1978). Selective reduction by noradrenaline and 5-hydroxytryptamine of nociceptive responses of cat dorsal horn neurones. Brain Res., 145, 185-189.

Herrmann, J.B. (1942). The pyretic action on rats of small doses of morphine. J. Pharmacol. Exp. Ther., 76, 309-315.

Herz, A. and Teschemacher, H-J. (1971). Activities and sites of antinociceptive action of morphine-like analgesics and kinetics of distribution following intravenous, intracerebral and intraventricular application. Adv. Drug. Res., 6, 79-119.

Heyman, J.S., Koslo, R.J., Mosberg, H.I., Tallarida, R.J. and Porreca, F. (1986). Estimation of the affinity of naloxone at supraspinal and spinal opioid receptors in vivo: studies with receptor selective agonists. Life Sci., 39, 1795-1803.

Home Office. (1876). Cruelty to Animals Act. Home Office, London.

Home Office. (1983). Cmnd. 8883, HMSO, London.

Home Office. (1985). Scientific procedures on living animals. (Supplementary White Paper May 1985). Cmnd. 9521, HMSO, London.

Home Office. (1986). Guidance on the operation of the Animals (Scientific Procedures) Act 1986. Home Office, London.

Howe, J.R., Wang, J.Y. and Yaksh, T.L. (1983). Selective antagonism of the antinociceptive effect of intrathecally applied a-adrenergic agonists by intrathecal prazosin and intrathecal yohimbine. J. Pharmacol. Exp. Ther., 224(3), 552-558.

Howe, J.R. and Yaksh, T.L. (1984). <3H> p- Aminoclonidine binding to multiple a_2-adrenoceptor sites in homogenates of cat frontal cortex and cat spinal cord. Eur. J. Pharmacol. 106(3), 547-559.

Howe, J.R., Yaksh, T.L. and Tyce, G.M. (1987). Intrathecal 6-hydroxdopamine or cervical spinal hemisection reduces norepinephrine content, but not the density of a_2-adrenoceptors, in the cat lumbar spinal enlargement. Neuroscience, 21(2), 377-384.

Hsu, W.H., Schaffer, D.D. and Hanson, C.E. (1987). Effects of tolazoline and yohimbine on xylazine-induced central nervous system depression, bradycardia, and tachypnea in sheep. J. Am. Vet. Med. Assoc., 190(4), 423-426.

Hughes, J., Smith T.W., Kosterlitz, H.W., Fothergill, L.A., Morgan, B.A. and (1975). Morris, H.R. Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature, 258, 577-579.

Irwin, S., Houde, R.W., Bennet, D.R., Hendershot, L.C. and Seevers, M.H. (1951). The effects of morphine, methadone and meperidine on some reflex responses of spinal animals to nociceptive stimulation. J. Pharmacol. Exp. Ther., 101, 132-143.

Jeans, M.E. (1983). The measurement of pain in children. In: Pain measurement and Assessment. Ed. Melzack, R. Raven Press, New York, 183-189.

Jephcott, E.H., McMillen, I.C., Rushen, J., Hargreaves, A. and Thornburn, G.D. (1986). Effect of electroimmobilisation on ovine plasma concentrations of b-endorphin/ b-lipotrophin, cortisol and prolactin. Res. Vet. Sci., 41(3), 371-377.

Jessell, T.M. and Iversen, L.L. (1977). Opiate analgesics inhibit substance P release from rat trigeminal nucleus. Nature, 268, 549-551.

Jezoua, D. (1985). Stimulation of ACTH release by naloxone: central or peripheral action? Life Sci., 37(11), 1007-1013.

Johnson, S.M. and Duggan, A.W. (1981). Evidence that opiate receptors of the substantia gelatinosa contribute to the depression by intravenous morphine of the spinal transmission of impulses in unmyelinated primary afferents. Brain Res., 207, 223-228.

Jones, D.J., Kendall, D.E. and Enna, S.J. (1982). Adrenergic receptors in the rat spinal cord. Neuropharmacology, 21, 191-195.

Jurna, I. and Grossmann, W. (1976). The effect of morphine on the activity evoked in ventrolateral tract axons of the cat spinal cord. Exp. Brain Res., 24, 473-484.

Jurna, I., Heinz, G., Blinn, G. and Nell, T. (1978). The effect of substantia nigra stimulation and morphine on a-motoneurones and the tail flick response. Eur. J. Pharmacol., 51, 239-250.

Kalpravidh, M., Lumb, W.V., Wright, M. and Heath, R.B. (1984). Effects of butorphanol, flunixin, levorphanol, morphine and xylazine in ponies. Am. J. Vet. Res., 45(2), 217-223.

Kania, B.F. (1985). Neurochemical changes in the brain and spinal cord of sheep: a basis for the immobilising action of etorphine. Journal of the South African Veterinary Association., 56(2), 89-92.

Katz, N.L., Sobaski, N., Sanchez, J., Young, J.E. and Schlemmer, R.F, Jr. (1989). Differential effects of clonidine analogs on food intake in rabbits and monkeys. Pharmacol. Biochem. Behav., 34(2), 433-439.

Kawasaki, K., Takesue, H. and Matsushita, A. (1978). Modulation of spinal reflex activities in acute spinal rats with a-adrenergic agonists and antagonists. Jpn. J. Pharmacol., 28, 165-168.

Kehlet, H. (1978). Influence of epidural analgesia on the endocrine-metabolic response to surgery. Acta Anaesth. Scand., 70, 39-42.

Kevetter, G.A. and Willis, W.B. (1984). Collateralisation in the spinothalamic tract: New methodology to support or deny phylogenetic theories. Brain Res. Rev., 7, 1-14.

Kirby, M.L. (1981). Development of opiate receptor binding in rat spinal cord. Brain Res., 205,400-404.

Kitzman, J.V., Booth, N.H., Hatch, R.C. and Wallner, B. (1982). Antagonism of xylazine sedation by 4-aminopyridine and yohimbine in cattle. Am. J. Vet. Res., 43(12), 2165-2169.

Kitchell, R.L. and Johnston, R.D. (1985). Assessment of pain in animals. In: Animal stress. Ed. Moberg, G.P., Bethesda, Maryland, USA. Am. Phys. Soc., 113-140.

Kivoy, W., Kruger, D. Zimmerman, E. (1973). Actions of morphine sulphate on segmental reflex of the decerebrate spinal cat. Br. J. Pharmacol., 47, 457-464.

Koll, W., Hause, J., Block, G. and Mulberg, B. (1963). The predilective action of small doses of morphine on nociceptive spinal reflexes of low spinal cats. Int. J. Neuropharmacol., 2, 57-65.

Kosuch, H. (1973). Studies on xylazine in sheep. Inaugural Dissertation, Tierarztliche Hochschule, Hannover.

Kosterlitz, H.W. (1985). Opioid peptides and their receptors. Proc. R. Soc., London-Biol., 225(1238), 27-40.

Kosterlitz, H.W and Walt, A.J. (1968). Kinetic parameters of narcotic agonists and antagonists with particular reference to N-allylnormorphone (Naloxone). Br. J. Pharmacol. Chemotherapeutics, 33, 266-276.

Krugalov, N.A. (1964). Effect of morphine group analgesics on the central inhibitory mechanisms. Int. J. Neuropharmacol., 3, 197-203.

Krueger, H., Eddy, N.B. and Sumwalt, M. (1941). The pharmacology of the opium alkaloids. Part 1. Public health rep., suppl 1, 165.

Krueger, H., Eddy, N.B. and Sumwalt, M. (1943). The pharmacology of the opium alkaloids. Part 2. Public health rep., Suppl 1, 165.

Kuraishi, Y., Harada, Y. and Takagi, H. (1979). Noradrenaline regulation of pain-transmission in the spinal cord mediated by a-adrenoceptors. Brain Res., 174, 333-337.

Kuraishi, Y., Hirota, N., Sugimoto, M., Satoh, M. and Takagi, H. (1983). Effects of morphine on noxious stimuli-induced release of substance P from rabbit dorsal horn in vivo. Life. Sci., 33 Suppl 1, 693-696.

Kuschinsky, K., Seeber, V., Langer, J. and Sontag, K.H. (1978). Effects of opiates and of neuroleptics on alpha-motoneurones in rat spinal cord: possible correlations with muscular rigidity and akinesia. In Characteristics and function of opioids, ed. Van Lee, J.M. and Terenius, L. Elsevier North, Amsterdam, Holland., 431-435.

Lal, H. (1977). Discriminative stimulus properties of drugs. Plenum Press, New York.

La Motte, C., Pert, C.B. and Snyder, S.H. (1976). Opiate receptor binding in primate spinal cord: distribution and changes after dorsal root section. Brain Res., 112, 407-412.

Langer, S.Z. (1974). Presynaptic regulation of catecholamine release. Biochem. Pharmacol., 23, 1793-1800.

Langer, S.Z. and Hicks, P.E. (1984). Alpha-adrenoceptor subtypes in blood vessels: Physiology and pharmacology. J. Cardiovasc. Pharmacol., 6, suppl 4, S547-S558.

Lasagna, L. (1954). Nalorphine (N-allylnormorphine): practical and theoretical considerations. Arch. Intern. Med., 94, 532-558.

Lasagna, L. (1964). The clinical evaluation of morphine and its substitutes as analgestics. Pharmacol. Rev., 16, 47-83.

Levine, J.D. and Taiwo, Y.O. (1989). Involvement of the mu-opiate receptor in peripheral analgesia. Neurosci., 32(3), 571-577.

Lewis, J.W., Chundler, E.H., Cannon, J.T. and Liebeskind, J.C. (1981). Hypophysectomy differentially effects morphine and stress analgesia. Proc. West. Pharmacol. Soc., 24, 323.

Lewis, J.W., Terman, G.W., Watkins, L.R., Mayer, D.J. and Liebeskind, J.C. (1983). Opioid and non-opioid mechanisms of foot-shock induced analgesia: role of the spinal dorsolateral funiculus. Brain Res., 267(1), 139-144.

Lewis, J.W., Tordoff, M.G., Sherman, J.E. and Liebeskind, J.C. (1982). Adrenal medullary enkephalin-like peptides may mediate opioid stress analgesia. Science, 217, 557-559.

Ley, S., Dash, A., Waterman, A. and Livingston, A. (1989a). A comparison of the analgesic effects of intrathecal a-adrenoceptor agonists and opioids in conscious unrestrained sheep. The Biosciences, 75, 495-498.

Ley, S.T., Livingston, A. and Waterman, A.E. (1989b). The effect of castration and taildocking on plasma catecolamines in lambs. Br. J. Pharmacol., 97, 570P, Abs No 6823.

Li, C.H., Barnafi, L., Chretien, M. and Chung, D. (1965). Isolation and amino-acid sequence of b-LPH from sheep pituitary gland. Nature, 208, 1093-1094.

Li, C.H. and Chung, D. (1976). Isolation and structure of an untriakontapeptide with opiate activity from camel pituitary glands. Proc. Natl. Acad. Sci., 73(4), 1145-1148.

Livingston, A., Nolan, A. and Waterman, A. (1986/87). The pharmacology of the a_2 adrenergic agonist drugs. J. Assoc. Vet. Anaesth., 14, 3-10.

Livingston, A., Nolan, A. Waterman, A. (1986). The relationship between analgesia induced by, and plasma concentration of, buprenorphine in the sheep. Br. J. Pharmacol., 88, 363P.

Lofgren, N. (1948). Studies on local anaesthetics (V) xylocaine. Haeggstroms, Stockholm.

Lombard, M.C. and Besson,J.M. (1989). Attempts to guage the relative importance of pre-and post-synaptic effects of morphine on the transmission of noxious messages in dorsal horn of the rat spinal cord. Pain, 37(3), 335-345.

Lundeberg, A. (1964). Supraspinal control of transmission in reflex paths to motorneurones and primary afferents. Prog. Brain Res., 12, 197-221.

MacDonald, R.L. and Nelson, P.G. (1978). Specific-opiate-induced depression of transmitter release from dorsal root ganglion cells in culture. Science, 199, 1449-1451.

McFadzean, I. (1988). The ionic mechanisms underlying opioid actions. Neuropeptides, 11(4), 173-180.

Ministry of Agriculture, Fisheries and Food (M.A.F.F.). (1988). Codes of recommendations for the welfare of livestock-sheep. M.A.F.F. Publications, Tolworth, Surbiton, Surrey.

Magnan, J., Paterson, S.J., Tavani, A. and Kosterlitz, H.W. (1982). The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. Naunyn Schmiedebergs Arch. Pharmacol., 319, 197-205.

Maier, S.F., Drugan, R.C. and Grau, J.W. (1982). Controlability, coping behaviour and stress-induced analgesia in the rat. Pain, 12, 47-56.

Mansour, A., Khachaturian, H., Lewis, M.E., Akil, H. and Watson, S.J. (1988). Anatomy of CNS opioid receptors. Trends. neurosci., 11(7), 308-14.

Martin, W.R. (1967). Opioid antagonists. Pharmacol. Rev., 19(4), 463-521.

Martin, W.R. (1984). Pharmacology of the opioids. Pharmacol. Rev., 35(4), 283-323.

Martin, W.R., Eades, C.G., Fraser, H.F. and Wilker, A. (1964). Use of hindlimb reflexes of the chronic spinal dog for comparing analgesics. J. Pharmacol. Exp. Ther., 144, 8-11.

Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E. and Gilbert, P.E. (1976). The effect of morphine and nalorphine like drugs in the nondependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther., 197, 517-532.

Mayer, D.J. and Price, D.D. (1976). Central nervous system mechanisms of analgesia. Pain, 2, 379-404.

McClane, T.K. and Martin, W.R. (1967). Effects of morphine, nalorphine, cyclazocine and naloxone on the flexor reflex. Int. J. Neuropharmacol., 6, 89-98.

McDowell, J. and Kitchen, I. (1987). Development of the opioid systems: peptides, receptors and pharmacology. Brain Res., 434(4), 397-421.

McFazdean, I. (1988). The ionic mechanisms underlying opioid actions. Neuropeptides, 11, 173-180.

McGrath, P. (1990). Assessment of pain in children. Pain, Suppl., 5, S2.

McGrath, P.J., Johnson, G., Goodman, J.T., Schillinger, J., Dunn, J. and Chapman, J.A. (1985). CHEOPS: A behavioural scale for rating postoperative pain in children. Advances in Pain Research and Therapy, 9, 395-402.

McGrath, J. and Wilson, V. (1988). a-adrenoceptor subclassification by classical and responce related methods same question, different answers. Trends in Pharmacol. Sci., 9(5), 162-165.

McIntosh, G.H., Baghurst, K.I., Potter, B.J. and Hetzel, B.S. (1979). Foetal brain development in the sheep. Neuropathol. Appl. Neurobiol., 5, 103-114.

Melzack, R. (1975). The McGill pain questionnaire: major properties and scoring methods. Pain, 1, 277-299.

Mellor, D.J. and Murray, L. (1989b). Changes in cortisol responses of lambs to tail docking, castration and ACTH injection during the first 7 days after birth. Res. Vet. Sci., 46(3), 392-395.

Mellor, D.J. and Murray, L. (1989a). Effects of tail docking and castration on behaviour and plasma cortisol concentrations in young lambs. Res. Vet. Sci., 46(3), 387-391.

Melzack, R. (1983). Concepts of pain measurement. In: Pain Measurement and Assessment. Ed, Melzack, R. Raven Press, New York, 1-7.

Mezey, E., Kiss, J.Z., Skirboll, L.R., Goldstein, M. and Axelrod, J. (1984). Increase of corticotropin-releasing factor staining in rat paraventricular nucleus neurones by depletion of hypothalamic adrenalin. Nature, 310(5973), 140-141.

Mitchell, G., Hattingh, J. and Ganhao, M. (1988). Stress in cattle assessed after handling, after transport and after slaughter. Vet. Rec., 22, 201-205.

Molony, V. and Duncan, I.J.H. (1986). Assessing pain in farm animals. Eds. Duncan, I.J.H. and Molony, V. (Proceedings of 1984 workshop), Commission of European Communities, Luxembourg, III-V.

Morton, D.B. and Griffiths, P.H. (1985). Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Vet. Rec., 116(16), 431-436.

Munck, A., Guyre, P.M. and Holbrook, N.J. (1984). Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev., 5(1), 25-44.

Munhall, R.J. Sukhani, R. Winnie, A.P. (1988). Incidence and etiology of failed spinal anaesthetics in a university hospital: a prospective study. Anaesthesia and Analgesia, 67(9), 843-849.

Nasmyth, P.A. (1954). Factors influencing the effects of morphine sulphate on the ascorbic acid contents of rat adrenal glands. Br. J. Pharmacol. Exp. Ther., 9(1), 95-100.

Ness, T.J. and Gebhart, G.F. (1989). Differential effects of morphine and clonidine on visceral and cutaneous spinal nociceptive transmission in the rat. J. Neurophysiol., 62(1), 220-230.

Nizielski, S.E., Levine, A.S., Morley, J.E., Hall, K.A. and Gosnell, B.A. (1986). Seasonal variation in opioid modulation of feeding in the 13-lined ground squirrel. Physiol. Behav., 37(1), 5-9.

Nolan, A.M. (1986). Investigations into the actions of opioids and alpha 2 drugs in sheep. Thesis. University of Bristol.

Nolan, A., Livingston, A., Morris, R. and Waterman, A. (1987). Techniques for comparison of thermal and mechanical nociceptive stimuli in sheep. J. Pharmacol. Meth., 17, 39-49.

Nolan, A., Livingston, A. and Waterman, A. (1987). Antinociceptive actions of intravenous a_2-adrenoceptor agonists in sheep. J. Vet. Pharmacol. Therap., 10(3), 202-209.

North, R.A. (1986). Opioid receptor types and membrane ion channels. Tins, march, 114-118.

Noueihed, R., Durant, P. and Yaksh, T.L. (1984). Studies on the effects of intrathecal sufentanil, fentanyl and alfentanil in rats and cats. Anaesthesiology, 60, A218, Supl.

Osburn, J.W., Taylor, R.F. and Schramm, L.P. (1990). Chronic cervical spinal cord injury and autonomic hyperreflexia in rats. Am. J. Physiol., 258(1 pt 2), R169-R175.

Pan, J.T. and Teo, K.L. (1989). Fentanyl stimulates prolactin release through mu-opiate receptors, but not the serotonergic system. Endocrinology, 125(4), 1863-1870.

Parsons, C.G , and Headley, P.M. (1989). Spinal antinociceptive actions of m- and k-opioids: the importance of stimulus intensity in determining selectivity between reflexes to different modalities of noxious stimulus. Br. J. Pharmacol., 98, 523-532.

Pasternack, G.W., Goodman, R. and Snyder, S.H. (1975). An endogenous morphine-like factor in mammalian brain. Life Sci., 16, 1765-1769.

Paul, D., Bodnar, R.J., Gistrak, M.A. and Pasternak, G.W. (1989). Different mu receptor subtypes mediate spinal and supraspinal analgesia in mice. Eur. J. Pharmacol., 168(3), 307-315.

Pettit, J., Taylor, J., Clarke, R.W. and Ford, D. (1989). Evidence that noxious stimulation of the toes evokes release of opioid peptides in rabbit spinal cord. British Opioid Colloquium, University of Reading 21-23 March, O.15.

Pippi, N.L and Lumb, W.V. (1979). Objective tests of analgesic drugs in ponies. Am. J. Vet. Res., 40(8), 1082-1086.

Plotsky, P.M., Cunningham, E.T. and Widmaier, E.P. (1989). Catecholaminergic modulation of corticotropin-releasing factor and adrenocorticotropin secretion. Endocr. Rev., 10(4), 437-459.

Pohl, J. (1915). Uber das n-allylnorcodein, einen antagonisten des morphins. Exp. Pathol. Ther., 17, 370-382.

Porecca, F., Heyman, J.S., Mosberg, H.I., Omnass, J.R. and Vaught, J.L. (1987). Role of mu and delta receptors in the supraspinal and spinal analgesic effects of <D-Pen 2; D-Pen 5> enkephalin in the mouse. J. Pharmacol. Exp. Ther., 241, 393-400.

Procacci, P., Zoppi, M. and Maresca, M. (1986). Clinical approach to visceral sensation. In: Cervero, F. and Morrison, J.F.B., eds. Progress in brain research. Amsterdam: Elsevier, 21-28.

Purchas, R.W. (1973). The response of circulating cortisol levels in sheep to various stresses and to reserpine administration. Aust. J. Biol. Sci., 26, 477-89.

Read, G.F., Fahmy, D.R. and Walker, R.F. (1977). Determination of cortisol in human plasma by radioimmunoassay. Ann. Clin. Biochem., 14(6), 343-349.

Reckitt and Coleman. (1982). Etorphine, preclinical and clinical studies (confidential). Report No. 28 (RC82137).

Reddy, S.V.R., Majerut, J.L. and Yaksh, T.L. (1980). Spinal cord pharmacology of adrenergic agonist mediated antinociception. J. Pharmacol. Exp. Ther., 213, 525-533.

Reddy, S.V.R. and Yaksh, T.L. (1980). Spinal noradrenergic terminal system mediates antinociception. Brain Res., 189, 391-401.

Reid, L.D. (1985). Endogenous opioid peptides and regulation of drinking and feeding. Am. J. Clin. Nutr., 42, 1099-1132.

Richardson, M.K.D., Van De Kar., L.D., Brownfield, M.S., Lorens, S.A., Napier, (1990). T.C. and Urban, J.H. Stress-induced renin and corticosterone secretion is mediated by catecholaminergic nerve terminals in the hypothalamic paraventricular nucleus. Neuroendocrinology, 51(3), 320-328.

Robertson, I.S. (1966). Castration in farm animals: Its advantages and disadvantages. Vet. Rec., 78(4), 130-135.

Rothman, R.B. and Westfall, T.C. (1982). Allosteric coupling between morphine and enkephalin receptors in vitro. Mol. Pharmacol., 21, 548-547.

Rowell, J.G. and Walters, D.E. (1976). Analysing data with repeated observations on each experimental unit. J. Agric. Sci., 87, 423-432.

Rudy, T.A. and Yaksh, T.L. (1977). Hyperthermic effects of morphine: set point manipulation by a direct spinal action. Br. J. Pharmacol, 61, 91-96.

Rushen, J. (1986). Some problems with the physiological concept of "stress". Aust. Vet. J., 63(11), 359-361.

Sanford, J. (1985). Recognising pain. Vet. Rec., 116(16), 429.

Sanford, J., Ewbank, R., Molony, V., Tavernor, W.D. and Uvarov, O. (1986). Guidelines for the recognition and assessment of pain in animals. Vet. Rec., 118(12), 334-338.

Sanford, J., Ewbank, R., Molony, V., Tavernor, W.D. and Uvarov, O. (1989). Guidelines for the recognition and assessment of pain. Universities Federation for Animal Welfare. Potters Bar, Herts.

Sar, M., Stumpf, W.E., Willer, R.J., Chang, K-J. and Cuatrecasas, P. (1978). Immunohistochemical localisation of enkephalin in rat brain and spinal cord. J. Comp. Neurol., 182, 17-38.

Satoh, M., Kawajiri, S-I., Ukai, Y. and Yamamoto, M. (1979). Selective and non selective inhibition by enkephalins and noradrenaline of nociceptive responses of lamina V type neurones in the spinal dorsal horn of the rabbit. Brain Res., 177, 384-387.

Sato, O., Osai, T., Amaro, Y., Hara, M., Tsugane, R. and Yagi, M. (1971). Formation of cerebrospinal fluid in subarachnoid space. Nature, 233, 129-130.

Sawynok, J., Pinsky, C., LaBella, F.S. (1979). Minireview on the specificity of naloxone as an opiate antagonist. Life Sci., 25, 1621-1632.

Schmidt, R.F. (1971). Presynaptic inhibition in the vertebrate central nervous system. Rev. Physiol. Biochem. Pharmacol., 63, 21-101.

Schmitt, H., Le Douarec, J.C. and Petillot, N. (1974). Antagonism of the antinociceptive action of xylazine an a-sympathomimetic agent, by adrenoceptor and cholinoceptor blocking agents. Neuropharmacology, 13, 295-303.

Schmauss, C. and Yaksh, T.L. (1984). In vivo studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. J. Pharmacol. Exp. Ther., 288(1), 1-12.

Segal, M. and Sandberg, D. (1977). Analgesia produced electrical by stimulation of catecolamine nuclei in the rat brain. Brain Res., 123, 369-372.

Selye, H. (1936). A syndrome produced by diverse nocuous agents. Nature, 138, 32.

Shiomi, H. and Takagi, H. (1974). Morphine analgesia and the bulbospinal noradrenergic system: increase in the concentration of normetanephrine in the spinal cord of the rat caused by analgesics. Br. J. Pharmacol., 52, 519-526.

Shorky, M., Morad, H.M. and Khalil, I.A. (1976). Studies on the effect of rompun (xylazine) in sheep. Vet. Med. Res., No2, 237-243.

Shutt, D.A., Fell, L.R., Connell, R., Bell, A.K., Wallace, C.A. and Smith, A.I. (1987). Stress-induced changes in plasma concentrations of immunoreactive b-endorphin and cortisol in response to routine surgical procedures in lambs. Aust. J. Biol. Sci., 40(1), 97-103.

Shutt, D.A., Fell, L.R., Connell, R. and Bell, A.K. (1988). Stress responses in lambs docked and castrated surgically or by the application of rubber rings. Aust. Vet. J., 65(1), 5-7.

Sinclair, J.G., Mais, C.D. and Lo, G.F. (1988). Spinal Vs supraspinal actions of morphine on the rat tail flick reflex. Pain, 33, 3P, 357-363.

Smits, S.E. and Takemori, A.E. (1970). Quantitative studies on the antagonism by naloxone of some narcotic and narcotic-antagonist analgesics. Br. J. Pharmacol., 39, 627-638.

Smythe, J.W. and Pappas, B.A. (1989). Noradrenergic and seretonergic mediation of the locomotor and antinociceptive effects of clonidine in infant and adult rats. Pharmacol. Biochem. Behav., 34(2), 413-418.

Solomon, R.E., Brody, M.J. and Gebhart, G.F. (1989). Pharmacological characterisation of a-adrenoceptors involved in the antinociception and cardiovascular effects of intrathecally administered clonidine. J. Pharmacol. Exp. Ther., 251(1), 27-39.

Solomon, R.E. and Gebhart, G.F. (1988). Intrathecal morphine and clonidine: antinociceptive tolerance and cross- tolerance and effects on blood pressure. J. Pharmacol. Exp. Ther., 245(2), 444-454.

Soma, R.A. (1971). Textbook of veterinary anaesthesia. The Williams and Wilkins Company, Baltimore, 461.

Spiller, W.G. and Martin, E. (1912). The treatment of persistent pain of organic origin in the lower part of the body by division of the anterolateral column of the spinal cord. J. Am. Med. Assoc., 58, 1489-1490.

Starke, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems. Rev. Physiol. Biochem., 77, 1-124.

Starke, K. (1981). Alpha-adrenoceptor subclassification. Rev. Physiol. Biochem. Pharmacol., 88, 199-236.

Stevens, C.W., Monasky, M.S. and Yaksh, T.L. (1988). Spinal infusion of opiate and a_2 agonists in rats: tolerance and cross tolerance studies. J. Pharm. Exp. Ther., 244(1), 63-70.

Straub, O.C. (1972). Rompun (xylazine) anaesthesia in sheep. Deutsche Tierarztliche Wochenschrift, 78(20), 537-538.

Taiwo, Y.O., Basbaum, A.I., Perry, F. and Levine, J.D. (1989). Paradoxical analgesia produced by low doses of the opiate-antagonist naloxone is mediated by interaction at a site with characteristics of the delta opioid receptor. J. Pharmacol. Exp. Ther., 249(1), 97-100.

Takemori, A.E., Hayashi, G. and Smits, S.E. (1972). Studies on the quantitative antagonism of analgesics by naloxone and diprenorphine. Eur. J. Pharmacol., 20, 85-92.

Takagi, H., Matsumara, M., Yanai, A. and Ogiu, K. (1955). The effect of analgesics on the spinal reflex activity of the cat. Jpn. J. Pharmacol., 4, 176-187.

Takemori, A.E. and Portoghese, P.S. (1984). Comparative antagonism by naltrexone and naloxone of mu, kappa, and delta agonists. Eur. J. Pharmacol., 104(1-2), 101-104.

Tasker, R.A.R. and Melzack, R. (1989). Different alpha-receptor subtypes are involved in clonidine produced analgesia in different pain tests. Life Sci., 44, 9-17.

Terenius,L. (1973). Stereospecific interaction between narcotic analgesics and a synaptic plasma membrane fraction of rat cerebral cortex Acta pharmacol. Toxicol., 32, 317-319.

Terman, G.W., Lewis, J.W. and Liebeskind, J.C. (1983). Opioid and non-opioid mechanisms of stress analgesia: lack of cross-tolerance between stressors. Brain Res., 260(1), 147-150.

Terman, G.W., Shavit, Y., Lewis, J.W., Cannon, J.T. and Liebeskind, J.C. (1984). Intrinsic mechanisms of pain inhibition: activation by stress. Science, 226, 1270-1277.

Teschemacher, H., Opheim, K.E., Cox, B.M. and Goldstein, A. (1975). A peptide-like substance from pituitary that acts like morphine. Life Sci., 16, 1771-1776.

Thorpe, W.H. (1965). The assessment of pain and distress in animals. In: Report of the technical committee to inquire into the welfare of animals kept under the intensive husbandry system. Command Paper 2836. London HMSO.

Torda, T.A. and Pybus, D.A. (1982). A comparison of four opiates for epidural analgesia. Br. J. Anaesth., 54, 291-295.

Toyoka, H., Kitahata, L.M., Dohi, S., Ohtani, M. and Hanaoka, K. et al (1978). Effect of morphine on the rexed lamina V11 spinal neuronal release to graded radiant heat stimulation. Exp. Neurol., 62, 146-158.

Traynor, J.R. and Wood, M.S. (1987). Distribution of opioid binding sites in spinal cord. Neuropeptides, 10(4), 313-320.

Tsou, K. and Jang, C.S. (1964). Studies on the site of analgesic action of morphine by intracerebral micro-injection. Sci. Sin., 13(7), 1099-1109.

Tyers, M.B. (1980). A classification of opiate receptors that mediate antinociception in animals. Br. J. Pharmacol., 69, 503-512.

Tyers, M. (1985). Detection of pain. BVA Animal Welfare Foundation (Symposium) Vet. Rec., 116(19), 509-511.

Uggala, A. and Lindquist, A. (1983). Acute pulmonary oedema as an adverse reaction to the use of xylazine in sheep. Vet. Rec., 113(2), 42.

Unnerstall, J.R., Fernandez, I. and Orensanz, L.M. (1985). The alpha-adrenergic receptor: Radiohistochemical analysis of functional characteristics and biochemical differences. Pharmacol. Biochem. Behav., 22(5), 859-874.

Unnerstall, J.R., Kopajtic, T.A. and Kuhar, M.J. (1984). Distribution of a_2-agonist binding sites in the rat and human central nervous system: Analysis of some functional, anatomic correlates of the pharmacologic effects of clonidine and related adrenergic agents. Brain Res., 319, 69-101.

Uvarov, O. (1989). Working definitions of pain. In: Guidelines for the recognition and assessment of pain. Eds. Sanford, J., Ewbank, R., Molony, V., Tavernor, W.D. and Uvarov, O. Universities Federation for Animal Welfare. Potters Bar, Herts.

Vainio, O. and Vaha-vahe, T. (1990). Reversal of medetomidine sedation by atipamezole in dogs. J. Vet. Pharmacol. Ther., 13(1), 15-22.

Vaught, J.L., and Rothman, R.B. and Westfall, T.C. (1982). Mu and Delta receptors: their role in analgesia and in the differential effects of opioid peptides on analgesia. Life Sci., 30, 1443-1445.

Veda, H. (1990). Molecular pharmacology of opioid receptor mechanisms. Folia Pharmacologica Japonica, 94(6), 339-351.

Vigouret, J., Teschemacher, H., Albus, K. and Herz, A. (1973). Differentiation between spinal and supraspinal sites of action of morphine when inhibiting the hindleg reflex in rabbits. Neuropharmacol., 12, 111-121.

Villiger, J.W., Taylor, K.M., and Gluckman, P.D. (1982). Ontogenesis of opiate receptors in regions of the ovine brain. Pediatric Pharmacology, 2, 349-356.

Von Braun, J. (1916). Untersuchungen uber morphium-alkaloide. III. Mitteilung. Ber. Deut. Chem. Ges., 49, 977-989.

Wall, P.D. (1988). The prevention of post-operative pain. Pain, 33(3), 289-290.

Wall, P.D. and Melzack, R. (1984). Textbook of pain. Churchill Livingston. Edin. Lond. Melb. NY.

Wamsley, J.K. (1983). Opioid receptors: Autoradiography. Pharmacol. Rev., 35(1), 69-83.

Waterman, A., Livingston, A. and Bouchenafa, O. (1988). Analgesic effects of intrathecally-applied a_2-adrenoceptor agonists in conscious, unrestrained sheep. Neuropharmacology, 27(2), 213-216.

Watkins, L.R. and Mayer, D.J. (1982). Organisation of endogenous opiate and nonopiate pain control systems. Science, 216, 1185-1192.

Waterman, A.E., Nolan, A. and Livingston, A. (1986/87). The effect of alpha 2 adrenergic agonist drugs and their antagonists on respiratory blood gases in conscious sheep. J. Assoc. Vet. Anaesth., 14, 11-13.

Webber, H. (1904). Uber anasthesie durch adrenalin. Ver. Ditsch. Ges. Inn. Med., 21, 616-619.

Weber, E., Evans, C.J. and Burchas, J.D. (1983). Multiple endogenous ligands for opioid receptors. Tins, Aug, 333-336.

Westlund, K.N., Bowker, R.M., Ziegler, M.G. and Coulter, J.D. (1982). Descending noradrenergic projections and their spinal terminations. Prog. Brain Res., 57, 219-238.

Westhues, M. and Fritsch, R. (1964). Principles of local anaesthesia. Animal anaesthesia, vol 1, 28-29.

White, S.R. and Neuman, R.S. (1980). Facilitation of spinal motoneurone excitability by 5-hydroxytryptamine and noradrenaline. Brain Res., 188, 119-127.

Wiesenfeld-Hallin, Z. (1987). Intrathecal noradrenaline has a dose-dependent inhibitory or facilitatory effect on the flexion reflex in the rat. Acta. Physiol. Scand., 130(3), 507-511.

Wikler, A. (1950). Sites and mechanisms of action of morphine and related drugs in the central nervous system. Pharmacol. Rev., (2), 435-506.

Wikler, A. (1953). Recent experimental studies on pain and analgesia. Neurology, 3, 656-660.

Willer, J.C. and Brussel, B. (1980). Evidence for a direct spinal mechanism in morphine-induced inhibition of nociceptive reflexes in humans. Brain Res., 287, 212-215.

Wilcox, G.L., Carlsson, K.H., Johim, A. and Jurna, I. (1987). Mutual potentiation of antinociceptive effects of morphine and clonidine on motor and sensory responses in rat spinal cord. Brain Res., 405(1), 84-93.

Woolf, C.J. (1980). Analgesia and hyperalgesia produced in the rat by intrathecal naloxone. Brain Res., 189, 593-597.

Wood, G.N., Mellor., D.J., Molony, V., Hodgson, J.C. and Fleetwood-Walker, S. (1988). Assessment of pain produced by castration and tailing of young lambs. Proceedings of the A.V.T.R.W. Scarborough, D.28.

Woolf, C.J. and Wall, P.D. (1983). Endogenous opioid peptides and pain mechanisms: A complex relationship. Nature, 306(5945), 739-740.

Woolf, C.J. and Wall, P.D. (1986). Relative effectiveness of C primary afferent fibres of different origins in evoking a prolonged facilitation of the flexor reflex in the rat. J. Neurosci., 6, 1433-1442.

Yaksh, T.L. (1979). Direct evidence that spinal seretonin and noradrenaline terminals mediate the spinal antinociceptive effect of morphine in the periaqueductal grey. Brain Res., 160, 180-185.

Yaksh, T.L. (1984). Multiple opioid receptor systems in brain and spinal cord. Eur. J. Anaesthesiol., part 1, 1(2), 171-199.

Yaksh, T.L. (1985). Pharmacology of spinal adrenergic systems which modulate spinal nociceptive processing. Pharmacol. Biochem. Behav., 22(5), 845-858.

Yaksh, T.L. (1987). Spinal opiates: a review of their effect on spinal function with emphasis on pain processing. Acta. Anaesthesiol. Scand., 31, supp. 85, 25-37.

Yaksh, T.L. (1978). Inhibition by etorphine of the discharge of dorsal horn neurones: effects on neuronal response to both high and low-threshold sensory input in the decerebrate spinal cat. Exp. Neurol., 60, 23-40.

Yaksh, T.L., Harty, G.J. and Onofrio, B.M. (1986). High dose of spinal morphine produce a nonopiate receptor-mediated hyperaesthesia: Clinical and theoretic implications. Anaesthesiology, 64(5), 590-597.

Yaksh, T.L., Jessell, T.M., Gamse, R., Mudge, A.W. and Leeman, S.E. (1980). Intrathecal morphine inhibits substance P release from mammalian spinal cord in vivo. Nature, 286, 155-157.

Yaksh, T.L. and Noueihed, R. (1985). The physiology and pharmacology of spinal opiates. Ann. Rev. Pharmacol. Toxicol., 25, 433-462.

Yaksh, T.L. and Rudy, T.A. (1976a). Chronic catheterisation of the spinal subarachnoid space. Physiol. Behav., 17, 1031-1036.

Yaksh, T.L. and Rudy, T.A. (1976b). Analgesia mediated by a direct spinal action of narcotics. Science, 192(76), 1357-1358.

Yaksh, T.L. and Rudy, T.A. (1977). Studies on the direct spinal action of narcotics in the production of analgesia in the rat. J. Pharmacol. Exp. Ther., 202(2), 411-428.

Yaksh, T.L. and Rudy, T.A. (1978). Narcotic analgetics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. Pain, 4, 299-359.

Yoshimura, M. and North, R.A. (1983). Substantia gelatinosa neurones hyperpolarized in vitro by enkephalin. Nature, 305(5934), 529-530.

Young, W.S. and Kuhar, M.J. (1980). Noradrenergic alpha 1 and alpha 2 receptors: Light microscopic autoradiographic localisation. Proc. Natl. Acad. Sci. USA., 77, 1696-1700.

Zayan, R. (1986). Assessment of pain in animals: epistemological comments. In: Assessing pain in farm animals. Eds. Duncan, I.J.H. and Molony, V. (Proceedings of 1984 workshop), Commission of European Communities, Luxembourg, 1-15.

Zhu, Y-S. and Szeto, H.H. (1989). Morphine-induced tachycardia in fetal lambs: a bell shaped dose response curve. J. Pharmacol. Exp. Ther., 249(1), 78-82.

Zieglgansberger, W. and Bayerl, H. (1976). The mechanisms of inhibition of neuronal activity by opiates in the spinal cord of the cat. Brain Res., 115, 111-128.

Zieglgansberger, W. and Tulloch, I.F. (1979). The effects of methionine and leucine enkephalin on spinal neurones of the cat. Brain Res., 167, 53-64.

Zimmerman, M. (1986). Behavioural investigations of pain in farm animals. Assessing pain in farm animals. Eds. Duncan, I.J.H. and Molony, V. Proceedings of 1984 workshop. Commission of European Communities, Luxembourg.

Zingoni, M.R., Garcia-Villar, R. and Toutain, P.L. (1982). Tolazoline as an antagonist of xylazine sedation in sheep. Revue De Medicine Veterinaire, 133(5), 335-339.

D28

ASSESSMENT OF PAIN PRODUCED BY CASTRATION AND TAILING OF YOUNG LAMBS. Wood, G.N.*, Mellor, D.J., Molony, V., Hodgson, J.C. and Fleetwood-Walker, S. Department of Preclinical Veterinary Sciences, R.(D.)S.V.S. and Animal Diseases Research Institute, Moredun, Edinburgh.

Previous studies have shown that castration and tailing are associated with behavioural and endocrinological changes which last for at least three hours. The magnitude and duration of such changes can be related to the intensity of pain assumed to be experienced in such procedures (Mellor & Murray, 1987). Experiments being carried out are designed to confirm the dependence of these changes on neural activity in the affected areas and to investigate the duration of this activity. Castration and tailing are being carried out on groups of lambs at 5 days of age as part of normal husbandry practice. Tight rubber rings are applied to the neck of the scrotum and tail with and without local anaesthesia in different groups. Local anaesthesia is obtained by prior infiltration of the spermatic cords, testis, scrotal skin and caudal epidural space with 2% lignocaine hydrochloride. Blood samples, behavioural and physiological measurements are taken at regular intervals before and after these treatments. The effects of this local anaesthesia on the behavioural indices measured will be presented.

Mellor, D.J. and Murray, L. (1987). J. Physiol. 386, 74P

C8

RESPONSES OF YOUNG LAMBS TO CASTRATION AND TAIL DOCKING AFTER EPIDURAL ADMINISTRATION OF MORPHINE OR XYLAZINE. G.N.Wood*, V.Molony J.C.Hodgson and S.M.Fleetwood-Walker, R(D)SVS and MRI,Edinburgh.

It has been shown previously that lambs castrated and tailed (C&T) at 5 days show changes in behaviour and plasma cortisol taken to be indicative of substantial pain and that these changes can be eliminated by treatment with local anaesthetic. In order to further test these indices and to see if the spinal mechanisms involved in nociception and pain in such lambs are similar to those of adult mammals, we have examined the effects of treatment with two analgesics on responses to C&T. Groups of six lambs were subjected to either 16mg of morphine (mainly μ agonist) or 40μg of xylazine (α_2 agonist), half introduced into the epidural space of the thoraco-lumbar and half into the lumbo-sacral spinal cord in 100μl of normal saline under halothane anaesthesia. Four groups of 5 lambs were used for each experiment, including treated and control groups. This xylazine treatment did not affect the changes in either plasma cortisol or behaviour; the morphine treatment produced complex effects on plasma cortisol and behaviour, but no clear evidence of analgesia. Ley et al. (1989) have shown that intrathecal clonidine (α_2 agonist) can produce marked analgesia, whereas intrathecal morphine was relatively ineffective in adult sheep. The significance of these results will be discussed

Ley,S., Dash,A., Waterman,A. and Livingston,A. (1989). Adv. in Biomed. Sciences (in press).

RESPONSE OF AFFERENTS IN THE SUPERIOR SPERMATIC NERVE OF RATS TO OCCLUSION OF THE TESTICULAR ARTERY AND VEIN.

B.D. Grubb*, V. Molony and G.N. Wood*, Department of Preclinical Veterinary Sciences, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, Scotland.

Poster 245
BLUE Th-Fri
Exhibit Hall
Abs No 785

AIM OF INVESTIGATION: To clarify the neural basis for the changes in plasma cortisol and behaviour produced by castration of lambs with tight rubber rings.
METHODS: Multiunit recordings were made from afferents in the main trunk of the superior spermatic nerve in 5 urethane (0.6 ml/25% w/v/100 g) anaesthetised rats. The presence of activity from the testis was confirmed by non-noxious and noxious mechanical stimulation of the testis. Neural activity was continuously recorded before and for up to 150 mins after the testicular artery and vein were clamped. The effectiveness of occlusion of the blood supply to the testis was determined by injection of Trypan blue dye into the systemic circulation. The spermatic cord at the recording site was fixed for microscopic examination in two rats.
RESULTS: Background afferent activity was present from the start of recording at frequencies up to about 40 i.p.s. and increased activity was produced by mechanical stimulation of the testis at qualitatively non-noxious and noxious intensity. Occlusion of the testicular artery and vein produced a gradual increase in afferent activity over the recording period of more than 300%. Experiments were stopped before afferent activity decreased. The fibre spectrum of the nerve was obtained from electronmicroscopic examination.
CONCLUSIONS: Activity in testicular afferents in anaesthetised rats continues and increases for more than two hours after occlusion of the blood supply. If the same response can be shown in lambs it will help to explain the signs of pain shown after castration of lambs by application of tight rubber rings to the neck of the scrotum. For this to occur the superior spermatic nerve(s) must continue to conduct through the rubber ring.

RESPONSES OF YOUNG LAMBS TO CASTRATION AFTER INTRATHECAL ETORPHINE OR XYLAZINE.

G.N. Wood*[1], V. Molony[1], J.C. Hodgson*[2] and S.M. Fleetwood-Walker[1], [1]Department of Preclinical Veterinary Sciences, R.(D.)S.V.S., The University of Edinburgh and [2] The Moredun Research Institute, Edinburgh, Scotland.

Poster 198
BLACK Mon-Tues
Exhibit Hall
Abs No 241

AIM OF INVESTIGATION: To produce spinal analgesia in young lambs in order to extend investigations of methods for the recognition and assessment of pain in lamgs produced by various methods of castration.
METHODS: Six groups of six (5 day old) lambs were treated intrathecally with saline, etorphine or xylazine at doses 75% of those which produced obvious ataxia and sedation. 100 µl of saline with or without xylazine or etorphine were injected intrathecally under halothane 1.5% anaesthesia at T_{10-11} and L_{6-7}. Twenty minutes after recovery from anaesthesia the lambs were handled as for castration or were castrated with tight rubber rings applied to the neck of the scrotum. Castration using this method is a common husbandry practice in the U.K. and elsewhere. These experiments were permitted as part of studies designed to improve regulation of such procedures. Blood samples for measurement of changes in plasma cortisol were taken before and during the three hours after treatment. Behaviour was also recorded.
RESULTS: Neither etorphine nor xylazine significantly changed the responses used here as indices of pain produced by castration. Both etorphine and xylazine did produce some sedation as indicated by changes in behaviour in both castrated and control groups.
CONCLUSIONS: Intrathecal etorphine (opioid) and xylazine (α_2 agonist) did not produce measureable analgesia in young lambs as assessed by their behavioural and plasma cortisol responses to castration with tight rubber rings. Alternative approaches must be sought for investigation of the reliability and sensitivity of the methods used for recognition and assessment of pain in young lambs.