DUODENAL SENSORY AND REFLEX MECHANISMS

BY

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To the memory of W.B.M.

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The rest of the work is mine.

signed :

FOREWORD

AMOR CON LE SUE MAN GLI OCCHI M'ASCIUGA,
PROMETTENDOMI CARA OGNI FATICA;
CHE VILE ESSER NON PUO CHI TANTO COSTA.

Michelangelo Buonarroti (1623), from Sonnet XXVII.

(Yet with his hands Love wipes my weeping eyes,
saying, this toil will end in happy cheer;
what costs the heart so much must needs be dear.)

DUODENAL SENSORY AND REFLEX MECHANISMS

RESULTS

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SUMMARY

Duodenal sensory and reflex mechanisms were examined during acute experiments with isolated loops of duodenum in anaesthetised rabbits and sheep.

Mechanical stimulation of the rabbit duodenum caused reflex motor responses in adjacent segments of the gut. The thresholds of these reflexes were the same as the thresholds of mechanoreceptors whose afferent activity was recorded in duodenal nerves during electrophysiological investigations. These mechanoreceptors were situated in the muscularis externa, the serosa and the mesentery. Mechanoreceptors in the muscularis externa were excited when the duodenum was perfused with chemical solutions containing glucose.

In the sheep duodenum the impulse activity of afferent units from slowly adapting mechanoreceptors in the muscularis externa coincided with local electromyographic (emg) and tension changes. Units were excited by passive distension, compression, active increases in muscle tension and alterations of temperature. The application of chemical solutions or light mechanical stimulation of the mucosa evoked intrinsic reflexes which modified impulse activity. Receptors were excited by injections of peptide hormone analogues and other drugs. With the exception of insulin, the responses evoked by drugs coincided with increases in muscle tension and emg activity.

Slowly adapting mechanoreceptors in the sheep duodenal mucosa were either silent in the absence of a mechanical stimulus or were spontaneously active. Mucosal receptor units were selectively sensitive to chemicals. One class of unit was excited by solutions of potassium chloride and another was excited by volatile fatty acids. No evidence was found for mucosal receptors sensitive to osmolality or temperature changes.

It was concluded that : (a) the mechanoreceptors in the muscularis externa of the sheep duodenum are tension receptors which occupy a position "in series" with longitudinal muscle; (b) post-prandially released alimentary hormones may increase impulse activity in duodenal tension receptors and thus, by a reflex mechanism, modify ingestive behaviour and gastric emptying; (c) spontaneously active mucosal receptors are associated with contractile elements in the muscularis mucosa; (d) the responses of mucosal receptors to chemicals in duodenal chyme makes them poor candidates for providing tonic afferent inputs to the central nervous system; (e) these mucosal mechanoreceptors do not initiate reflexes requiring large mechanical stimuli; and (f) the sensory receptors studied do not play a part in duodenal reflex mechanisms initiated by osmotic changes.

GENERAL INTRODUCTION

This thesis concerns the study of sensory receptors (enteroceptors) in the mammalian duodenum. The main experimental approach has been to use acute electrophysiological techniques. A better understanding of duodenal sensitivity is needed to improve our knowledge of the involvement of the duodenum in alimentary reflexes particularly those modifying gastric emptying. This understanding of duodenal sensitivity is important also for clinical reasons : selective vagotomy for the relief of gastric and duodenal peptic ulceration in man has often given unsatisfactory side effects (Dragstedt & Woodward, 1968; Schrock, 1975; Ward, 1976), and although many of these effects are due to the interruption of extrinsic motor control, many of the projecting afferent axons from sensory receptors are also divided. Under these conditions there develops a duodenal osmotic overload which together with intestinal distension contributes to the post-vagotomy "dumping syndrome" (Roberts, 1967).

Control by the duodenum involves both nerves and hormones. These systems are best considered as an integrated mechanism (Dochray & Gregory, 1980). The intraluminal stimuli which initiate hormonal release and neural reflexes are often the same (Makhlouf, 1974). Both mechanisms work cooperatively to effect the ordered progress of digesta along the alimentary canal.

THE INVOLVEMENT OF THE DUODENUM IN VEGETATIVE AND SOMATIC REFLEXES

In common with other segments of the alimentary canal the duodenum initiates reflexes in both oral and aboral contiguous, as well as distant, structures. Its own activity can also be modified by events in these and other structures. It is therefore possible to formulate the concept of the duodenum as both a reflexogenic as well as a reflex target organ.

The development of the concept of duodenal control of alimentary functions has been based upon experiments in which reflex events were observed. The term "reflex" has often been used loosely in gastrointestinal physiology, to mean "cause and effect" and does not necessarily implicate neural pathways, which confuses both the Sherringtonian concept as well as our understanding of the mechanisms involved. The reflex events may or may not involve both afferent and efferent nerves, for example, they may be neurohormonal. Neural involvement may be implied when the latency is shorter than the local circulation time, and when reflexes are absent following selective neurotomy. The question arises whether reflexes are caused by "myenteric", "axon reflex" or "long extrinsic" neural mechanisms. "Myenteric" implies an intact submucous and myenteric plexus with short chain transmission (Hirst & McKirdy, 1975); "axon reflexes" are peripheral reflexes occurring outside the intestinal wall, but not necessarily projecting to the CNS and may involve ganglia (Szurszewski & Weems, 1976); "long reflexes" involve either an afferent or efferent limb or both, and the CNS (Thomas & Baldwin, 1968).

APPROACHES USED TO DEMONSTRATE REFLEX PATHWAYS

Evidence for the involvement of nerves in duodenal activity has accumulated from a number of sources; from non-invasive techniques which include serial test meals, oral catheterisation and X-radiology, and invasive techniques which may be minimally disruptive, like electromyography (emg), and more grossly disruptive cannulation and cross-perfusion studies. Extensive surgery must affect the normal function of the area and often, when preparations are reported as being totally denervated, they are probably only partly so, because the interconnections in the alimentary canal are complex and do not follow "classical" pathways. Total functional neurotomy probably only occurs when the viscus is removed and transplanted to a remote site. Therefore the techniques of truncal vagal and splanchnic neurotomy are of limited analytical value, because they cannot answer the questions concerning enteroceptor specificity and location, nor whether these extrinsic nerves are involved in an afferent or efferent role. Local ganglionectomy is only of value when the afferent and efferent connections to it are fully known, and cordotomy may be of value in eliminating intersegmental reflexes when these are suspected. The significance of electrical excitation of nerves must be interpreted with caution because afferent and efferent axons of many unrelated organs are excited unnaturally. Drugs, combined with reflex and electrophysiological studies, have been used to sensitise, stimulate or anaesthetise enteroceptors; to reversibly block the afferent limb; to excite or depress central function; and either mimic transmitters at various levels of the autonomic nervous system or to specifically block effector pathways. The many different techniques each in their turn provide some insight into the same mechanism. They have been used to examine, or allow us to conclude, the probable role of the duodenal enteroceptors in the integrated control of alimentary function.

There is a large body of knowledge which relates to the problem of duodenal sensation. In this literature survey the following points have been examined in particular detail :

1. The extrinsic and intrinsic neurones of the alimentary canal function as part of an integrated system, in which putative neurotransmitters and hormones combine to modulate smooth muscle excitability.

2. The sensitivity and the location of sensory receptors may be concluded from experiments involving reflex events.

REVIEW OF THE LITERATURE

THE DUODENUM AS A REFLEXOGENIC ORGAN

INTRINSIC REFLEXES AND THE CONTROL OF MYOGENIC ACTIVITY

In common with most of the alimentary canal the duodenum undergoes peristalsis. That is, moderate distension causes the reflex, or series of reflexes, associated with an aboral progress of a wave of relaxation followed by a wave of contraction (Bayliss & Starling, 1899). The intrinsic nerves integrate gut motility which occurs independent of extrinsic nerves and of the mucosa (Diament, Kosterlitz & McKenzie, 1961). There are mechanoreceptors associated with muscularis externa and those in longitudinal muscle have a relatively lower tension threshold than those associated with circular muscle, and both muscles behave independently (Hukuhara, Nakyrama & Namba, 1959; Kosterlitz, 1968). Models have been proposed for the neural network involved (Hirst & McKirdy, 1975; Daniel, 1977). Included in Auerbach's (myenteric) and Meissner's (submucous) plexuses are small ensembles of ganglion cells which contribute nerve fibres to a synaptic neuropil within each ganglion. These neuronal connections compose an integrative circuitry which processes sensory information and generates nervous output that is immediately appropriate for control of a localised section of the gastro-intestinal musculature (Wood, 1976). There may be two separate descending nervous pathways in the small intestine concerned with peristalsis which mediate descending inhibition and excitation of the smooth muscle. The descending excitatory pathway may involve both the myenteric and submucous plexus. The submucous plexus may not be involved in "long" ascending or descending pathways in the wall of the gut but its role may be confined to a more local integrative action (Hirst, Holman & McKirdy, 1976).

It is firmly established that the inhibition of circular muscle at the end of the myenteric plexus chain is by non-adrenergic inhibitory neurones (Bennet, Burnstock & Holman, 1966). The major control exerted by sympathetic nerves is inhibition by nor-adrenaline, acting on α-receptors. They inhibit the activity of intrinsic cholinergic neurones (Furness & Costa, 1974). In addition, the intestine contains neural inhibitory pathways which are neither adrenergic nor cholinergic which may be involved in descending inhibition (Burnstock, 1979). The substance released may be a purine nucleotide, although in the dog in vivo intestine close intra-arterial injections of adenine nucleotides cause excitation (Burks & Grubb, 1978). There is also evidence that 5-hydroxytryptamine-containing myenteric neurones are present and these may be associated with vagal inhibitory pathways to the stomach (Gershon & Dreyfuss, 1977). Probably the inhibitory intrinsic neuronal control of the gut is similar everywhere and differences in its manifestation depend upon the presence or absence of "active tone". In the absence of tone, relaxation cannot occur, and only inhibition of an evoked contraction can be observed (Daniel, 1977).

Gastro-intestinal muscle undergoes an oscillation of membrane potential without an external driving or known input. This is called the basal electrical rhythm (BER) or electrical control activity (ECA). These oscillations occur in the stomach and intestine continually whether motor activity is present or not. When motor activity occurs it is at the frequency of the oscillations and the mechanical response is always associated with spiking and the release of acetylcholine (electrical response activity, ERA, or motor profile). Increasing the rate of acetylcholine release increases the amplitude of ERA and contractions, but not the frequency of contractions. In summary, the occurrence of contractile events depends on the nervous activity and acetylcholine release (ERA) whereas the timing of contractions is myogenically controlled (BER) (Sarna, 1975).

The most commonly recognised recurring spiking activity of ERA has been called the migrating myoelectric complex (MMC) (Szurszewski, 1969). There are three distinct phases : 1. a prolonged period of irregular spiking activity (ISA) associated with propulsive movements; 2. regular spiking activity (RSA) associated with non-propulsive segmental activity; 3. quiescent phase (Grivel & Ruckebusch, 1972; Bueno, Fioramonti & Ruckebusch, 1975). The MMC possibly originates in circular muscle layers (Ruckebusch, 1972).

In the dog the MMC occurs in the starvation and post-prandial states and the term "interdigestive MMC" was introduced (Szurszewski, 1969; Carlson, Bedi & Code, 1972; Code & Marlett, 1975). In the ad libitum fed pig, rabbit, and sheep the MMC appears at all times (Grivel & Ruckebusch, 1972; Bueno, & Ruckebusch, 1976). It is probable that the MMC is a basic pattern of activity of the gastrointestinal tract in all mammals which is interrupted in some species during feeding and post-prandial states (Ruckebusch & Bueno, 1977a). Gastric distension and liquids and nutrients introduced into duodenum interrupt interdigestive activity (Code & Marlett, 1975). The local reflexes to the bulk of digestive contents seem to be of prime importance in regulating ISA duration, the role of the vagus nerve being that of local reinforcement and adjustment of the response (Ruckebusch & Bueno, 1977a) presumably involving both afferent and efferent nerves.

THE COORDINATION OF THE GASTRODUODENAL JUNCTION

The BER is low in the pyloric antrum (5 min^{-1}) and high in the duodenum (18 min^{-1}) (Bass, Code & Lambert, 1961). The BER is transmitted myogenically along longitudinal muscle because in some species there is an electrical insulating barrier in circular muscle at the pylorus (Edwards & Rowlands, 1968). No relationship has been shown between BER in the antrum and duodenum of the dog, however, coordination has been seen between antral BER and duodenal ERA in fed dogs (Edwards & Rowlands, 1968). Feeding therefore seems to be a phase-locking mechanism. The extrinsic nerves do not appear to play a decisive part in the correlation between the electrical activities of the dog stomach and duodenum (Atanassova, 1976). There is coordination of ERA of the gastric and duodenal walls during hunger peristalsis and balloon inflation of the stomach and after feeding, but not BER after thoracic vagotomy and sympathectomy. Gastroduodenal section abolishes ERA coordination and also increases the frequency of duodenal ERA (Atanassova, 1976). Presumably there is some restraint by the pylorus upon duodenal ERA in the normal animal. Hexamethonium blocks the ERA and probably myenteric plexus neurones are involved.

EXTRINSIC REFLEXES

In reviewing the role of extrinsic nerves in duodenal reflexes, particular emphasis is paid to the reflex threshold, in an attempt to determine the enteroceptor sensitivity.

Mechanically Stimulated Reflexes

A reduction in gastric emptying and tone, and slowing of antral rhythm, "the enterogastric reflex", can be induced by mechanical stimulation of the duodenum (Thomas & Mogan, 1931; Herrin, Meek & Mathews, 1933; Lalich, Meek & Herrin, 1936; Thomas, 1957). The threshold for the reflex in the dog is 95 - 177 cm water and involves the vagus and splanchnic nerves (Lalich et al, 1936). Prolonged distension (102 cm water) reduces the threshold of the reflex (Lalich, Herrin & Meek, 1936). The threshold is probably lower because active contraction of the duodenum also causes temporary slowing of antral rhythm in dogs (Daniel & Wiebe, 1966), by a reflex which is reserpine-sensitive, abolished by bilateral vagotomy and sometimes pyloric section. Daniel & Wiebe (1966) suggest that the afferent nerves for this reflex run in the vagus, and efferent nerves run in the sympathetic system, and that active firing of sympathetic nerves is probably involved rather than inhibition of vagal excitation.

The duodenal bulb may be the site of organisation of the phase of regular spiking activity of the migrating myoelectric complex (MMC) in the sheep, and the starting point of an inhibitory effect on antral motility (Ruckebusch & Bueno, 1977b) which may result from an enterogastric reflex similar to that produced by duodenal distension in dogs (Daniel & Wiebe, 1966). This reflex inhibition has also been observed in calves (Bell & Watson, 1976).

Diathermy of the duodenal wall and blunt mechanical probing cause gastric relaxation by a vagal route (Abrahamsson, Glise & Glise, 1979). Blunt mechanical stimulation in the antral-duodenal area causes gastric relaxation by a mechanism which is insensitive to anti-adrenergic (guanethidine) and anti-cholinergic (atropine) drugs and which is abolished by vagotomy (Abrahamsson, Glise & Glise, 1979).

Duodenal distension also causes pyloric closure (Luchardt, Phillips & Carlson, 1919; Thomas, Crider & Mogan, 1934). Thomas & Crider (1935) found that the reflex was stimulated by physiological levels of intra-duodenal pressure (10 - 20 cm water). Intraluminal pressures above 30 cm water cause pyloric spasm (Thomas & Crider, 1935).

Rapid (2 - 3 seconds) inhibition of electrical and mechanical activity in the aboral small intestine is also caused by distension in the range 81 - 109 cm water (Herrin et al, 1933; Youmans, 1944; Thomas, 1957). The reflex is sensitised by prolonged distension at 48 - 136 cm water (Peterson & Youmans, 1945) and is independent of the vagi but involves the splanchnic nerves (Youmans, 1944). The reflex is possibly ipselateral, homosegmental and involves the spinal cord, but may also be due to axon reflexes at high thresholds (Youmans, 1968).

In anaesthetised and decerebrate preparations, the reflexes of pupillodilation, raised blood pressure, limb movement and the panniculus reflex are caused by stretching of the mesentery and spasm of the duodenum (induced by barium chloride) (Irving, McSwiney & Suffolk, 1937), handling and pinching (Downman & McSwiney, 1946), compression, cutting serosa and muscle, scratching, rubbing and distension with a balloon (Downman, McSwiney & Vass, 1948). There is however considerable threshold variation (Irving et al, 1937) making quantitation by this method difficult (Downman & McSwiney, 1946). The splanchnic nerves are important (Downman & McSwiney, 1946). Cutting, burning, and scraping the serosa or mucosa do not cause these reflexes (Irving et al, 1937).

Dobromyslova (1957) found that transmural compression was more effective than balloon distension in causing blood pressure changes. Venous occlusion in the mesentery may cause tachypnoea (Bean, 1952) and vomiting may result from mechanical irritation of the duodenal mucosa (Luckhardt et al, 1919).

In conscious man duodenal distension causes vasoconstriction of the skin (Carmichael, Doupe, Harper & McSwiney, 1939) and it is possible to cause painful sensations by distension of the duodenum (Payne & Poulton, 1923, 20 - 30 cm water; Bloomsfield & Polland, 1931, 82 - 163 cm water; both quoted by Irving et al, 1937).

There is synchronisation between duodenal tone and the tone of the sphincter of Oddi causing changes in bile flow (Wyatt, 1967). The correlation of ERA along the cystic and common bile ducts of sheep, with the duodenal propulsion of the bolus, so that bile is delivered into the duodenum just before the arrival of the bolus at the level of the sphincter, strongly suggests the involvement of extrinsic nerves (Bueno & Praddaude, 1979).

Chemically Stimulated Reflexes

There is a great deal of interest in the reflex effects of various chemicals in the duodenal lumen, particularly the reflex inhibition of gastric motility "the chemical enterogastric reflex". Experimental design has been to take each particular chemical and study its effect on gastric emptying, motility, electrical activity or secretion. Quite often the stimulus used appears to bear little resemblance to the normal composition of chyme.

Many of the long latency effects involve hormones but also the extrinsic nerves are important. The evidence for the role of the vagus nerve in "chemical reflexes" is still unsettled. Neurectomy alone cannot decide whether afferent or efferent axons or hormones are involved. Vagotomy abolishes the enterogastric reflex to acid (Thomas et al, 1934), proteose and peptone (Crider & Thomas, 1938), fat, acid, hypertonic and hypotonic solutions (Quigley & Meshan, 1938) and stretch and chemicals (Cragg & Evans, 1960). However, selective duodenal extragastric vagotomy does not effect the duodenal "brake" in the dog to HCl, dextrose, water and fat (Shahidullah, Kennedy & Parks, 1975).

In general, the evidence cited below suggests that the chemistry of the duodenal chyme plays an important role in the intestinal phase of gastric digestion, both by stimulating duodenal sensory receptors and by releasing duodenal hormones. The reflex effect of this is to modulate the efferent activity of the vagus nerve to the stomach differentially, either by excitatory (cholinergic) or inhibitory (non-adrenergic, non-cholinergic) nerves, which either increases gastric secretion and motility or causes slowing and receptive relaxation. In addition, the splanchnic nerves and preganglia may be involved.

Reflexes Induced by Fats, Fatty Acids and Soaps

Fats introduced into the duodenum delay gastric emptying (Shay & Gershon-Cohen, 1934; Quigley, Zettleman & Ivy, 1934). This reflex is abolished by procaine on the duodenal mucosa (Quigley & Meshan, 1937; Best & Cohnheim, 1910, quoted by Thomas, 1957) and vagotomy (Quigley & Meschan, 1938; Roze, Couturier, Chariot & Debray, 1977). Myristic acid is most effective in man (Hunt & Knox, 1968b). It is probable that the putative hormone "enterogastrone" is involved, and that a local reflex mechanism for release exists which is facilitated by vagal action (Thomas & Baldwin, 1968). Fats also inhibit gastric secretion of acid (Pavlov, 1910; Shay, Gershon-Cohen & Fels, 1939) after a long latency of 15 - 30 minutes and prior digestion is necessary (Sircus, 1958).

The nervous system has been implicated by Hunt & Knox (1954) who demonstrated a short-latency slowing of gastric-emptying by fatty acid and a short persistence of the inhibition. Olive oil causes a rapid reflex in the pig which is abolished by vagotomy (Roze et al, 1977). Fat receptors seem to be absent from the first 5 centimeters of the duodenum in the dog (Cooke & Clarke, 1976).

Acid Induced Reflexes

Hirsch (1893) and von Mering (1893) first demonstrated that acid introduced into the duodenum causes a delay in gastric emptying (quoted by Shay & Gershon-Cohen, 1934). It is possible that Serdjukov (1898/9), a pupil of Pavlov first appreciated its mechanism and that Lutvarev (1901) understood the role of the pancreas in titration (quoted by Shay & Gershon-Cohen, 1934). The reflex inhibition of the pars pylorica and change in pyloric sphincter tone was first called "enterogastric reflex" by Thomas & Mogan (1931) who subdivided the reflex into: a rapid, primary increase in tonus of the pyloric sphincter, a relaxation of the sphincter by a myenteric mechanism and a later inhibition of the pars pylorica which they thought was mediated by a vagal efferent mechanism as it was abolished by vagotomy (Thomas et al, 1934). The primary reflex was not abolished but was augmented by vagotomy; when the stomach was full the primary contractions were less easily demonstrated (Thomas et al, 1934).

The acid enterogastric reflex is rapid and possibly involves a neural mechanism which is abolished by vagotomy (Cragg & Evans, 1960), although Schapiro & Woodward (1955, 1959) were only able to abolish the effect of large quantities of acid in the jejunum (N/10 HCl, 100 ml) by coeliac-ganglionectomy. The proximal 5 cm of the dog duodenum is very sensitive to acid at concentrations above 0.24 - 0.36 mEq HCl, less than those necessary to cause significant secretin release. The reflex is rapid in onset (less than 1 minute), suggesting a neural mechanism (Cooke, 1974, 1975; Cooke & Clarke, 1976). Acid in the jejunum, but not the second-to-fourth segments of the duodenum, slows gastric emptying (Cooke, 1977). It is possible that intra-jejunal acid reduces a jejunal flow rate due to increased contractile activity, probably in circular muscle (Summers, 1978). The acid reflex has also been demonstrated in the ruminant (Salmin, 1960; Bell & Grivel, 1975) and the pig (Roze et al, 1977). In the pig there is a rapid (1 - 2 min) antral response which is reduced by vagotomy, the gastric body response being completely abolished.

It is unlikely that gastric emptying is influenced by the pH of an acid (Hunt & Knox, 1962). Probably the titratable acidity is important, the mechanism titrating to about pH 6.5, the pancreas and Brunner's secretion neutralizing the chyme and thus abolishing the cause of the reflex, so that a steady-state occurs (Hunt & Knox, 1962). High molecular weight strong acids are more effective than low molecular weight strong acids, possibly due to an osmotic effect; high molecular weight weak acids are less effective than low molecular weight weak acids (Hunt & Knox, 1969, 1972). The oil-water partition coefficient is apparently unimportant (Hunt & Knox, 1969).

The reflex is unaffected by intravenous hexamethonium (a nicotinic blocker) and may be unchanged or slowed by propanolol (beta$_1$ and beta$_2$ blocker), and is inhibited by intravenous guanethidine (which prevents nor-adrenaline release) and intramuscular reserpine (adrenergic depleter) (Cooke & Clarke, 1976). The inhibitory effect is reduced by subcutaneous atropine (muscarinic blocker), intravenous phenoxybenzamine (alpha$_1$ blocker) and phentolamine (alpha$_2$ blocker) (Cooke & Clarke, 1976). Thus the efferent limb of the reflex is probably adrenergic but neither alpha nor beta receptor mediated (Cooke & Clarke, 1976).

Acid in the duodenum also inhibits gastric acid secretion, a phenomenon probably first demonstrated by Sokolov (quoted by Babkin, 1950; Shay, Gershon-Cohen & Fels, 1942) and Pincus, Thomas & Rehfuss (1942) showed that a threshold of pH 2.5 and below in the duodenum is necessary. The rapidity in dogs (Code & Watkinson, 1955; Konturek & Johnston, 1971) and goats (Singleton, 1951) suggests a neural mechanism. The innervated gastric mucosa is important (Code & Watkinson, 1955) and the reflex may be inhibited by intraduodenal procaine (Sircus, 1958). Andersson believes that the duodenal bulb is most important and that a hormone, bulbogastrone, is produced to cause this effect (Andersson, 1972; Andersson, Nilsson & Sjodin, 1976), although Konturek & Johnson (1971) suggest that a neural mechanism from the duodenal bulb exists which is sensitive to 8 mEq HCl per hour. Cooke (1974) has also shown that the proximal 5 centimeters is involved.

Under some conditions duodenal acid may stimulate the secretion of gastric acid (Sircus, 1953; Code & Watkinson, 1955). Pepsinogen secretion is also stimulated by duodenal acid (Brooks, Isenberg & Grossman, 1969; Nakajima & Magee, 1970; Wormsley, 1971; Bell and co-workers, see below).

Rune (1968) has suggested that the raised tension of CO_2 generated from bicarbonate buffering of acid and subsequent dehydration of carbonic acid may be responsible for some of the effects of H^+ ions in the duodenum. A more detailed suggestions has been made by Hunt & Knox (1972).

Hunt & Knox's model for receptor responding to acid (1972) :

On the basis of human reflex experiments Hunt & Knox have proposed a model for an alimentary enteroceptor which responds to acid, with a threshold of 5 - 10 mM HCl and titrating to end point pH 6.5 Such a model has two components :

1. Either a molecular sieve which makes weak acids with high molecular weight less effective than weak acids with low molecular weight, or an aqueous diffusion pathway along which protons diffuse. The walls of this pathway (or sieve) are held at pH 6.5 so that acids with large oil/water partition coefficients, which might otherwise diffuse away, are charged and poorly lipid soluble. Chloride ions may be lost from the diffusion pathway, taking H^+ ions with them which do not therefore reach the titration site if the mucosal threshold concentrations is not reached. H^+ ions in close proximity to walls may be neutralised by HCO_3^- ions or a chloride/bicarbonate exchange mechanism occurs, activated by H^+ ions, and the resulting CO_2 produced diffuses away.

2. A titrating chamber, possibly 500 µm below the mucosal surface which estimates the acid concentration by titration of protons to pH 6.5, and recognises acids by their molecular weight. Bicarbonate ions enter the chamber to maintain the standard pH 6.5. Protons are absorbed and excreted unchanged, or metabolised to bicarbonate. The p CO_2 produced by titration produces a graded receptor potential in the receptor cell.

Thus all acids become carbonic acid which explains why the response is largely independent of pK of the acid in question.

Reflexes Initiated by Protein Digestion Products, Proteoses, Peptone and Amino-Acids

Protein duodenal meals cause reflex inhibition of gastric motility (Thomas & Crider, 1935, 1939). The reflex is abolished by vagotomy (Crider & Thomas, 1938). Monoamino and monocarboxy acids only are effective, 49 mM tryptophan is more effective than glycine by a mechanism which is not due to osmoreceptors (Thomas, 1942; Stephens, Woolson & Cooke, 1975). The potent CCK-PZ liberators L-phenylalanine, L-valine and L-methionine do not effect gastric emptying; in dogs the L-tryptophan threshold is 4 mM (with maximum effect at 40 mM) (Stephens et al, 1975). Other amino acids possibly work as osmoreceptors and are effective because of molar concentrations and not molecular weight. In oral trials Cooke & Moulang (1972) suggest that a threshold of 200 mM and upwards is due to osmotic pressures, whereas duodenal perfusion studies in dogs show the threshold is 80 mM and above (Stephens et al, 1975). Forty mM tryptophan causes reflex changes in the first 5 centimeters and second-to-fourth part of the duodenum and the jejunum in dogs (Cooke, 1977). The amino acid composition of normal chyme is unknown.

Twenty percent peptone solutions (1600 mOsm KG^{-1}; pH 5.7; Na, 203 mM; K, 350 mM; Cl, 135 mM) in the duodenum raise the lower oesophageal sphincter pressure after a long latency (15 - 20 min), by a mechanism which may involve gastrin (Lepsien, Koelz, Weiser, Blum & Siewart, 1978). Duodenal amino acids have no effect on gastric motility or emg in pigs (Roze et al, 1977). Semidigested meat in chyme, introduced into the jejunum stimulates secretion of acid from gastric pouches (Sircus, 1953).

Carbohydrate Induced Reflexes

Cannon (1898, 1904, quoted by Thomas, 1957) noted that glucose inhibited gastric emptying, although not to the same extent as acid or fat. Twenty-five percent glucose into the canine duodenum (Lecompt, 1900, quoted by Day & Komarov, 1939), 20 - 30% glucose in adult goats (Singleton, 1951) and 10% glucose in pigs (Roze et al, 1977) inhibit gastric motility. Day & Komarov (1939) suggest that a peripheral osmoreceptor phenomenon caused by 10 - 40% glucose may be due to hypertonicity of body fluids which is actually "sensed" centrally. The effect in pigs is completely abolished by vagotomy (Roze et al, 1977).

Glucose also inhibits gastric secretion (Day & Komarov, 1939; Shay et al, 1939) by a mechanism which is not abolished by duodenal procaine (Sircus, 1958) nor by vagotomy (Quigley & Halloran, 1932).

Duodenal Osmotic Reflexes

Thomas & Baldwin (1968) remind us that "the evidence for the existance of osmoreceptors in the intestine has been widely accepted by physiologists but one feels that there is a tendency to over emphasise their importance, particularly their versatility. For example, attempts have been made to explain the effects of carbohydrates, products of protein digestion and even fats on the rate of gastric emptying on the basis of the action of these substances on osmoreceptors ... It is difficult to believe that protein and products of protein digestion occur in the intestine in concentrations sufficient to have significant osmotic activity". The suggestion has been made that isotonic (300 mOsm Kg^{-1}) solutions are ineffective and that hypertonic and hypotonic solutions are effective. "It is discussed, without evidence, that both solutions stimulate the same receptor"(Thomas & Baldwin, 1968).

The evidence for the initiation of duodenal reflexes by osmotic activity is substantial (Carnot & Chassevant, 1905, 1906, quoted by Thomas & Baldwin, 1968; Hunt & Pathak, 1960; Bell & Razig, 1973a; Cooke, 1975). Apperly (1926) states that the osmotic or salt values of the duodenum, determine the acidity and rate of evacuation of the stomach, and Shay & Gershon-Cohen (1934) found that isotonic solutions of salts were unable to initiate the duodeno-pyloric reflex, while hypertonic and hypotonic solutions did so readily. Sircus (1958) suggested threshold values of below 275 mOsm Kg^{-1} and above 425 mOsm Kg^{-1}. There may be species differences, for in adult goats there is a reduction in abomasal motility by 20 - 30% glucose but isotonic glucose solutions are not effective (Singleton, 1951), whereas isotonic glucose may inhibit abomasal emptying in pre-ruminant calves (Bell & Razig, 1973a). Hypotonic and isotonic NaCl and $NaHCO_3$ increase abomasal emptying. Hypertonic solutions and any solution of KCl, $CaCl_2$, glucose and HCl inhibit emptying (Bell & Mostighani, 1975).

Osmotic enterogastric reflexes are abolished by vagotomy (Quigley & Meshan, 1938), and Sircus (1958) states that it is a nervous reflex in the dog, which is inhibited by mucosal anaesthetics. Konturek & Grossman (1965a, 1965b) suggest that the whole of the duodenum is osmosensitive, although it is possible that receptors are not present in the first 5 centimeters in the dog duodenum (Cooke & Clarke, 1976). Hypertonic solutions are effective in canine jejunum but not duodenum (Cooke, 1977). Elias, Gibson, Greenwood, Hunt & Tripp (1968) provide evidence that the site of osmoreceptors is postpyloric in man, because they find that 1 mole of maltose or lactose is equivalent in slowing emptying to 2 moles of monosaccharide, and as disaccharidases are in the brush border of the small intestine then osmoreceptors must be located deep to these hydrolytic enzymes. Meeroff, Go & Phillips (1974) with steady-state perfusion in man, support this view by showing osmoreceptor reflexes from the second-to-fourth part of the duodenum, but not jejunum. The stomach was excluded because hypertonic gastric solutions leave at control rates when the duodenum is isotonic. Because of the long latency of the response it is not known whether this is a neural or hormonal reflex in man (Meeroff, Liang & Phillips, 1975).

Serosal Chemoreceptor Reflexes

Reflex pupillodilation and limb movements are caused by application of KCl (348 mOsm Kg^{-1}), NaCl (3418 mOsm Kg^{-1}) and HCl (1 - 0.1 N) to the intestinal serosa (Downman et al, 1948), but the physiological significance of this reflex is unknown.

Nutritive Density

It has been suggested that duodenal receptors are sensitive to the energy content of food in man (Hunt & Stubbs, 1975). The greater the nutritive density of the meal the less is the volume transferred to the duodenum. There is equal slowing by iso-caloric amounts of fat, protein and carbohydrate.

Reflexes Caused by Alkali

Serdjukov (1889, 1899, quoted by Shay & Gershon-Cohen, 1934) was able to retard alkali emptying by acid in the duodenum. Alkali increases gastric emptying (Shay & Gershon-Cohen, 1934) and intraduodenal alkali causes a rapid fall in gastric pressure which is abolished by vagotomy (Cragg & Evans, 1960). Alkalinisation of the duodenal bulb (pH = 11) in fasted dogs, causes increased ERA in small intestine, and in sheep increases MMC activity throughout the infusion time (Bueno & Fioramonti, 1980).

Reflexes Initiated by Thermal Stimuli

Thermal stimulation of the exposed intestine in the anaesthetised, decerebrate and spinalised cat causes dilation of the pupil, leg movements and pressor reflexes at thresholds on the serosa of 42^{O}C and the mucosa of 80^{O}C (Downman et al, 1948).

An alteration of ventilation rate in the conscious sheep is caused when peritoneal heating plaques increase the local temperature to 42 - 44^{O}C although the cutaneous surface temperature rises only 1^{O}C above core temperature (Rawson & Quick, 1970). Cutaneous heating is without effect. Alteration of intra-ruminal temperatures, when the environmental conditions are changed, induces or stops panting or shivering (Rawson & Quick, 1972). These reflexes can be abolished ipselaterally by splanchnotomy - a suggestion that the splanchnic innervation of the ruminant gut is organised viscerotopically. It was suggested that thermoreceptors with a threshold of 0.75^{O}C alterations of core temperature may be in the mesenteric veins or gastrointestinal tract walls. The possibility that the splanchnic innervation of the parietal peritonium (which is possibly segmentally arranged) is involved was not discussed or excluded. These thermoreceptors might well be sensitive to the temperature gradient which exists across the rumen wall; the rumen temperature is 0.5 - 1.0°C above vaginal temperature in non-fasting sheep (Rawson & Quick, 1970).

In the cat both warm and cold changes in the duodenum reduce emg activity in the gastric antrum, a "reflex" which is inhibited by bi-vagotomy, but not bisplanchnotomy (El Ouazzani & Mei, 1979).

Serial Test-Meals, Their Use in Ruminants

The serial test-meal method used by Hunt & Knox and co-workers has contributed extensively to our knowledge of the mechanism of gastric emptying in man. The technique provides only indirect evidence for the location of receptors, for it is concluded that the volume of the original meal remaining in the stomach is the volume which has not passed into the duodenum, or been absorbed. The indicator phenol red is neither absorbed or secreted in the stomach, even at pH 1.

Calculation

1. Volume of original meal = volume recovered + volume emptied into the duodenum + volume absorbed through the gastric wall

2. Mass of phenol red in the original meal = mass recovered + mass lost into the duodenum

Duodenal efflux does not affect concentration of phenol red recovered, and concentration changes are due only to absorption, or secretion, from the stomach. When considering concentration alone, 2 becomes 3 :

3. $$\text{Volume of test meal} \times \text{concentration of phenol red} = \text{volume recovered} \times \text{concentration of phenol red recovered}$$

or :

$$\text{Volume of test meal} = \frac{\text{volume recovered} \times \text{concentration of phenol red recovered}}{\text{concentration of phenol red in the test meal}}$$

When the calculated volume recovered differs from the actual volume recovered the difference represents the duodenal efflux.

Phenol red estimations are based on the assumption that duodenal reflux does not influence the calculations. This phenomenon is known to occur in man (Edwards & Rowlands, 1968) and the ruminant (Singleton, 1961). Because of the long collection delays involved, the method does not distinguish between sensory receptors involved in either nervous or humoral reflexes, or if the same receptors influence both systems.

The serial test-meal technique has been used in the ruminant, with abomasal perfusion only (Bell & Razig, 1973a, 1973b) and with direct perfusion into the cannulated duodenum, in an attempt to analyse duodenal effects on gastric motility and gastric emptying. Bell (see table 1) finds a variation in results both within and between animals, which suggests either a large range of possible sensitivities of receptors, or methodological problems. Bell makes

Table 1 Evidence for Ruminant Duodenal Receptors

ABOMASAL MEAL	DUODENAL STIMULUS	EMPTYING INHIBITED	EMPTYING INCREASED	NO ABOMASAL EFFECT	INVESTIGATION
water	$NaHCO_3$ (mOsm Kg^{-1})	up to 600 (400-1200)	200-300 (100-1100)		
"	NaCl	up to 600 (700-1100)	200-300 (100-600)	100,600	
"	KCl	100 - 700			
"	$CaCl_2$	100 - 800			
"	NH_4Cl	200 - 700	100 - 200	200	
"	urea	150 - 1200	100 - 700		
"	glucose	250 - 1250		250	
"	lactose	100 - 550	100	100	
"	HCl (mEq/L)	10 - 60	10		
"	acetic	20 - 60	10 - 20		
					Bell & Mostaghni 1975

Table 1 (continued)

ABOMASAL MEAL	DUODENAL STIMULUS	EMPTYING INHIBITED	EMPTYING INCREASED	EMG FUNDUS	EMG ANTRUM
glucose 3, 7.5, 10, 15%	NaCl, 300 mOsm Kg^{-1}		+ve		
water	$NaHCO_3$, 250		+ve		
glucose 900 mOsm Kg^{-1}	$NaHCO_3$, 250		+ve		
KCl, 600	$NaHCO_3$, 250		+ve		
$CaCl_2$, 600	$NaHCO_3$, 250		+ve		
HCl, 60 mEq/L	$NaHCO_3$, 250		+ve		
water	KCl, 600	+ve			
NaCl, 250	$CaCl_2$, 250	+ve			
$NaHCO_3$, 250	HCl, 10 mEq/L	+ve			
					Bell & Mostaghni 1975
water	$NaHCO_3$, 300 mOsm Kg^{-1}		++ve	++ve	++ve
water	HCl, 60 mEq/L	+ve		quiescent	quiescent
water	water		+ve		
					Bell & Grivel 1975
water (1-4 litres)	HCl, 60 mM	+ve partial only when volume abomasum large		quiescent some emg activity	
					Bell & Watson 1976

Table 1 (continued)

ABOMASAL MEAL	DUODENAL STIMULUS	EMPTYING INHIBITED	SECRETION
saline	lactose, 66 g/L	+ve (compared to $NaHCO_3$, (concentration not given)	N/S
saline	casein, 20	+ve	N/S
saline	lactose and casein	+ve	N/S
saline	milk replacer 100 g/L	++ve	reduced
saline	milk fat	++ve	reduced
saline	cows milk	++ve	reduced

Bell & McLeay, 1978

Table 1 (continued)

ABOMASAL MEAL	DUODENAL STIMULUS	EMPTYING INHIBITED	EMPTYING INCREASED	GASTRIC ACID	PEPSINOGEN
saline	HCl, 60 mM	+ve		+ve low levels or -ve	+ve low levels or -ve
saline	$NaHCO_3$, 300 mOsm Kg^{-1}		++ve	++ve	++ve
saline	*tryptophan + $NaHCO_3$ (isotonic)	50 mM		N/S	-ve 25 mM
saline	*tryptamine + $NaHCO_3$ (isotonic)	0.1 mg $Kg^{-1}min^{-1}$		-ve 0.1 mg Kg^{-1} min^{-1}	-ve 0.1 mg Kg min^{-1}
saline	*5HT + $NaHCO_3$ (isotonic)	0.1 mg $Kg^{-1}min^{-1}$		-ve	-ve 0.1 and 0.05 mg $Kg^{-1}min^{-1}$
					Bell & Webber 1979

(*solutions of variable osmolality, 312 - 390 mOsm Kg^{-1})

the assumption that phenol red only travels onward into the duodenum, which may be incorrect for it is now established that abomasal contents in the adult may also pass back into the omasum and reticulo-rumen (Ehrlein, 1979). This will presumably affect the disappearance of phenol red. This unknown quantity probably varies for different test meals and so a suitable correction-factor is difficult to establish. It seems unwise to ignore it, as its consideration might reduce the apparent range of responses which are found. Possibly, this route is non-functional in the pre-ruminant animal.

DUODENAL REFLEXES IN THE RUMINANT

The evidence from ruminant studies supports the concept that the duodenum is a profoundly reflexogenic organ. The results from the work of Bell and colleagues are presented in detail in table 1. Other workers have found that duodenal acid inhibits abomasal emptying by a rapid mechanism (Singleton, 1951) and increases abomasal motility (Smith, 1964). Acid also inhibits reticulo-rumen motility and rumination (Salmin, 1960) which also occurs when abomasal contents are perfused (Phillipson, 1952). Abomasal emptying is inhibited by 20 - 30% glucose, 2% protein and vegetable oils (Singleton, 1951). Oils and hydrolysate products inhibit rumen motility and depress food intake (Hill, 1965; Titchen, Reid & Vlieg, 1966; McLeay & Titchen, 1976; Fitzgerald & McLeay, 1978 (see : Bell & McLeay, 1978)) and reduce abomasal acid secretion (Hill, 1965). Hypertonic saline (2%) (Singleton, 1951) and drainage (Singleton, 1951; Phillipson, 1952) increase emptying whereas duodenal distension inhibits emptying (Phillipson, 1952) and rumination (Salmin, 1960). Bueno & Ruckebusch (1974) find that lactic acid (200 mM L^{-1}) and isobutyric (400 mM L^{-1})

but not amino acids, glucose and C_6 and C_8 fatty acids are effective in increasing omasal motility, depressing reticulo-rumen amplitude, rhythm and emptying. The effect of acid has an optimum pH of 3 - 4 and is not due to osmotic effects.

Bell and co-workers have studied the rate of emptying of abomasal test meals while perfusing a cannulated duodenum. The assumption is made that abomasal emptying is by the duodenal exit only. When duodenal re-entrant cannulae are used this eliminates the contamination of the distal duodenum with abomasal contents (although not distal duodenal, biliary and pancreatic secretions). Abomasal emptying measurements can be made either directly, by collecting effluent, or indirectly, by marker analysis. Direct perfusion of the duodenum has distinct advantages over the oral route given to Hunt's subjects.

Bell's work establishes that : hypotonic and isotonic (300 mOsm Kg^{-1}) saline and $NaHCO_3$ (250 mOsm Kg^{-1}) increase abomasal emptying of test meals, and that hypertonic $NaHCO_3$, saline, KCl, glucose, HCl, acetic and milk products inhibit emptying; also that NH_4Cl, urea, and lactose have little effect; milk products, oleic, linoleic acids and olive oil, and amino acids inhibit gastric acid production; and pepsinogen secretion is stimulated by $NaHCO_3$, and inhibited by amino acids. The $NaHCO_3$ (300 mOsm Kg^{-1}) inhibitory effect is abolished by vagotomy (Bell, 1978) but acid inhibition is not affected by vagotomy and probably involves hormones (Bell, 1978). Intraluminal duodenal infusion of lactate inhibits food intake (Bell, 1980). The receptors sensitive to isotonic $NaHCO_3$ and HCl (60 mM) and which are involved with the inhibition of abomasal emptying in calves are probably located throughout the duodenum (Bell & Holbrooke, 1979). Abomasal emptying of milk is at first retarded by vagotomy in calves, but is restored to "normal" later (7 - 29 days) (Bell, Holbrooke & Titchen, 1977).

In general, perfusion studies have given us little guide to the nature of the environment which affects the duodenal receptor, and often effects are produced by unnatural chemical concentrations. They have not yet been successfully utilised in the ruminant to show whether nerves or hormones, or both, are responsible for these reflexes. The long latency responses to oleic, linoleic (McLeay & Titchen, 1976) and myristic acid (Fitzgerald & McLeay, 1978) and vegetable oils (Titchen et al, 1966) suggest hormonal involvement.

Bell & Watson (1975) find that transthoracic vagotomy of abdominal branches causes slowing of abomasal test meal emptying to duodenal perfusion with water and $NaHCO_3$ (300 mOsm Kg^{-1}), which indicates that duodenal alkaline and osmoreceptor reflexes act via the vagus, but apparently acid reflexes do not. The reflex effect of duodenal acid is not altered by this technique. The work does not provide information about the role of the abdominal vagus nerve, whether as an afferent or efferent or exclusive route. Bell & Watson (1975) find that acid, but not alkali, causes the release of secretin, motilin and pancreatic glucagon, which either singly or collectively may be responsible for abomasal atony and reduced gastric emptying. Secretin causes inhibition of amplitude and frequency of rumen contractions in sheep (Bruce & Huber, 1973). When the duodenum is perfused with lactate (5.6 mM min^{-1}, pH 2) there is a long latency inhibition of amplitude and frequency of rumen contractions. Gastro-duodenal vein blood from these sheep produce similar effects in transfused recipients (Bruce & Huber, 1973).

From reflex studies therefore the ruminant duodenal receptors seem to have the following thresholds : HCl, 10 mEq/L; $NaHCO_3$, 250 mOsm Kg^{-1}; NaCl, 100 mOsm Kg^{-1}; acetic, 20 mM L^{-1}; tryptamine, 0.1 mg Kg^{-1}; tryptophan, 25 mM L^{-1}.

THE DUODENUM AS A REFLEX TARGET ORGAN

The duodenum is subject to both inhibitory and motor influences mediated by the extrinsic nerves via long and short reflex loops. Gastric distension inhibits duodenal motor activity, the "mechanical gastroenteric inhibitory reflex", by a mechanism which is inhibited by reserpine and guanethidine, and which is not affected by vagotomy or pyloric section (Daniel & Weibe, 1966), but is prevented by coeliac ganglionectomy and chemical sympathectomy (Daniel & Irwin, 1968). Laparotomy causes temporary inhibition of duodenal motility by a mechanism which is inhibited by atropine and guanethidine (Abrahamsson et al, 1979).

Distension of the gall bladder and the bile ducts (to pressures 68 - 218 cm water) cause inhibition of the spontaneous contracting duodenum in anaesthetised dogs (Nakayama & Mori, 1967). This reflex, initiated by an apparently excessive stimulus, is inhibited by thoraco-lumbar splanchnotomy. Semba (1956) has demonstrated, in the dog, that squeezing a full urinary bladder causes a reduction of duodenal motility by a nervous reflex involving the hypogastric nerves as an efferent limb. When nicotine is painted on the coeliac plexus the response is reversed and an increase in motility results by a mechanism which involves both the splanchnic and vagus nerves.

Close antral intra-arterial infusion of drugs which release acetyl-choline from preganglionic sites (pentagastrin, motilin) or which act on nicotinic (acetylcholine) or muscarinic receptors (acetylcholine, McNeil A-343), and which induce local muscular contraction of the antrum cause transient inhibition of the duodenum (Daniel & Sarna, 1975). These atropine-sensitive antro-duodenal inhibitory responses may involve non-adrenergic inhibitory neurones. The exact mechanism however has not been further examined. Because both passive distension (Daniel & Weibe, 1966) and active contraction (Daniel & Sarna, 1975) cause duodenal inhibition, this suggests the presence of mechanoreceptors in the gastric antrum with an "in-series" location.

THE NATURE OF THE DUODENAL ENVIRONMENT OF RABBIT AND SHEEP

Any logical attempt to examine the sensitivity of enteroceptors in the viscera, must commence from a knowledge of the physiological parameters of the receptor environment. The literature provides some information, but often the measurements are made upon preparations where surgical manipulation has altered the conditions. The receptor environment depends upon its histological location. In a viscus as complex as the alimentary canal the influence of the "internal milieu", as well as the digesta, must affect the response of the receptor.

THE MECHANICAL ENVIRONMENT

It is possible to assume that the various components of the gut are subjected to different mechanical changes : the mucosa to particulate matter and volume; the muscularis externa to stresses and tensions, active propulsive forces, passive transmural compression and distension; the serosa and mesentery to the proximation of nearby loops, stretch and touch, arterial pulsations, venous congestion and lymphatic dilatation. Little is known about many of these parameters in the species under study and other sources only are available.

The chyme of the rabbit and sheep contains small particulate matter including undigested lignins and other plant material. There are rhythmic contractions of the muscularis mucosa of man which are powerful near the pylorus (Walder, 1953). Abbott, Hartline, Hervy, Inglefinger, Rowson & Zetzel (1943) found that the intraluminal pressure changes in the human small intestine, above the surrounding serosal pressures, are in the order of 8 - 10 cm water, with values up to 50 cm water, for brief periods. Magee (1962) shows records of intraluminal pressure in man reaching up to 85 cm water and Hightower (1968) gives similar values. Duodenal pressures in the dog seldom exceed 10 cm water (Thomas & Crider, 1935) and in the calf values are found up to 20 cm water (Bell, 1978).

We are beginning to understand the mechanical role of the duodenal bulb in the ruminant. In the sheep the flow of chyme into the duodenum is rapid and almost continuous regardless of feeding pattern (Hogan & Phillipson, 1960; Harrison & Hill, 1962; Ruckebusch, 1970; Ruckebusch & Bueno, 1977b). Radioscopic studies in sheep have shown that gastric emptying is not immediately followed by duodenal propulsive waves as occurs in the dog (Quigley & Louches, 1962). Distension of the duodenal bulb is required before it empties by a propagated burst of spikes (Ruckebusch & Bueno, 1977b). In the sheep, gastric emptying and duodenal bulb evacuation are not synchronised, and it seems probable that the duodenal bulb has a "reservoir" function and emg "spikes" are not initiated until above a certain threshold. It has been suggested that this function may be related to the ascending configuration of the proximal duodenum and the continuous emptying of the abomasum (Ruckebusch & Bueno, 1977b).

THE CHEMICAL ENVIRONMENT

Different parts of the alimentary canal are stimulated by different chemical influences : the mucosa is bathed by chyme which is a mixture of digestive products both particulate and non-particulate, these include carbohydrates, volatile fatty acids, fats, soaps, proteins, amino acids, various ions, solutions of varying osmolality, pH, titratable acidity and alkalinity, viscosity, pCO_2; the submucosa and muscularis externa are bathed in solutions diffusing from the mucosa, changes in pO_2 and pCO_2; the vascular tissues by the absorbed chemicals, digestive products and pO_2 and pCO_2.

A summary of the chemical composition of chyme of rabbits and sheep, together with uncontaminated secretions, is given in table 2. A knowledge of whole-viscus chemical composition is not necessarily helpful about the conditions at the level of the receptor membrane.

THE THERMAL ENVIRONMENT

It is probable that the thermal environment is relatively stable at body core temperature. The fermentative process may raise the rumen temperature and its surrounds by $1^{o}C$ (Rawson & Quick, 1970). It is therefore unlikely that the observations in which reflex events are caused by much larger temperature changes are indicative of physiological responses which are usually operating.

Table 2A The Chemical Composition of Chyme

RABBIT	STOMACH			SMALL INTESTINE	
	ACUTE EXPERIMENT	MIXED DIET	GREENS DIET	FED, ACUTE EXPERIMENT	INVESTIGATION
pH (m = mean)	1.0 - 1.3, 1.1 m	1.0 - 4.3, 2.4 m	1.0 - 4.8, 2.5 m	7.6 - 8.0, 7.9 m	B
volatile fatty acids, mEq/L (m +/- sd)	33.3 +/- 5.04	-	-	8.51 +/- 2.40	B
lactic acid mEq/L (m +/- sd)	4.01 +/- 1.10	3.43	3.49	-	B
bicarbonate mEq/L (m +/- sem)	8.6 +/- 1.4	-	-	57.0 +/- 8.0	A
potassium ions mEq/L (m +/- sem)	19.0 +/- 2.2	-	-	39.0 +/- 9.7	A
sodium ions mEq/L (m +/- sem)	14.0 +/- 0.3	-	-	128.0 +/- 10.4	A
chloride ions mEq/L (m +/- sem)	153.0 +/- 9.4	-	-	82.0 +/- 10.2	A
phosphates mEq/L (m +/- sem)	15.0 +/- 1.9	-	-	19.0 +/- 2.5	A

Table 2B

SHEEP	ABOMASUM			DUODENUM	INVESTIGATION
	MIXED	HOLLANDER POUCH	PAVLOV POUCH		
pH (m +/- sem)	-	-	-	2.48 +/- 0.15	G
	-	-	-	3.13 +/- 0.21	G
	-	-	-	2.7 +/- 0.02 (proximal)	E
	-	-	-	3.81 +/- 0.04 (distal)	E
	-	1.05 - 1.30, 1.19 m	up to 6 and over	2.3 - 4.7, 3.95 m	H
titratable acid mEq/L	8 - 14	-	-	-	E
	10 - 110	-	-	-	E
	-	66 - 117, 94.7 m	2 - 88, 30.7 m	39 - 74, 54.7 m	H
volatile fatty acids, mEq/L	-	-	-	2 - 19, 7.8 m	H
	-	-	-	4 - 14	E
phospholipid total (T) particulate free (PF) mEq/L	-	-	-	(T) 63.4 +/- 10.8	G
	-	-	-	(T) 74.1 +/- 5.9	G
	-	-	-	(PF) 40.0 +/- 12.7	G
	-	-	-	(PF) 49.0 +/- 12.2	G
chloride ions mEq/L	114 - 129	153 - 165, 157.5 m	148 - 158, 152.8 m	109 - 135, 121 m	H
fatty acid total (T) particulate free (PF) (mg/100 ml)	-	-	-	(T) 155.5 +/- 9.1	G
	-	-	-	(T) 125.0 +/- 5.3	G
	-	-	-	(PF) 17.7 +/- 0.9	G
	-	-	-	(PF) 20.6 +/- 1.6	G

Table 2C

	SHEEP BILE (D)	PANCREATIC JUICE (F)	BRUNNERS GLANDS (C)
pH	-	-	8.2 - 9.3
titratable alkaki (nM)	- -	- -	4 - 20 (sheep) 50 - 80 (rabbit)
bicarbonate mEq/L	-	25 - 170	14 - 22
sodium ions mEq/L	196.6 +/- 4.8	154 +/- 7	136 - 150
chloride ions mEq/L, (m +/- sem)	73.6 +/- 3.7	-	130 - 140
potassium ions mEq/L	8.03 +/- 0.27	4.8 +/- 0.9	4.5 - 8.0
carbon dioxide	-	154 +/- 10	-
nitrogen (mg/100 ml)	247.7	-	-

KEY : A Alexander, 1965 B Alexander & Chowdhury, 1958 C Cooke, 1967
D Harrison, 1962 E Harrison & Hill, 1962 F Janowitz, 1967
G Leat & Harrison, 1969 H Masson & Phillipson, 1952

THE ANATOMY OF THE SENSORY INNERVATION OF THE SMALL INTESTINE

Both the vagus and splanchnic nerves innervate the duodenum of the dog (Mizeres, 1955) and ruminant (Duncan, 1953; Habel, 1956; Ghoshal & Getty, 1970). In all animals studied the left and right vagus nerves divide at the level of the the posterior thoracic oesophagus into, usually, two main trunks which recombine into dorsal and ventral abdominal vagal branches. In the cat the left vagus may contribute more to the ventral vagus and the right vagus to the dorsal branch (Agostini, Chinoch, Daly & Murray, 1957), a situation which Duncan (1953) has observed in ruminants.

Until recently most has been known about the projection of the heavily myelinated axons from pacinian corpuscles. They project via the splanchnic nerve in the cat (Sheehan, 1932) and into the rami communicantes (Downman & Hazarika, 1962). More recently horse radish peroxidase injected in the gastroduodenal junction has been used in the cat and retrograde labelled neurones counted in the nodose and spinal ganglia (El Ouazzani & Mei, 1978), but this technique cannot define the receptor modality nor the character of the afferent axon.

Most of the evidence for the relative proportion of afferent axons and efferent axons in major nerve trunks is from degeneration studies after "selective" neurotomy. The relative proportions are probably as follows : afferent:efferent, 9:1 vagus, 3:1 splanchnic, 1:1 pelvic nerve (Leek, 1977).

Most fibre number estimates have been derived from total or sample counts using the light microscope. Recently EM studies have shown that light microscopy omits a large number of small myelinated 1 - 4 µm, and unmyelinated <3 µm axons (Ranieri, Crousillet & Mei, 1975). Consequently most studies have under-estimated the total axon number. This confirms the view of Gasser (1955) who first discovered by electron microscopy that unmyelinated axons are present in groups of Schwann sheaths and who suggested that individual axons may not be distinguished by the older silver-staining methods.

The data derived from selective neurotomy has inherent problems. Apart from questioning the effectiveness or otherwise of the surgical technique, it is now known that many visceral enteroceptors not only have their cell bodies in peripheral organs or in adventitious ganglia, but that they may project along non-classical pathways, for example, entering the CNS via ventral roots (Ryall & Piercey, 1970; Coggeshall, Coulter & Willis, 1974; Clifton, Vance, Applebaum, Coggeshall & Willis, 1974; Willis & Coggeshall, 1978: Clifton, Coggeshall, Vance & Willis, 1976), at least in the pelvic region. Numbers derived from cats and rabbits, although showing some qualitative agreement, cannot be extrapolated to include other species. Counts often refer to one nerve, for example the right vagus (Agostini et al, 1957) and these may not be representative. Mei (1970a) has shown that there is variation between vagus nerves in the same animal and between different animals. There is less variability in the laryngeal nerves (Mei, 1970a). For these reasons the efficacy of neurotomy for denervation should be questioned. The techniques of neurotomy and ganglionectomy have provided some useful information about numbers and possible function of nerves, especially when combined with reflex and neurophysiological investigations.

Supranodose vagotomy can only de-efferent the distal nerve if all preganglionic fibres descend through the nodose. Fibres joining the vagus below the nodose will not be sectioned, and also the postganglionic nerves remain intact. Whether infranodose vagotomy both de-afferents as well as de-efferents the cervical vagus depends on the location of cell bodies elsewhere. They may be in the periphery or have cell bodies in pre- or paraganglia and have joined the nerve (Schofield, 1960). It may be that "adventitious fibres" illustrated by Agostini et al (1957) in cat abdominal vagus, and Evans & Murray, (1954) in rabbit abdominal vagus following infranodose vagotomy, were visceral afferent axons with cell stations elsewhere. Paintal (1963) states that the source of these fibres remain obscure. It is possible that they are aberrant sympathetic axons because some sympathetic fibres do join the vagus nerve (Youmans, 1968). Evans & Murray (1954) suggest that "adventitious fibres" may be visceral afferent axons leaving the thoracic vagus to join the intercostal nerve close to their exit from the intervertebral foramina and entering the spinal cord by dorsal roots, and Harper, McSwiney & Suffolk (1935) provide reflex evidence to support this concept.

Similarly, there is difficulty in the interpretation of neurotomy and ganglionectomy in the non-vagal nerves. Selective de-efferentation can only be achieved by sectioning both the dorsal root proximal to the dorsal root ganglion and ventral rhizotomy, because preganglionic fibres may exit from the CNS via dorsal or ventral roots. In addition, adventitious efferent fibres may join some nerves more peripherally. Total abdominal counts must also allow for efferent fibres distributed with the segmental nerves. Dorsal ganglionectomy does not selectively cause de-afferentation, because some enteroceptors projecting to the CNS have cell bodies peripheral to this and will not degenerate and many afferents may enter ventral roots; some dorsal root preganglionic (efferent) axons will be interrupted, and so will be included in degeneration counts, and those preganglionic fibres distributed via segmental nerves are not included, so total counts are likely to be underestimates.

Splanchnotomy will cause degeneration of preganglionic sympathetic fibres, and visceral afferent axons whose cell body is in the dorsal root ganglion or paraganglia, but the visceral afferent axon whose cell body is in the periphery will remain. The greater splanchnic nerve of the cat occasionally includes a bundle of postganglionic fibres arising from the sympathetic trunk ganglia (Ranson & Billingsley, 1918; Kuntz, Hoffman & Schaeffer, 1957). Ganglion cells have been demonstrated in the splanchnic nerve and must be regarded as the source of many postganglionic fibres in the distal parts of the nerve (Kuntz et al, 1957). There are numerous ganglion cells throughout the abdominal vagus nerve of the sheep and goat, which may also affect degeneration counts (Habel, 1956).

THE CONCEPT OF SELECTIVE SENSITIVITY OF SENSORY RECEPTORS

An "afferent unit" has been defined as "the complex formed by an afferent axon, its receptor terminals or endings, and any associated non-nervous structural elements" (Iggo, 1966b). This implies a functional rather than an anatomical unit. Iggo (1974) has also introduced the term "selective sensitivity" for afferent units in the somatosensory system. The term is to be understood as meaning a heightened and not an exclusive sensitivity to one form of energy - mechanical, thermal or chemical, because receptors are not absolutely specific in a biophysical sense of responding exclusively to one form of energy. Implicit in the concept of selective sensitivity is the concept of coding. That is, the possibility that the characteristic discharge pattern set up by the afferent unit is important in informing the central nervous system of peripheral events (Iggo, 1965). There are two hypotheses concerning this second concept. The first states that the incoming information is coded in a spatial and temporal pattern of impulses in a population of afferent fibres with nerve endings which are individually similar, if not identical, but which because of their morphological disposition may respond differently to identical stimuli (Hagen, Knocke, Sinclaire & Weddell, 1953). And secondly that the coding, although depending in part on the temporal pattern of impulses, arises in peripheral receptors that display a considerable degree of dissimilarity, so that the receptors play a significant part in determining or selecting the afferent fibres that are active in any particular situation.

In the somatosensory system, mechanoreceptors with myelinated afferent axons have distinct physiological behaviour which correlates with distinct anatomical differences in receptor morphology (Brown &

Iggo, 1967; Iggo & Muir, 1969; Chambers, Andres, Duering & Iggo, 1972; Iggo & Ogawa, 1977) and this evidence supports the second hypothesis. Cutaneous thermoreceptor units, also with myelinated axons, show selectively sensitive responses (Iggo, 1969) but an histological receptor correlate is yet to be established.

Iggo (1958b, 1959a, 1959b, 1960, 1961, 1963, 1965) has demonstrated that a fine degree of selective sensitivity exists in somatosensory afferent units with unmyelinated axons. This implies either that a dissimilarity of receptor morphology exists or that their "morphological disposition" plays a significant role in the transduction process. Except for the sensitive C-fibre mechanoreceptors (Andres & Hensel, unpublished observations, see Iggo & Young, 1975) no morphological distinctions have been found between C-fibre endings to determine these possibilities. Therefore, there is some support for both hypotheses, but only as yet for receptors with myelinated axons.

In the gut, where, with few exceptions, unmyelinated axons innervate enteroceptors, there remains speculation about these alternative hypotheses. The evidence supports the concept of selective sensitivity for many of the mechanoreceptors, although in many instances the precise stimulus has been difficult to quantify. There appears to be an apparent conflict between the concept of selective sensitivity and the demonstration of multimodal sensitivity of the mucosal mechanoreceptors which also respond to chemicals in the lumen. This conflict cannot be resolved until more is known of the association of axons and cells in the wide variety of tissues constituting the alimentary well, and the physiological investigator has recognised and quantified the precise nature of the stimulus-response relationship.

No firm electrophysiological evidence exists for a separate population of alimentary enteroceptors which respond only to stimuli of noxious intensities, therefore the concept of specific visceral nociceptors responding above a threshold which is actually or potentially damaging has no support. In the gut, pain may arise from the same population of receptors which at raised thresholds show more intense activity in their fibres and which at moderate frequencies of discharge signal other modalities (Douglas & Ritchie, 1960). We do not yet know to what extent the central analysis of patterns of activity in populations of afferent fibres responding to peripheral stimuli affects sensation (Weddel, Palmer & Taylor, 1959).

Hence many of the conflicting concepts which existed in the somatosensory nervous system in the early 1960's, and which to some extent have been resolved (Iggo, 1974), remain in our understanding of the behaviour of the gastro-intestinal enteroceptors.

THE ELECTROPHYSIOLOGY OF ALIMENTARY ENTEROCEPTORS

The mesentery is composed of a central mesodermal derived structure consisting of connective tissue, blood and lymph vessels and a thin visceral peritoneum covering each side. The alimentary tube, in addition, has mesodermally derived smooth muscle elements and an endothelial-derived mucosa. The nerves are derived from neurectoderm (Romer, 1970). Whether the ontogenic derivation of a tissue determines completely its sensitivity, cannot as yet be fully judged. The electrophysiological evidence suggests that receptor modalities may differ in different organs. This section is concerned with the electrophysiological evidence for alimentary enteroceptors. The classification is similar to that of Leek (1977) and is based upon the three major anatomical divisions : (a) mucosa, submucosa and muscularis mucosa, (b) muscularis externa, (c) mesentery and serosa. Alimentary enteroceptors are usually mechanosensitive with rare exceptions. Slowly adapting mucosal mechanoreceptors are rarely found, and there is evidence for both selective and multiple chemical sensitivity of mucosal receptors. In the muscularis externa, mesentery and serosa mechanoreceptors are present whose activity is related to mechanical distortion.

There are three important *in vivo* techniques which have been used to record unitary afferent activity from alimentary enteroceptors : 1. In acute experiments Iggo's modification of Adrian's dissection technique (1933) has been used extensively. Mostly large nerve trunks have been studied. The nerves include the cervical vagus (Iggo, 1954a, 1954b), splanchnic (Morrison, 1973), hypogastric (Floyd, Hick & Morrison, 1976b), superior laryngeal (Andrew, 1956), dorsal roots

(Leitner & Perl, 1964), ventral roots (Clifton et al, 1974), thoracic ramus communicans (Downman & Hazarika, 1962), hepatic (Niijima, 1969a) and mesenteric (Bessou & Perl, 1966). 2. Mei (1962) introduced the technique of recording extracellularly from paraganglia, preganglia and the nodose ganglion. This technique has the advantage that the axon remains intact, which is not possible with the dissection technique. 3. Recently Rousseau & Falempin (1979) have devised an important new approach which is a modification of the technique used by Dussardier (1960). Rousseau & Falempin (1979) used chronic preparations in which there was supra-nodose cross anastomosis with the cleidomastoidus motor innervation, and recorded unitary emg activity with concentric needle electrodes. This technique has provided new information about the central transmitter in the afferent projecting system. Because there was functional reinnervation this suggests that this is a cholinergic synapse.

Further information about the unitary activity of mechanosensitive neurones is provided from in vitro preparations. Both intracellular and extracellular microelectrode studies have been conducted in the myenteric and submucous plexus (Nishi & North, 1973; Hirst, Holman & Spence, 1974; Wood & Mayer, 1978) and intracellular activity has been recorded in abdominal preganglia (Szurszewski & Weems, 1976; Kreulin & Szurszewski, 1979).

MUSCOSAL MECHANORECEPTORS

There appear to be two types of mechanoreceptor, rapidly adapting (RA) and slowly adapting (SA) types. It is interesting that RA units with C-fibres exist in the alimentary canal for they have not been found in somatosensory investigations (Iggo, 1959b and onwards).

Leek & Harding (1975) have called the superficial receptors in the reticulo-rumen "epithelial" because of the absence of mucous membrane in this location.

The threshold of RA mechanoreceptors is high (150 mg, Davison, 1972). A variety of sizes of mechanical receptive fields have been reported, the smallest in the rat stomach (2 - .5 mm diameter, 10 x 3 mm, Clarke & Davison, 1978), intermediate in the sheep reticulum and rumen (2 - 3 cm diameter consisting of 5 ridges or 12 papillae, Harding & Leek, 1972a) and large in the cat stomach (5 cm^2, Iggo, 1957c; 1.5 - 4.0 cm^2, Davison, 1972). The response to a mechanical stimulus can range from a brief excitation by discrete placement of a small probe, to a more-prolonged discharge following light brushing with cotton wool (Harding & Leek, 1972a, 1972b; Leek & Harding, 1975). The receptive field for brushing may be greater than that for discrete probing (Clarke & Davison, 1978). The position and sensitivity makes them suitable for recording particulate matter large enough to displace the mucosa.

The mechanically sensitive mucosal receptors are not homogeneous. There are at least two functional groups : those which are silent in the absence of applied stimuli which have a low mechanical threshold (most workers), and a group with a bursting discharge with variable interburst intervals (Paintal, 1957b) and which have a higher mechanical threshold (Paintal, 1957b; Clifton et al, 1976) and are possibly associated with the muscularis mucosa. Because spontaneously active units are not excited by distension of the gut, without mechanical distortion the receptor elements may be radially orientated, possibly "in series" with the muscularis mucosa and are only excited when the muscularis mucosa is distorted or actively contracts

(Paintal, 1957b). Such a receptor may behave as a "flow receptor" (Paintal, 1957b). The criterion upon which this conclusion is based - excitation with 30% saline - seems inadequate to substantiate this conclusion. Sporadic bursts are also seen in the abomasum (Harding & Leek, 1972a).

The diameter of the afferent axon depends upon its location. Those in the cat oesophagus and compound stomach are myelinated, and those in the monogastric stomach and intestine are unmyelinated. Feline "flow receptors" were not examined for conduction velocity but are "probably" myelinated (Paintal, 1957b).

Mucosal receptors can easily be excited electrically, providing evidence for their superficial location (Harding & Leek, 1972a) and are rapidly inhibited by mucosal application of lignocaine (Bitar, Mei & Michelucci, 1975; Leek, 1977) and easily destroyed by mucosal excision (Iggo, 1957c).

RA mucosal mechanoreceptors in the gastro-intestinal tract have only been described in the vagus nerve. Slowly adapting mucosal mechanoreceptors have also been described in the rectum responding to high threshold pinching and irritant chemicals, but not 30% saline (Clifton et al, 1976). These units project via ventral sacral roots, and are also excited by thermal stimuli.

Sensitive mucosal mechanoreceptors are also excited during excessive stretching of the viscus (Iggo, 1957c; Mei, 1970b; Clarke & Davison, 1978), causing reflex inhibition of the homonymous organ (Iggo & Leek, 1967a). These responses may be experimental artifacts caused by the presence of intraluminal balloons (see below, page 168).

Most mucosal mechanoreceptors are sensitive to chemicals applied to the receptive fields. Some are not. A population in the cat caudal oesophagus examined by Harding & Titchen (1975) were not acid sensitive. Those found in the lower oesophageal sphincter mucosa by Biter et al (1975) were not tested with chemicals. A proportion of sheep mucosal receptors are not excited by chemicals (Harding & Leek, 1972a).

MECHANORECEPTORS IN THE MUSCULARIS EXTERNA

Mechanoreceptors exist in the deep layers of the alimentary canal and their activity is closely associated with the contractile apparatus of longitudinal and circular muscle. In the toad stomach vagal receptors are 5 x 5 mm^2 and are generally smaller than splanchnic projecting units (Niijima, 1962) and in sheep, diameter about 20 mm (Leek, 1977). In mammals, most evidence suggests a vagal projection although splanchnic units have been described (Ranieri, Crousillet & Mei, 1970; Ranieri, Mei & Crousillet, 1973; Clifton et al, 1974, 1976). Generally the receptors in the oesophagus have faster afferent axons than elsewhere.

The Concept of "In Series" Tension Receptors

Matthews (1933) introduced the concept of "in series" and "in parallel" mechanoreceptors for skeletal muscle, and Iggo (1955) extended the "in series" concept to include slowly adapting mechanoreceptors in hollow viscera, which respond both to active isometric contraction and passive distension. This was later supported by Leek (1969). Leek (1972a) states that a tangent at the circumference of the muscle is equivalent to the linear situation in skeletal muscle.

However, no preparation has validated this concept because the recording of tension in small segments of hollow viscera, while coincidentally recording from afferent axons, has not been achieved.

Tension receptors do not respond accurately to sustained intraluminal pressure, indeed longitudinal section of the bowel does not abolish activity. They respond to the rate of distension, rapid distension causing a greater impulse activity than slow distension (Iggo, 1957b; Davison & Clarke, 1977). They probably cannot respond accurately as volume detectors (see below). The non-nervous elements of the gut transmit the intensity of the stimulus by causing mechanical deformation of the receptive field. Mechanoreceptors are thus stimulated by the mechanical deformation of the wall, rather than the intraluminal pressure changes per se (Paintal, 1972).

Tension receptors are particularly sensitive to the "tone" of the smooth muscle. Thus the frequency of discharge during distension depends firstly upon the dynamic properties of the receptor itself - that is by its tendency to discharge most rapidly while the intensity of stimulation is increasing, and secondly on the response to stretch of the smooth muscle in which it is embedded. Visceral smooth muscle adjusts its length during and after distension so that at different times a hollow viscus may contain different volumes at the same pressure. This ability of smooth muscle to adjust its length so that the tension is kept about the same probably accounts in part for the "adaption" of the discharge in the receptors (Iggo, 1966a). The extent to which the mechanoreceptors, or the fibroelastic tissues to which they are presumably connected, will become stretched for a given transmural pressure depends upon two factors : (a) the geometry of the gut wall - that is the internal radius (R) and the wall thickness, and (b) the elastic and viscus properties of the wall, which to a large extent will be determined by the tissue composition. When the wall thickness (D) is small compared with the internal radius, the magnitude of the mean stretching stress ($\overline{S}$) across the wall that results from transmural pressure (P) is given by : $\overline{S} = P \cdot \frac{R}{D}$ (Kirchheim, 1976). The dynamic response of the receptor will be determined by : (a) the amplitude of the applied pressure step, (b) the rate of rise of pressure (dP/dt), and (c) the mean pressure level at which a pressure step is applied (the operating level) (see Davison & Clarke, 1977, fig. 4) - this is why it is often difficult to compare units from different preparations.

There is little evidence from electrophysiology to support the concept that different populations of tension receptors exist with different mechanical thresholds, a concept suggested but not demonstrated by Iggo & Leek (1967b). The best evidence appears to be the observation of Iggo (1955) that electrical stimulation of extrinsic motor nerves at low strengths caused a single unit to respond, whereas raising the level of excitation caused a further unit to fire, presumably when the tension in the tissue had raised above a higher threshold level. Davison & Clarke (1977) describe threshold differences in rat stomach.

The mechanical threshold of mechanoreceptors in circular muscle (which reflexly inhibit longitudinal muscle contraction) is higher than mechanoreceptors in longitudinal muscle which each cause their respective muscles to contract during peristalsis *in vitro* (Kosterlitz, Pirie & Robinson, 1956; Kosterlitz & Lees, 1964). Intraluminal pressures of 1.0 - 3.0 cm water are necessary to stimulate peristalsis (Kosterlitz et al, 1956), a threshold which bears little relationship to the intraluminal pressures which have been applied in vivo (34 cm water, Iggo, 1957b), but are similar to more recent investigations in the ferret (Andrews, Grundy & Scratcherd, 1980).

The "in series" arrangement may have an important consequence for the control of reflex activity in the viscera (see : Leek, 1972a). In the unobstructed viscus the receptors may elicit propulsive movements, but if the lumen is obstructed they may excite very powerful and prolonged contractions (Iggo, 1966a).

It has recently been suggested that the same receptor type in ferret stomach may well respond to tension in one location, and stretch in another (Andrews et al, 1980).

The Concept of "In Parallel" Distension Receptors

Matthews (1933) showed that some mechanoreceptors are "in parallel" with the contractile elements of skeletal muscle. They are off-loaded when active contraction occurs and excited when stretch is applied. Such a receptor responds therefore to length rather than any other movement parameter. There is some evidence to support such a receptor in the alimentary tract. Often stretch and distension are reported as exciting receptors (Paintal, 1957a; Mei, 1970b; Falempin, Mei & Rousseau, 1978).

It is unlikely that the mesenteric and omental units closely associated with the gut can logically be included in this group because they are not intimately involved in the contractile apparatus, and should be excluded from the concept (Iggo, 1957c; Morrison, 1973, 1977). They are clearly mechanoreceptors which will be excited when there is distortion of the mesentery, and depending upon the relative positions of tissues, they can therefore behave as tension or stretch monitors. It is unnecessary to confuse the concept (Floyd, Hick & Morrison, 1976a, 1976b) as they are quite different from "in series" tension receptors.

Two investigations in dog antrum (Takeshima, 1974) and cat oesophagus (Harding & Titchen, 1975) have used the term "in parallel". In the skeletal muscle of the anal sphincter SA afferent units were silenced by contraction of the sphincter, thus possibly occupying an "in parallel" location (Todd, 1964; see also Leek, 1977, p 166).

RAPIDLY ADAPTING MECHANORECEPTORS IN THE MUSCULARIS EXTERNA

Rapidly adapting mechanoreceptors in the muscularis externa have also been described, although less frequently. Ranieri et al (1973) have observed what they have called "on-off" receptors from the gastric antrum of cat, and Mei (1965) uses this term for some cat thoracic oesophageal units. The location of "on-off" units in the sheep oesophagus is unknown (Falempin et al, 1978).

MECHANORECEPTORS IN THE SEROSA AND MESENTERY

The function of the pacinian corpuscle - a rapidly adapting mechanoreceptor, has not been satisfactorily resolved although it has been extensively studied both in vivo and in vitro (Hunt, 1974). The afferent axon projects via the splanchnic nerves through the dorsal root (Ranieri et al, 1973) by a rapidly conducting axon and monitors either arterial pulse, venous volume (Gammon & Bronk, 1935) or "contact" (Leitner & Perl, 1964) and is common in the cat mesentery and found also in dog and man. They may also be present in the rabbit (Downman & Hazarika, 1962).

A distinct "movement detector" has been described in the cat intestine (Bessou & Perl, 1966) characterised by rapid adaptation, following sinusoidal vibrations below 100 Hz, 1 - 5 sensitive points less than 1 mm diameter and situated on the sub-serosa commonly as the artery enters the intestinal musculature. Units have conduction velocities which indicate a myelinated axon. In all respects, except its rate of adaptation, this receptor resembles the slowly adapting mechanoreceptor described by Morrison and his colleagues (1973 et seq.) which is sensitive to mechanical distortion of the mesentery, traction, stretch and usually closely associated with the vascular junction. However, some of Morrison's units had tonic activity, especially in the dog (Floyd & Morrison, 1974a). Units have multiple punctate receptive fields and individual points can be separated by up to 4 cm. Some units are not associated with blood vessels (Floyd, Hick, Koley & Morrison, 1977a) and some have single receptive fields (Floyd et al, 1976b). Units sometimes have rhythmic discharges and are associated with respiratory, cardiac and intestinal movements, depending upon the method of mesenteric distortion. The mechanical threshold to touch varies considerably (5 - 100 gm). These units have a wide variety of conduction velocities, from C-fibres to Aδ-fibres which project to the spinal ganglia (Crousillat, Ranieri & Mei, 1970; Ranieri et al, 1973) and via the ventral roots (Floyd, Koley & Morrison, 1976).

An omental or gastric serosal unit was found by Iggo (1957c) which projected via the cervical vagus of the cat which was also excited when the greater curvature of the stomach was distorted. No further details of receptive field characteristics were given. Splanchnic projecting omental units in the sheep have 1 - 6 punctate receptive fields (Floyd & Morrison, 1974b).

ALIMENTARY CHEMORECEPTORS

There is little single unit evidence that specific chemoreceptors exist in the alimentary canal. Mei (1978) has recently described intestinal glucose receptors projecting in the vagus nerve of the cat. It was assumed that receptors were situated in the mucosa because of the "short latency" (the range was 1 - 10 sec). Units were not mechanically sensitive to mucosal stroking, although how this was accomplished in an intact intestine is not disclosed. Units were not spontaneously active. The glucose response was modulated by intravenous insulin : an increased activity was caused by IV insulin or isotonic saline, and intravenous glucose decreased the activity. The number of receptor sites may be greatest in the duodenum and proximal small intestine. Single unit evidence also exists for hepatic glucose receptors (Niijima, 1969a) whose tonic activity is inhibited by glucose and glucosides.

A number of multi-unit investigations have been undertaken which demonstrate a change in discharge activity as a result of chemical perfusion of the gut. These results are difficult to interpret because they have often been combined with experiments which poorly monitor mechanical parameters. This is necessary for it is well known that mechanoreceptors in the muscularis externa are sensitised by chemicals : glucose intra-arterially sensitises the stretch receptors in the stomach (Paintal, 1954a) and mucosal application of glucose, amino acids and hydrochloric acid sensitise intestinal mechanoreceptors (Zamiatina, 1957).

However, no chemical response has been seen in some tension receptors (Iggo, 1957b; Harding & Leek, 1972a; Clarke & Davison, 1975). It is therefore difficult to evaluate the work of Sanford (Sanford, 1976; Hardcastle, Hardcastle & Sanford, 1978) who reversibly inhibited the glucose excitation of multi-unit preparations in vitro with 5 x 10^{-4}M phylorrhizin and who conclude that the enteroceptor site is beyond the phylorrhizin-sensitive glucose entry mechanism which is located at the luminal side of the mucosal epithelial cells (Parsons, Smyth & Taylor, 1958). Phylorrhizin possibly prevents movement of glucose into cells from mucosal fluid, and at high concentrations (10^{-3}M plus) interferes with oxidation of glucose by cells (Parsons et al, 1958).

ALIMENTARY OSMORECEPTORS

Electrophysiological evidence supports the concept of hepatic osmoreceptors (Niijima, 1969b; Adachi, Niijima & Jacobs, 1976). There are probably two different populations of osmoreceptors responding to either hypotonic or hypertonic solutions with sensitivities +/- 17 mOsm Kg^{-1} in the liver. It is possible that the water receptors described in the larynx (Storey & Johnson, 1975; Harding, Johnson & McLelland, 1978) may also be osmoreceptors.

The multiple sensitive mucosal/epithelial receptors which have osmotic sensitivity are discussed in the next section. Their activity is rapidly abolished by local anaesthetics, it is therefore difficult to believe they are the same receptors which are involved in osmotic reflexes (see Sircus, 1958).

MULTIMODAL ALIMENTARY ENTEROCEPTORS

Most mucosal mechanoreceptors are also excited by chemicals. The range of chemical excitants varies between species, and in some investigations a degree of selective sensitivity is found. In others it is not and receptors respond to a wide variety of chemicals.

The RA mucosal mechanoreceptors of the cat are sensitive only to single chemicals. One population is excited by HCl below pH threshold 3, and another by alkali above pH 8.2 (Iggo, 1957a, 1957d; Davison, 1972). The chemical response is long lasting and stimulus related. A reinterpretation of Iggo's results suggests that pH alone may not have been the adequate stimulus, because buffered solutions of similar pH gave less response than unbuffered solutions. Davison's paper shows that, like Iggo, he did not measure the pH of test solutions in contact with the gastric mucosa. Borate buffer (pH 2.0) was almost ineffective on acid receptors responding vigorously to HCl and acetic acid at the same pH. $NaHCO_3$ was ineffective when NaOH was effective on alkali receptors (Davison, 1972). The change in osmolality of the different solutions was not considered to influence the response (Iggo, 1957a, 1957d; Davison, 1972).

Andrews & Andrews (1971) were the first electrophysiologists to consider whether titratable acidity was more important than pH. From multi-unit records they describe two duodenal classes, rapidly and slowly adapting, in rabbits, each responding to perfused solutions with thresholds below pH 2. Because buffered solutions at pH 1 (trisodium citrate and HCl) had similar responses to unbuffered HCl of the same pH, they conclude that receptors respond to pH and not titratable acidity, and they therefore support the pH concept introduced by Iggo (1957a).

These responses contrast with receptors found in other locations and other species (sheep, rat and cat oesophagus). Harding & Leek (1972a) demonstrated a multimodal receptor in the sheep gastroduodenal area which is excited both by acid and alkali. The response is independent of pH and pK, and is related to the normalities and molecular weights of the chemicals tried, and their aqueous diffusion coefficients. Further, titratable acidity or titratable alkalinity and not pH per se is important (Harding & Leek, 1972b). Harding & Leek (1972a, 1972b) find that strong acids of high molecular wieght, in saline solution behave as HCl. Harding & Leek expand the interpretation of these data later (Leek & Harding, 1975; Leek, 1977) and consider that the reason why high molecular weight acids are less effective a stimulus, having a longer latency, is because of their diffusion coefficient, the length of the diffusion pathway and the buffering which occurs there. Acids probably diffuse in the form of undissociated weak acids or as hydrogen-ion-anion couples. The multimodal receptors also respond to hyperosmotic and hypoosmotic solutions (Harding & Leek, 1972b) with a widely variable latency (sec → min, Leek, 1977).

The chemical threshold for acid and alkali is about 15 - 50 mEq L^{-1} (Harding & Leek, 1972b; Clarke & Davison, 1978) and the mechanical receptive field is 25 mm - 3 cm diameter. Clarke & Davison (1975) say that there is no relationship between effectiveness of the acid and molecular size, and also that the size of the undissociated radical affects diffusion rate to the receptor site (Clarke & Davison, 1978). Although Clarke & Davison (1978) say that there is little change in latency to different acids, their figures (figures 7 and 8) suggest that the latency to peak frequency is dependent on molecular weight, units reaching higher frequencies earlier with low molecular weight acids.

No explanation or model of the transduction mechanism which allows sensitivity to a wide variety of chemicals and mechanical stimuli has been given. In the absence of chemical and mechanical stimuli these units are usually silent. It is interesting to note here that glossopharyngeal taste units respond best to a combination of mechanical and chemical stimulation, but not to the mechanical stimulus alone (Iggo & Leek, 1967c). This sensitisation phenomenon has not been reported elsewhere in the alimentary canal.

ALIMENTARY THERMORECEPTORS

Three electrophysiological investigations of single unit C-fibre activity modulated by temperature changes in the abdomen have been reported. Vagal units have been described in the stomach and duodenum of the cat (El Ouazzani & Mei, 1979), splanchnic units in the dorsal abdominal wall of the rabbit (Riedel, 1976), and rectal mucosa (Clifton et al, 1976).

It is stated that thermoreceptor units in the cat are mechanically and chemically insensitive, although a great many units were not tested, and one was sensitive to glucose (El Ouazzani & Mei, 1979). Two populations were found : cold units had peak frequencies of discharge up to 15 imp sec^{-1} with optimum temperatures 10 - 12^{o}C and range 10 - 36^{o}C; and warm units had peak frequencies up to 35 imp sec^{-1} with optimum temperatures 46 - 49^{o}C and range 38 - 51^{o}C (El Ouazzani & Mei, 1979).

Two populations of warm receptor which were silent at 30^{o}C were found in the rabbit : type A have a temperature threshold of 32 - 34^{o}C and optimum of 46^{o}C; and type B have temperature threshold 35 - 36^{o}C and optimum 40^{o}C, and are silent at 44 - 45^{o}C (Riedel, 1976). Rabbit units are mechanically insensitive and situated in the region of the adrenals and superior mesenteric artery and have peak frequencies of 4 - 6 imp sec^{-1}. Both rabbit and cat units have low frequency discharges at 37^{o}C.

High threshold rectal mucosal mechanoreceptors, which are also excited by cantharadin, are excited by cold (3 - 8^{o}C) or hot (44 - 50^{o}C) water enemata (Clifton et al, 1976). Three rectal units were excited by both temperatures.

ALIMENTARY NOCICEPTORS?

Many C-fibres in the somatosensory nervous system are attached to nociceptors with free nerve endings. In the gut many of the afferent axons are unmyelinated but the response characteristics suggest that it is unlikely that many subserve a nociceptive function.

Some units respond to high threshold stimulation. For example, pinching, chemicals and thermal stimuli excite anal and rectal mucosal receptors in the cat (Clifton et al, 1976). The very sensitive mucosal mechanoreceptors can also be stimulated during over-distension or firm pressure (Iggo, 1957c; Iggo & Leek, 1967a; Mei, 1970b; Clarke & Davison, 1978) but to what extent this is an artifactual response is not known. It seems unlikely that such a receptor would subserve both functions. Colonic high threshold mechanoreceptors with tonic responses have been described (Floyd et al, 1976).

Rapid and slowly adapting mechanoreceptors throughout the alimentary tract are excited by local injections of bradykinin, but as movement occurs during this response, to what extent this drug is affecting the receptors indirectly is not known (Floyd, Hick & Morrison, 1975). Their action is probably indirect (Floyd et al, 1977a, 1977b). It remains a matter of speculation whether chemoreceptors in the gastric mucosa might be responsible for the pain of gastric ulceration (Iggo, 1962).

In the absence of any conclusive electrophysiological evidence, our knowledge of alimentary nociceptors has accumulated from other sources : human subjective description, and pseudo-affective reflex techniques combined with neurotomy. These suggest that pain may be a consequence of mechanical disturbance in the hollow organ, distension and contraction, traction, compression and twisting of mesentery, or altered chemical state - inflammation, potassium ion release, ischaemia, and tissue acidity (Ayala, 1937; Moore, 1938; both quoted by Keele & Armstrong, 1964). A number of pharmacological reagents injected close intra-arterially, although not intravenously, are potent algogenics : substance P, bradykinin (Potter, Guzman & Lim, 1962); KCl and acetylcholine (Braun, Guzman, Horton, Lim & Potter, 1960); histamine and 5HT (Guzman, Braun & Lim, 1962). Whether these drugs are acting directly on the receptor mechanism is not known. That some drugs act indirectly (bradykinin, Paintal, 1964) must raise doubts about the mechanism of excitation caused by any other applied substance.

Leek (1972a) discusses the possibility that endogenous substances like substance P, bradykinin and 5HT may be released by high threshold stimulation, not only exciting sensitive enteroceptors but also sensitising or exciting adjacent putative nociceptors by an axon reflex mechanism. These nociceptors may have afferent axons which project along the splanchnic nerves.

THE POSSIBLE HISTOLOGY OF ALIMENTARY ENTEROCEPTORS

The difficulty of ascribing an histological structure to enteroceptors in the gut is that the nervous networks are complex and the afferent axons often unmyelinated and that even if specific receptor elements exist they have not successfully been identified. Putative receptor structures have been described in a number of locations and the duodenojejunal segment is particularly well innervated by "sensory fibres" (Sharma, 1967).

MESENTERY

In the mesentery of the cat and man a number of structures have been observed which may subserve an enteroceptive function. These include encapsulated endings, free nerve endings attached to myelinated axons, and fine nervous plexuses attached to unmyelinated axons (Sheehan, 1932).

MUSCULARIS EXTERNA AND MYENTERIC PLEXUS

Carpenter (1918) describes "sensory endings" in the longitudinal muscles of the cat stomach which appear as skeins or networks of very fine varicose fibrils running parallel with muscle and arising from unmyelinated nerves. In longitudinal muscle of the small intestine they have typical end-tufts. Langworthy & Ortega (1943) describe spindle-shaped nerve endings that have individual bulbous terminations that penetrate and run parallel to the long axis of the stomach. These are continuations of myelinated fibres. Langworthy & Ortega (1943) also describe subserous club-shaped terminations. Histological examination of rumen wall by Stephen & Marshall (1970) reveal that nerve fibres are restricted to muscle layers and to the vicinity of vessels in the sub-epithelial space. They speculate that muscle stretch receptors may be present as unspecialised endings within nerve trunks bound by loops of collagen to adjacent muscle fibres.

Some histological evidence suggests a possible receptor function for a different cell. Daniel (1977) speculates whether a neuroglial cell (which Gabella calls "interstitial cells" in the rabbit, 1974, and Daniel calls "hybrid cells") may fulfil the function of a tension receptor. These are interspersed between dense and smooth circular muscle cells of circular muscle, having a gap junction with smooth muscle and each other, but not with dense smooth muscle. They are also closely associated with nerve axons. Daniel suggests that they may act as intermediaries between electrical activity in smooth muscle cells and nerves and that their "in series" location would allow distortion during distension or muscular contraction. They may generate potential changes which initiate impulses in visceral afferent axons. They may also behave as chemoreceptors (Daniel, 1977).

Nonidez (1946) has described what he believes are mechanoreceptors in myenteric ganglia of the dog oesophagus attached to thick myelinated vagal afferent axons. They consist of endings with single or multiple arborizations which envelop each myenteric ganglion. Hukuhara et al (1959) suggest that the cell body of the receptor involved in the in vivo intestino-intestinal inhibitory extrinsic reflex is probably in the spinal ganglion, whereas the cell body of the intestino-intestinal intrinsic reflex is in the myenteric plexus. In both cases the receptor is probably located in the longitudinal muscle or myenteric plexus (Hukuhara et al, 1959).

MUCOSA AND SUBMUCOSA

There are fine unmyelinated fibres arising from submucous ganglia which reach the muscularis mucosae. Leek (1972b) describes fine unmyelinated nerve fibres with no special nerve ending in the conical papillae surmounting the ridges of the reticulum. Light microscopy could not determine whether axons passed through the basement membrane (Leek & Harding, 1975). Some of the unmyelinated nerve endings in the intestinal villus of the cat degenerate after unilateral "afferent" vagotomy (Mei, 1978). Laryngeal receptors responding to water are probably not associated with taste buds but with epithelial and subepithelial nerve fibres (Harding et al, 1978). It has been suggested that non-chromaffin epitheliod bodies in the rat and mouse are chemoreceptors associated with the vagus nerve (Hollingshead, 1946).

Duodenal osmoreceptors may have a structural basis. It is possible that the lateral intercellular spaces around enterocytes, which expand and contract under different osmotic conditions, may be associated with such a receptor (Barker, Cockrane, Corbett, Dufton, Hunt & Roberts, 1978). This concept implies that osmoreceptors are mechanosensitive, but has no histological support.

EVIDENCE FOR THE ALIMENTARY LOCATION OF SOME ENTEROCEPTOR CELL BODIES

A large number of myelinated and unmyelinated axons have their cell bodies in dorsal root ganglia or homologous spinal ganglia. There is also evidence to support a peripheral location for many unmyelinated axons.

Ross (1958), using selective neurotomy and post-degenerative histology of intestinal neurovascular bundles in rabbit, concluded that at least 30% of axons are centripetal (afferent) with cell bodies in intestine and not in periarterial ganglia discrete from intestine, evidence for which exists in the rat, cat and man (Schofield, 1962). Schofield (1960) found similar survival in the rat and cat. Kuntz (1940) showed persistence of terminal networks in the inferior mesenteric ganglion (IMG) after chronic decentralization and demonstrated histologically that fine C-fibres leave the colon in the colonic nerves. These fibres did not degenerate in the peripheral end of the nerve after it had been sectioned.

Job & Lundberg (1952), with electrophysiological methods, showed that the hypogastric nerves contain C-fibres which form synapses in the IMG that do not degenerate after the ganglion is decentralized, nor after degenerative section of the ventral and dorsal roots T_{11} - L_6. McLennan & Pascoe (1954), with in vitro IMG-colon preparations after selective neurotomy and degeneration, suggest that the cell body of "C" fibres is in the PNS, possibly in the enteric plexus. Further, excitatory inputs to the IMG from colonic mechanoreceptors have been observed electrophysiologically (Crowcroft, Holman & Szurszewski, 1979; Kreulien & Szurszewski, 1979). It is not known whether the afferent axon projects beyond preganglia towards the CNS, nor whether they are found in locations other than colon or of rat, cat and rabbit small intestine, or whether non-mechanoreceptors are involved. Nor is there conclusive evidence for the projecting afferent axons being a primary afferent one.

Intracellular electrophysiological records have been made in both myenteric and submucous plexuses. Two functional categories of cell have been observed : those with and those without synaptic input. The cells with no synaptic input are possibly mechanoreceptor cell bodies.

They consist of about 30% of the sample of myenteric plexus neurones (Hirst et al, 1976) but are not found in the submucous plexus. These cells have a characteristic prolonged after-hyperpolarisation (AH-cells) (Hirst et al, 1974) and are probably the same neurones as the Type 2 cells of Nishi & North (1973). Nishi & North (1973) propose that the Type 3 cell may correspond with the glial cells seen in histological studies by Gabella (1972a, 1972b). They may cause a rapid (1.2 sec inhibitory) and slower (11 sec excitatory) response in aborally recorded cells involved in peristalsis (Hirst et al, 1976). On the other hand, Wood (1979) suggests that AH-cells are the same as "tonic type enteric neurones" which may be interneurones, and not sensory cells.

THE AIMS OF THE INVESTIGATION

The preceding account shows that there are many gaps in our knowledge of duodenal sensory and reflex mechanisms. The limitations of "selective" neurotomy make it difficult to establish whether extrinsic nerves are involved in reflex mechanisms, and if they are, whether in an afferent or efferent capacity, or both. Studies of reflex events have often involved the use of stimuli greater than the physiological range and therefore rarely provide information about the sensitivity of individual enteroceptors. The concept of selective sensitivity (specificity) of alimentary enteroceptors has recently been questioned by the isolation of multimodal receptors. The evidence which exists for the presence of "in series" tension receptors in the gut lacks the direct support of tension measurements of isolated parts of the muscle. There is limited evidence for alimentary thermoreceptors, and the thermal sensitivity of other receptors has not been tested.

It was the aim of this study to further the investigation of the sensitivity of duodenal enteroceptors in two herbivorous species, the rabbit and the sheep. Two approaches have been used. One was attempted in the rabbit. This was the analysis of reflex phenomena which occurred during natural mechanical stimulation of isolated duodenal loops. There were difficulties encountered with this approach in acute experiments in the rabbit. Another approach was to develop an electrophysiological technique to enable the isolation of single unit afferent activity in mesenteric nerves and to examine the sensitivity to controlled natural stimulation of isolated in vivo loops. This technique has the advantage of predetermining the organ innervated, which is not possible when recording from the more central nerve trunks. The dissection method was chosen. This preparation would enable an analysis of the mechanical, chemical and thermal sensitivity of isolated single units and could be used to test the concept of selective sensitivity, and in particular :

(a) to test the concept of specificity of duodenal osmoreceptors;

(b) to test the concept of "in series" and "in parallel" mechanoreceptors in the muscularis externa by utilising force transducers and emg records from small segments of the duodenum, while recording from single afferent units;

(c) to test the concept of threshold differences in tension receptors; and

(d) to test for the presence of duodenal thermoreceptors.

A chemical analysis was made of the composition of chyme, in order to establish a range of physiological values suitable for the stimulation of putative duodenal chemoreceptors. This was necessary because some parameters of the chemical environment are not readily available in the literature.

A study of the histology of the nerves used in the electrophysiological investigation was undertaken.

MATERIALS AND GENERAL METHODS

58 rabbits and 52 sheep were used during these experiments.

RABBITS

Because of the habit of copraphagia it was not easy to starve rabbits and so they were fed ad libitum.

ANAESTHESIA : Lean New Zealand White rabbits of either sex weighing 2 - 3 Kg were anaesthetised with 25% urethane (1.75g Kg^{-1}, 7 ml Kg^{-1}) administered intravenously into the caudal marginal ear vein. Maintenance doses of urethane (one third the induction dose) were given as required.

SHEEP

Hay was withdrawn for 24 hours before an experiment but water was available ad libitum. On one occasion, for control purposes (see Chapter 1), a sheep was not starved.

ANAESTHESIA : Sheep of various Scottish breeds, of either sex and approximately 30 Kg body weight were anaesthetised with 1% chloralose (70 mg Kg^{-1}) after induction with either intravenous pentabarbitone sodium (24 mg Kg^{-1}) or fluothane inhalation (3.5% in 50:50 nitrous oxide and oxygen). Chloralose was thereafter supplemented at intervals of approximately two hours with one third the induction dose. After eight hours chloralose was ineffective and pentabarbitone was used as necessary.

GENERAL SURGICAL MAINTENANCE

Fluid requirements for adult mammals up to 60 Kg body weight are approximately 40 ml Kg^{-1} water each day (Hall, 1967). These figures were used as a basis for fluid maintenance during prolonged anaesthesia. When fluids in excess of maintenance requirements were necessary to maintain blood pressure above an acceptable minimum, Dextran 110 B.P. injection was used (6% solution 10 - 20 ml Kg^{-1}) after pre-hydration with normal saline (0.9% NaCl). In later experiments Haemaccel (Hoechst) was used. Prophylactic doses of procaine penicillin were given pre-operatively to some animals to avoid the so-called "intestinal factor" contributing to a condition of irreversible shock. A high level of surgical care was maintained and tissue trauma, exposure and evaporative heat losses were minimised.

MONITORING OF PHYSIOLOGICAL PARAMETERS

The trachea was cannulated and end-tidal CO_2 was monitored with an infra red gas analyser (P.K. Morgan Ltd.) calibrated over the range 0.05 - 10.5%. When it was necessary to provide positive pressure ventilation end-tidal CO_2 was maintained at 4% with a pump (C.F. Palmer Ltd.). The deep body temperature was maintained at 39°C with an homeothermic heating blanket with feedback control from a thermistor, placed in the abdomen.

An heparinised catheter was positioned in the femoral artery in the rabbit and a carotid artery in the sheep to record systemic blood pressure with a Stathem transducer. A saphenous femoral vein in the rabbit and a jugular vein in the sheep was also catheterised for the administration of anaesthetics and systemic drugs.

SURGICAL PREPARATION

A surgical technique was developed to isolate a portion of the duodenum in vivo. In the rabbit two separate preparations were possible : (a) a distal, and (b) a proximal loop. Only the distal loop could be used for electrophysiological experiments. In the sheep a duodenal bulb preparation was made. In both animals the receptive field and dissecting pool were visible through the binocular microscope.

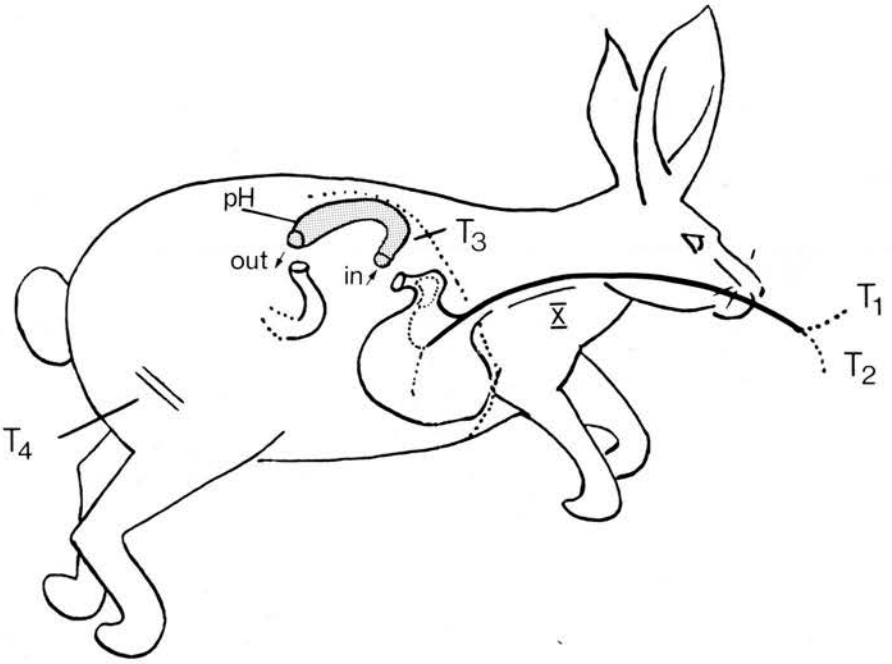
THE RABBIT PREPARATION

A mid-line abdominal incision through the linea alba exteriorised the stomach and a 3 cm long gastrotomy was performed along the greater curvature. Gastric evacuation of, usually, copraphagic faecal pellets was made. The gastric incision was closed using separate inversion suture patterns in the mucosa and muscle coats. A wide-bored polythene tube (8 mm) was passed into the stomach per os to decompress the stomach throughout the experiment. The mid-line incision was closed. The rabbit was repositioned in left lateral recumbency and a horizontal, longitudinal incision extending from the costo-chondral arch to a point below the level of the tuber coxae was made and a 110 mm diameter brass ring was sutured to the edges of the incision.

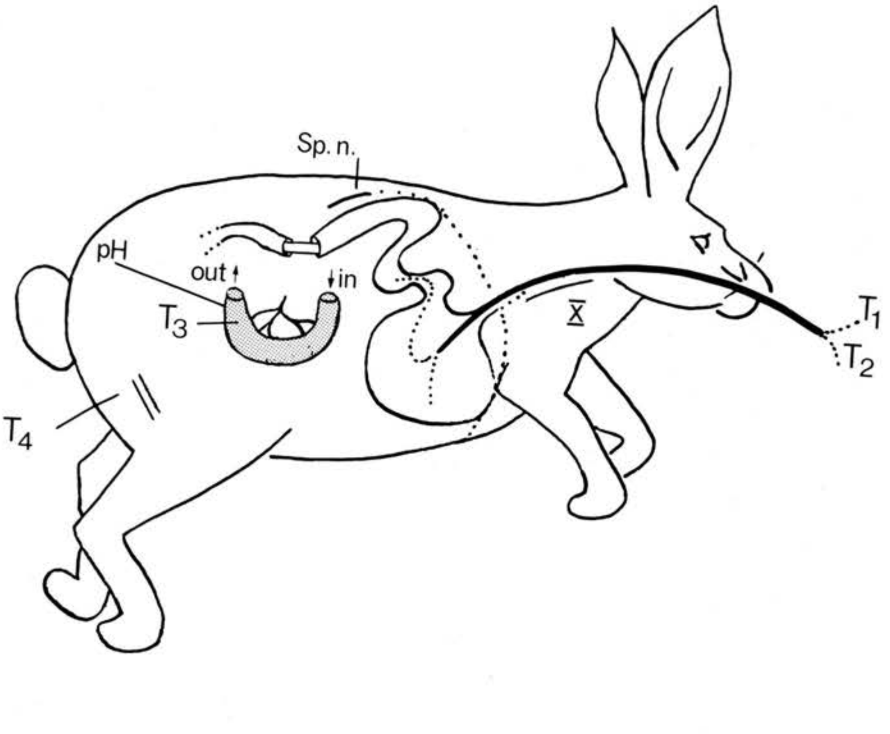
In the distal preparation a double enterotomy was performed and an isolated pancreatic and distal duodenal loop was secured inside a perfusion pool. An end-to-end anastomosis was made in the remaining duodenum with a 1 cm section of catheter tubing (8 mm diameter) and sutures. In separate animals a proximal duodenotomy was made in order to isolate the second-to-third part of the duodenum. No attempt was made to re-anastomose the proximal loop (see figure 1).

Figure 1 The two preparations used in the rabbit. (A) The proximal duodenum. No anastomosis was made between the first and last parts of the duodenum. (B) The distal duodenal preparation, also used for electrophysiological studies. In both preparations pressure transducers could be located in the stomach (T_1, T_2), the isolated loop (T_3) and the femoral artery (T_4). The loop was perfused in the aboral direction (in and out) and a pH electrode was secured in the effluent arm.

A


pH
T_3
out
in
T_1
$\overline{X}$
T_2
T_4

B


Sp. n.
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T_3
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T_1
T_2
T_4

The perfusion pool was made of 3 mm plate polyvinyl chloride (PVC) and had a single polyacetyl drawbridge (see figure 2). The intestine was secured with 8 mm (outside diameter) PVC tubing and the mesenteric fan was supported by a 30 mm diameter nickel-plated brass mushroom. A separate transparent perspex horseshoe prevented the duodenum from floating in the liquid paraffin. The lower edge of the sluice, through which the mesentery was passed, was made paraffin-tight with the aid of cotton wool and vaseline and filled with liquid-paraffin at 39^{o}C. Thus an isolated pool was created, free of all unwanted abdominal movements while allowing normal blood supply and lymphatic drainage.

Normal saline or mammalian Tyrode solutions, with and without glucose, were routinely used as perfusion fluids. Drugs and test solutions could be applied intraluminally by a separate catheter. An open-tipped catheter or small balloon (capacity 0.25 ml) connected to a pressure transducer was used to record local changes in intraluminal pressure. A pH electrode (Russel pH Ltd.) was secured in the effluent fluid. The pH meter was calibrated with standard buffer tablets dissolved in distilled water at 20^{o}C (BDH Chemicals Ltd.).

The perfusion apparatus was designed to deliver a controlled volume of solution and to provide rapid changes in pressures to preset maxima from a 5-litre bell-jar. By clamping the outlet of the apparatus (outlet-clamping) abrupt (square-wave) and slow (ramp-wave) approximately isotonic intraluminal pressure changes could be applied to the loop. A safety valve prevented the intraluminal pressure from exceeding 30 cm water, and usually values below 20 cm water were used. It was important to prevent syphoning from the effluent tube which otherwise collapsed the loop (see figure 3). The perfusion apparatus was regularly washed in chlorhexidine gluconate solution (Hibitane, ICI).

Figure 2 (A) The rabbit perfusion bath. (a) plate polyvinyl chloride bath, (b) polyacetyl drawbridge, (c) nickel-plated brass mushroom, (d) transparent perspex horseshoe.

(B) The perfusion bath *in situ*. The perfusate was introduced at the oral end of the duodenal loop and passed from right to left. A pH electrode was situated in the effluent limb. A thermistor (t) was placed into the abdomen and provided feedback control to the heating blanket.

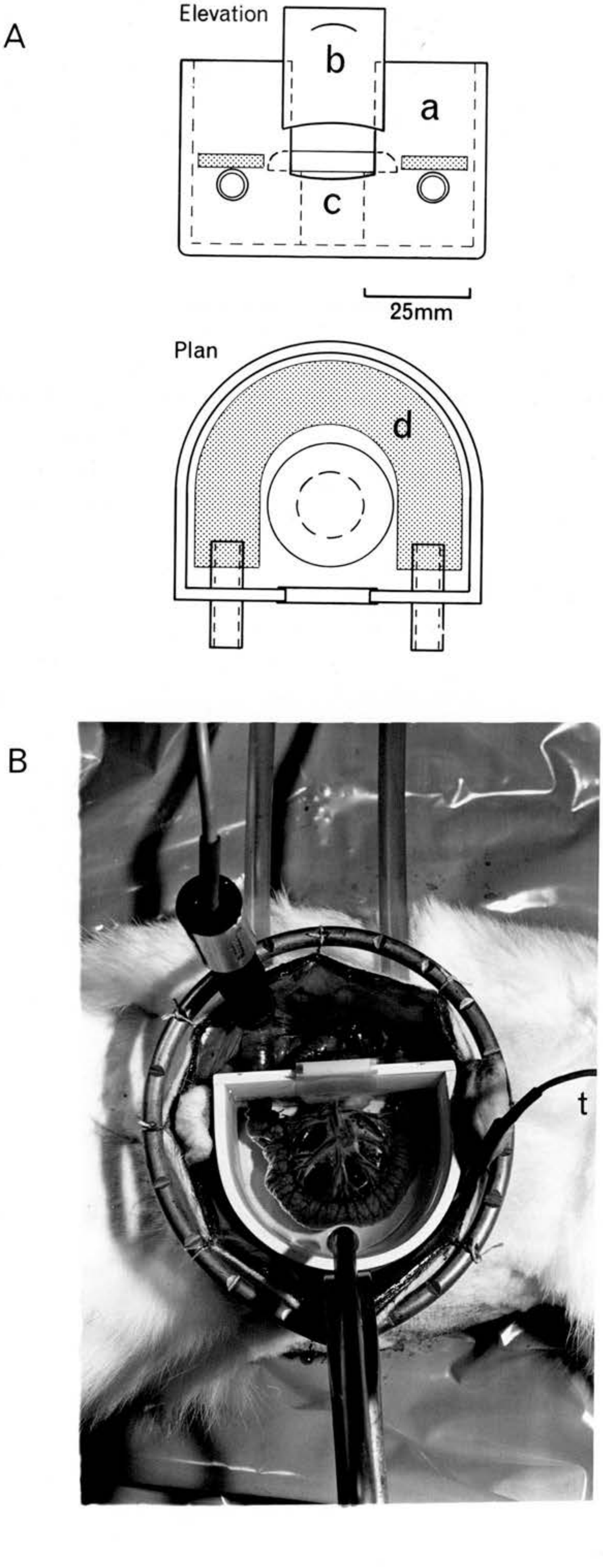
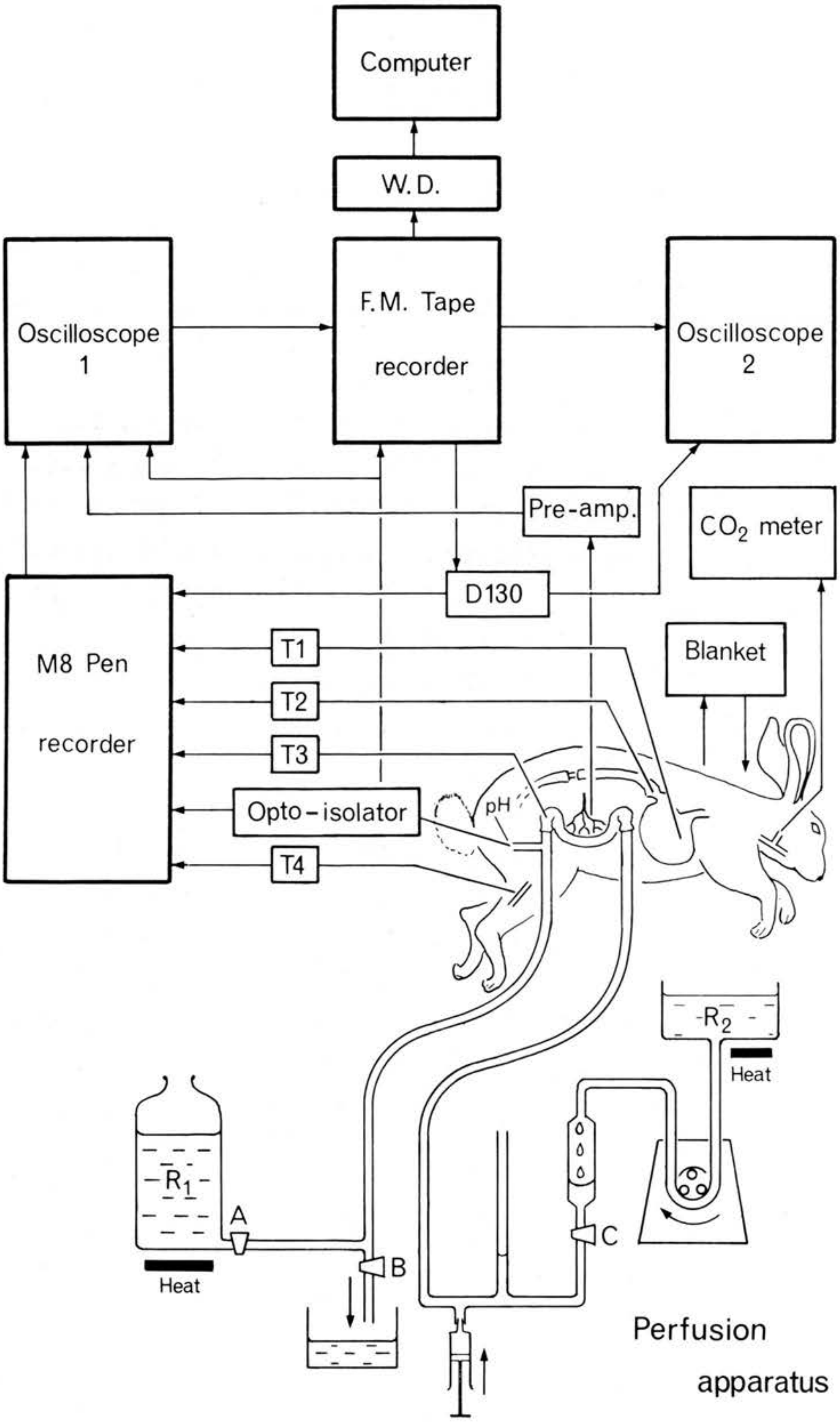

A
Elevation
b
a
c
25mm
Plan
d
B
t

Figure 3 A schematic diagram of the recording and perfusion apparatus, for use in the rabbit. Pressure transducers (T_1, T_2, T_3 and T_4); spike processor (D130); reservoirs (R_1 and R_2); three-way valves (A, B and C). The window discriminator (W.D.) and PDP-12 computer (Computer) were used off-line. Nervous and emg activity went to the pre-amplifier (Pre-amp).

In the sheep experiments the perfusion apparatus was not used and, in addition, tension changes in the outer muscle coat were collected and stored on a separate two-channel pen recorder (Devices Ltd) with suitable amplification.


Computer
W. D.
Oscilloscope 1
F. M. Tape recorder
Oscilloscope 2
Pre-amp.
CO$_2$ meter
D130
M8 Pen recorder
T1
T2
T3
Opto-isolator
T4
Blanket
pH
R$_2$
Heat
R$_1$
A
B
C
Heat
Perfusion apparatus

THE SHEEP PREPARATION

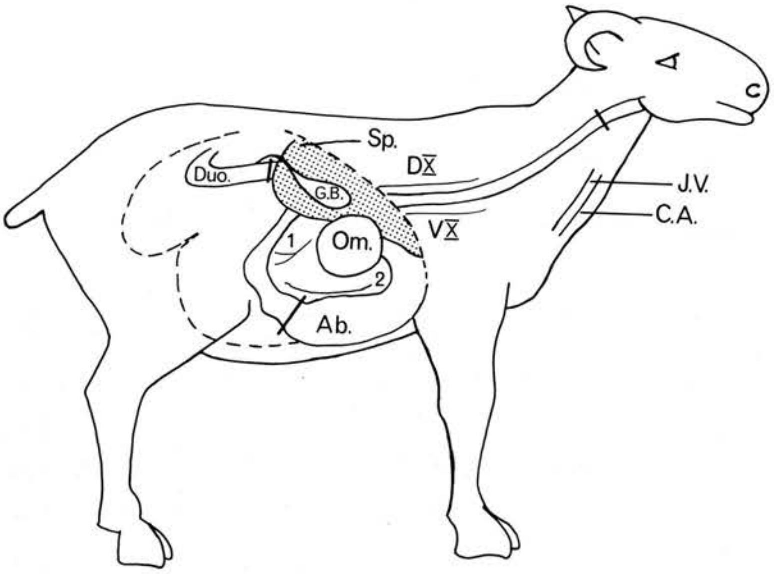
The oesophagus was ligatured in the cervical region. A rumenotomy was performed and reticulo-rumen contents evacuated and the wound sutured. With the sheep in left lateral recumbency a flap incision was made following the right costo-chondral arch from which the pylorus could be exteriorised. The preparation was ligatured between the fundus and antrum and also at the duodenum just oral to the entrance of the bile duct to prevent chyme and bile reflux. After sections through the superficial sheet of the greater omentum and lesser omentum the preparation could be firmly secured in a polyvinyl chloride organ bath (see figures 4 and 5). In situ the long pyloric (hepatoduodenal) branch of the right vagus nerve (Duncan, 1953; Habel, 1956) together with the veins, lymphatics, arteries and a prominant fibrous bundle were lain across an isolated dissection pool by selecting suitable apertures. This was made paraffin-tight with polyacetyl drawbridges, cotton wool and vaseline. The dissection pool was filled with liquid paraffin maintained at 39°C. The pylorus and proximal duodenum were laid across the cork dissection board of the larger compartment.

Two preparations could be made : either (a) the duodenum was left intact except for the introduction of small perfusion catheters, open-tipped catheters and balloons (expanded volume 4 ml), attached to Stathem pressure transducers, or (b) a longitudinal incision was made to expose the mucous membrane of the torus pyloricus, pylorus and duodenum. This second preparation was most often used. To prevent heat loss by evaporation and to reduce condensation onto the lens of the dissecting microscope, the abdominal cavity was packed with sterile foam rubber.

Figure 4 (A) Schematic drawing of the anatomy of the right side of the abdomen of the sheep. (1) long pyloric (hepatoduodenal) nerve, (2) continuation of ventral vagus; J.V. jugular vein; C.A. carotid artery; DX dorsal and VX ventral abdominal branches of the vagus nerves; Sp. right splanchnic nerve; G.B. gall bladder; Om. omasum; Ab. abomasum; Duo. duodenum. Thick bars indicate the position of alimentary ligatures.

(B) The sheep duodenal recording bath. (a) polyvinyl chloride bath, (b) polyacetyl drawbridges, (c) nickel plated brass base of dissecting pool, (d) cork base.

A



B

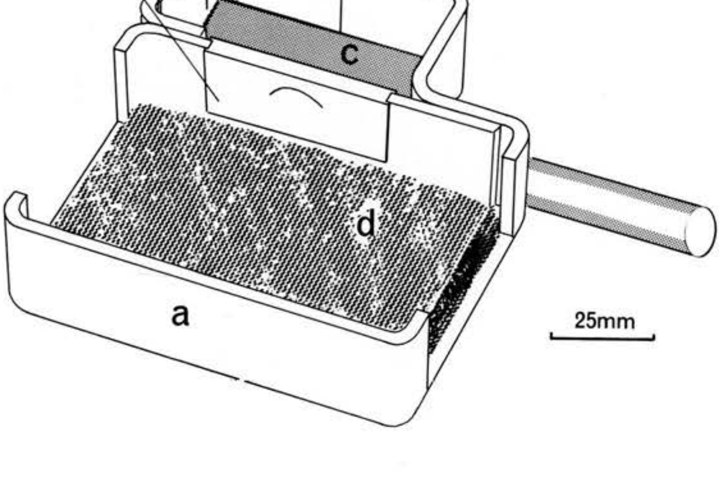
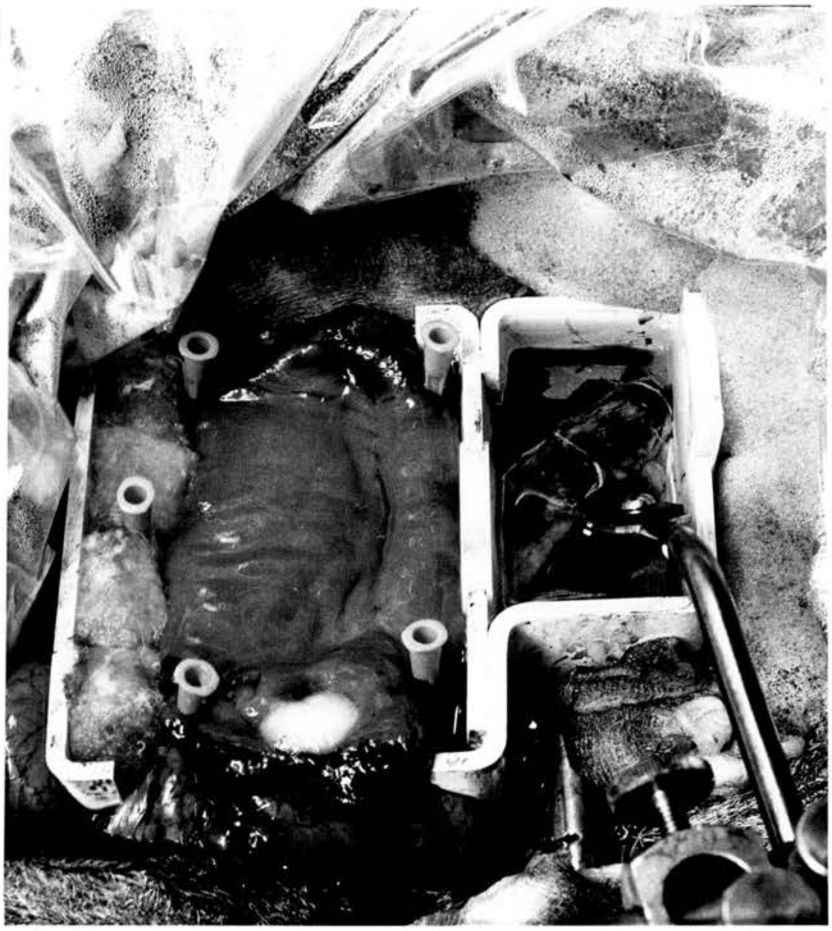


Figure 5 The sheep duodenal recording bath *in situ*. (A) illustrates the intact duodenal preparation, a perspex catheter has been introduced through a small puncture incision in the pyloric bulb. (B) The duodenal segment has been opened longitudinally and secured with needles on the cork board. Note the accumulation of Brunner's gland secretion which keeps the preparation moist.

A

B



MECHANICAL RECORDING AND STIMULATION

In the rabbit, the wide-bored polythene tube used to decompress the stomach contained two pressure-recording catheters with either open-tipped ends or liquid-filled balloons (capacity 5.0 ml). These were secured in the duodenal bulb, pyloric sphincter, pyloric canal or the fundus by small sutures. Pressure recording catheters could also be introduced into the duodenal bulb or pyloric sphincter, or into the aboral duodenum via the transverse enterotomy wounds. The duodenal bulb and aboral duodenum could be distended with intraluminally placed balloons, distended volume 5 ml.

In the sheep closed-loop preparation, balloons and catheters were secured as described above. In open-loop preparations, the receptive fields were explored with glass probes, cotton-wool buds and Von Frey hairs. The tip diameter of hairs was measured with a microscope and an eye-piece micrometer. The hairs were calibrated against isometric force transducers and gram weights. The receptive field could be stretched at a distance with blunt forceps and compressed with a probe attached to an isometric force transducer. The muscularis externa was made to contract with transmurally applied electric currents, electrical excitation of the continuation of the ventral vagus (sic) and the administration of drugs intravenously and close intra-arterially. The tension developed in the muscularis externa was recorded by two isometric (force) transducers (Devices Instruments Ltd.) positioned to monitor longitudinal and circular (tangential) tensions by thin cotton sutures and displayed on a 2-channel recorder (Devices Ltd., transducer 3559).

Intraluminal pressures have been expressed as units of centimeters of water ("cm water") above atmospheric pressure which could be read directly from a water manometer open to atmosphere (see figure 3).

THERMAL STIMULATION

The temperature of the receptive field was altered either by using a thermal device which was a copper thermode through which water was delivered from three thermostatically controlled reservoirs kept at different temperatures (Cottrell, Iggo & Kitchell, 1978), or by flooding with solutions of various temperatures and measuring the surface temperature with a centigrade thermometer (range 0 - $50^{o}C$). In later experiments a temperature probe with a 1 mv $1^{o}C^{-1}$ calibrated output was used (type 650-01, Digitron Inst. Ltd.).

DRUGS AND CHEMICALS

The pharmacological preparations used, their routes of administration and chemicals applied may be found in the relevant chapters.

The composition of mammalian Tyrode solution was :

NaCl (8 g); KCl (0.2 g); $CaCl_2$ (1.8 g); $MgCl_2$ (0.1 g); NaH_2PO_4 (0.05 g); $NaHCO_3$ (1 g); and glucose (1 g) (to make 1 litre).

ELECTRICAL RECORDING

Nerves were dissected from neurovascular bundles and divided transversely. The perineurium was threaded off and the exposed nerve dissected longitudinally using micro-techniques similar to Iggo (1955). The distal end was used for afferent recording. Electrical activity was recorded with silver electrodes monopolarly, the second arm of the bipolar electrode was earthed to non-nervous tissue of similar size to the fibre under investigation. The preparation was earthed by a large nickel-plated brass plate situated in the dissection pool. Impulses were differentially amplified with a preamplifier (3160 Digitimer Ltd.) displayed on a storage oscilloscope (Tektronix D13 dual beam) and stored on an FM tape recorder (T.E.A.C. Corporation).

The conduction velocity was measured by the peripheral stimulus technique (Iggo, 1958a) over different sections of the nerve. Isolated stimulators were used (Mark IV, Devices) and triggered from a digitimer (Devices Instruments Ltd.). Conduction distances were measured by accurate placement of thin cotton thread along the nerve, which was then measured on a meter rule.

Electromyographic activity was recorded differentially from the outer muscle coats with plastic-covered stainless steel wires (150 µm diameter Diamel coated) which were placed with stilettes made from 25 mm gauge needles. The emg was stored on magnetic tape and displayed on an 8 channel recorder (Devices Ltd.).

Voltages from the pH electrode were passed through an analogue opto-isolator system (Donald Ensor) to enable permanent storage on the 8-channel recorder and magnetic tape.

DATA ANALYSIS

Spike train analysis was made both on-line and off-line with a spike processor (D130, Digitimer Ltd.) and histograms were stored permanently on heat-sensitive paper together with pH, emg and pressure records (M-8 channel recorder, Devices Ltd.). Off-line analysis was performed with a PDP-12 computer and spike height discriminator (Ramsey, 1975) to transform the nerve impulses into standard voltage pulses.

During routine examination of unitary spike trains the minimum interspike interval was measured by triggering the oscilloscope from the impulse and by choosing suitable sweep speeds to measure the time to the peak of the second impulse. Intervals were read directly from the storage trace of the oscilloscope. Long spike trains, together with up to two analogue channels, were displayed on an RM 565 dual beam oscilloscope (Tektronix) and photographed with a continuous recorder camera (Nikon Kohden, Kogyo Co Ltd.) on Kodak 2495 RAR film.

Interspike intervals were collected in real time and stored on linctape as dwell and interval histograms with a PDP-12 computer and a programme Collect 72 (D Young, 1974). Sequential dwell histogram material was viewed and analysed with Data 72 programme (D Young, 1974). Data stored on separate dwell histograms were combined with a Focal programme and stored as interval histograms (F Cervero, 1978). Stored and unstored material could be analysed with a Kate programme package (Hugh Bostock) and results displayed with an X-Y plotter (Complot, Digital Displays Ltd.).

The distribution of histograms was compared with the theoretical normal distribution :

$$f(x) = 1/(sd.\sqrt{2\pi}) . \exp -1/2 (<X - X>/sd)^2,$$

and using χ^2 calculation : $\chi^2 = \Sigma(O - E)^2/E$. For this a computer programme was written in Fortran (G McConnell, 1981).

Linear regressions were fitted by the method of least squares.

Student's t test was used to determine the significance of differences between data.

CHEMICAL ANALYSIS

For the analysis of biological and stimulating solutions the following methods were used :

Osmolality : A depression of freezing point osmometer (Vogel) after calibration against standard 300 mOsm Kg^{-1} solution and distilled water.

pH : A glass electrode (Russel Ltd.) and pH meter (E.I.L.) after calibration against standard buffer solutions (BDH Chemicals Ltd.).

In addition the following methods were used only for biological material :

Sodium and potassium : Solutions were transferred into dialysis tubing (Scientific Instruments Centre Ltd.) and suspended in distilled water in a refridgerator overnight. The resulting solution was analysed with a flame photometer (Evans Electroselenium Ltd.) calibrated against standard solutions of sodium chloride and potassium chloride. Twenty-four hour samples were not significantly different from 48 hour controls.

Chloride : Chloride levels were estimated coulometrically with a chloride titrator (CMT 10, Radiometer Copenhagen).

Titratable acidity and alkalinity. Unfiltered solutions were left to stand and the supernatant titrated against standard pure solutions of NaOH or HCl (BDH Chemicals Ltd., concentrated volumetric solutions) suitably diluted with distilled water (N/10, N/100). Solutions were titrated to end point pH 6.5 with pH electrode (Russel Ltd.) and pH meter (E.I.L.). The end point was chosen from the data of Hunt & Knox (1972).

Volatile fatty acids. Two analytical techniques were used for estimating volatile fatty acids either in free solution (method 1) or adsorbed to particulate digesta (method 2). The details may be found below. A Gas Chromatogram (Hewlett-Packard, 5700 A) was used to analyse the final samples and calibrated against standard solutions (2 - 4 $\mu g \ \mu l^{-1}$) (see figure legend 6). The assumption was made that the peak height of the chromatograph was a good indication of area, therefore :

$$\begin{array}{l}\text{weight VFA in original} \\ \text{sample } (\mu g)\end{array} = \frac{\text{sample peak (mm)}}{\text{calibration peak (mm)}} \times \begin{array}{l}\mu g \text{ in calibration} \\ \text{sample volume } (3\mu l)\end{array} \times \frac{\begin{array}{c}\text{total residual} \\ \text{sample } (\mu l)\end{array}}{\begin{array}{c}\text{calibration} \\ \text{volume } (\mu l)\end{array}}$$

$$= \frac{S}{C} \times (3 \times 2 \text{ or } 4) \times \frac{2000}{3} \ \mu g$$

Method 1

Adjust pH of sample to approximately pH 1 with N HCl

Steam distil to give approximately 100 ml

Extract in 200 ml ether (twice original volume)

Shake well in a separating flask, allow to settle and draw off water

Add anhydrous sodium sulphate to the ether/VFA mixture to remove remaining water

Pour off the ether to leave VFA

Dissolve VFA in 2 ml acetone and use directly in gas chromatograph

Method 2

Check original pH

Centrifuge the sample

Remove the supernatant and treat as method 1

To solid add N NaOH, mix well and centrifuge

(Any VFA adsorbed will now be converted to soluble sodium salts)

Acidify with N HCl to approximately same as original sample

Treat as method 1. Bound VFA will show on the gas chromatograph.

CHAPTER 1

THE CHEMICAL ENVIRONMENT OF THE DUODENUM OF THE RABBIT AND SHEEP

INTRODUCTION

This investigation was initiated to determine whether the chemical composition of abomasal and duodenal chyme of starved sheep, and the gastric and duodenal chyme of laboratory rabbits obtained during acute experiments were similar to the values reported in the literature from a number of different sources. A variety of previously unreported parameters were studied, in addition to an examination of sheep Brunner's gland secretion and sheep bile. These analyses were an attempt to define some of the parameters of the intraluminal chemical environment in order to choose values for the electrophysiological studies to which the afferent units might respond. Measurements were run in parallel with the electrophysiological investigations and were an integral part of them.

METHODS

The same animals used for the electrophysiological and reflex experiments were also used during this investigation. Fresh material was obtained at the first convenient opportunity during the surgical preparation and was rapidly transferred to stoppered bottles. Either fresh solutions were analysed, or solutions were stored for a short time in air-tight refridgerated conditions. Some solutions were allowed to sediment and the supernatant was used. Sheep bile was aspirated directly from the gall bladder with a wide-bored needled and syringe and the puncture wound was closed by a modified purse-string suture.

RESULTS

The analysis of the chemical environment of the stomach and duodenum is presented in tables, 3, 4 and 5.

Two large peaks were found in the rabbit samples for which calibration solutions were unavailable (see figure 6).

A separate analysis of rumen contents was made. The total VFA (mM Kg^{-1}) for starved sheep was 2.07, 3.41 and 1.18 in three separate sheep. One sheep, allowed access to hay and crushed oats, had a value of 13.8 mM Kg^{-1}. A detailed analysis is shown in table 3B.

Table 3 Volatile Fatty Acid Analysis

A. RABBIT STOMACH

VOLATILE FATTY ACIDS (mean +/- sd) (N > 8)

	MW	In solution, mM L^{-1}	Adsorbed, mg Kg^{-1}
acetic (C_2)	60.05	0.113 +/- 0.049	26.21 (other values not quantified but high)
propanoic (C_3)	74.08	0.152 +/- 0.011	1.13 +/- 1.30
isobutanoic		0.019 +/- 0.012	0.27 +/- 0.30
butanoic (C_4)	88.12	0.84 +/- 0.53	8.76 +/- 6.87
pentanoic (C_5)	102.13	0.052 +/- 0.03	2.38 +/- 3.21

B. SHEEP RUMEN

(N > 8)	mM Kg^{-1}		mM Kg^{-1}	
	starved (m+/-sd)	ratio	fed	ratio
acetic	0.62 +/- 0.41	1	3.9	1
propanoic	0.75 +/- 0.47	1.2	5.8	1.5
isobutanoic	0.18 +/- 0.07)	1.2	3.6)	2
butanoic	0.57 +/- 0.25)		3.6)	
pentanoic	0.09 +/- 0.03	1.05	0.1	-

Table 4 Chemical Analysis of Bile and Brunner's Gland Secretions

SHEEP (mean +/- sd) (N > 8)

	Brunner's Gland	Bile
osmolality (mOsm Kg^{-1})	306 +/- 2.8	293.3 +/- 14.2
pH	7.85 +/- 0.28	7.33 +/- 0.77
Na (mM L^{-1})	172.3 +/- 23.5; 58.3	149.2 +/- 18.9; 228
K (mM L^{-1})	7.10 +/- 1.61	7.54 +/- 1.73
Cl (mM L^{-1})	117.0 +/- 8.3	73.2 +/- 13.9

Table 5 Chemical Analysis of Rabbit and Sheep Chyme

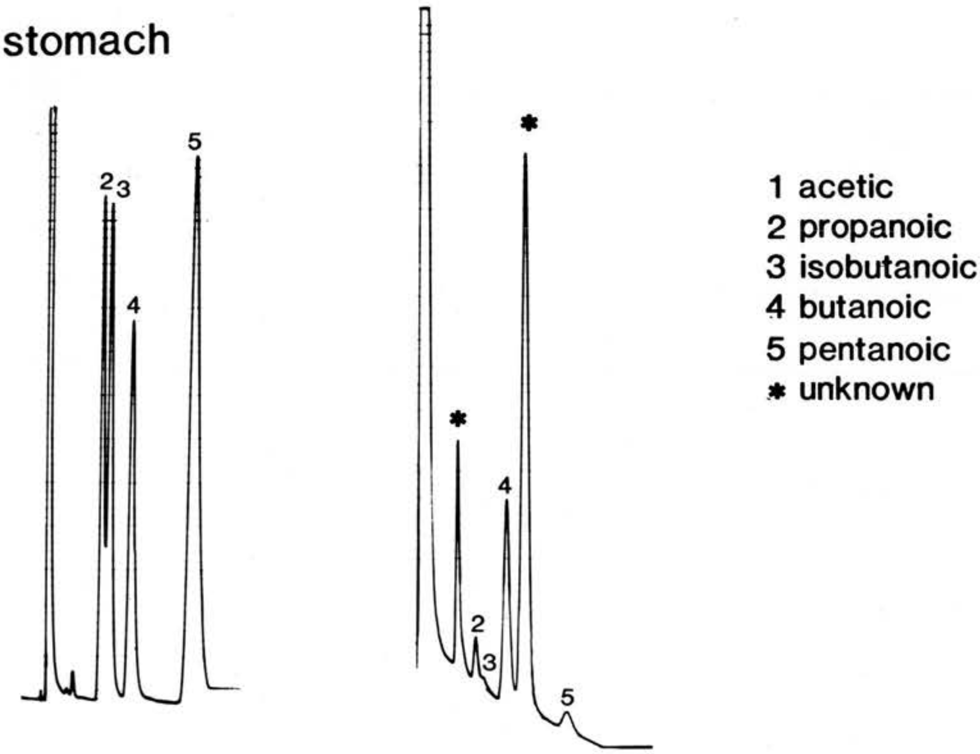
	STOMACH/ABOMASUM		DUODENUM	
(N > 8)	RABBIT	SHEEP	RABBIT	SHEEP
pH (m +/- sd)	1.4	3.4 +/- 0.3	6.7	6.5 +/- 1.3
volatile fatty acids free (mM L^{-1})	1.11 +/- 0.62	-	-	0.32
volatile fatty acids adsorbed (mg Kg^{-1})	21.27 +/- 19.67	-	-	-
titratable acid mEq L^{-1}	-	15.82 +/- 12.4	zero	8.6 +/- 9.9
titratable alkali mEq L^{-1}	-	-	-	14.2 +/- 12.2
potassium ions mEq L^{-1}	26.4 +/- 12.6	20.5 +/- 24.6	20.6 +/- 3.5	7.4 +/- 3.8 (one value 122.9)
sodium ions mEq L^{-1}	17.4 +/- 5.6	80.9 +/- 58.9	53.4 +/- 14.8	61.7 +/- 29.9
chloride ions mEq L^{-1}	156.5 +/- 29.2	86.8 +/- 26.0	82.5 +/- 7.8	156.5 +/- 29.2
osmolality mOsmol Kg^{-1}	267.3 +/- 41.5	286 +/- 28.1	-	284.7 +/- 14.3

Figure 6 Representative traces from gas chromatography. In each pair of traces the first is of a standard mixture in acetone of 6 μg propanoic, isobutanoic, butanoic and 12 μg pentanoic acids. The sheep standard also contained 6 μg acetic acid.

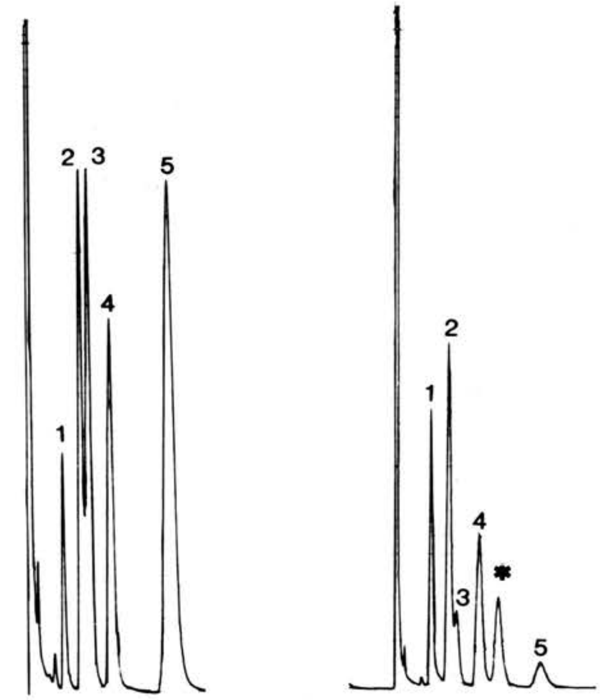
The chromatography took place in a 5-foot length column containing a stationary phase of 5% carbawax with 0.35% H_3PO_4, and a standardised flow rate of a carrier gas, helium. The oven temperature was 125^oC and the injection port temperature was 150^oC.

In the rabbit stomach sample (A) the sensitivity of the sample reading was x 20 the standard. In the sheep rumen sample (B) the sensitivities of the standard and sample were the same. The volume in all cases was 3 μl. Note : the unknown peaks denoted by an asterisk.

Rabbit stomach



Sheep rumen



DISCUSSION

These results show close agreement with those found under other experimental conditions (see table 2). Because the pH of the duodenal contents in the sheep was high, the corresponding titratable acid values were low. Also, because some samples were alkaline, values were found for titratable alkalinity. This variation may have been due to the manipulation of the animal during surgical preparation, which must be avoided for precise measurements. This is particularly the case when recording low pH in the duodenal bulb.

The analysis confirms the low values of volatile fatty acids reaching the duodenum under normal conditions. The values however are lower than are expected from the literature. It was necessary to test the method with samples known to contain greater concentrations of VFA's. For this rumen contents were used, which shortly after feeding are known to contain large amounts (Annison, 1954; Gray, Pilgrim, Rodda & Weller, 1952). The fed sheep showed significantly larger values than the starved sheep. The ratio acetic : propionic : butyric was 1 : 1.2 : 1.2 in the starved sheep, and 1 : 1.5 : 2 in the fed sheep. Because the expected values are of the order 3 : 2 : 1 then it is probable that the technique was insufficient to obtain all the acetic acid in solution. The large peak preceding the propanoic peak in the rabbit was probably lactic acid (Alexander & Chowdhury, 1958). The second peak may have been isopentanoic, but this was not confirmed.

There was close agreement with the results of Harrison (1962) who measured the ionic composition of bile obtained from the gall bladder (Na^+, 197; K^+, 8.0; Cl^-, 74 mEq L^{-1}). In other species the bile is isotonic (Wheeler, 1968).

These results, together with others in the literature, confirm that the duodenal environment has the following characteristics : a stable osmolality; little volatile fatty acids in the sheep, although possibly higher values of adsorbed VFA's in the rabbit; large changes in pH, titratable acid and alkali; and wide fluctuations in potassium and sodium ions. The electrophysiological evidence presented elsewhere (Chapter 3) demonstrates that duodenal enteroceptors respond to the physiological ranges found in the chemical environment with a sensitivity of +/- 5.0 to 10 mM L^{-1}. It would appear that the stimuli used in reflex studies are often outside the probable physiological ranges encountered.

CHAPTER 2

THE MECHANICAL SENSITIVITY OF THE DUODENUM OF THE RABBIT
A REFLEX STUDY

INTRODUCTION

The duodenum is known to be profoundly reflexogenic (Thomas & Baldwin, 1968) and it is also a reflex target organ (Daniel & Weibe, 1966; Daniel & Irwin, 1968; Daniel & Sarna, 1975). The enteric ganglia play an important role in local intestinal reflexes (Wood, 1976), but the precise role of the extrinsic nervous system remains to be clarified.

The aim of this series of experiments was to test the mechanical sensitivity of isolated loops of the duodenum in the rabbit, by monitoring mechanical changes induced in the loops and other segments of the alimentary canal. These reflex mechanisms have not been previously investigated in the rabbit. A knowledge of reflex thresholds was needed in order to establish the probable threshold and location of single mechanoreceptors which were to be investigated electrophysiologically.

It was found that urethane-anaesthetised rabbits could not tolerate gross surgical interference: the preparation deteriorated early in each experiment. Also, the rabbit was unable to withstand the effects of drugs normally utilised for reflex blocking purposes. For these reasons, the study was incomplete. A more robust species is needed to enable the reflex mechanisms to be investigated thoroughly. In general, the mechanical reflex thresholds agreed closely with the sensory thresholds found in mechanosensitive afferent units, which are discussed in Chapter 3.

METHODS

Proximal and distal duodenal loops were prepared in separate animals. The following neurotomies were performed during the investigation : 1. abdominal dorsal and ventral vagotomy, either via the abdomen or transthoracically; 2. left and right cervical vagotomy; 3. right proximal splanchnotomy; 4. selective mesenteric neurotomy. The splanchnic nerve was exposed either by the method of Liddell & Sherrington (1929) or via the abdomen during the preparation of duodenal loops. The main nerve trunks were left in continuum and secured with thin cotton thread for later location.

The distal ends of sectioned vagus and splanchnic nerves could be excited from isolated stimulators with bipolar silver electrodes using ranges 1 - 10 V; 1 - 10 Hz and 0.1 - 5 msec. These parameters are similar to those used during experiments utilizing electrical excitation of the cervical vagus nerves in the cat (Martinson, 1959; Brooks & Carr, 1975). The right saphenous femoral nerve was sectioned and prepared for electrical stimulation at its central end. Electromyographic recording electrodes were placed in the muscle of the pyloric sphincter area in some experiments.

The following drugs were used intravenously : atropine (2.5 mg Kg^{-1}, muscarinic blocker); hexamethonium (10 mg Kg^{-1}, nicotinic blocker); propanolol (1.0 mg Kg^{-1}, $beta_1$ and $beta_2$ blocker); phentolamine (0.5 mg Kg^{-1}, $alpha_2$ blocker). In addition, lignocaine (2%) and cocaine (3%) were applied to the mesentery or added to the perfusion fluid.

RESULTS

Reflex responses in the rabbit were not easily quantified and therefore limited analytic progress was made. After the first response to the application of a stimulus there often followed periods during which further responses to the same stimulus were either absent or reduced. Also, the preparation did not maintain physiological stability : deterioration was progressive and refractory to treatment with positive pressure ventilation with oxygen enriched air (20 - 40% O_2), pressor drugs and intravascular fluids. For these reasons, it was often difficult to decide whether abolition of a reflex followed surgical or pharmacological neurotomy.

In all preparations complete transverse enterotomy was performed and therefore myenteric mechanisms were eliminated as a possible reflex pathway. The duodeno-gastric portal system was not involved because all responses vanished when selective mesenteric neurotomy was performed, or when local anaesthetics were applied to the mesentery. These procedures left the vascular system intact.

THE GENERAL ACTIVITY OF THE STOMACH AND DUODENUM

When the intraluminal pressure was raised from 0.5 to 2.0 cm water, peristalsis occurred in the duodenal loop. This coincided with increases in the amplitude of pyloric contractions, as indicated by pressure changes in locally placed balloons as well as increased electromyographic activity. Pyloric contractions had a phase lead of about 2.5 seconds over similar duodenal contractions. The general level of motor activity in the duodenum and stomach also increased during depression of systemic blood pressure which occurred during long experiments or following doses of hexamethonium (10 mg Kg^{-1}). Anaesthetic levels were also important : maintenance doses of urethane often inhibited previously-active preparations for several hours.

ORALLY DIRECTED REFLEXES

1. Duodeno-Duodenal Bulb Inhibition

In 18 trials in 6 preparations a square-wave stimulus (onset ramp 10 cm water sec^{-1}), applied to proximal duodenal loops, caused a fall in intraduodenal bulb pressure after a short latency (1 - 5 sec). In different preparations the threshold was between 7.5 and 9 cm water. When a ramp waveform was used (onset ramp 0.6 cm water sec^{-1}) to the same maxima shown to give a response with square-wave stimulation, there was no change in bulbar pressure. Figure 7A demonstrates this reflex response. Note that repetition of a supra-threshold square-wave stimulus, within several minutes of a slow ramp, gave no response. Ten minutes later the response returned. Local anaesthetic solutions applied to the mesentery, and selective mesenteric neurotomy abolished the response.

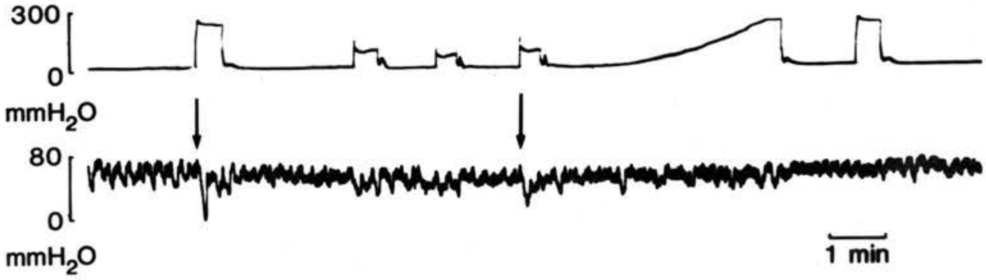
2. Duodeno-Pyloric Contraction

In most experiments rapid distension of the proximal duodenal loop, from mean values of 0.5 up to 25 cm water (at 10 cm water sec^{-1}), caused a contraction of the pyloric sphincter. The latency was less than 10 seconds and the threshold was 4 cm water. The pressure changes were usually tri-phasic when the square-wave stimulus was held for 1 minute or longer (see Figure 8). The characteristic contractions were recorded only by a balloon placed across the

Figure 7 (A) Duodeno-duodenal bulb inhibition reflex. Upper Trace : The intraduodenal pressure in a proximal loop. Lower Trace : The intraduodenal bulb pressure. Six stimuli were delivered with peak intraluminal pressures of 27, 9, 6, 9, 27 and 27 cm water. The threshold for the reflex was above 9 cm water (arrows). After a slow ramp stimulus a square-wave stimulus of 27 cm water failed to cause the reflex change, which returned after 10 minutes. The latency of the onset of duodenal bulb relaxation was 5 sec and the maximum relaxation occurred after a delay of 12 sec.

(B) The duodeno-depressor reflex. Upper Trace : intraduodenal pressure; Middle Trace : systemic arterial blood pressure; Bottom Trace : pyloric pressure. A fall in blood pressure occurred during the onset of the square-wave (denoted by arrows). Note that the large square-wave stimulus produced a tri-phasic duodeno-pyloric reflex (see figure 8A) denoted by asterisks, which was not present when stimuli below 9 cm water were used.

A


300
0
mmH$_2$O
80
0
mmH$_2$O
1 min

B

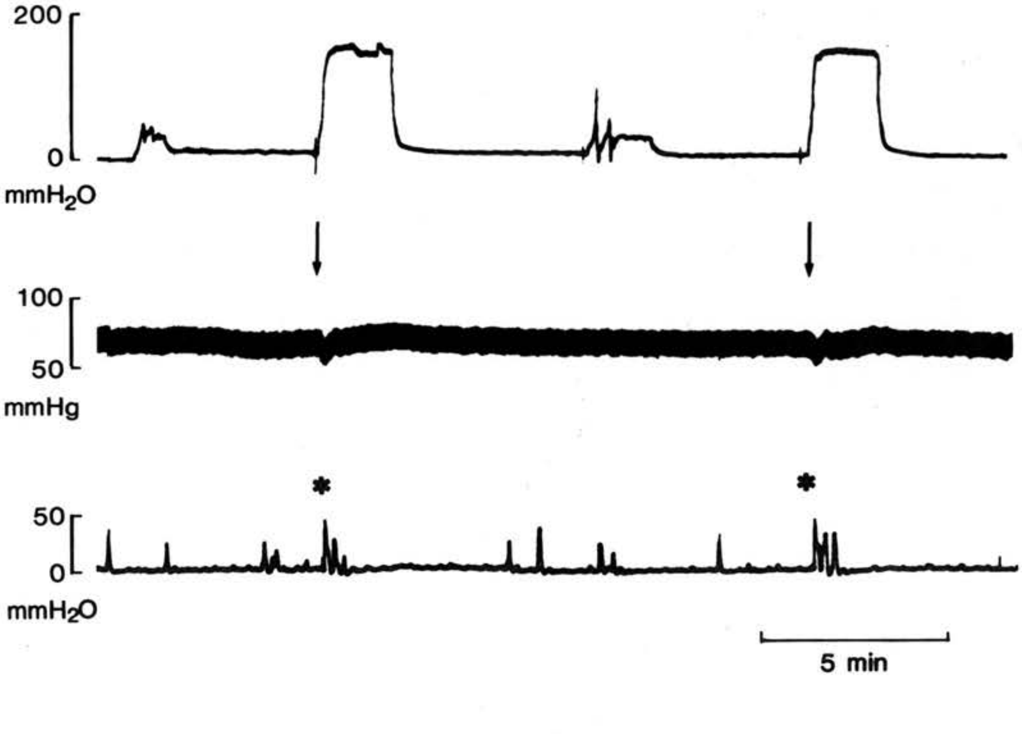
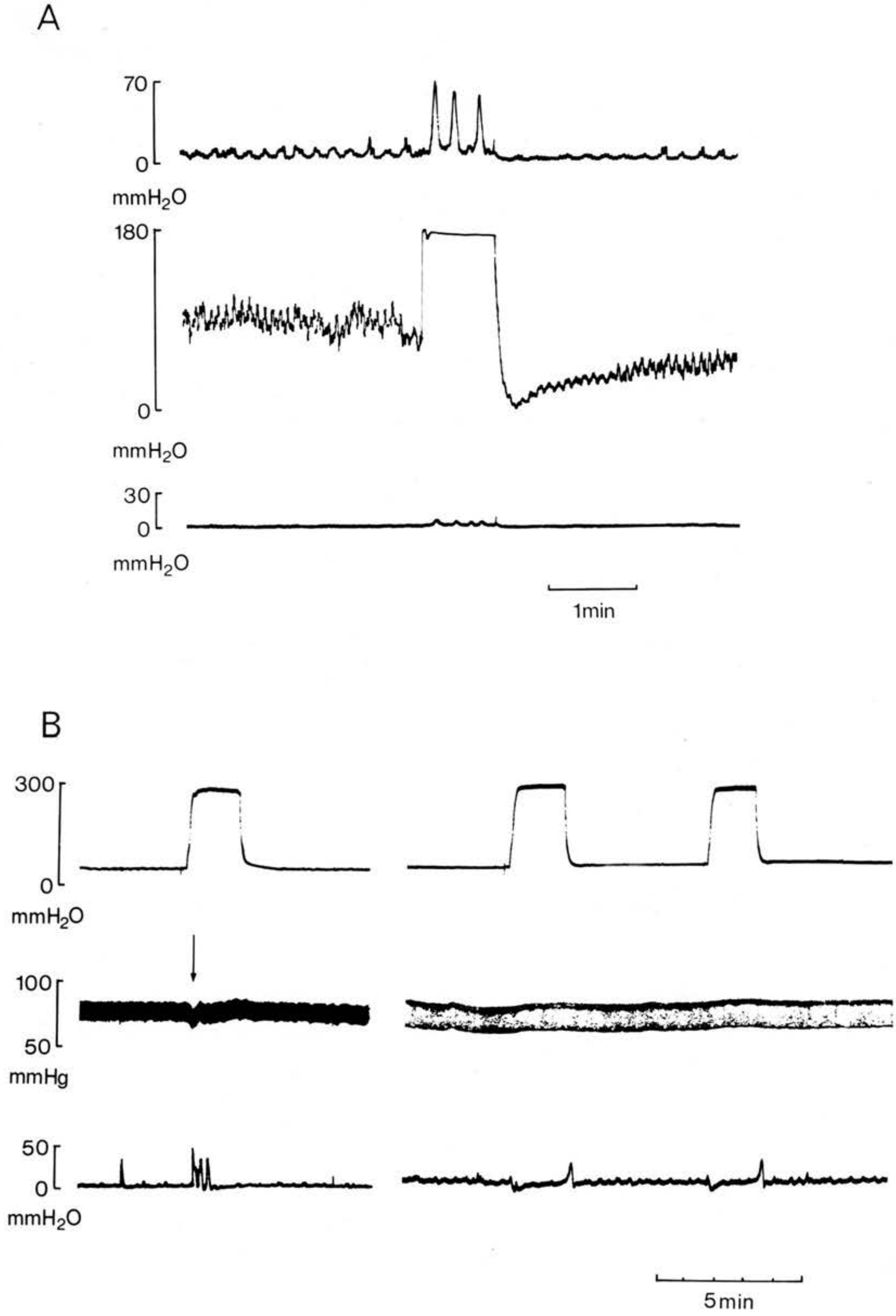

200
0
mmH$_2$O
100
50
mmHg
*
*
50
0
mmH$_2$O
5 min

Figure 8 The duodeno-pyloric reflex. (A) A square-wave increase in intraluminal pressure in the proximal loop (middle trace) causes three contractions in the pyloric sphincter (upper trace). A fundic balloon (lower trace) shows much smaller changes in pressure of, probably, a passive nature. The pre-stimulus intraduodenal pressure in this record is larger than usual. The record was chosen because it also illustrates that duodenal peristalsis, as indicated by the pressure in an open-tipped catheter, is stimulated at values greater than approximately 2.5 cm water. Note also how the peristaltic waves in the pylorus, which are of lower frequency, also occur when duodenal peristalsis becomes more active.

(B) The effect of atropinisation. On the left the square-wave increase in intraluminal pressure coincides with a depressor reflex (middle record, arrow) and a tri-phasic pyloric refex (lower record). The right records commence 60 minutes after the administration of atropine. Note that the depressor reflex is absent and that the character of the pyloric contraction has changed (see text). The triphasic contraction altered 35 minutes after the application of atropine (see Andrews & Scratcherd, 1980, figure 9).


A
70
0
mmH2O
180
0
mmH2O
30
0
mmH2O
1min
B
300
0
mmH2O
100
50
mmHg
50
0
mmH2O
5 min

sphincter and not by a recording balloon situated in the duodenal bulb or the gastric fundus.

The duodeno-pyloric contraction reflex was consistently demonstrable. The reflex response was most easily provoked when pyloric contractions occurred spontaneously (80% of trials), although it could also be evoked when pyloric activity was infrequent (40% of trials). Pyloric contractions reduced in amplitude, or vanished for more than 20 minutes when pressures of 20 cm water were maintained for more than 5 minutes, or when shorter stimuli were repeated at invervals less than several minutes. On 10 occasions, repetitive square-wave stimulation caused the inherent pyloric rhythm to reduce from 1.6 to 0.1 min^{-1}.

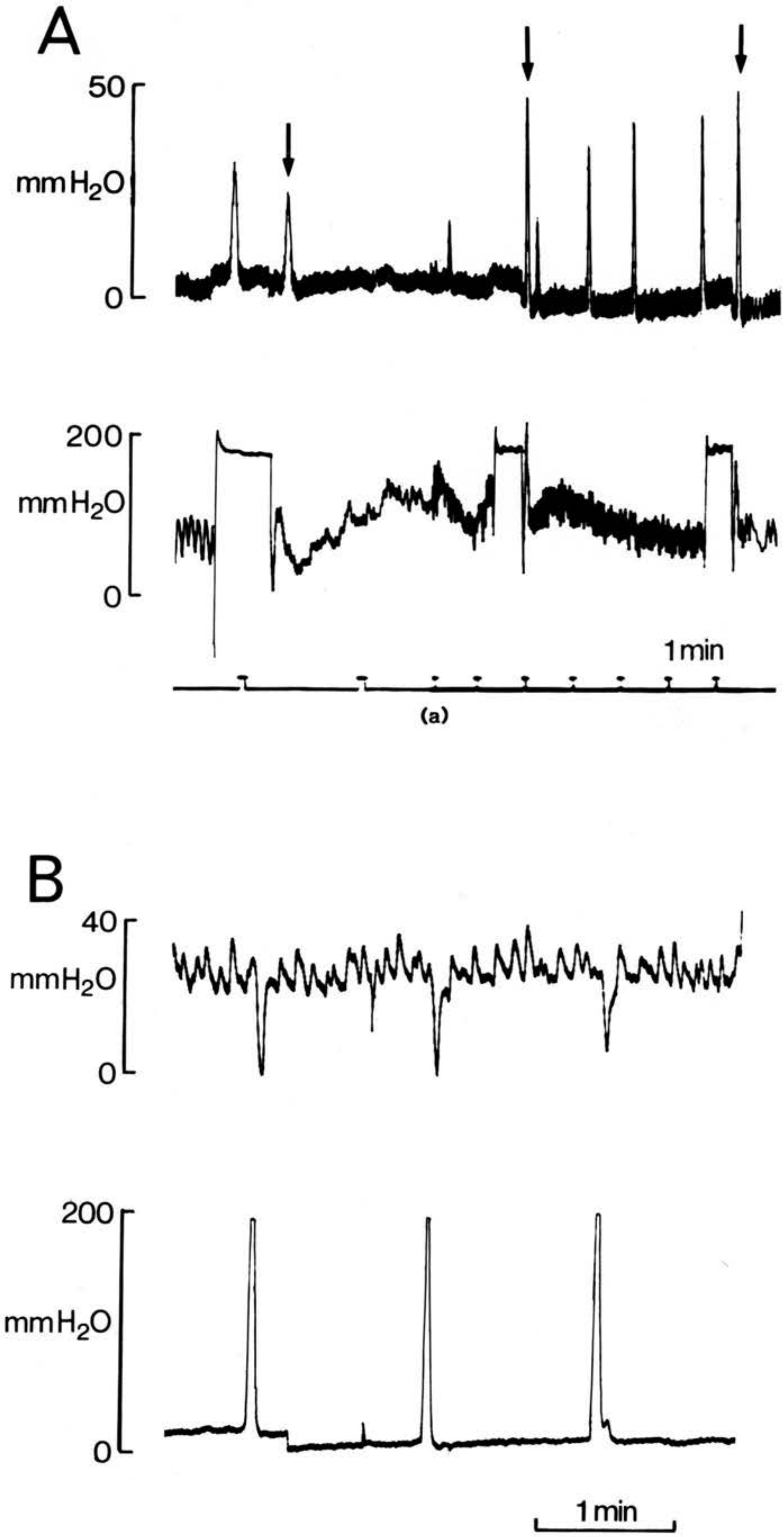
Intraluminal perfusions of cocaine (20 mg) and lignocaine (20 mg) were without effect on the response. Injections of atropine (2 mg Kg^{-1}) did not inhibit the reflex response which remained in a modified form : there was at first an early relaxation of the pylorus, a slow return to tone and an "off" contraction when the stimulus was removed (see figure 8B). Systemic doses of hexamethonium (10 mg Kg^{-1}) gave inconclusive results, as did combined treatments with atropine. Maintenance doses of urethane often inhibited the reflex response. In three experiments, during the first 25 minutes after administration of propanalol (2 mg Kg^{-1}) each post-distension contraction of the duodenum was followed, after 4 - 6 seconds, by an evoked pyloric contraction (see figure 9A). The post-distension contraction of the duodenum and the duodeno-pyloric contraction were both inhibited 30 minutes after phentolamine treatment (0.5 mg Kg^{-1}).

Figure 9 (A) The duodeno-pyloric reflex.

Upper Trace : pyloric pressure; Lower Trace : duodenal pressure. The record was selected from a series of trials after the administration of propanalol (2 mg Kg^{-1}). Note that the triphasic contraction of the pyloric area was absent and that a single pyloric contraction (arrows) occurred within 5 seconds of an active duodenal contraction which followed the release of the square-wave stimulus. Note the change in recording speed at (a). (see Andrews & Scratcherd, 1980, figure 11.)

(B) Aboral duodenal inhibition reflex.

A square-wave increase in intraluminal pressure in the duodenal loop (lower trace) caused a rapid fall in pressure in the aboral duodenal segment (upper trace) from which it had been isolated by a transverse enterotomy.


A
50
mmH$_2$O
0
200
mmH$_2$O
0
1 min
(a)
B
40
mmH$_2$O
0
200
mmH$_2$O
0
1 min

The effects of neurotomy were straightforward. The reflex was abolished when either a left or right abdominal vagotomy was performed, or when the loop was completely denervated by sectioning the mesenteric nerves. Indeed, when the intact abdominal vagus nerve had been exposed for use in the experiment, the duodeno-pyloric reflex response was difficult to demonstrate.

3. Duodeno-Gastric Inhibition

In all cases prolonged mechanical distension of isolated duodenal loops, as well as repeated stimuli of short duration, caused a fall in intragastric tone and reduced amplitude of fundic contractions. The latency was greater than 5 minutes and the response persisted for up to one hour. Duodeno-gastric inhibition was unaffected by local anaesthesia of the mesentery or selective mesenteric neurotomy.

ABORALLY DIRECTED REFLEXES

1. Duodenal Bulb Contraction

In 10 experiments spontaneously occurring duodenal bulb contractions were followed, after less than 1 second, by a fall in intraluminal pressure in the proximal duodenal loop. Both duodenal segments had been isolated by a transverse enterotomy. This co-ordination was removed by mesenteric nerve section.

2. Duodeno-Duodenal Aboral Inhibition

In twenty trials, distension of the duodenal bulb, the distal or proximal duodenal loop, caused a fall in pressure in aboral duodenal segments. The latency was less than 5 seconds. The threshold was greater than 9 cm water and the response was abolished by mesenteric nerve section. This reflex response is illustrated in figure 9B.

DUODENO-DEPRESSOR REFLEX

Pinching the ear, the interdigital cleft, or repetitive electrical excitation of the saphenous nerve, caused the blood pressure to fall rapidly and then return slowly. Square-wave changed in intraduodenal pressure up to 20 cm water also caused this response.

In twenty animals during a total of 60 trials, a square-wave stimulus (onset ramp 10 cm water $\sec^{-1}$ ± 2 (s.d.) to a maximum intraduodenal pressure of 20 cm water, and maintained for 20 seconds, caused the mean carotid B.P. to fall from 80 mm Hg (± 8 (s.d.)) to 60 mm Hg (± 10 (s.d.)), reaching maximum hypotension 15 seconds (± 5 (s.d.)) after the peak of the intraduodenal pressure. Depression of systemic B.P. started within 3 sec of the onset of the stimulus and returned to basal levels 60 seconds (± 20 (s.d.)) after the maximum intraduodenal pressure had been reached (see figure 7B). Neither pulse pressure nor heart rate changed during these events. Unless periods of rest greater than 5 minutes were allowed between trials, the B.P. fell to dangerous levels and the animal entered refractory hypotension. Ramp stimulation of the proximal duodenum (0.6 cm water $\sec^{-1}$ ± 0.1 (s.d.)), to the same maximum pressure, produced no noticeable blood pressure change in a total of 20 trials conducted in six animals. The duodeno-depressor reflex remained after bilateral cervical vagotomy. It was abolished for about 60 minutes by a single injection of atropine.

On one occasion only, in a series of hundreds of trials, a pressor response was elicited.

MISCELLANEOUS PHENOMENA

Perfusion of the loop with solutions of NaOH, pH 10, always caused an increase in intraluminal tone and peristalsis. Both acid (HCl, pH 3.8) and gastric juice (see Chapter 1) caused a reduction in intraluminal pressure. Acidic solutions perfused into the duodenum also reduced pyloric pressure and fundic tone for periods up to 2.5 hours. The latency exceeded 2 minutes. The response was not altered by section of mesenteric nerves. This reflex response has been called "the chemical enterogastric reflex" (Thomas & Mogan, 1931).

Repetitive electrical excitation (1 - 10 Hz) of the peripheral end of the cervical vagus nerve caused an increased amplitude of antral contractions, which at high voltages led to regurgitation. There were coincidental increases in duodenal contractions. During 5 seconds electrical excitation, with pulse widths ranging from 1 - 5 msec, the amplitude of pyloric contractions increased for stimulus strengths up to 7.5 volts. Thereafter, from 8 - 10 volts, there was a reduction in the amplitude of contractions. This differential effect was not seen in the duodenal recording balloon. There was no alteration in fundic tone.

Splanchnic nerve excitation using the same parameters always caused a reduction in both fundic tone and in the amplitude of spontaneously occurring duodenal contractions.

DISCUSSION

These results establish that mechanical changes in the isolated duodenal loop of rabbits influence mechanical activity in adjacent gut segments with which no myenteric communication is possible. The latencies of the response were short and the mechanism must involve extrinsic nerves because most reflex responses were abolished by local anaesthesia and section of the mesenteric nerves. A rapid portal circulation was therefore unlikely. Two exceptions were the long latency duodeno-gastric reflex caused by mechanical stimulation and the chemical enterogastric reflex in which hormones were presumably involved (Thomas & Baldwin, 1968).

The experiments were complicated by the progressive deterioration of the anaesthetised rabbit; the more extensive the surgery, the less responsive was the preparation. Downman & McSwiney (1946) also noted a lack of constancy in responses to noxious pinching of the gut in decerebrate, cordotomised and decapitate cats, which made quantitative study difficult. They found that handling the intestines caused a rapid deterioration of reflex responses, and that exposing the splanchnic nerve by the retroperitoneal approach caused profound and prolonged depression. They concluded that the effect of section of the splanchnic nerve could not be investigated in acute experiments. Reflexes in the cat are also sensitive to excessive trauma or haemorrhage (Downman & McSwiney, 1946), laparotomy procedures and noxious stimulation of the gut (Abrahammson et al, 1979).

In the rabbit preparation, reflexes were demonstrated which were excited by low mechanical thresholds. When the intraluminal pressure was maintained at 0 - 0.5 cm water the duodenal loop and adjacent gut were often quiescent. By raising the intraluminal pressure above this value, peristalsis was provoked in the loop and corresponding pressure changes were also seen in both oral and aboral segments. The mechanical thresholds of the reflexes studied compare well with those established during in vitro investigating of peristalsis (Kosterlitz et al, 1956). Repeated mechanical stimulation, or supra-threshold mechanical excitation caused a reduction in the amplitude of contractions elsewhere. The sensitivity of the reflexes varied, and this has been demonstrated during intestino-intestinal inhibition (Peterson & Youmans, 1945).

Coordination of duodenal motility and gastric peristalsis was first noted by Joseph & Mestzer (1910 - 1911, quoted by Thomas, 1957) in rabbits. They observed relaxation of the duodenal bulb when the pylorus contracted, a reflex which was studied by Daniel & Wiebe (1966) in the dog. This reflex was not investigated here, but similar phenomena were seen in the proximal loop when the duodenal bulb actively contracted or was passively distended, and also in the aboral duodenal segment which relaxed when the duodenal loop was distended. Because the gut was divided transversely, the coordination of pressure changes in adjacent segments implies that the extrinsic nerve supply was involved. There is electromyographic evidence for this : the propagation of the migrating myoelectric complex across transverse enterotomies and anastomoses occurs, after some delay, in a variety of chronic preparations of intestine (Carlson et al, 1972; Grivel & Ruckebusch, 1972).

It has been suggested that the duodeno - pyloric reflex may control regurgitation of duodenal chyme in the dog (Thomas & Crider, 1935). The mechanism of the reflex in the dog seems different from the rabbit, because in the dog it is abolished by duodenotomy (Thomas, 1957).

However, other workers (Thomas et al, 1934) have found that the division of all extrinsic nerves is necessary to abolish the response. The mechanism in the rabbit was mediated by α-receptors because the response was abolished by phentolamine. The pyloric contraction which followed active contraction in the duodenal loop, especially after propanolol treatment, suggests that active as well as passive changes were involved in reflex initiation and implies that mechanoreceptors "in series" with the duodenal muscularis externa may be involved. It is interesting to speculate whether the "rebound off" contraction of the pylorus, following natural stimulation of the duodenum and atropinisation, was mediated by prostaglandin release; often this interpretation is made when "off" responses are seen in electrically stimulated preparations (Burnstock, Cocks, Paddle & Staszewaka-Barczak, 1975).

Depressor reflexes are a phenomenon of lagomorphs and they seem to be a general vasomotor response to noxious influences in these species. In other animals pressor reflexes are common. Distension of the ileum in decerebrate cats causes a pressor response, which is not abolished by bi-vagotomy (Downman & McSwiney, 1946). Thresholds above 13.6 - 40.8 cm water are required and bi-splanchnotomy abolishes the reflex (Downman & McSwiney, 1946). In spinal cats the threshold is much higher (Irving et al, 1937). However, depressor reflexes may sometimes occur in the cat, for example when 5 mls of solution are injected into the duodenum (see Cragg & Evans, 1960, figure 6). The findings in the rabbit support the probable splanchnic afferent route of the reflex. This was also concluded by Anand (1980) in unilaterally vagotomised cats.

The differential effects of electrical excitation upon pyloric motility is interesting. These responses are similar to observations in the cat (Martinson, 1959; Brooks & Carr, 1975) and suggest that two classes of vagal efferent fibre, classified by their electrical thresholds, also exist in the rabbit. The effect such a technique produces upon the stomach, as a result of antidromic excitation of vagal afferent fibres, is unknown. The phenomenon requires further study.

It is difficult to conclude which duodenal enteroceptors population initiated these reflex responses, because as well as the increase in intraluminal pressure, a number of other changes occurred in the loop : distension; increase of transmural pressure; stretching of the mesentery and stretch of the loop near the securing ligatures (see figure 2B). Also, active tension changes were present in the muscularis externa because intraluminal pressures above 2.0 cm water caused contractions to appear (see Chapter 3). The possibility that there were alterations in duodenal circulation at the pressures used is unlikely (Lawson & Chumley, 1940). The mechanical stimulus was therefore not precise enough to limit the excitation to one population of duodenal receptors. The putative mechanoreceptors involved were not in the mucosa or submucosa because mucosally applied anaesthetics did not abolish reflexes (see Chapter 5), and these findings are therefore in agreement with Downman et al (1948). They are not in agreement with Osadchy (1956) who found that reflexes excited by large intraluminal pressures were inhibited by mucosally applied anaesthetics.

The threshold measurements obtained from the study of duodenal reflexes could now be used as a basis for the electrophysiological investigation of duodenal mechanoreceptors, and this is discussed in Chapter 3. The results of the single-fibre study support the suggestion that the mechanoreceptors involved in these reflexes are associated with the muscularis externa and the mesentery.

CHAPTER 3

THE SENSITIVITY OF SINGLE AFFERENT UNITS IN RABBIT DUODENAL NERVES

INTRODUCTION

The technique of recording impulses in closely-associated mesenteric nerves has been successful for single-unit activity in the cat where axons are mostly myelinated (Gammon & Bronk, 1935; Gernandt & Zotterman, 1946; Brown & Gray, 1948; Bessou & Perl, 1966). More often an analysis of multi-unit recordings has been made either in vivo (Zamiatina, 1957; Sharma & Nasset, 1962; Andrews & Andrews, 1971; Itina, 1971; Andrews, Andrews & Orbach, 1972; Hardcastle et al, 1978, 1980) or in vitro (Sirotin, 1961; Clothier, Green, Hardcastle, Hardcastle & Sanford, 1975; Sanford, 1976). The multi-unit records are unhelpful in clarifying whether selective sensitivity of particular enteroceptors occurs and therefore cannot resolve the conflicting concepts which exist.

It was the aim of this investigation to isolate single unit afferent activity in the mesenteric nerve of the rabbit. The identification of enteroceptors with unmyelinated axons in the duodenal nerves was made possible by adequate mechanical stabilisation and controlled natural stimulation provided by the perfusion apparatus described above.

METHODS

The distal duodenal preparation was used. In most preparations the pancreatic duct entered into the isolated loop but its position near to the effluent side of the preparation did not grossly influence the pH of the loop. Pancreatic secretion is continuous in the rabbit (300 - 500 µl min^{-1}, Solberg & Brooks, 1969). Control ligation of the duct did not have a noticeable effect on pH measurements when compared with open-duct preparations. Presumably the perfusion rate (usually 4 ml min^{-1}) was rapid enough to wash pancreatic juice away.

The following perfusion solutions were used (at 39°C, mM L^{-1}) : HCl (50 - 100); acetic (10 - 100); propanoic (10 - 100); pentanoic (10 - 100); D-glucose (5.5 - 2000); L-glutamate (50); glycine (150); L-tryptophan (12.5 - 50); NaCl (100 - 1200); myristic acid (33); sodium citrate (33).

The contralateral femoral artery was catheterised and opened 10 mm on the cardiac side of the coeliac artery for close arterial injection of drugs. The position was confirmed later during the surgical preparation, and also post mortem.

DRUGS

Close intra-arterial injections of phenyldiguanide (100 µg Kg^{-1}) and veratrine (10 µg Kg^{-1}) were made. Lignocaine (2%) and cocaine (5%) were perfused into the duodenal loop. Nor-adrenaline (10 µg ml^{-1}) was given intravenously and was also perfused into the loop.

RESULTS

Twenty-eight duodenal afferent units were isolated as single-unit preparations and a further 31 units were identified in few-unit preparations. Three separate classes of mechano-sensitive afferent units were identified : slowly adapting units associated with the muscularis externa (17 units); "vasoserosal" units (5 units) and "vasomesenteric" units (2 units). Some units were mechanically insensitive (4 units). No pacinian corpuscles were seen and no electrophysiological evidence for myelinated axons was found.

MECHANORECEPTORS IN THE MUSCULARIS EXTERNA

This class of unit had increased activity both during passive increases in intraluminal pressure (which in these experiments also caused distension), and also during electrically evoked contractions and naturally occurring peristalsis (see figure 10). Activity was slowly adapting during short square-wave and pulse-wave steps in intraluminal pressure (see figure 11). The slowly adapting nature of the response was not always easily correlated with intraluminal pressure throughout a sustained stimulus, the impulse frequency waxed and waned at a frequency similar to observed changes in the movement of the loop (see figure 11C). When the stimulus was removed there was, at first, a brief silence and later a burst of activity which coincided with observed duodenal contraction.

The sensitivity of units, as judged by the intraluminal pressure threshold, varied within and between trials. The peak frequency recorded for the first second of the response varied from 23 - 40 imp sec^{-1}, and the minimum interspike interval from 9 - 50 msec.

Figure 10 Mechanoreceptor in the muscularis externa of a rabbit. Upper trace electrical activity, lower trace intraluminal pressure recorded with open-tipped catheter placed near the receptive field. Conduction velocity of the unit 0.8 $msec^{-1}$ ($36^{o}C$). (A) During pendular movement, defined as rhythmic lengthening and shortening of the loop. (B) During peristaltic activity. Note that the pressure record shows a poor correlation with electrical activity. (C) The response of a muscularis externa unit to maintained intraluminal (square-wave) pressure of 25 cm water (indicated by the horizontal bars). Note that the prolonged stimulus caused an increased oscillating activity, the bursts coincided with observed peristalsis in the loop. After removal of the stimulus a "rebound" active contraction of the loop occurred which coincided with increased impulse activity. (The intraluminal wave form was not permanently recorded with this unit but was similar to Figure 11B for the period of the bar.) (see Takeshima, 1975, p 107).

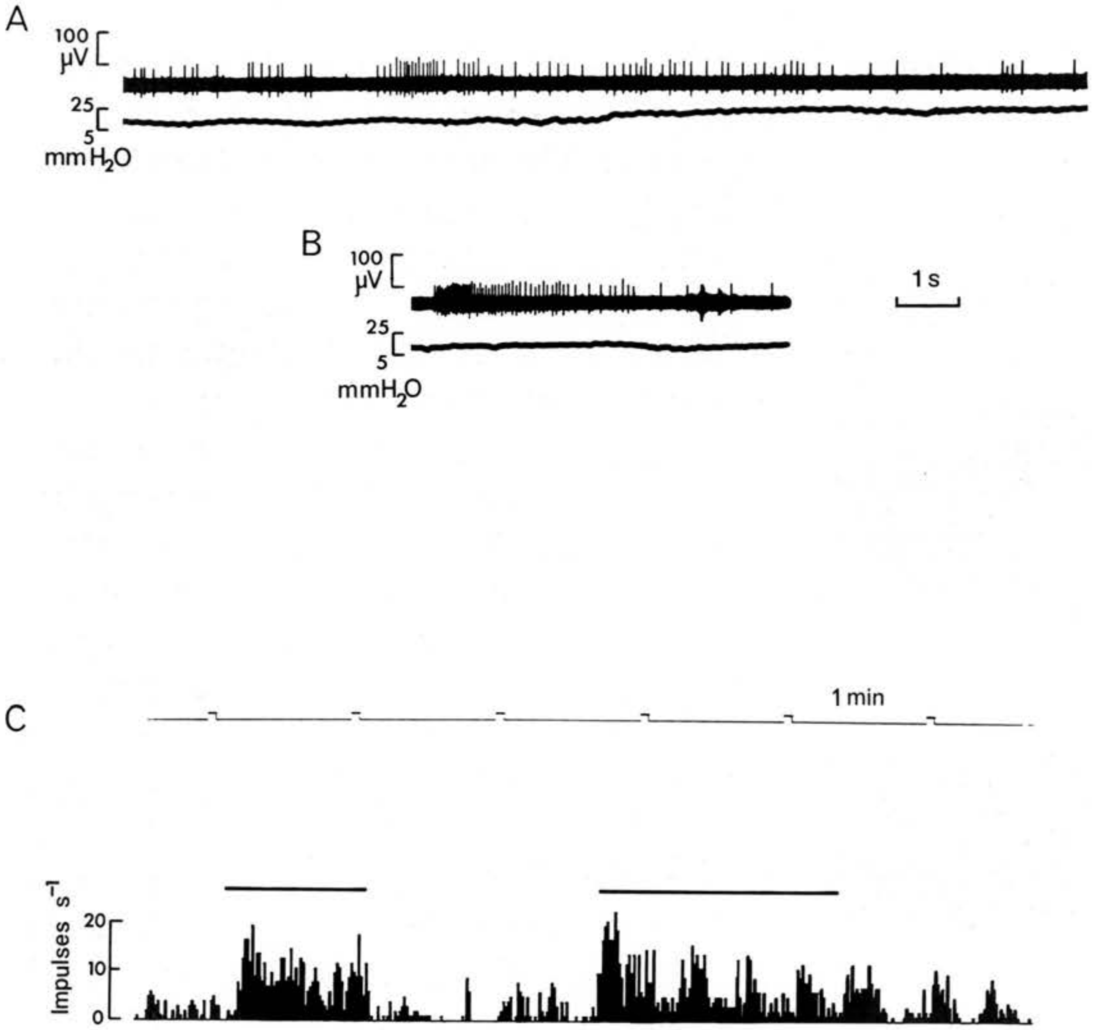
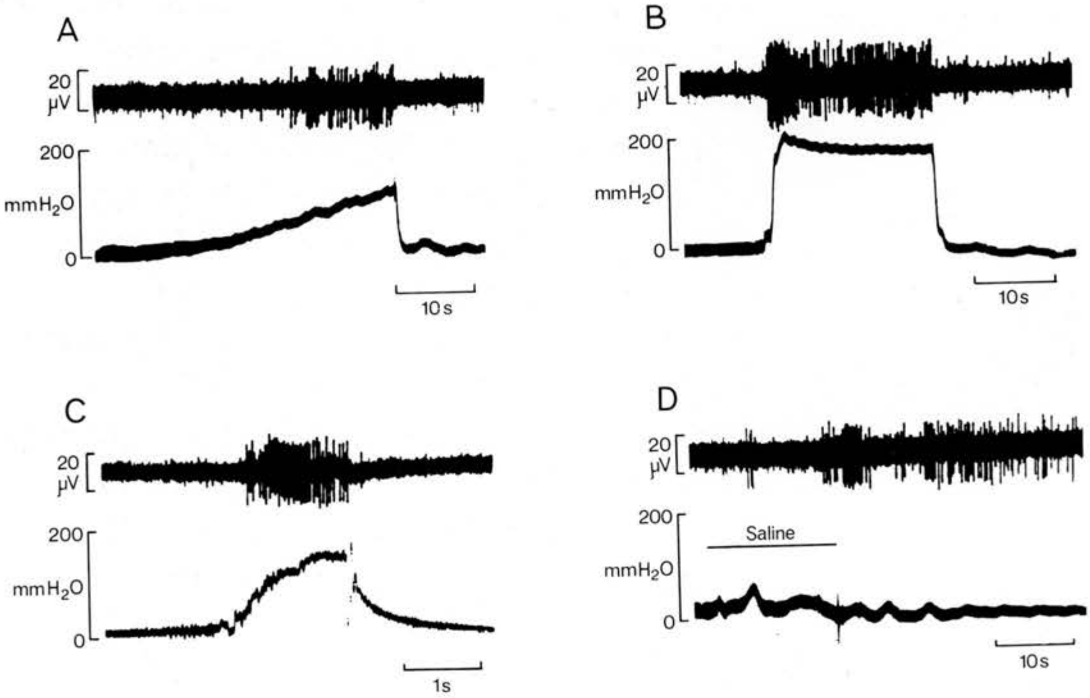

A
100
μV
25
5
mm H₂O
B
100
μV
25
5
mmH₂O
1 s
C
1 min
Impulses s⁻¹
20
10
0

Figure 11 Response of a muscularis externa mechanoreceptor when stimulated by changes in intraluminal pressure measured by an open-tipped catheter. By adjustment of suitable valves in the perfusion apparatus it was possible to apply (A) ramp-wave, (B) square-wave and (C) pulse-wave pressure changes. In (D) active contraction of the receptive field was induced by perfusion with 1200 mOsm Kg^{-1} NaCl at the bar. In (D), at lower intraluminal pressures, the nervous activity was more vigorous than A, B and C, which indicated that the unit responded to active contraction as well as passive distension.


A
20 µV
200
mmH2O
0
10 s
B
20 µV
200
mmH2O
0
10s
C
20 µV
200
mmH2O
0
1s
D
20 µV
200
Saline
mmH2O
0
10s

When the intraluminal pressure was maintained below 0.5 - 1.0 cm water, the units had few spontaneous discharges. Above 2.0 cm water, peristalsis was evoked and rhythmical activity was stimulated. This was not invariably the case and often low intraluminal pressure records were associated with vigorous discharges (see figure 11D). Four units only became noticeably more active when the intraluminal pressure was above 4.0 cm water. Units were not examined above a maximal intraluminal pressure of 30 cm water and so the possibility of further units responding to pressures above this threshold was not excluded.

Receptive Fields

All units had single receptive fields which appeared to be a 1 cm wide annular ring which, when mechanically stimulated, evoked peristalsis with short latency. Oral and aboral mechanical and electrical transmural stimulation up to 4 cm distant from the short-latency field, caused a delayed response, which was related to the time taken for an observed peristaltic wave to travel from the stimulus point to the receptive field. The unit responded with a high frequency (up to 40 imp sec^{-1}), but poorly sustained, discharge when tangential stretching was applied with two smooth-tongued forceps held 15 mm apart near the receptive field. Transmural pressure applied to the serosal, with the lumen filled with a semi-solid, caused a greater and more sustained response. Serosal probing with von Frey hairs gave inconsistent responses and thresholds between 0.132 g (8.6 g mm^2) and 4 g were found, often in the same unit at different times. Such stimuli usually evoked a peristaltic annular constriction and the response outlasted the stimulus application. The "after-discharge" was associated with visually observed active contraction of the receptive field.

Activity in these receptors was not abolished by intraluminal lignocaine (2%) or cocaine (5%), and remained when the intestine was sectioned longitudinally along its antimesenteric border, and also when the mucosa and serosa were removed locally. Close intra-arterial injections of phenyldiguanide (100 μg Kg^{-1}) and veratrine (10 μg Kg^{-1}) excited units in a way similar to those previously reported (Paintal, 1957a; Iggo, 1957a; and see Chapter 4). Inhibition of spontaneous activity sometimes occurred with phenyldiguanide.

The mean conduction velocity of these units was 0.64 $msec^{-1}$, and ranged from 0.32 - 1.1 $msec^{-1}$ (see Chapter 6, figure 39).

Response to Chemicals

When solutions containing 55 mM L^{-1} glucose, 550 mM L^{-1} glucose in normal saline and Tyrode solution containing 5.5 mM M^{-1} glucose were perfused, there was an increased peak frequency in rhythmically responding units, with shortening of the inter-burst interval. This coincided with increased observed mechanical activity in the loop. When the glucose solution was removed and replaced with normal saline, the frequency and rhythm returned to prestimulus levels. Twice normal saline (600 mOsm Kg^{-1}) also stimulated peristalsis and increased the impulse frequency (see figure 11D).

The following solutions caused no change in activity :
10 - 100 mM L^{-1} acetic, propanoic and pentanoic acid in normal saline; myristic acid 33 mM L^{-1}; sodium citrate 33 mM L^{-1} in normal saline; citrate HCl buffer; Tyrode HCl pH 5.5 - 1.4; HCl 50 - 100 mM L^{-1};

L-glutamate 50 mM L^{-1} in normal saline; glycine 150 mM L^{-1}; L-tryptophan 12.5 - 50 mM L^{-1} in normal saline.

VASOSEROSAL UNITS

One group of afferent unit was not directly excited by smooth muscle contraction and was closely associated with blood vessels, usually arteries, on the mesenteric border. They responded with bursts of 8 - 12 impulses at a frequency of 60 - 100 imp sec^{-1} when a mechanical probe, or von Frey hair above 4 g threshold, was applied to the receptive field (see figure 12). Units were not spontaneously active at intraluminal pressures below 6.0 cm water, were insensitive to mechanical stimulation of the mucosa and were not excited by stretching of the mesentery distinct from the mesenteric border. They were stimulated only briefly during peristalsis during observed distortion of the receptive field. No quantitative measurement of this distortion was made. Single receptive fields had an area of 1 mm^2, and axons had a mean conduction velocity of 0.58 $msec^{-1}$.

Selected units were tested for frequency following characteristics using trains of electrical stimuli. They followed consistently one-to-one prolonged (5 sec) electrical stimulation at frequencies up to 50 Hz, and shorter bursts up to 120 Hz. The phenomenon of slowing of the conduction velocity was demonstrated at higher frequencies, early impulses in the train had larger interspike intervals than those found later in the record (see figure 12B).

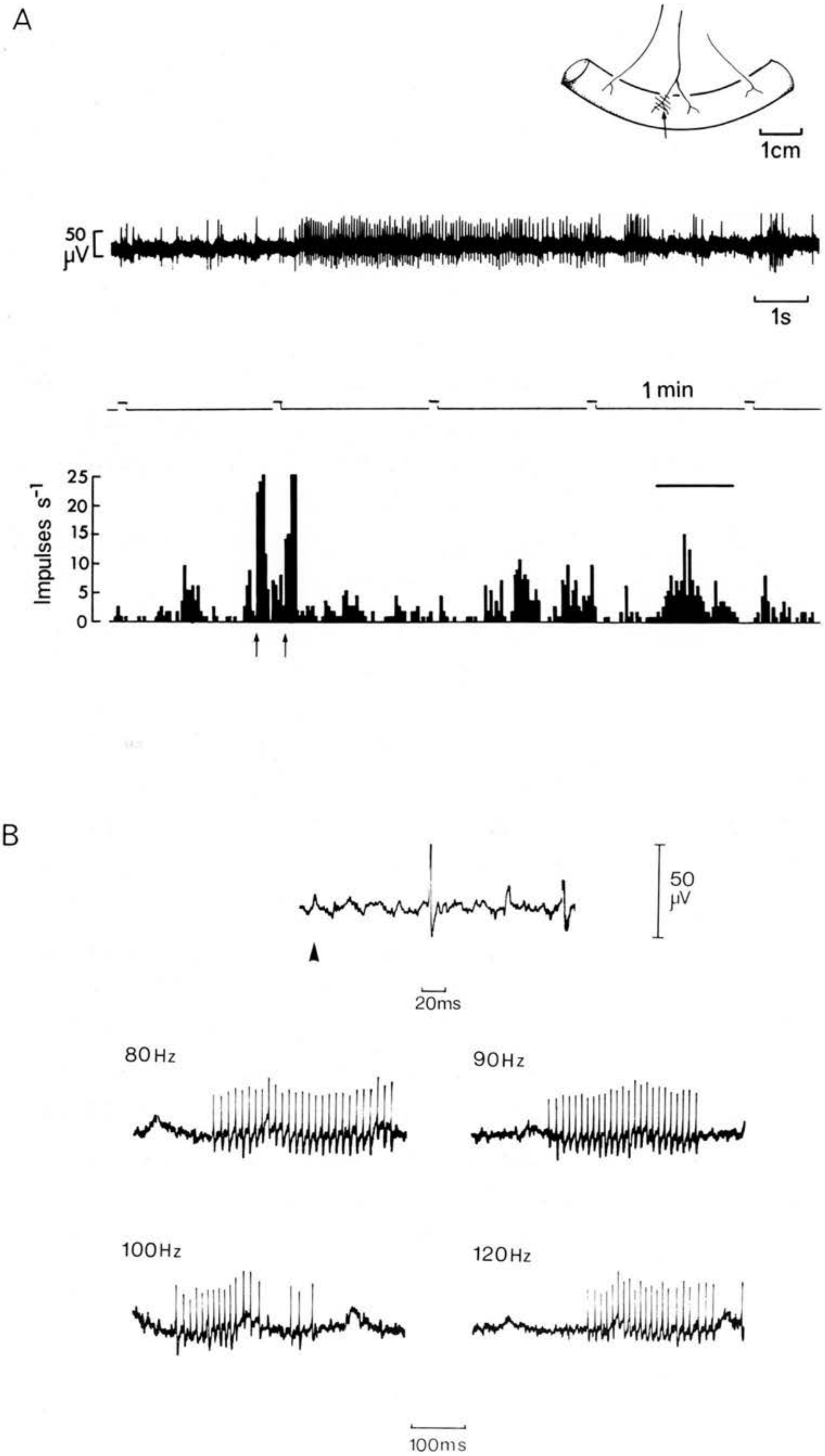
VASOMESENTERIC UNITS

Two units had receptive fields unassociated with duodenal smooth muscle and were located in the mesentery near the bifurcation of blood

Figure 12

(A) A vasoserosal unit. Upper Trace. The response to discrete probing with a von Frey hair of 0.132 g threshold (8.6 g mm^{2}). Inset, the receptive field.
Lower Trace. The response to maintained discrete probing of the receptive field (arrows) and maintained (square-wave) intraluminal pressure of 25 cm water (bar), which gave a lower frequency response.

(B) The frequency following characteristics of a vasoserosal unit. The receptive field had an electrical threshold of 4.5 V, 0.2 msec and a receptive field near blood vessels similar to inset. The unit was entrained up to a maximum frequency of 120 Hz for bursts of 10 spikes. Above this frequency the discharge broke down. The conduction velocity was 0.44 msec^{-1} (34^{o}C).


A
1cm
50
μV
1s
1 min
Impulses s^{-1}
25
20
15
10
5
0
B
50
μV
20ms
80Hz
90Hz
100Hz
120Hz
100ms

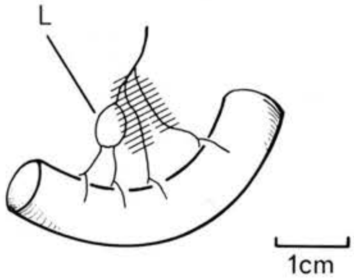
vessels. They had a von Frey threshold of 1 gram. When the mesentery in a discrete area was stretched, units had a rapidly adapting response with a minimum interspike interval of 15 msec, and a peak frequency during one second of 50 imp sec^{-1}. Units were silent unless either pressures in the loop exceeded 5.0 cm water, when vigorous peristalsis occurred, or during outlet-clamping - a procedure which distended the loop and stretched the mesentery. They had a single receptive field of area 30 mm^2. The unitary activity was unaltered by venous and arterial occlusion, or by raised blood pressure caused by the systemic administration of nor-adrenaline. The conduction velocity of the two units was 0.4 $msec^{-1}$ (34^oC) and 1.0 $msec^{-1}$ (35^oC) (see figure 13).

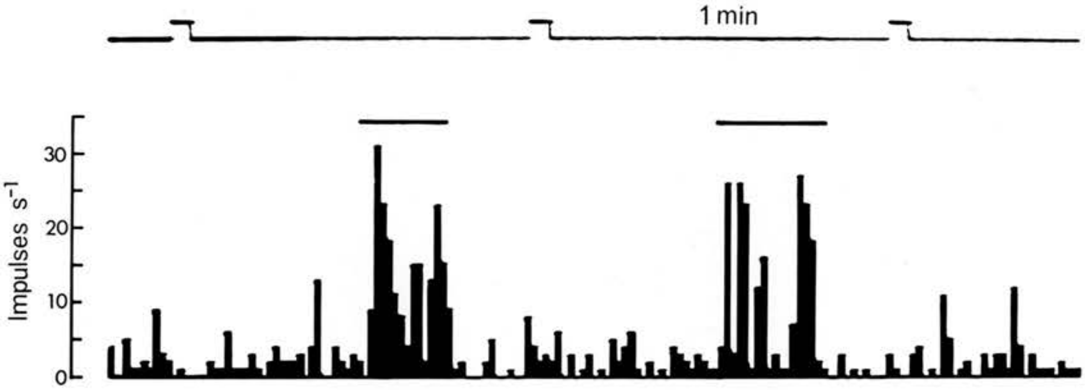
The activity of neither vasoserosal nor vasomesenteric units could be made to change with systemically applied drugs or chemicals perfusing the loop.

FURTHER AFFERENT UNITS

Four units were not excited by intraluminal pressure changes up to 20.0 cm water, by squeezing the loop through the serosa, by direct mucosal mechanical stimulation nor stretching and probing the mesentery. Units had a bursting discharge with 5 - 6 impulses per burst, a mean interspike interval of 80 - 100 msec and interburst intervals varying from 5 to 20 seconds. Activity was unassociated with peristalsis. One unit had a consistent response to intraluminal nor-adrenaline, causing a burst of activity following a 25 second delay. Units were not otherwise stimulated by intraluminal changes in pH 1.2 - 8.0; glucose 150 - 2000 mM L^{-1}; tryptophan 12.5 - 50 mM L^{-1}, or

Figure 13 A vasomesenteric unit. The response to probing with von Frey hairs with threshold above 1 g is shown during the bars. The receptive field was not punctate and only stretching of the mesentery, in the shaded area of the inset, caused a response. Rhythmic lengthening and shortening movements of the intestine caused movement of the mesenteric fan and also excited the unit. Local arterial and venous occlusion did not excite the unit or alter its activity. The conduction velocity was 1.0 $msec^{-1}$ ($35^{o}C$).
(L = lymph node).


L
1cm


1 min
Impulses s^{-1}
30
20
10
0

saline 10 - 1200 mOsm Kg^{-1}. The conduction velocity of two units measured over the nerve was 1.1 $msec^{-1}$ and 1.2 $msec^{-1}$, both at 36.8^{o}C.

DISCUSSION

The three types of mechanosensitive units found in the rabbit duodenum and associated mesentery resemble those found in other species. In many respects the units excited by contraction of the muscularis externa resemble the "tension" receptors described in the cat and ruminant (Iggo, 1957a; Leek, 1969). But the method of illustrating mechanical activity (intraluminal pressure records and visual observations) was not sufficient to confirm changes in tension in the muscle needed to support the hypothesis of an "in series" location.

The vasomesenteric units were similar to mesenteric receptors described by Morrison (Morrison, 1973; Floyd & Morrison, 1974b) in the cat and dog. Morrison's sample however, contained mostly faster conducting fibres with conduction velocities which ranged between C-fibres and Aδ fibres. The vasoserosal units had characteristics which were similar to those found in the cat and called "movement detectors" (Bessou & Perl, 1966). However, although some slowly conducting afferent units are found in the cat, the conduction velocities range between 2 - 21 msec^{-1} (Bessou & Perl, 1966). The conduction velocities of the rabbit sample were therefore similar only to the slowest conducting units found in cats and dogs.

The non-mechanosensitive units most resemble the units described by Paintal (1957b) but, unlike those, they were not excited by high-threshold squeezing of the loop. It was not possible to confirm whether these units were associated with the muscularis mucosa (Paintal, 1957b). Because Paintal (1957b) did not report conduction velocity measurements it is not possible to compare the rabbit and cat units in this way.

It is surprising when one considers the implications of multi-unit reports of mesenteric nerve afferent activity, that the putative chemosensitive mucosal population was not represented in this single unit sample (Hardcastle et al, 1978, 1980). None were seen in multi-unit and few-unit preparations isolated during surveying of the mesenteric bundles. Neither direct mechanical stimulation of the mucosa nor changes caused by chemicals with short-latency responses were seen in the preparation. If mucosal chemoreceptors represent an important component of the mesenteric nerves then one would expect to see some indication of change using impulse discrimination techniques on multi-unit preparations. The most numerous units were those associated with the muscularis externa, and unless there are considerable differences between species and location, they must always provide a substantial contribution to any multi-unit record of mesenteric nerves. The observation that mechanoreceptors were stimulated by glucose-containing solutions makes it necessary to eliminate them rigorously when investigating possible chemoreceptor activity.

From other investigations it is possible that duodenal chemoreceptors responding to titratable acid may only be located within 50 mm of the stomach in the dog (Cooke, 1974). Fat receptors may not be present in the first 50 mm in the dog duodenum (Cooke & Clarke, 1976) but may be present in the second to fourth part of the duodenum in man (Meeroff et al, 1975). The duodenum of the calf may be sensitive to chemicals throughout its length (Bell & Holbrooke, 1979). Unfortunately, the proximal segment was inaccessible to electrical recording using the present technique in the rabbit and so the most chemically sensitive region may not have been investigated.

If the vasoserosal and vasomesenteric units are the same as splanchnic projecting units found in other species with multiple receptive fields (Morrison, 1973), then the results suggest that axon collaterals must converge more centrally than the limit of the pool because only single receptive fields were found. It is interesting that there is such a distinct sampling difference between vagal (Iggo, 1966a) and splanchnic (Morrison, 1973) investigations. Units with "in series" characteristics have not been described projecting through the splanchnic nerves. Both receptors associated with the muscularis externa which had some "in series" characteristics, and the mesentery associated with arterial bifurcations were found at the level of the duodenum. Is it possible that there is functional viscerotopic isolation of enteroceptor projections? This hypothesis is supported by evidence from reflex studies. Low threshold mechanical changes during contraction of the duodenum cause reflex changes in the gastric antrum via the vagal nerves (Daniel & Wiebe, 1966). High threshold intestinal distension of the intestine cause reflex inhibition of the aboral small intestine by a reflex which is independent of the vagus and involves the splanchnic nerves (Youmans, 1944). The splanchnic nerve is also important in reflex pupillodilation, pressor reflexes and limb movement in decerebrate cats when the intestine is handled and pinched (Downman & McSwiney, 1946). The final destination of the afferent units investigated here was not known.

These results establish that single afferent activity from unmyelinated axons can be prepared from duodenal nerves in the rabbit.

The technique, however, restricted access to the duodenum for recording tension events in the muscularis externa and the direct examination of any putative mucosal receptive fields. It was therefore necessary to devise an experiment where these two parameters could also be studied, and a suitable preparation was found in the sheep.

CHAPTER 4

TENSION RECEPTORS IN THE PROXIMAL DUODENUM AND PYLORIC SPHINCTER OF THE SHEEP

INTRODUCTION

Matthews (1933) introduced the concept of "in series" and "in parallel" mechanoreceptors for skeletal muscle. While recording single unit activity he was able to monitor changes in tension during isometric contraction and passive stretching. Iggo (1955), during single unit afferent recording from hollow viscera, demonstrated the activity of slowly adapting mechanoreceptors which responded both to active isometric contraction and passive distension. Although his technique did not enable him to record localised tension in smooth muscle, but only in the whole viscus, the response characteristics of these units suggested an "in series" location, analogous with those in skeletal muscle. Later, Leek (1969) demonstrated directly that tangential stretching of the receptive field was an adequate stimulus. Beyond these observations, it has been difficult to provide well-quantified stimuli to test this concept further.

There is support from a number of sources for the concept of tension receptors with different mechanical thresholds. Iggo (1955) first demonstrated that some single units appear to have a higher tension threshold because contraction induced by electrical stimulation of extrinsic motor nerves causes units to fire at apparently different thresholds. Reflex studies of reticulo-ruminal motility have suggested the possibility of two receptor populations with high and low thresholds (Iggo & Leek, 1967b) with either excitatory or inhibitory reflex effects. It is assumed these are tension receptors. In vitro experiments demonstrate that the sensory mechanoreceptors causing circular muscle contraction during peristalsis have a higher distension threshold than those in longitudinal muscle (Kosterlitz & Lees, 1964; Kosterlitz et al, 1956). Because these muscles contract independently presumably two separate receptor populations in different locations exist. These concepts have been further explored here. It has been possible to combine in vivo records of tension and electromyography with the activity of alimentary mechanoreceptors. The preparation provides further support for the "in series" nature of tension receptors, which are possibly located in longitudinal muscle.

The single unit preparation of tension receptors was used to test their responsiveness to locally and systemically applied drugs, particularly the peptide hormone analogues. Gastro-intestinal peptides may play a role as hormonal factors in regulating gastrointestinal motility via acetylcholine release, and their effect may be controlled by the tonic activity of the sympathetic nervous system which reduces acetylcholine release (Vizi, 1976). They may also act directly on the smooth muscle membrane (Szurszewski, 1975) and may be alimentary neurotransmitters (Hokfelt, Johansson, Ljungdahl, Lundberg & Schultzberg, 1980). Some may be important as transmitters in sensory neurones (Lundberg, Hokfelt, Nilsson, Terenius, Rehfeld, Elde & Sami, 1978; Burnstock, 1979). The results suggested that postprandially released hormones alter the activity of tension receptors and may therefore play an important peripheral role in appetite control and gastric emptying.

METHODS

Both open-loop and closed-loop preparations were used. Close arterial injections were made via a catheter passed up the right gastroepiploic artery. The use of this artery restricted the administration of I.A. drugs to that section of the preparation supplied by the artery, which varied between preparations. Intra-aortic catheters were tried but gave inconsistent results.

DRUGS

Drugs were selected from those known to cause changes in sensory receptor activity when administered locally and systemically (Paintal, 1964; Daniel, 1968); and those now known to alter alimentary motor activity (Bennet, 1972), and to test the response to the newly available analogues of gastro-intestinal hormones which modulate smooth muscle tonus (Grossman and others, 1974). The activity of single afferent units was closely studied while observing local mechanical and electromyographic changes surrounding the receptor, in order to test the concept that : "when secondary (indirect) effects are excluded it follows that the drug must have a direct action on the (sensory) ending" (Paintal, 1964).

The following preparations were used : lignocaine (2%); phenyldiguanide (5 - 90 μg); veratrine (1 - 100 μg); prostaglandin E_2 and $F_{2\alpha}$ (50 μg Kg^{-1}); pentagastrin (1 μg Kg^{-1}) (*); nor-adrenaline (50 μg); acetylcholine (50 μg); insulin BP (0.1 - 10 IU); 5-hydroxytryptamine (4×10^{-6}M); papaverine (10^{-3}M); atropine SO_4

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* Peptavlon, ICI

(0.26 M); hexamethonium (0.19 M); bradykinin acetate (1 - 50 μg) (**); glucose saline (1 - 30%); CCK-8 (***); CCK-33, CCK-39 (1 - 50 μg). Ammonium chloride was injected close intra-arterially (1.5 - 2.0 gm/100 ml).

CHEMICAL STIMULI

Chemicals were selected from a knowledge of the range of composition of chyme (see Chapter 1), and to test specifically the response to those which have been used in gastric emptying studies and which implicate the presence of duodenal sensory receptors (Hunt & Knox, 1962 et seq.; Bell and others, 1973 et seq.).

The following chemicals were applied to the receptive field on its mucosal surface : HCl (10 - 75 mM L^{-1}); NaOH (10 - 25 mM L^{-1}); butanoic acid (10 - 50 mM L^{-1}); NaOH (289 - 815 mOsm Kg^{-1}); tyrosine (50 mM L^{-1}); tryptophan (50 mM L^{-1}); bile. Where appropriate the osmolality was adjusted to about 300 mOsm Kg^{-1} with saline and measured with an osmometer.

** Cambridge Research Biochemicals Ltd.

*** Squibb & Son Ltd.

RESULTS

Fifty-six tension receptors were isolated as single units in the hepatoduodenal nerve of the sheep and their activity was studied in closed and open-looped preparations.

Units had a receptive field size which, in response to perpendicular exploration, was always a single discrete area which was usually ellipsoidal with a greater longitudinal axis. The largest was 5 by 15 mm and the smallest 3 by 2 mm when measured from the mucosal surface. The mean area was 33 mm^2 (+/- 16.5 sd). Units always had resting activity which coincided with observed electromyographic (emg) and tension changes in the receptive field. The spontaneous activity in the longitudinally-exposed loop was not different from closed-loop preparations. The mean frequency during bursts was 7.86 imp sec^{-1} (+/- 4.1 sd) and the minimum interspike interval was 91.4 sec (+/- 57.8 sd).

The conduction velocity measurements of the units indicated two fibre populations: eighteen units were unmyelinated (conduction velocity 0.68 +/- 0.24 $msec^{-1}$), and six units were probably myelinated (4.53 +/- 1.69 $msec^{-1}$). There was no consistent difference in response characteristics between these two groups.

MECHANICAL STIMULATION

In closed-loop preparations the inflation of a balloon caused an impressive, although poorly sustained, discharge which declined as a powerful constriction pushed the balloon aborally, and was silent while an annular constriction remained in the receptive field. When a suprathreshold compression was applied from the mucosal surface in open-looped preparations, the response was slowly adapting and the frequency during the first second of the response was 38.8 imp sec^{-1} (+/- 17.5 sd), and the minimum interspike interval was 24.6 ms (+/- 11.3 sd). The compression threshold was 6.12 grams (+/- 2.93 sd) (0.5 g mm^2). The longitudinal tension threshold varied between preparations and was of the order of 100 mg (see figures 14, 18, 22). When longitudinal tensions were applied the frequency increased by 150% (see also the drug effects below). After prolonged perpendicular pressures there followed a silent phase before spontaneous activity resumed. Occasionally the units showed a continuing "after discharge".

The characteristic response during compression had a regular interspike interval with a coefficient of variation, during periods of stationarity, which in 4 units was 0.18, 0.21, 0.12 and 0.24. The discharge adapted slowly over a period of minutes. An example is given in figure 14. The emg near the receptive field at this time demonstrated dramatic changes and so it was difficult to interpret the actual stimulus provided. This could have been a primary (passive) distortion of the receptive field as well as a secondary (myogenic) effect (see figure 15B).

The response of two units in the same preparation to compression is illustrated in figure 15. The relationship between tension and adapted spike frequency was linear, although in unit (.) there is a greater variability in the response. Unit (.) was approximately three times more sensitive at all levels of applied tension, and had a lower threshold (3 gm) than unit (+) (10 - 12 gm).

A compression-sensitive receptive field could not be found for two units. Their phasic discharge pattern and excitation by drugs was otherwise similar to the rest of the sample. Both units were

Figure 14 The impulse train of a tension receptor during compression of 25 grams on the mucosal surface. The error bar in the frequency histogram (left) represents the sd of the frequency converted from the sd of the interspike interval and assumes a Gaucian distribution of intervals for each 5 sec period. The response during the first 20 sec was statistically stationary and an interval histogram (right) was constructed for this period. The distribution of intervals during 20 sec was compared with the theoretical normal distribution :
$f(x) = 1/(sd.\surd(2\pi))\ .\ \exp -\frac{1}{2}((X - \overline{X})/sd)^2$,
with $\chi^2 = \Sigma(0 - E)^2/E$, and was found not to be normally distributed.

The spike train shows a representative section of the discharge taken during the period of stationarity.

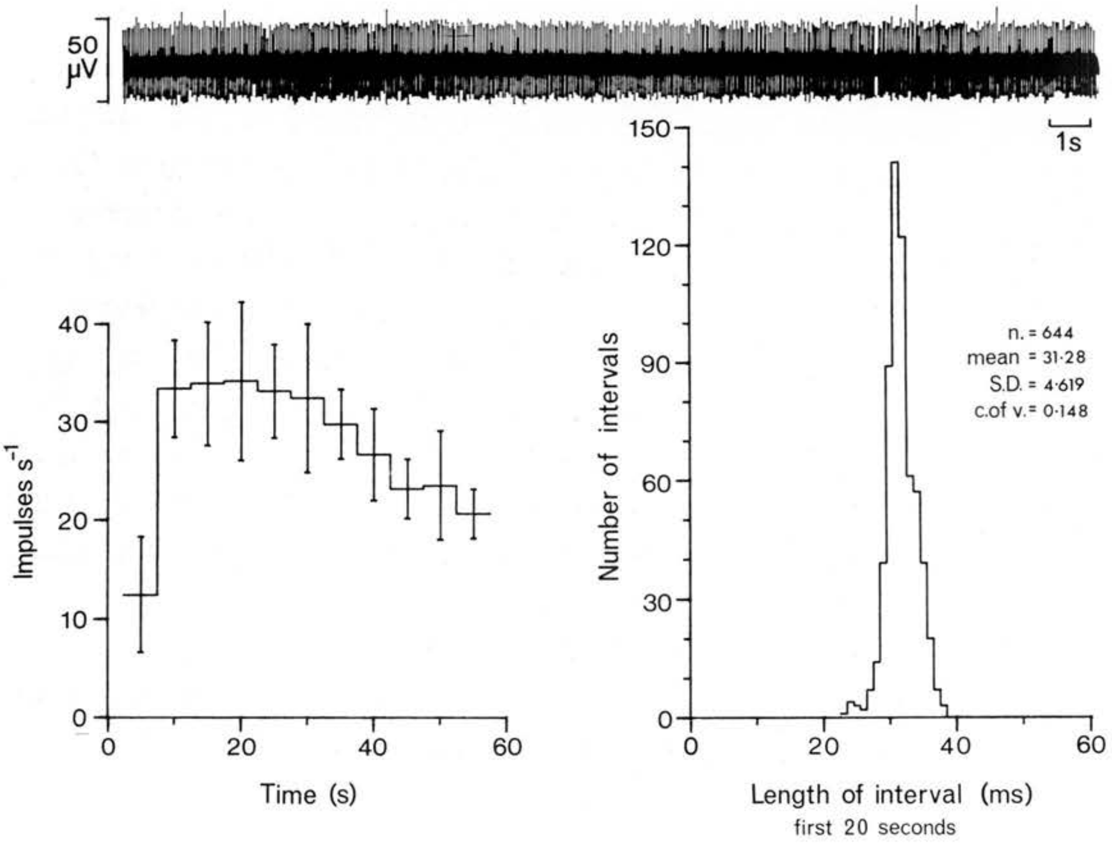
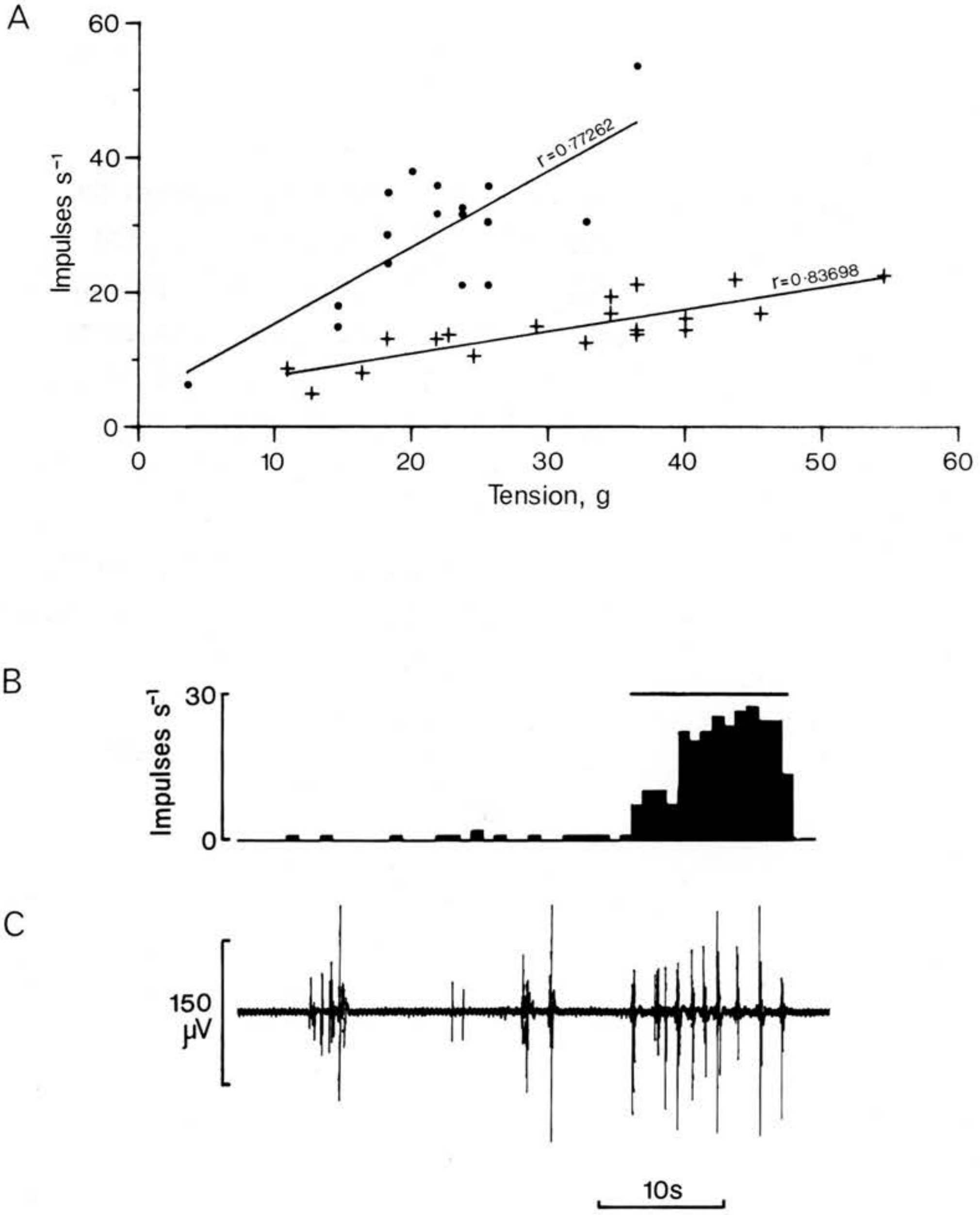

50 µV
1s
Impulses s^{-1}
40
30
20
10
0
0
20
40
60
Time (s)
Number of intervals
150
120
90
60
30
0
0
20
40
60
Length of interval (ms)
first 20 seconds
n. = 644
mean = 31·28
S.D. = 4·619
c.of v. = 0·148

Figure 15 (A) The adapted response to compression from two tension units in the same preparation. Unit (+) was from the torus pyloricus and had a conduction velocity of 0.72 $msec^{-1}$. Unit (.) was from the mid-duodenum with a conduction velocity of 6.0 $msec^{-1}$.

(B) An example of impulse activity during the application of transmural compression indicated by the bar.

(C) Emg activity in the region of the receptive field.


A
Impulses s⁻¹
60
40
20
0
r=0·77262
r=0·83698
0
10
20
30
40
50
60
Tension, g
B
Impulses s⁻¹
30
0
C
150
μV
10s

situated in the pyloric sphincter and were characteristically inhibited by close IA 5-HT.

ELECTRICAL STIMULATION

Transmural and local motor nerve excitation caused a vigorous increase in impulse activity which generally, but not always, coincided with emg changes and was always followed by a silent phase whose length varied with the stimulus applied (see figure 16).

THE RESPONSE TO DRUGS

Tension receptors were tested with a number of pharmacological preparations (see table 6). Their responses were clearly different from mucosal mechanoreceptors (see Chapter 5), particularly in their sensitivity to peptides and their insensitivity to mucosally applied local anaesthetics. 50 mM solutions of glucose, lactic acid and acetic acid solutions close IA did not alter the responsiveness of units.

Units increased their activity to a wide range of drugs. Local arterial injections of phenylbiguanide, veratrine, 5-hydroxytryptamine and acetylcholine caused an increase in activity with a short latency (less than 2 seconds). There was, in addition, a later (secondary) response to prostaglandins $F_{2\alpha}$ and E_2, acetylcholine and nor-adrenaline which was characteristically repetitive bursts coincident with mechanical movement (peristalsis) and emg activity in the receptive field. The response to nor-adrenaline close IA was typically a reduction in activity coincident with decreased tone of the loop. During this atonic phase there was no response to close arterial phenylbiguanide, 5-HT or pentagastrin.

Figure 16 The response of a tension receptor in the pyloric sphincter to transmural electrical excitation (50 Hz, 1 msec, 20V). The most powerful contraction and greatest impulse frequency followed the 4 sec excitation. Note the period of silence following each period of activity.

The stimulus artefact is represented by the bar.

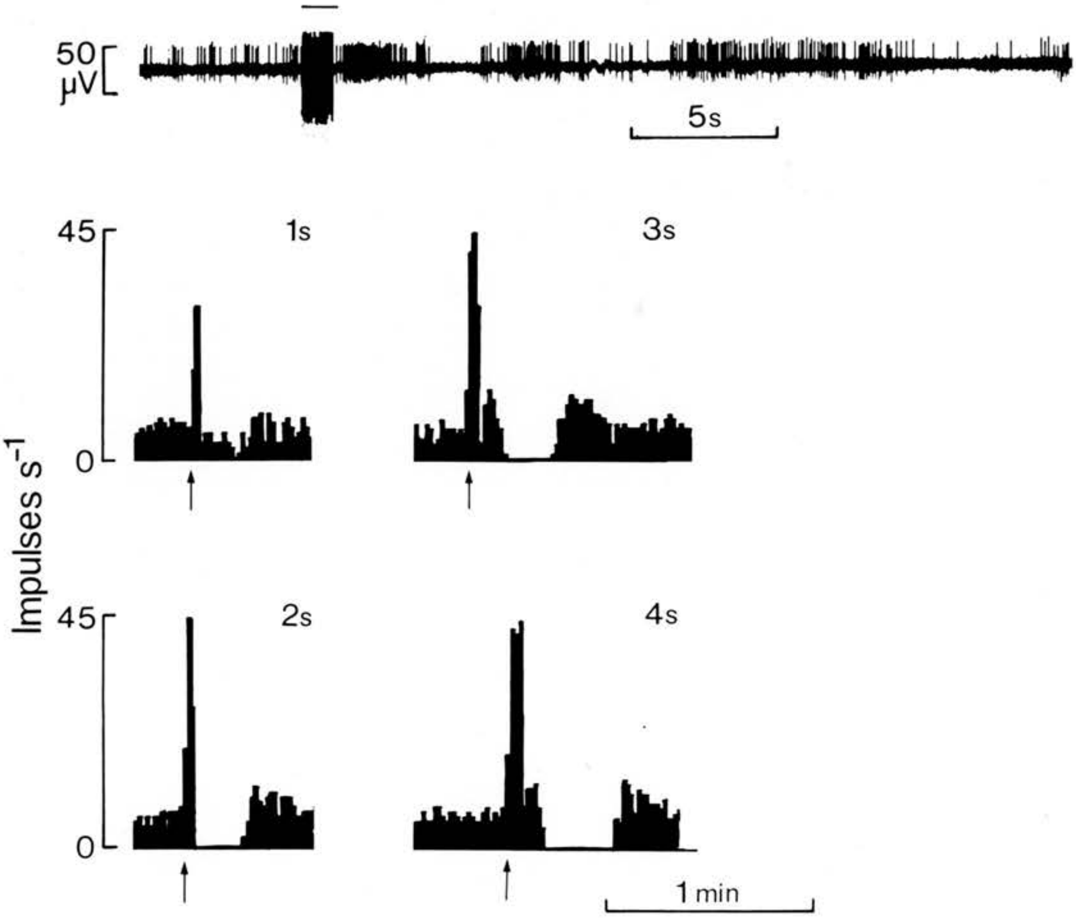

50 µV
5s
45
0
1s
3s
Impulses s⁻¹
45
0
2s
4s
1 min

Table 6

PHARMACOLOGY OF SHEEP TENSION RECEPTORS

Drug	Dose	Route	Response	Trials
acetylcholine	50 µg	close IA	primary and secondary excitation	5/6
adrenaline	0.25 ml 10^{-6}M	"	reduced activity, gut atony, refractory to drugs including 5-HT, pentagastrin, phenylbiguanide	6/6
atropine	200 µg	"	no change	8/10
			increased activity	2/10
atropine and hexamethonium		"	relaxation of gut tone and abolished drug evoked responses	4/4
bradykinin	1 µg	"	no change	3/3
	25 µg	"	excitation	4/4
bradykinin and PG $F_{2\alpha}$	1 µg + 10 µg	"	no change, prolonged blanching	2/2
CCK-8	1 - 2 µg	"	vigorous activity lasting <15 min phasic discharge pattern	10/10
CCK-33	2 - 5 µg	"	no change; no movement seen	5/5
CCK-39	2 - 5 µg	"	increased number of spikes per burst	4/8
glucose	1 - 10%	"	no change	5/5

Table 6 (continued)

hexamethonium	100 μg	close IA	one unit only increased activity	1/5
5-hydroxytryptamine	1 ml, 10^{-4}M	"	excitation, a short burst of activity at frequencies up to 44 imp sec^{-1}, excitation may last <90 sec	8/10
			2 units inhibited in pyloric sphincter	2/10
insulin	1 - 5 I.U.	"	increased activity with short latency no mechanical changes	12/20
lactic acid, acetic acid	50 mM L^{-1}	"	no change	8/8
lignocaine	few drops, 2%	topically on to mucosa	no change	n/n
nor-adrenaline	50 μg	close IA	primary and secondary excitation, inhibition as adrenaline	3/7 4/7
papaverine	10^{-3}M	"	increased activity decreased activity	4/7 3/7
pentagastrin	1 μg Kg^{-1}	IV jugular	dramatic increase after circulation delay	10/10
	"	close IA	moderate increase in activity	6/8
phenylbiguanide	50 - 80 μg	IV jugular	increased activity coincident with mechanical and emg events	4/5
	5 - 90 μg	close IA	increased activity, peak frequency 30 imp sec^{-1} associated with increased tonus	8/10

Table 6 (continued)

phenylbiguanide (cont)	5 - 90 μg	mucosal	no change	4/4
prostaglandin E_2	50 μg Kg^{-1}	close IA	5 sec delay, then repetitive bursts lasting 10 min, peak frequencies 71 imp sec^{-1}	10/10
prostaglandin $F_{2\alpha}$	"	"	as PG E_2	10/10
veratrine	20 μg Kg^{-1}	IV, jugular	little change	5/5
	1 - 100 μg	close IA	vigorous activity with later repetitive discharge and desensitisation to peak frequency 54 imp sec^{-1} compression	5/6

Tension receptors were excited by close IA bradykinin (>25 μg) but lower doses (1 μg) were ineffective. The larger dose caused movement of the receptive field (see figure 17).

The response to phenylbiguanide is illustrated in figure 18 in which it is compared with a mixed population from a rabbit. The increases in intraluminal pressure and impulse activity were coincident. When the gut became atonic after the administration of nor-adrenaline or atropine and hexamethonium, the excitation was abolished.

Prostaglandin E_2 caused the greater discharge frequency when compared with PG $F_{2\alpha}$ and the bursts and interburst intervals were of longer duration. The accummulated impulse total of a six minute sample period were approximately equal, with a mean rate of 8 imp sec^{-1}. The enhanced activity after prostaglandin administration usually disappeared after ten minutes. Following the return to pre-stimulus levels of activity the receptors were more sensitive to applied mechanical stimuli. Also, close intra-arterial ammonium chloride (1.5 - 2.0 mg), which had previously caused no response, caused an increased discharge, which was characteristic bursts of activity. The response to prostaglandins is illustrated in figures 19 and 20.

A rapid injection of intravenous pentagastrin (1 μg Kg^{-1}) caused either an increase in peak frequency in spontaneously active units, or caused a de novo discharge in previously silent units, which coincided with increased emg and tension changes after a delay of approximately 25 seconds (see figure 22). This activity coincided with an active contraction of the pylorus and duodenum and the delay could be accounted for by the circulation time. The same drug close intra-arterially was effective on 6/8 occasions with a short (<5 second)

Figure 17 The response of tension receptors to close intra-arterial injections of drugs. (A) 5-hydroxytryptamine (25 μg) causes contraction in the longitudinal and circular direction. The impulse activity increases during the early and late phases of the longitudinal contraction. (B) Bradykinin (25 μg) excites the receptor after a long delay (>10 sec). The impulse activity increases during an increase in longitudinal tone (middle trace) which develops during a reduction in systemic BP (lower trace).

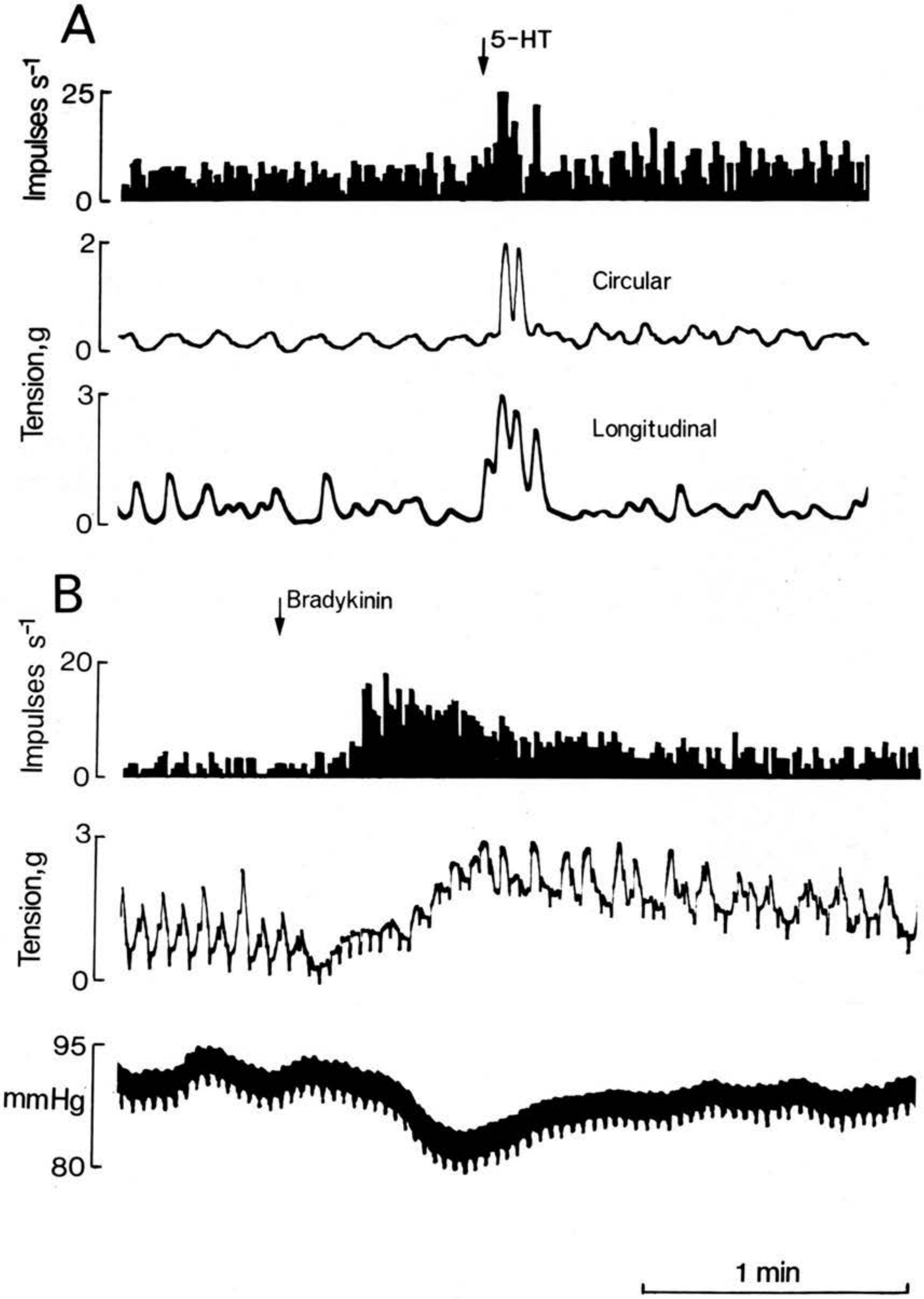

A
5-HT
Impulses s⁻¹
25
0
2
Circular
0
Tension,g
3
Longitudinal
0
B
Bradykinin
Impulses s⁻¹
20
0
3
Tension,g
0
95
mmHg
80
1 min

Figure 18 The response of mechanoreceptors to phenylbiguanide.

(A) A mixed population in a rabbit excited by 50 μg IV into the jugular vein. Upper Trace : spike train; Second Trace : proximal intra-duodenal pressure; Third Trace : systemic blood pressure; Lower Trace : duodenal loop intraluminal pressure. Note that impulse activity increases during contraction of the duodenal loop (indicated by the rising phase of the lower trace) and that BP and proximal duodenal bulb changes precede these events.

(B) Sheep tension receptor unitary activity following 20 μg close IA. There is concurrent relaxation of the circular muscle and increased longitudinal tension.

(C) 100 μg was injected close IA at the first arrow, and 5 μg at the second arrow. There was an increase in the tone of circular muscle (change in baseline of upper trace) and the activity of the receptor was increased but the phasic pattern of discharge is reduced, until the baseline levels have returned. The increased activity caused by phenylbiguanide was blocked by atropine and hexamethonium,and nor-adrenaline.

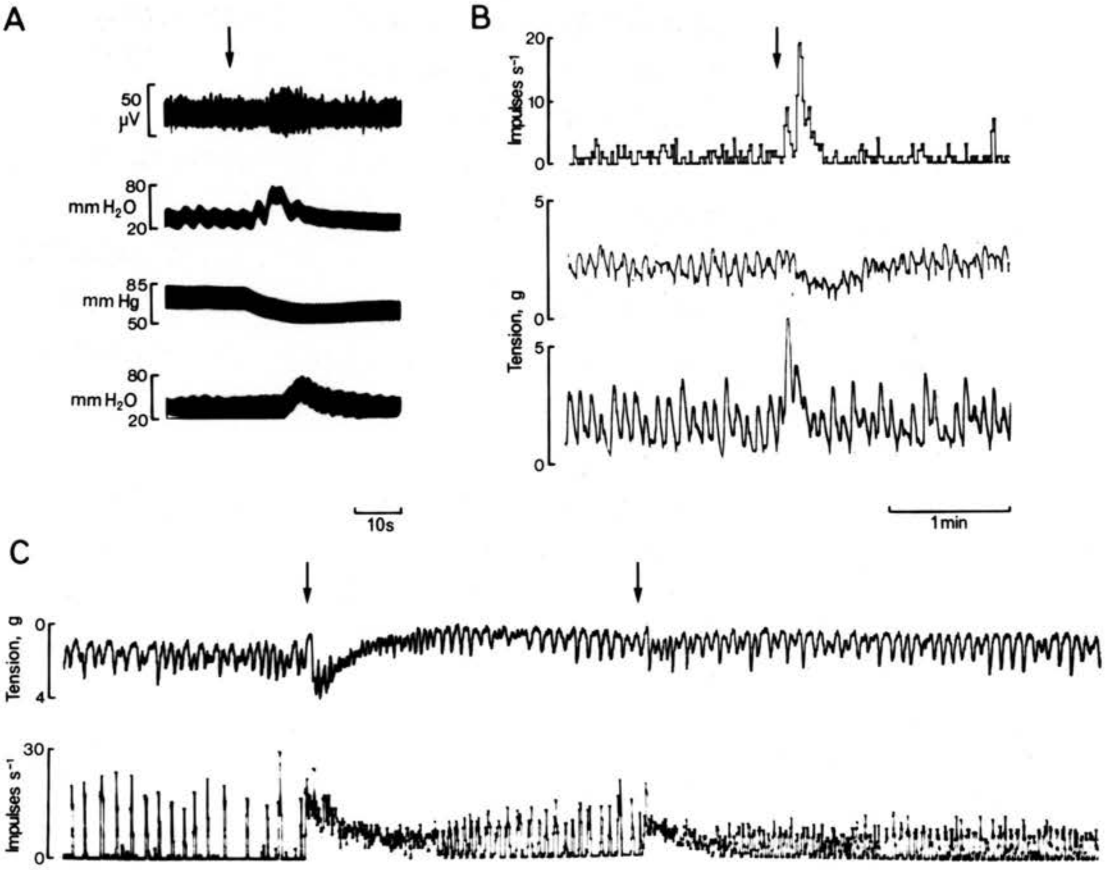
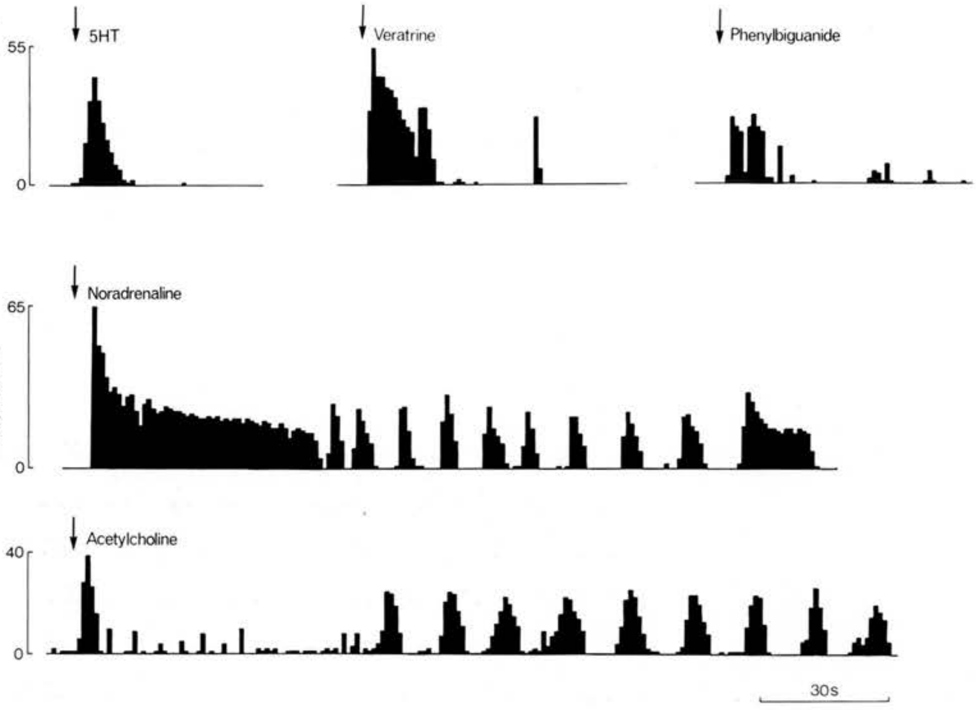

A
50
μV
80
mm H₂O
20
85
mm Hg
50
80
mm H₂O
20
10s
B
Impulses s⁻¹
20
10
0
5
Tension, g
0
5
0
1 min
C
Tension, g
0
4
Impulses s⁻¹
30
0
1 min

Figure 19 I The response of a single tension receptor to injection of drugs. 50 μg has been injected close intra-arterially at each arrow and the total response is shown. Note that nor-adrenaline and acetylcholine produce a primary as well as a secondary discharge. At least 10 min delay was allowed between trials.

II The response to close intra-arterial injection of prostaglandin E_2 and $F_{2\alpha}$ in a single tension receptor. In trace (A) the total response to $PGF_{2\alpha}$ (50 μg Kg^{-1}) is shown. In trace (B) this response has been expressed as peak frequency per burst (+) and mean frequency per burst (*) and is compared with the response to the same dose of PGE_2. The mean length of the burst for $PGF_{2\alpha}$ was 7.6 sec and PGE_2 was 4.3 sec.

I


5HT
Veratrine
Phenylbiguanide
55
0
Noradrenaline
65
0
Impulses s⁻¹
Acetylcholine
40
0
30s

II

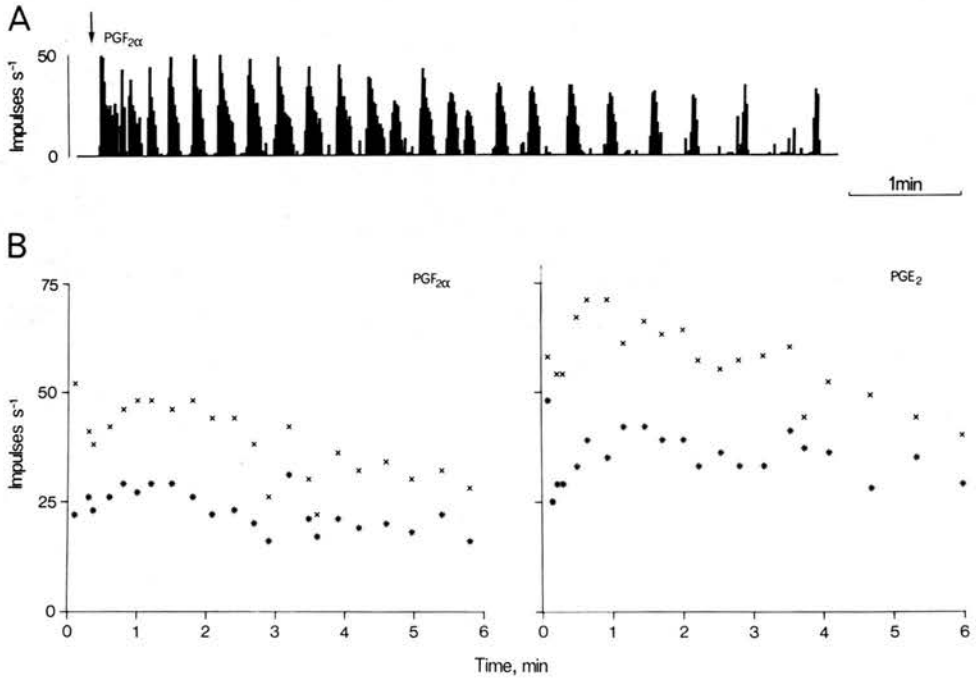

A
PGF2α
50
Impulses s⁻¹
0
1min
B
PGF2α
PGE2
75
50
25
0
Impulses s⁻¹
0
1
2
3
4
5
6
Time, min

Figure 20 Sensitisation of a sheep tension receptor by $PGF_{2\alpha}$. The response to light stroking of the mucous membrane is shown (arrow) five minutes after the close IA injection of $PGF_{2\alpha}$ (50 µg) which caused no change in this relatively inactive unit. Prolonged rhythmic activity persisted following this brief mechanical stimulus. The break in the record was 1 min duration.

Figure 21 (A) The response of a sheep tension receptor to close IA synthetic CCK-8 (5 µg). Notice the induced phasic discharge and increased frequency, with distinctive silent periods between bursts. The increased activity was sustained for a further five minutes.

(B) The response of a spontaneously active mucosal receptor (see Chapter 5). The unit had periods of mechanical insensitivity. At the bar perpendicular pressure was applied to the mucosa. Later synthetic CCK-8 (5 µg) was injected close IA. Note that there is some increase in impulse activity but the rhythmic nature of discharge typical of tension receptors was not seen. The total response is shown.


Impulses s^{-1}
50
0
30s

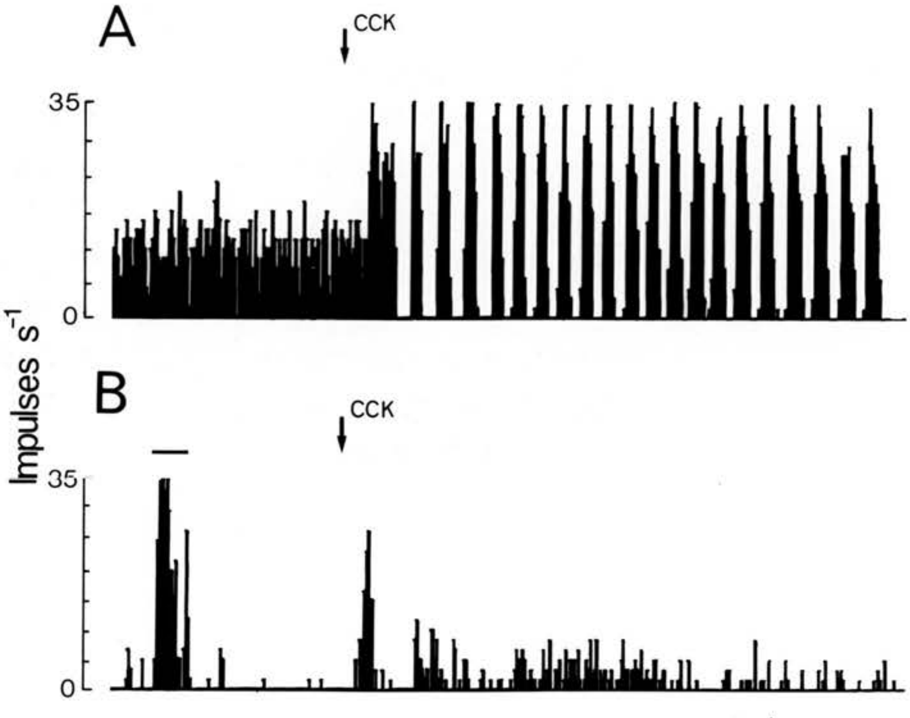
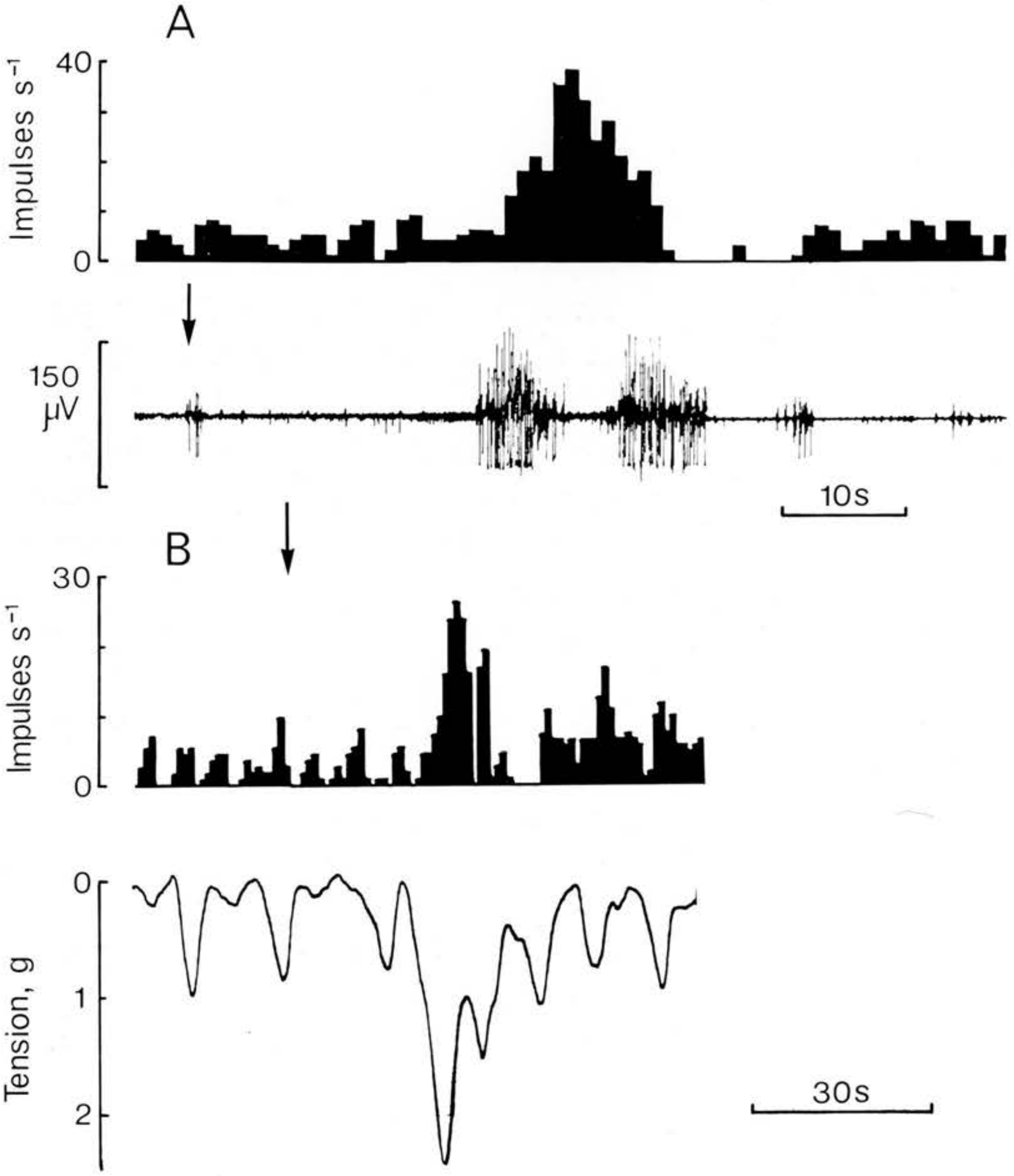

A
CCK
35
0
Impulses s^{-1}
B
CCK
35
0
1 min

Figure 22 The response of two separate tension receptors (A) and (B) to the intravenous injection of pentagastrin (1.0 μg Kg^{-1}) at the arrow. (A) Note the increase in the emg recording which precedes the increase in impulse frequency and also note the pause in the impulse activity before resuming background activity. In (B) the longitudinal tension has been measured. Note the close correlation between the tension and the impulse activity. Because there was a 1 sec delay in counting spikes both histograms are displaced 1 sec to the right.

(Note : the similarity of the trace (A) with figure 4B, duodenum, Bell, Titchen & Watson, 1977, where 0.1 μg Kg^{-1} was used.)


A
Impulses s⁻¹
40
0
150
µV
10s
B
Impulses s⁻¹
30
0
Tension, g
0
1
2
30s

latency (see figure 23A).

Close IA injections of cholecystokinin (synthetic CCK-8) caused activity very similar to the PG E_2 response. Silent units were sensitised and rhythmic discharges started, whereas active units had enhanced discharge frequencies. An example is shown in figure 21.

The response to close IA insulin B.P. was inconsistent. Often (8/20) there was no change in activity. When units changed their activity (12/20) there was always an increase in discharge which was never associated with muscle tension changes. The threshold was below 3 I.U. Often there was a vigorous discharge with less than approximately 0.1 I.U. insulin (flushing the catheter) in the most sensitive units (see figure 23B and C).

Close intra-arterially, atropine caused inhibition of the secondary response to acetylcholine and reduced the response to the prostaglandins. Close intra-arterial hexamethonium inhibited the primary response to acetylcholine. Combinations of atropine and hexamethonium abolished all drug-evoked responses which had previously excited the receptor. One unit responded biphasically to IA doses of hexamethonium. There was an initial excitation of the unit, lasting 12 sec, followed by a 12 sec inhibition until pre-stimulus discharge rates returned. The response was consistently demonstrated over six trials.

CHEMICAL SENSITIVITY

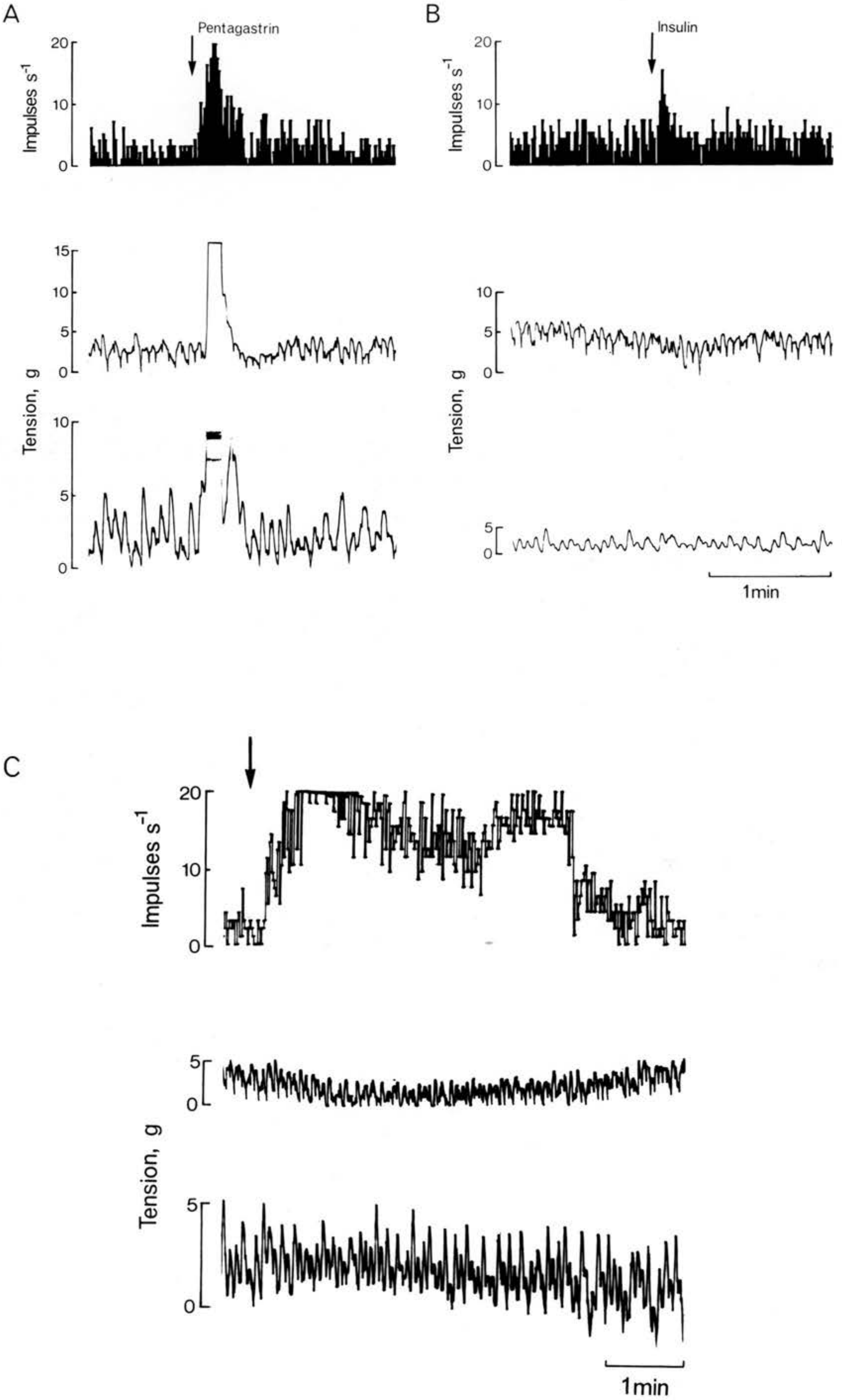
Flooding the mucosa with some solutions at 39°C caused an increase in the impulse frequency of spontaneously active units. The following solutions gave positive responses : HCl 25 - 75 mM L^{-1},

Figure 23

(A) The response of a sheep tension receptor to close IA pentagastrin (25 μg) at the arrow. Note an increased impulse activity during raised tension in circular (middle trace) and longitudinal (bottom trace) direction.

(B) The same unit responded to close IA insulin (1 IU B.P.). Note the increased impulse activity without changes in muscle tension.

(C) A sheep tension receptor response to less than 0.1 IU of insulin. This was the greatest response to insulin from this unit. There was no change in longitudinal or circular muscle tension.


A
Pentagastrin
Impulses s-1
20
10
0
Tension, g
15
10
5
0
10
5
0
B
Insulin
Impulses s-1
20
10
0
Tension, g
10
5
0
5
0
1min
C
Impulses s-1
20
10
0
Tension, g
5
0
5
0
1min

50 mM in normal saline; acetic acid 50 - 100 mM L^{-1} in normal saline; butanoic 22 mM L^{-1}; lactic 100 mM L^{-1} in saline; NaOH 25 mM L^{-1} in saline; $NaHCO_3$ 299 - 815 mOsm Kg^{-1} and tyrosine 50 mM L^{-1}. Normal saline itself caused no changed in activity, but saline reduced the activity induced by acids and so did bile (see figure 24). Otherwise, the response persisted for up to ten minutes.

Increased activity was always coincident with observed movements or tension changes with delays of 5 - 20 sec. Flooding the receptive field with lignocaine increased the latency of the acid-induced responses suggesting an indirect mechanism of excitation. In 4/10 units the response was greatest for acetic acid (50 mM L^{-1} in saline, 340 mOsm Kg^{-1}) and less for HCl (25 - 50 mM L^{-1} in saline, 346 - 384 mOsm Kg^{-1}). Figure 25 I demonstrates this response in which there were smaller pH changes with acetic (0.8 units) than HCl (2.4 units) in the receptive field.

THERMAL SENSITIVITY

Tension receptors altered their response when solutions of varying temperatures were applied to the mucosa, or the gut was stimulated by the thermal device. An example is shown in figure 25 II. The unit responded with an increased discharge when solutions of $NaHCO_3$ (293 mOsm Kg^{-1}) at 20°C and saline (300 mOsm Kg^{-1}) at 20°C were added to the mucosal receptive field. The surface temperatures were respectively 28 and 26°C. No changes were seen when the solutions were 39°C. The threshold was therefore 11°C. Ten units showed a reduction in impulse activity when the receptive field was either warmed or cooled away from 39°C. The response continued after application of lignocaine to the mucosa.

Figure 24

The response of tension receptor to mucosal flooding by acid. In the upper trace 25 mM HCl saline (346 mOsm Kg^{-1}) and in the lower trace 50 mM HCl in saline (384 mOsm Kg^{-1}) was added at the arrow. The activity was removed by washing with saline and was delayed by mucosal application of lignocaine 2%. Note the greater latency of the response in the lower trace, a phenomenon also seen in mucosal receptors after repeated acid stimulation.

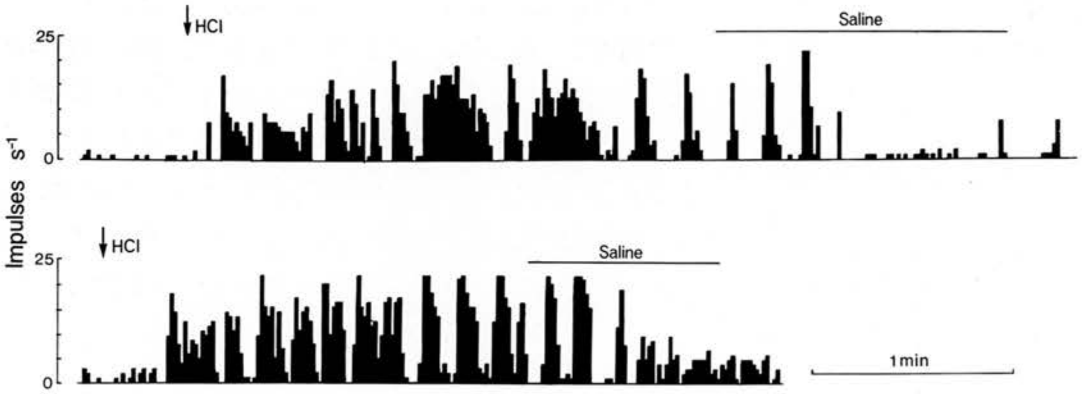
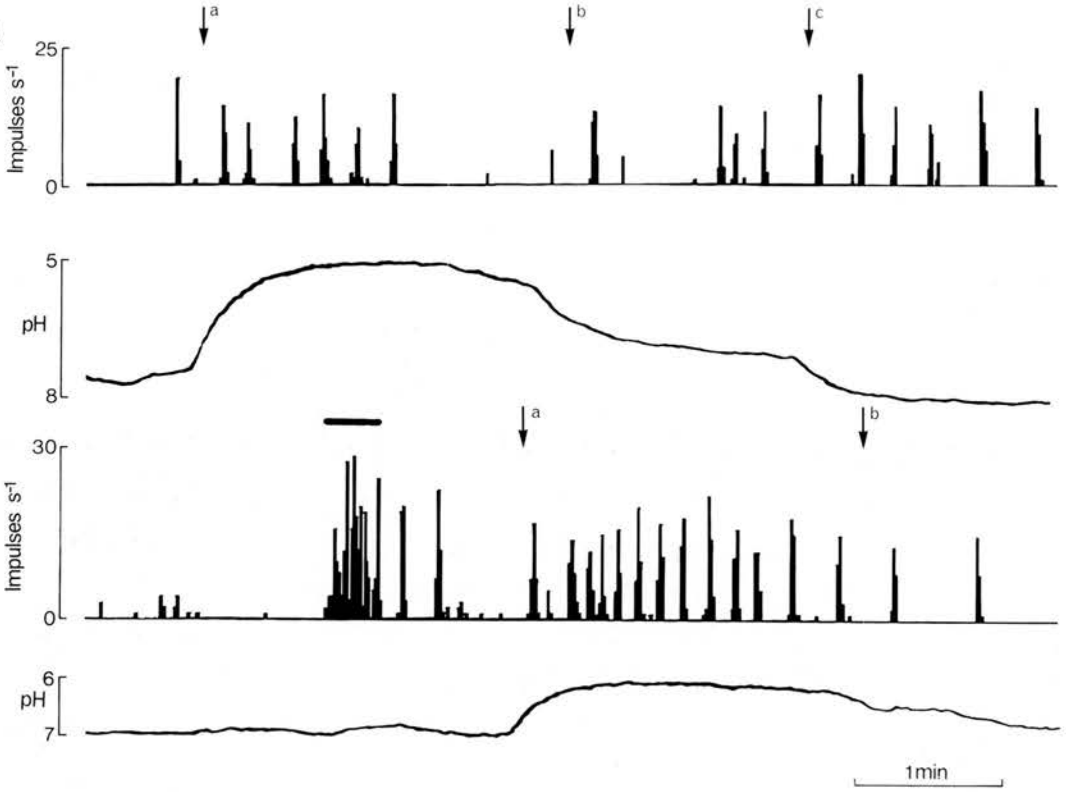
HCl
Saline
25
0
Impulses s⁻¹
HCl
Saline
25
0
1 min

Figure 25 The response of a sheep tension receptor with low levels of spontaneous activity to flooding the mucosal receptive field with solutions of different pH and temperature.

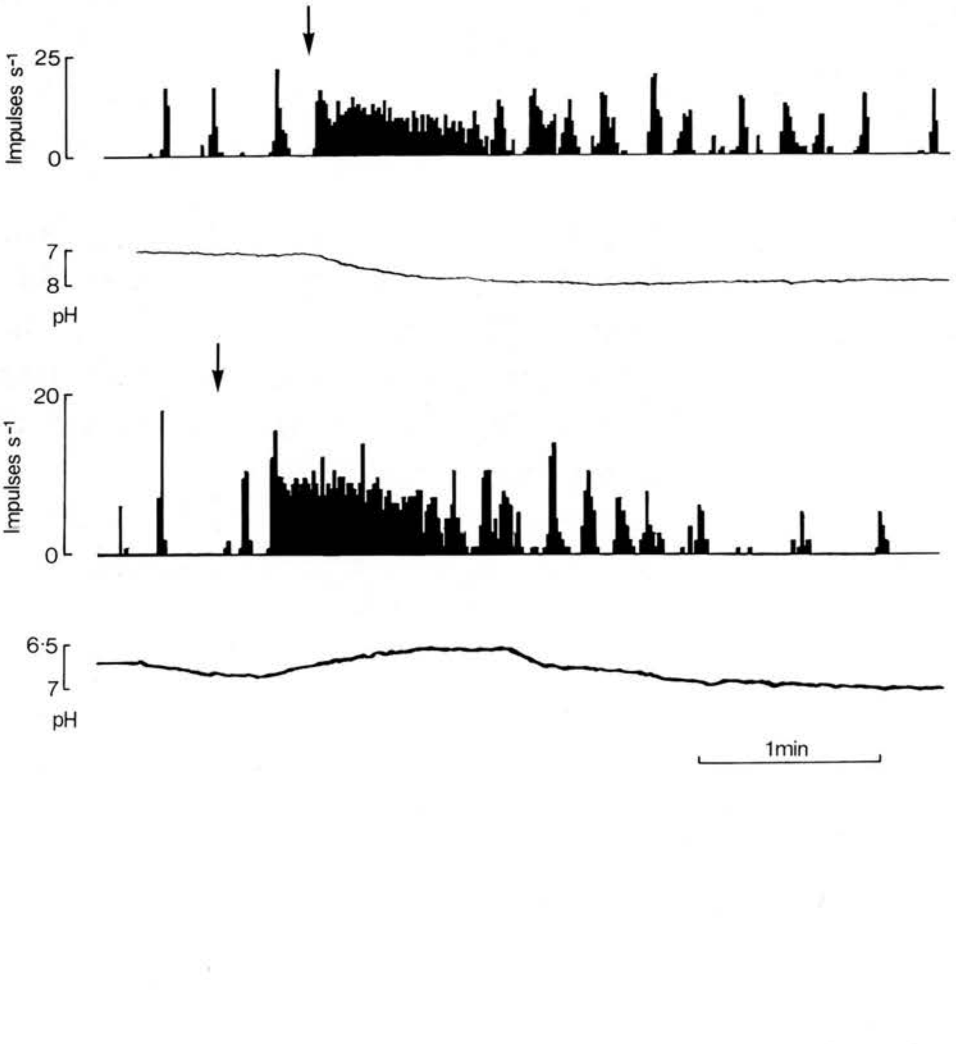
(I) The effect of pH. Solutions at 39°C.
(A) At arrow (a) 25 mM HCl in saline (346 mOsm Kg^{-1}) was applied; at (b) washing with saline; at (c) $NaHCO_3$ (297 mOsm Kg^{-1}).
(B) Digital compression at the bar; (a) 50 mM L^{-1} acetic acid in saline (321 mOsm Kg^{-1}); (b) washing with saline. Note that pH changes in (B) were less than (A) and that more vigorous activity was provoked by acetic than HCl. (See also figure 24).

(II) The effect of temperature. (A) $NaHCO_3$ (293 mOsm Kg^{-1}) at 20°C was added at the arrow. (B) Normal saline at 20°C at the arrow. Units were insensitive to these solutions at 39°C. The surface temperature during the record rose from 26 - 32°C.
The lower trace in both records is the surface pH, recorded with a glass electrode.

I


A
a
b
c
25
Impulses s⁻¹
0
5
pH
8
B
a
b
30
Impulses s⁻¹
0
6
pH
7
1min

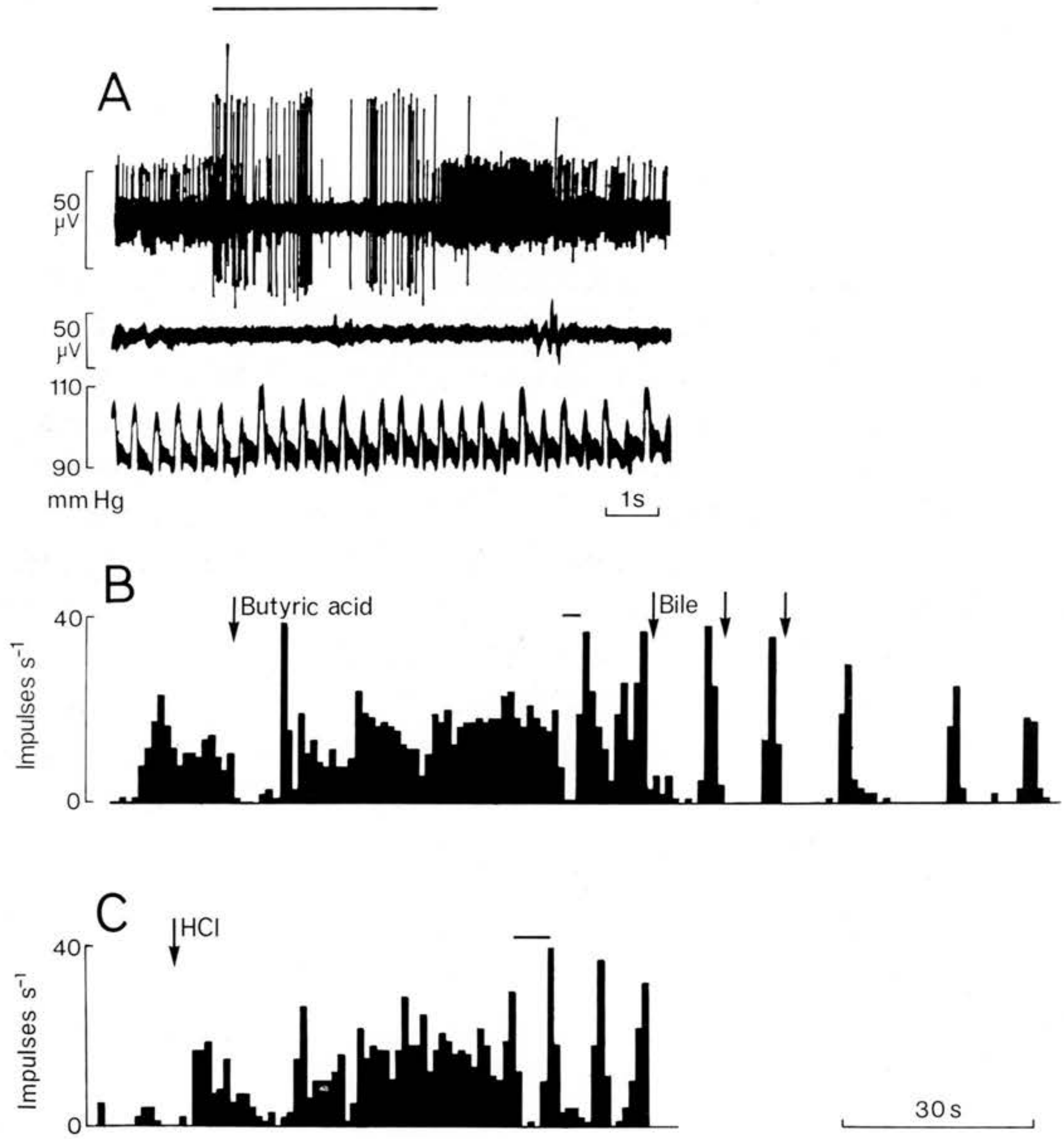
II


A
25
Impulses s⁻¹
0
7
8
pH
B
20
Impulses s⁻¹
0
6·5
7
pH
1min

RECEPTOR INTERACTION

A characteristic feature of most tension receptor units was the brief inhibition of activity during light mechanical brushing or jetting of solutions on to the mucosal receptive field. This is demonstrated in figure 26. During light mechanical stimulation sufficient to excite only mucosal receptors directly (see Chapter 4) the tension receptor activity was inhibited before returning with an increased frequency. The mucosal stimulus itself was insufficient to affect the tension of the muscularis externa, although it will be seen later (figure 34) that the excitation may be due to an evoked contraction mediated by intrinsic nerves.

Figure 26 The response of tension receptors to light mucosal mechanical stimulation. In (A) application of a cotton-wool ball on the receptive field at the bar excites a mucosal mechanoreceptor (large spike) during which the activity of the tension receptor is inhibited. (Note : the accelerated impulse activity in (A) after the mucosal stimulation and its coincidence with emg activity.) In traces (B) and (C) the activity of the units have been excited by butanoic acid and HCl. Note that brushing (at the bars) inhibits the activity.


A
50 µV
50 µV
110
90
mm Hg
1s
B
Butyric acid
Bile
40
0
Impulses s⁻¹
C
HCl
40
0
Impulses s⁻¹
30 s

DISCUSSION

These results establish that mechanoreceptors of the muscularis externa have characteristics which fit them into the proposed concept of "in series" tension receptors (Iggo, 1954b; Leek, 1969). Although the measurement of tension changes in the outer muscle coat was not precise enough to demonstrate which outer muscle layer was involved in the development of tension, with few exceptions, the longitudinal tension changes closely paralleled the activity seen, suggesting that tension receptors are associated with the longitudinal muscle. The response to electrical stimulation indirectly supports this location because if the receptor occupies a position in the longitudinal muscle, then its activity would be inhibited during reflex contraction of the circular muscle during natural peristalsis, and following co-axial electrical stimulation (Kottegoda, 1970). Under conditions when the circular muscle is not contracting the response would be slowly adapting as long as the stimulus lasts (Kottegoda, 1970). This response was consistently seen in these units when local compression was applied. But when peristalsis was evoked by electrical stimulation, as well as by drugs, the increased activity coincided with the earliest mechanical and emg activity, and was followed by a prolonged silence which corresponds to the inhibition during circular muscle contraction. This accounts for the poorly sustained discharge during propulsive movements caused by distension of an ultra-luminal balloon, when an annular constriction could be observed following behind the balloon, and for the phasic discharge pattern during duodenal motility.

The sensitivity of tension receptors to compression was first demonstrated by Iggo (1954a) in the oesophageal groove of goats, and it has been utilised here to produce a reproducible response for quantitative analysis. Because of differences between individual preparations it has only been possible to compare the sensitivities of individual units in the same animal, when there was often marked differences between units in similarly located receptive fields. Compression is apparently not the natural stimulus because longitudinal increases in tension, evoked by drugs, caused bursts with peak frequencies much larger than those caused by transmural compression and at much lower tensions. When transmural compression was combined with naturally or artificially occurring activity, the frequencies increased. Therefore it seems probable that transmural pressures are important to the sensitivity of the receptor and that the pressure changes at different parts of the abdomen must influence their activity. This response to compression may play an important role in the reduction in food intake during pregnancy or excessive abdominal fat (see Forbes, 1980).

Whether visceral mechanoreceptors respond to transmural pressure or radial stretching varies with different viscera. Some tension receptors in the reticulo-rumen are insensitive to transmural compression (Leek, 1969). Radial stretching is important in the isolated intestine of the guinea pig as a stimulus for peristalsis (Kosterlitz, 1968) and in the carotid sinus in the baroreceptor reflex (Hauss, Kreuziger & Asteroth, 1949). However, aortic baroreceptors respond to transmural pressure (Angell James, 1971) and so do atrial baroreceptors (Goetz, Hermreck, Slick & Starke, 1970). If the present receptors are the same population that are involved in the peristalsis reflex in the isolated gut, the observation that peristalsis is absent when a constricting cuff is applied

(Kosterlitz et al, 1956; Kosterlitz & Lees, 1964) can be interpreted in the following way : the persistence of tension receptor excitation by compression prevents the onset of peristalsis, and when radial stretch is not prevented peristalsis is provoked. Subsequently longitudinal muscle tension is reflexly inhibited during circular muscle contraction.

The regularity of the adapted discharge suggests that a single spike-initiation locus exists in tension receptors, which is therefore similar in structure and function to the Ruffini ending in the somatosensory system (Chambers et al, 1972).

The response to drugs confirms the close association of tension receptors with the mechanical changes in the muscularis externa seen by others (Paintal, 1954a, 1954b; Iggo, 1957b, 1957d). Drugs may act by directly altering the sensitivity of receptor endings; indirectly by either modulating neurotransmitter release in the enteric plexus which changes gut motility, or by acting directly on the smooth muscle membrane and affecting the rate of oscillation of membrane potential. Some drugs may act in more than one way and may involve indirect long reflexes.

With the exception of insulin, it has been difficult to agree with the suggestion that some drugs cause primary excitation of the tension receptor, because both mechanical activity and electromyographic changes always accompanied impulse activity. Indeed under conditions of smooth muscle atony (after nor-adrenaline) the loss of response to phenylbiguanide, 5-HT and pentagastrin suggested that these drugs do not cause a primary receptor excitation as is suggested elsewhere (see Daniel, 1968). Local and systemic atropine and hexamethonium administration did not totally abolish the spontaneous motor or afferent activity. However, when the preparation allowed direct arterial perfusion, atropine was found to block spontaneous and evoked activity suggesting that the action of drugs causing secondary responses was mediated by muscarinic pathways. Even with massive doses of both blocking drugs it was difficult to uncouple the excitation contraction mechanism and it was therefore difficult to analyse whether drugs acted directly on either the generator or regenerative region of the receptor.

Acetylcholine is the excitatory transmitter to intestinal smooth muscle and any drug which increases its release would be expected to increase mechanical activity and therefore tension receptor activity.

The excitatory effect of adrenaline and nor-adrenaline on some receptors in the region of the sheep pyloric sphincter is not entirely unexpected. The presence of adrenergic α-excitatory pharmacological receptors in alimentary smooth muscle sphincters has been reported elsewhere (see Lee, 1970). In the goat, Ehrlein (see Bell & Watson, 1975, p 342) reports contraction of the pyloric sphincter to both adrenaline and atropine, so presumably α-receptors are dominant here. Their presence has been reported in the intestinal wall, away from sphincters, but they are more difficult to demonstrate. They have been shown in longitudinal muscle of guinea-pig ileum (Lee, 1970) but not duodenum. The effect may be caused by a direct action on the muscle, or indirectly by inhibiting release of acetylcholine as a type of presynaptic inhibition (Vizi, 1976). Tension receptors are also excited by nor-adrenaline in the cat stomach (Paintal, 1953, 1954b, 1954c; Iggo, 1957b, 1957d) and cat intestine (Leitner & Perl, 1964).

Bradykinin is a powerful vasodilator causing rapid depressor and later pressor effects, possibly as a result of contraction of veins and large arteries (Goodman & Gilman, 1975). There is also increased capillary permeability and local oedema. It is a potent algogenic substance. Floyd, Hick, Koley & Morrison (1977b) have demonstrated excitation of splanchnic mechanoreceptors by intra-arterial bradykinin (1 - 10 μg) in cats, and conclude that the effect is secondary to contraction produced indirectly by excitation of efferents to the gut (Floyd et al, 1977a). The results presented here demonstrate that duodenal tension receptors were also excited by close IA bradykinin which coincided with increased motility. The latency of ten seconds or more supports a possible indirect mechanism. Evidence is presented below that bradykinin also excites superficial mucosal receptors with short latency, by an atropine-sensitive mechanism, probably by causing smooth muscle to contract (see Chapter 5).

In the sheep duodenum increases in afferent activity following adrenaline always accompanied tension changes. Some tension receptors, not necessarily distant to the sphincter, were not excited and often their activity was reduced. It is therefore difficult to conclude that adrenaline excitation may be due only to receptor depolarisation or reduction of threshold of the regenerative region, as is suggested by Paintal (1964).

5-Hydroxytryptamine causes alimentary smooth muscle contraction _in vivo_ and _in vitro_ (Born, 1970) via indirect (neural) and direct muscle excitation. There is evidence for 5-HT receptors throughout the alimentary canal in nerves and muscle (Kottegoda, 1970) but there is limited evidence for its action as a transmitter (Gershon, 1970).

The results in the sheep duodenum suggest that 5-HT does not primarily excite tension receptor membranes as is suggested by Daniel (1968), because of the demonstration that smooth muscle motility always occurred and when atony was induced with nor-adrenaline the receptors were insensitive to the drug.

The increase in guinea pig longitudinal motor activity *in vitro* by gastrin, pentagastrin and CCK-like peptides is blocked by atropine, which indicates that they act indirectly by releasing acetylcholine from intramural myenteric plexuses via non-nicotinic receptors, because inhibition is caused by tetrodotoxin (which blocks nerve conduction) but not hexamethonium (Vizi, Bertaccini, Impicciatore & Knoll, 1973). The increase in slow-wave complex by pentagastrin (1×10^{-6}M) *in vitro* dog gastric longitudinal muscle is not affected by atropine or tetrodotoxin and hence directly affects the smooth muscle membrane (Szurszewski, 1976). The effect of peptide hormones is continuously controlled by the tonic activity of the sympathetic nervous system at pre-synaptic sites via α-receptors which reduce acetylcholine release (Vizi, 1976). It is therefore possible that gastro-intestinal hormones released after a meal act to enhance or depress the release of acetylcholine by intrinsic nerves and alter smooth muscle membrane excitability. Szurszewski (1975) concludes that pentagastrin may regulate the frequency of occurrence, and acetylcholine determines the strength of these phasic and tonic contractions. The results presented here do not provide support for this hypothesis because the response to pentagastrin was of phasic nature, causing a short-lasting increase in emg and tension activity often followed by prolonged reduction in mechanical activity. Clearly CCK did not increase the frequency of contraction, but each contraction excited the receptor to a greater extent. This was possibly due to acetylcholine release because the response was antagonised by nor-adrenaline and by atropine.

The response of the duodenal-pyloric region to the gastric homologue pentagastrin confirms a number of reports in conscious ruminants. Ruckebusch, Fargeas & Bueno (1969) increased antro-pyloric tone after rapid jugular injection (6 $\mu g\ Kg^{-1}$) with a delay of 18 - 20 seconds. Bell, Titchen & Watson (1977) obtained increased emg activity in the duodenum of calves with 0.1 $\mu g\ Kg^{-1}$ also with rapid IV injection and the responses here confined to the duodenum only, have similar latencies and emg activity. The likely mechanism of this excitation has already been suggested, however, there is an additional possibility that there may be an indirect long reflex involved (Bell et al, 1977), because the relaxation of some parts of the forestomach (reticulo-rumen and abomasum) by pentagastrin seen in sheep (Ruckebusch, 1971) may "off load" tension receptors in these sites which provide tonic input to central regulating centres. When this tonic activity is reduced there follows a reflex increase in motor activity in structures like the duodenum and omasum. This indirect reflex may explain why local IA injections of pentagastrin did not cause as spectacular a response as the same dose intravenously.

The systemic post-prandial levels of gastrin 34 (75% of total gastrin) in man, range from 20 - 50 fmol/ml (pmol/L) (Jensen, McCallum & Walsh, 1978). This value gives little idea of the concentration in the duodenum, so it is difficult to know whether the pharmacological doses utilized in this study are grossly different from physiological values.

The complex interneuronal influence upon the muscularis externa was demonstrated by hexamethonium injected close IA. It was not possible to inhibit duodenal motility with this drug alone, and even when combined with atropine total atony was not always demonstrated. The excitation of tension receptor activity by the drug was unexpected. It is known that hexamethonium when given in strong concentrations *in vitro* may itself produce regular, rhythmic contractions (Feldberg, 1951). Vladimirova (1976) also demonstrated a biphasic response to hexamethonium in the amplitude of electrically evoked epsp's in gastric muscle strips, which at first increase for 3 - 5 minutes, then decrease and stabilise after 10 - 15 minutes, despite the continual presence of the drug. Thus the *in vivo* mechanism of the phenomenon demonstrated in sheep duodenum may be due to an altered release of excitatory transmitter which at first increases and later declines; the time course was short *in vivo*.

Prostaglandins stimulate cholinergic neuroeffector transmission in the intestinal tract probably acting at prejunctional sites on acetylcholine release, or possibly indirectly by inhibiting the release of nor-adrenaline from adrenergic nerves (Hedqvist, 1977). Petkov & Radomirov (1977) find that PG $F_{2\alpha}$ causes strong contraction in the cat jejunum by a mechanism abolished by atropine. PG $F_{2\alpha}$ causes contraction of both longitudinal and circular muscle, and PG E_2 inhibits circular muscle in most tissues (Bennet, 1972). Prostaglandin E_1 reduces the inhibitory effect of sympathetic nerve stimulation on intestinal motility and enhances the responses to both nerve stimulation and exogenous acetylcholine in guinea pig longitudinal muscle strips and whole ileum preparations. Indomethacin, an inhibitor of endogenous prostaglandin synthesis, markedly reduces the contractions of whole ileum in responses to nerve stimulation. It seems likely that the ability of prostaglandins to remove nor-adrenergic restraint on acetylcholine release (by inhibiting the release of nor-adrenaline), also plays some role in the enhancement of gastro-intestinal motility by prostaglandins (Vizi, 1976). It has not been possible to draw strong conclusions about the location of sheep duodenal tension receptors using PG $F_{2\alpha}$ and PG E_2. Whether these drugs selectively excite or inhibit contraction in longitudinal and circular muscle in this species is not known. Their effect seemed to be to lower the threshold of the peristalsis reflex to natural stimuli. Tension receptor activity was enhanced during increased tension in the external muscle coat. The mechanism probably involved enteric neurones because the interburst interval was not altered, and this is probably dependent on direct myogenic depolarisation.

The excitatory response to insulin in some receptors was particularly interesting. Insulin has a direct or indirect effect on practically all organs and metabolic process of the body. In this case, the excitatory process did not involve active contraction of muscle and strongly suggested a direct sensitisation of the receptor terminal. Clearly the tension receptor was not primarily excited by glucose, although insulin will increase the uptake of glucose and volatile fatty acids from the receptor environment. Mei (1978) has shown that glucose receptor activity increases with intravenous insulin, but the phenomenon seems not to have been demonstrated before in tension receptors. In the taste system the electrophysiological threshold is unchanged by insulin (Pfaffmann & Hagstom, 1955).

The idea that increased post-prandial tension receptor activity may disrupt the MMC by an inhibitory feedback reflex has been suggested by Davison (1978, see Bueno & Ruckebusch, 1978, discussion). These results suggest that receptor activity may also be due to excitation independent of tension changes. In ruminants VFA's release insulin, and high doses of insulin (1 I.U. Kg^{-1}) intravenously disrupt the MMC and cause uniform spiking activity in sheep jejunum (Bueno & Ruckebusch, 1975). Whether insulin acts peripherally or centrally is not known. These results suggest that a peripheral sensitisation by insulin may be partly responsible.

The chemical response, particularly to acid, was to alter the mechanical sensitivity of the unit. The changes were considered to be secondary to intramural tension changes as a result of intrinsic reflexes, because they were delayed by mucosal lignocaine which does not otherwise affect the response of tension receptors. The response probably also involves hormones. It is curious that no chemical response has been seen in other preparations of tension receptors (Iggo, 1957b; Harding & Leek, 1972a; Clarke & Davison, 1975). The chemical modification of mechanoreceptor activity by glucose was seen in the rabbit duodenum (Chapter 3) and there is other reflex evidence for increased circular muscle motility caused by acid (Summers, 1978).

The demonstration of an interaction between "primary afferent" discharges in tension receptors and mucosal receptors during mechanical stimulation of the mucosa emphasises a methodological problem in gastroenterology, which is that the presence of an intraluminal recording device will alter the physiology of the viscus.

It is well known from reflex studies that a duodenal catheter will cause brief inhibition of gastric peristalsis (Thomas & Baldwin, 1968; Davison, 1972) in the enterogastric reflex, and these results show that such a cannula will also affect the activity of the duodenum itself.

The thermal sensitivity of tension receptors makes it necessary to exclude tension receptors in an analysis of putative alimentary thermoreceptors, and also to be rigorous in maintaining thermal neutrality in reflex experiments when testing the effects of chemicals. It has recently been reported that hot and cold solutions perfused into the duodenum alter the electromyographic activity of the duodenum and cause reflex changes in antral motility (El Ouazzani & Mei, 1979). The possible involvement of sensitised tension receptors, and not thermoreceptors, has not been excluded. The temperature threshold of tension receptors was large. This may be due to the thermal buffering of the intestinal wall between the lumen and receptor-containing layer.

It is concluded from these results that tension receptors are associated with one or other of the muscles in the muscularis externa. They are not associated with both, because individual receptors fire phasically and not continuously as peristalsis passes along the receptive field. Most of the increased impulse activity was associated with longitudinal tension changes, but also activity related to circular tension changes was seen. In addition, one drug, phenylbiguanide, usually caused changes in impulse activity when longitudinal and not circular tension increased; PG E_2 inhibits circular muscle in most tissue, providing indirect evidence that tension receptors may be located in longitudinal muscle, and the observation that electrical stimulation first excited and then inhibited activity suggests that the silent phase may be related to the time when there is reflex inhibition of longitudinal muscle by circular muscle. The results therefore suggest that sheep duodenal tension receptors are probably situated in the longitudinal muscle.

CHAPTER 5

MUCOSAL ENTEROCEPTORS IN THE PROXIMAL DUODENUM OF THE SHEEP

INTRODUCTION

Knowledge of duodenal sensitivity is important for the understanding of the reflex control of gastric emptying (Hunt & Knox, 1968a). The role of chemoreceptors in duodeno-gastric reflexes has been implicated by a number of investigations. Hunt & Knox (1964) demonstrated a short latency slowing of gastric emptying of fatty acids and a short persistence of the inhibition. Cooke (1974) demonstrated that acid in the proximal duodenum inhibits gastric emptying with a latency of less than one minute in dogs, by a neural reflex (Cooke & Clarke, 1976), an observation similar to pigs (Roze et al, 1977). Konturek & Johnson (1971) and Code & Watkinson (1955) suggest that the inhibition of gastric secretion by the proximal duodenum is by a nervous reflex in dogs.

Studies of reflex events have shown that the ruminant duodenum can influence abomasal emptying and motility, as well as gastric acid and pepsinogen secretion. Bell and his colleagues have suggested that many of these abomasal functions are controlled by putative osmoreceptors distributed throughout the length of the duodenum (Bell & Mostigini, 1975; Bell & Grivel, 1975; Bell & Holbrooke, 1979), a distribution that is similar to dog (Konturek & Grossman, 1965a). In the dog the location of receptors responding to acids is mainly in the proximal duodenum (Cooke, 1974). The mid-duodenum is an important location of osmoreceptors in man (Meeroff et al, 1975) and the jejunum contains a variety of receptors in dogs (Cooke, 1977). It is probable that the proposed chemoreceptors are situated in the mucosa.

The role of the visceral afferent nerves cannot be deduced from reflex studies because of the difficulties of interpreting selective neurotomy and latency measurements. For this, electrophysiological recording is necessary. The technique used in this study allowed mechanical stability of the duodenal bulb area in the sheep and the electrical recording of single afferent units in closely associated nerves. Until recently, experimental conditions at this level of the gut have not allowed more than a multifibre analysis of unmyelinated axons (Andrews & Andrews, 1971).

The results in general, support the observations briefly reported in the cervical vagus from the same species (Harding & Leek, 1972a; Leek & Harding, 1975; Leek, 1977). There are however differences in detail in this chapter which considers the sensitivity of enteroceptors in the mucosa.

METHODS

The duodenal open-loop preparation was used. Chemicals were washed away with normal saline and 5 - 10 minutes were allowed between trials.

Application of Chemicals

In the open-preparations the accumulation of mucus and Brunner's gland secretion, particularly following the application of acid, kept the mucosa moist. Before applying test solutions this barrier was aspirated, washed in warm saline and gently swabbed with cotton wool, so that the latency of the chemical response was not affected by diffusion through it. The accumulation of mucus has been shown to interfere with the sensitivity of drugs in other preparations (Walder, 1953). It is probable that the mucus barrier plays an important function in modifying the chemoreceptor response to chyme.

If units became spontaneously active or sensitive to chemicals, when previous applications had shown no response, or the chemical sensitivity was lost, the units were abandoned. These criteria were needed in order to test the concept of selective sensitivity (specificity) of enteroceptors. Preliminary studies demonstrated that treatment with HCl and NaOH with concentrations greater than 50 mM L^{-1}, caused visible alterations in the surface of the duodenal mucosa which persisted for periods up to 2 hours. These changes ranged from small white blebs, petecheal haemorrhages and generalised inflammatory responses. For these reasons the range of chemical concentrations and osmolalities was generally kept within the normal range (see Chapter 1) and the application of unnatural concentrations to unprotected mucous membrane was avoided. Under these conditions multiple sensitivity of mucosal receptors was rarely found.

Distilled water was not routinely used because it can injure the intestinal absorption of NaCl and damage ileal mucosa (Blichenstaff, 1964). When tap water was flooded over the mucosal receptors there followed a prolonged desensitisation of the chemical receptive field, and so this unphysiological stimulus was avoided.

The following chemical solutions were used after characterisation of the mechanical receptive field : all solutions at $39^{o}C$, (mM L^{-1}) : HCl (10 - 100); acetic (10 - 150); lactic (50 - 200); butanoic

(10 - 100); propanoic (10 - 100); myristic (10 - 40); NaOH (10 - 50); $NaHCO_3$ (100 - 450); KCl (10 - 200); D-glucose (50 - 2000); L-glutimate (50); glycine (150); L-tryptophan (12.5 - 50); tyrosine (50); $BaSO_4$ (100% w/v); saline (100 - 1200); bile; olive oil; $MgSO_4$ (150); choline chloride (150); NH_4Cl (859 mOsm Kg^{-1}); urea (917 mOsm Kg^{-1}).

Drugs Used

The following drugs were applied systemically via the jugular vein (s), close intra-arterially (IA), and topically on the receptive field (t) : lignocaine (2%, t); phenylbiguanide (20 - 100 μg, s IA); pentagastrin (1 μg Kg^{-1}, s IA); prostaglandin $F_{2\alpha}$ (5 mg ml^{-1}, t IA); protaglandin E_2 (20 μg, t IA); acetylcholine (100 μg, t IA); nor-adrenaline (30 - 100 μg ml^{-1}, t IA); papaverine (10^{-6}M, t IA); atropine (2 mg ml^{-1}, t); hexamethonium (1 mg ml^{-1}, t); veratrine (50 μg, IA); CCK-8, CCK-33, CCK-39 (2 μg IA, t); insulin BP (1 - 4 IU); 5-HT (10 - 20 μg IA); bradykinin (1 - 50 μg, IA); glucose saline (1 - 10%, IA).

RESULTS

Forty-six mucosal receptors were examined as single unit preparations. The criteria used for their identification were : low mechanical threshold and excitation by local electrical currents. Lignocaine (2% with and without 1/80,000 nor-adrenaline) rapidly abolished the mechanical and chemical response (within 10 seconds). The response returned within 3 - 4 minutes when they were washed with saline, otherwise the inhibition persisted. Many drugs shown to excite tension receptors were without effect. The mechanical and chemical sensitivity of units was unaffected by temperature changes over the range 20 - $54^{o}C$. In addition, no unit studied was specifically sensitive to temperature.

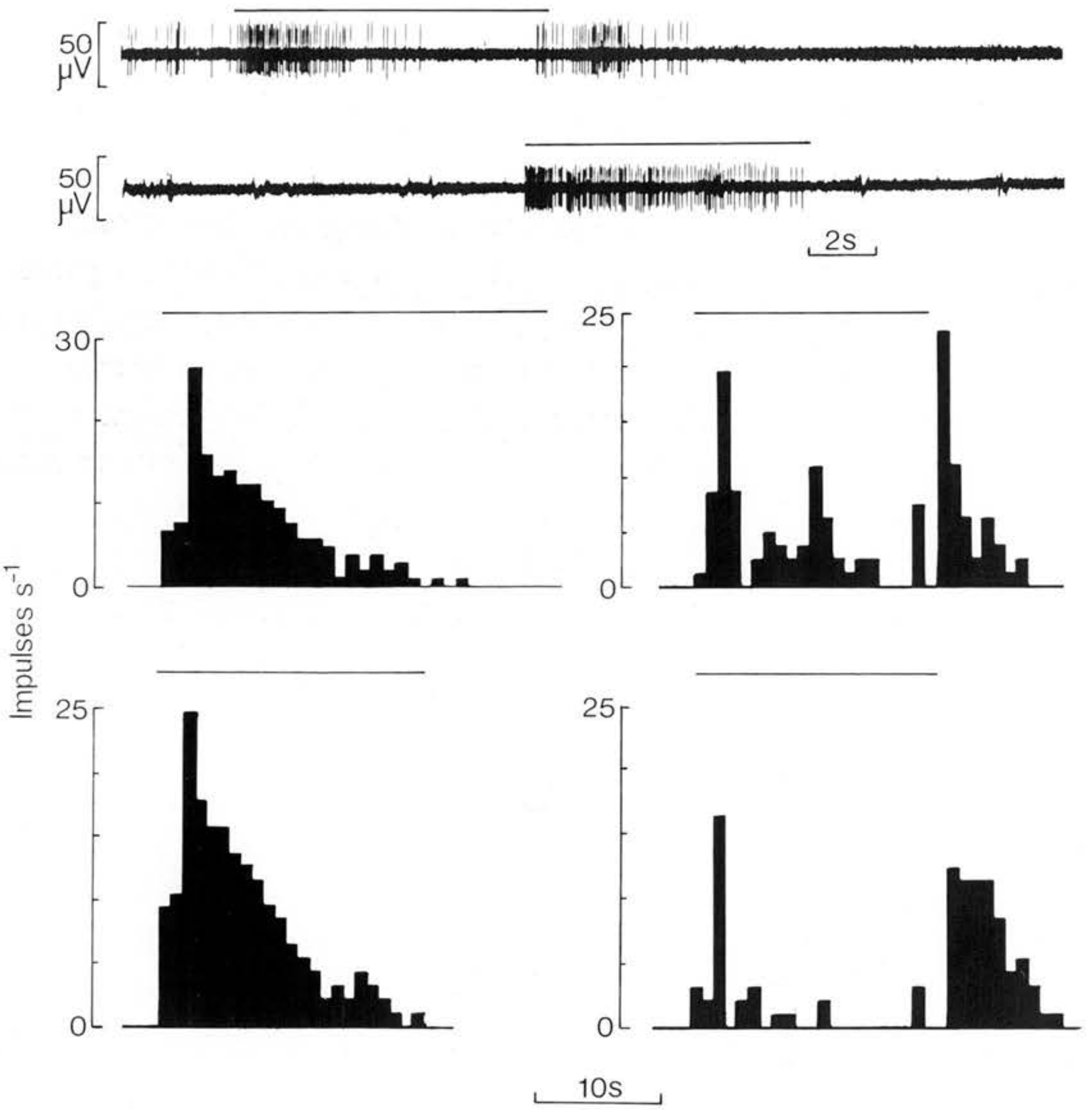
Two classes of receptor were distinguished. Twenty-nine units were silent in the absence of mechanical stimulation, and seventeen units had "spontaneous" activity. The term "spontaneous" is used for background activity.

"SILENT" MUCOSAL RECEPTORS

Units had a discrete symmetrical mechanical receptive field with a Von Frey hair threshold of 132 mg (a force of 8.6 g mm^2) and isometric transducer threshold between 80 - 150 mg which caused a discharge of a few spikes from an area 1 - 5.5 mm^2. Stretch over an area of 1 cm^2 infrequently caused units to fire. Brushing with a fine paintbrush caused a rapidly adapting response of 4 - 5 spikes, often at frequencies of 80 imp sec^{-1}. A sustained slowly adapting response was elicited by precise probing with a small glass rod. When the receptive field was mechanically excited eccentrically a rapidly adapting response with an "on" and "off" component occurred. This phenomenon is illustrated in figure 27. For five receptors only, a slowly adapting discharge could not be found and so it was possible that a rapidly adapting sub-class existed.

Receptors usually had no resting activity unless peristalsis caused villi to brush against each other. In these circumstances a resting discharge 1 - 3 imp sec^{-1} occurred but had no clear relationship with the emg or tension changes in the loop. Systemic pentagastrin, CCK, and 5-HT, caused no change in activity unless

Figure 27 The response of a slowly adapting mucosal receptor to sustained pressure with a glass probe (bar) placed eccentrically (upper spike trace) or accurately (lower spike trace). The corresponding frequency histograms of a number of responses in the same unit is shown below. Left histograms accurate probe placement, right histograms eccentric placement. Conduction velocity 0.87 msec^{-1}.


50 µV
50 µV
2s
30
0
25
0
Impulses s-1
25
0
25
0
10s

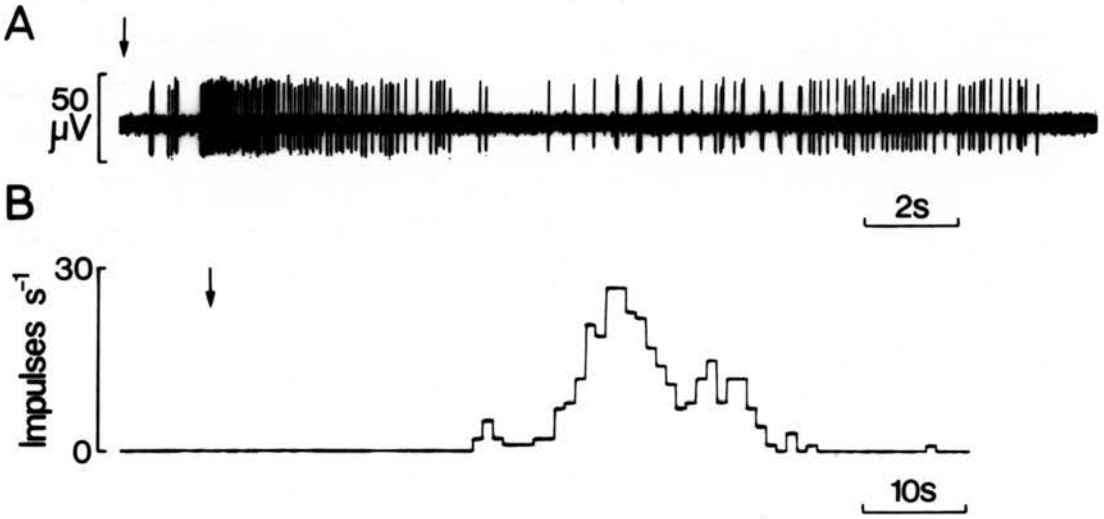
movement caused receptive fields to touch.

Receptors usually adapted to silence during applied mechanical stimulation and the response was sustained for up to 20 seconds. After a prolonged mechanical stimulus there was often an "after-discharge" associated with a depression in the receptive field, but unlike the "spontaneous" units described below, this activity was not as pronounced as the adapting response. The discharge frequency, for the first second of the response, was 28.3 imp sec^{-1} (+/- 11.1 sd) and the minimum interspike interval was 29.6 ms (+/- 20.2 sd). The conduction velocity of this class of afferent units was 0.90 $msec^{-1}$ (+/- 0.33 sd) (N = 23).

Chemical Sensitivity :

Nine units were insensitive to the chemicals used. Units which were sensitive to chemicals responded to a variety of solutions, although in the case of acetic and KCl their sensitivity with one exception did not overlap. When receptors responded to chemicals often the response was not reproducible nor did increasing concentrations necessarily cause increases in response (see figure 28, which illustrates the response to sodium hydroxide). Units were always insensitive to saline over the range (200 - 1200 mOsm Kg^{-1}). Distilled water in five units caused prolonged desensitisation to applied chemicals. No unit responsed to lactic acid (50 - 100 mM L^{-1}) or glucose solutions. Chemically responding units were excited for approximately 30 sec, although in three units $NaHCO_3$ (299 mOsm Kg^{-1}) caused discharges of 16 imp sec^{-1} for 1.5 min.

Figure 28 An example of the response of a sheep mucosal receptor to 50 mM L^{-1} NaOH in saline, applied at the arrow. (A) The first response illustrated as a spike train. (B) The second response as an histogram. Note the long latency of the second response. The unit afterwards became insensitive to further application of NaOH. Between stimulus (A) and (B) the unit was washed in saline for 5 min.


A
50 μV
B
2s
Impulses s⁻¹
30
0
10s

"Silent units" were divided into two sub-groups on the basis of chemical sensitivity and responses to drugs.

Sub-Group 1

In fourteen units the response to KCl increased with increasing molar concentrations (17 - 450 mM L^{-1}, made up to 300 mOsmol Kg^{-1} with saline where appropriate) and one example is shown in figure 29. The latency was consistently below one second and the mean threshold for the response was 12.5 mM (at 300 mOsm Kg^{-1}). The latency of the response and threshold for other chemicals is summarised in table 7. Barium sulphate (100% w/v), $MgSO_4$ (320 mOsm Kg^{-1}), olive oil, fresh rumen contents and glucose (55 mM L^{-1}) applied topically were without effect.

Response to Drugs :

Topical application of PG $F_{2\alpha}$ but not PG E_2 caused spontaneous activity in units previously only responding to mechanical stimuli. Topical applications of acetylcholine and nor-adrenaline also caused silent units to fire, but without altering the mechanical sensitivity of the receptive field to mechanical stimuli. Papaverine caused at first an excitation and reduced the mechanical response without altering the sensitivity to KCl (see figure 30). Topical atropine also reduced or abolished the mechanical sensitivity of the units.

Sub-Group 11

Seven units were insensitive to KCl at all concentrations but were sensitive to volatile fatty acids. The response was not

Figure 29 Mucosal receptor response to KCl. Upper right response to 50 and 400 mOsm Kg^{-1} applied at arrows. Other traces the mean of four responses for each treatment (bar = sd) with KCl at approximately 300 mOsm Kg^{-1} (except 300 mM KCl), threshold 17.5 mM, conduction velocity 1.5 msec^{-1}. Mechanical receptive field 25 mm^{2}. The unit was not excited by VFA's.

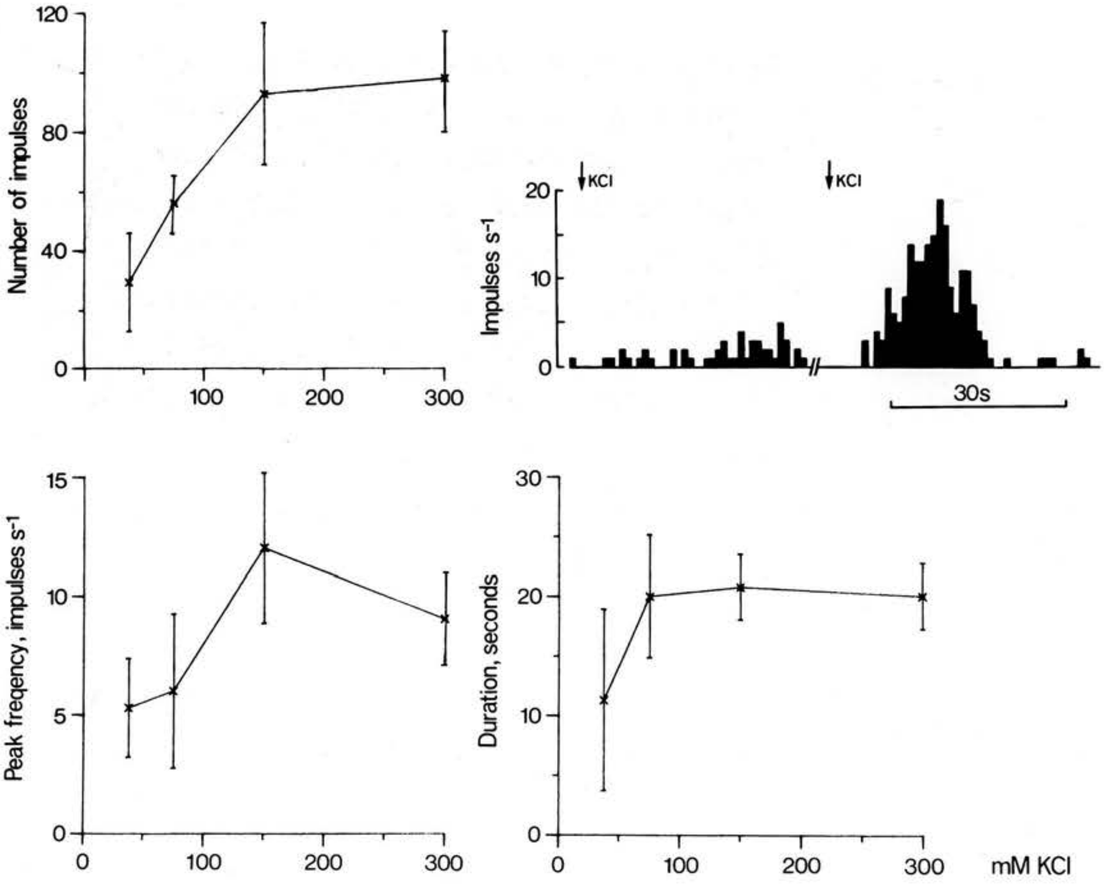
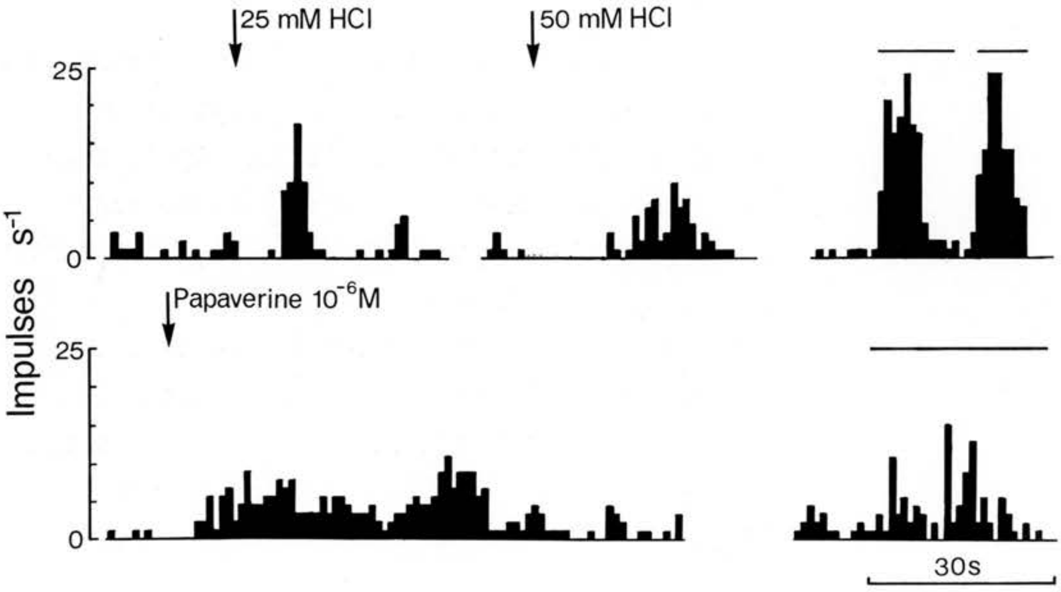

120
80
40
0
Number of impulses
100
200
300
KCl
KCl
20
10
0
Impulses s-1
//
30s
15
10
5
0
Peak freqency, impulses s-1
0
100
200
300
30
20
10
0
Duration, seconds
0
100
200
300
mM KCl

Table 7 Threshold of Sheep Mucosal Receptors

	THRESHOLD		LATENCY	
Chemical	"silent" units mM L^{-1}	spontaneous units mM L^{-1}	sec (mean +/- sd)	 (range)
HCl	12.5 (4 units 50)	10	34.5 +/- 27.2	4 - 72
NaOH	25	-	1, 3, 30	1 - 30
$NaHCO_3$	150	145	3, 4, 5	-
KCl	12.5	12.5	<1.0	<1.0
glucose	-	-	-	-
acetic	25	25	21.9 +/- 10.4	4 - 38
propanoic	18.5	NT	20.7 +/- 2.9	19 - 24
butanoic	11	NT	18.0 +/- 15.9	6 - 36
bile	+/-	+/-	-	-
$BaSO_4$ (100% w/v)	-	+ve	16	-

(- = negative)

Figure 30 Mucosal receptor response to acid and mechanical stimulation. Upper trace the response to 25 mM HCl (with saline to 346 mOsm Kg^{-1}) and 50 mM HCl (384 mOsm Kg^{-1}) demonstrates the shorter latency of the response (8 - 10 sec) and greater peak frequency at lower concentration. Lower trace papaverine (10^{-6} M) added to the mucosal receptive field causes the otherwise relatively silent unit to discharge. Probing (bar) shows mechanical sensitivity before (upper right) and after (lower right) drug treatment.


25 mM HCl
50 mM HCl
25
0
Impulses s⁻¹
Papaverine 10⁻⁶M
25
0
30s

correlated with either pH or osmolality, and was closely related to the molecular weight and titratable acid. In figure 31, the response of one unit has been examined for the parameters : peak frequency, latency, total impulse count and total duration of the response. Note that the latency of the response for acetic acid (for which this unit was most closely tested) shows an independence of molarity. One unit was excited both by KCl and acetic acid.

Response to Drugs :

Topical application of papaverine was without effect, and atropine and hexamethonium applied to the receptive field did not alter their mechanical response.

SPONTANEOUSLY ACTIVE MUCOSAL RECEPTORS

Seventeen mucosal receptors had background activity which was correlated with tension and emg recordings in the muscularis externa, and were in some ways similar to tension receptors (Chapter 4, see figure 32). Units had a resting discharge of 1 - 5 imp sec^{-1}, and 19 imp sec^{-1} was seen in one unit.

When lightly abraided with a cotton-wool swab, units produced a high frequency discharge which always outlasted the stimulus, often with a high frequency (see figures 33, 34, 35C). The receptive field was usually a single spot 1 mm^2, but on one occasion, two distinct receptive fields 0.5 mm apart were found. The response to perpendicular pressure with von Frey hairs and isometric transducers was poorly sustained and had a threshold of 750 mg (+/- 120 sd). A characteristic feature of the receptive field was that its sensitivity

Figure 31 Mucosal receptor response to volatile fatty acids. The impulse relationship to the molar concentrations is shown. A very similar relationship existed for titratable acid, but not osmolality, nor pH. Latency of response to acetic acid in this unit supports the possibility of an aqueous diffusion pathway approximately 200 µm long. The unit was not excited by KCl solutions.

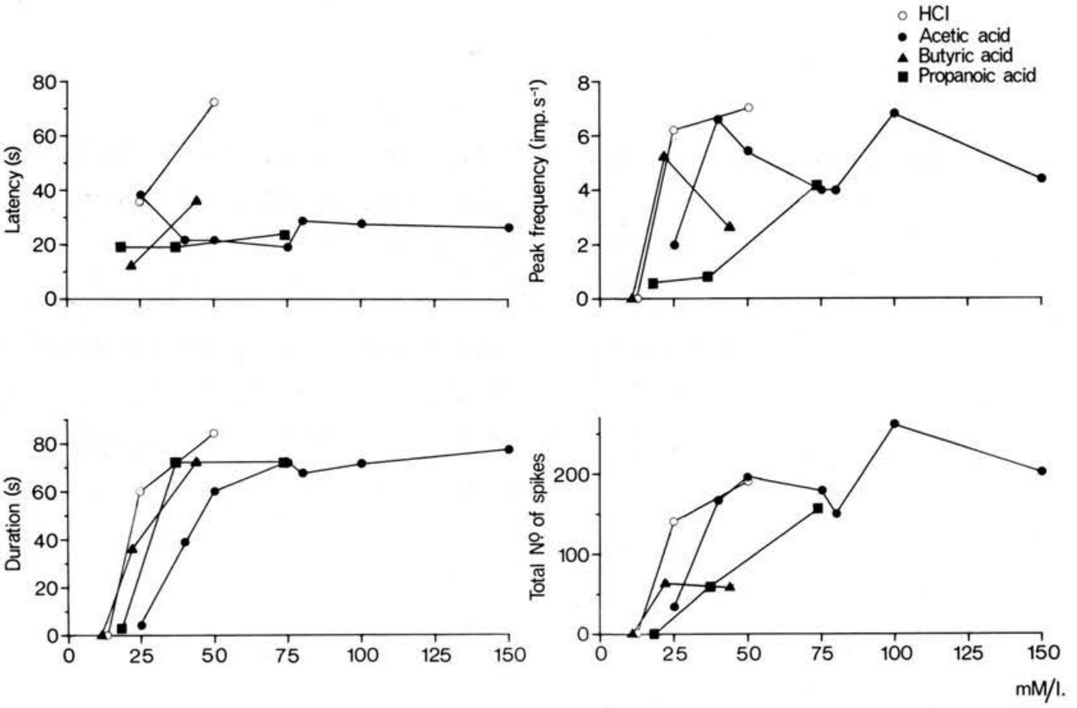

○ HCl
● Acetic acid
▲ Butyric acid
■ Propanoic acid
Latency (s)
80
60
40
20
0
Peak frequency (imp.s⁻¹)
8
6
4
2
0
Duration (s)
80
60
40
20
0
0
25
50
75
100
125
150
Total Nº of spikes
200
100
0
0
25
50
75
100
125
150
mM/l.

Figure 32 A "spontaneously" active mucosal receptor. In this unit there was a close relationship between the impulse activity (upper trace), longitudinal tension (middle trace) and the emg in the muscularis externa. Note that the tension develops quickly after the emg, but that the impulse activity reaches a peak frequency after the muscle tension has started to decline. The conduction velocity (0.9 $msec^{-1}$) does not explain the delay between tension and impulses which at other times in the same record was greater.

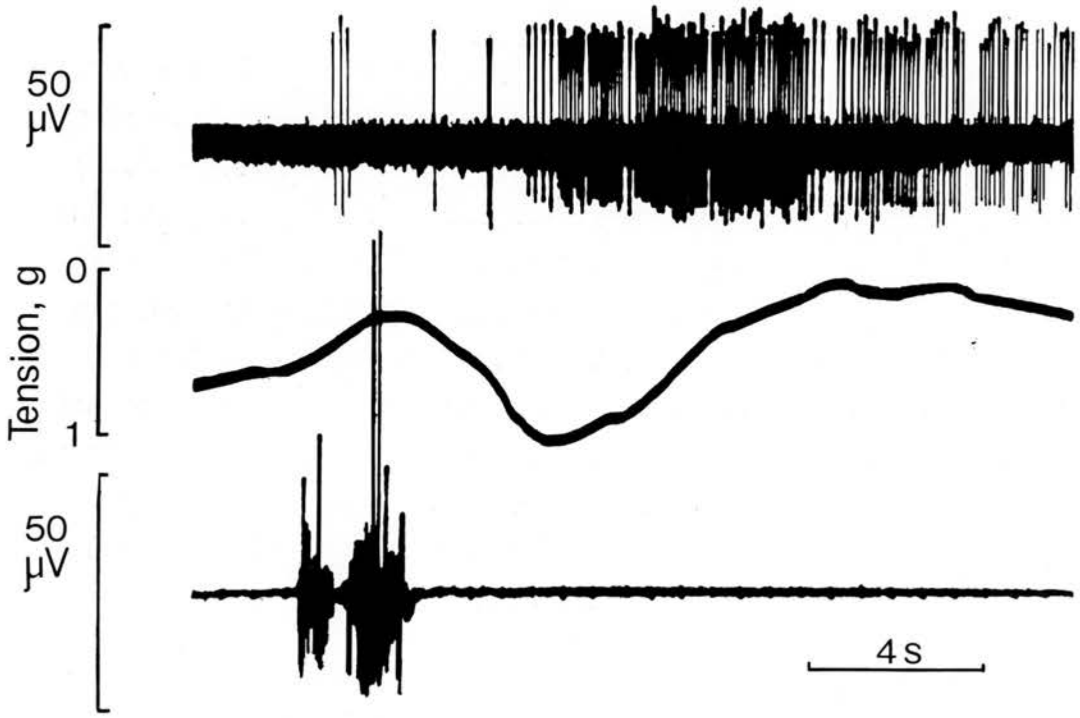

50
μV
Tension, g
0
1
50
μV
4 s

Figure 33 The same unit as figure 32. Note : the insensitivity to IV pentagastrin (3 μg Kg^{-1}) At the bar the receptive field was stroked with cotton wool causing a prolonged "after discharge". This response demonstrates that actively produced tension changes were incapable of firing the mucosal units.

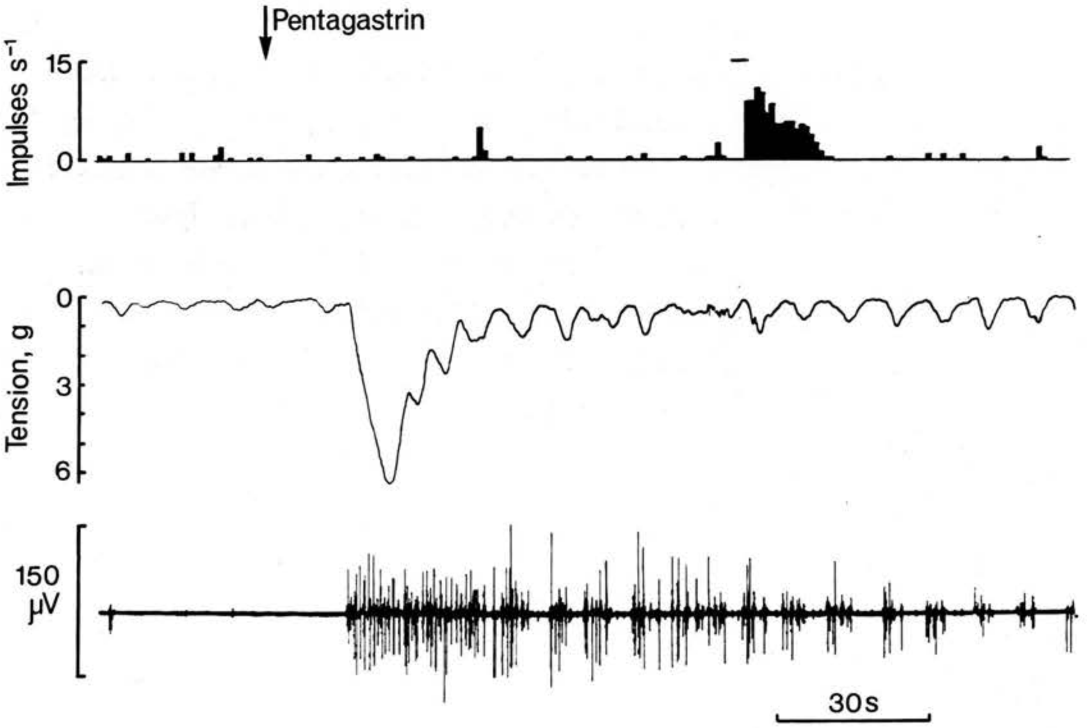

Pentagastrin
Impulses s^{-1}
15
0
Tension, g
0
3
6
150
µV
30s

Figure 34 Changes in tension in the outer muscle coat (middle trace, recorded in a longitudinal direction) were associated with regularly occurring emg activity (lower trace). The activity in a spontaneously active mucosal receptor (top trace) did not have a steady relationship with tension changes and the unit apparently fired randomly. On other occasions the tension changes were more closely related to nervous activity. Stroking with a fine paintbrush (arrows) produced an early phasic response with a slowly adapting "after discharge" which was associated with changes in tension. The 4 sec delay of the provoked emg and tension traces after the spike train suggested that the receptor excitation mechanism was independent of the emg and tension changes. This response demonstrates that light mechanical stimulation excites tension receptors probably by an intrinsic reflex (see : Leek, 1959, p 606; Iggo, 1957b).

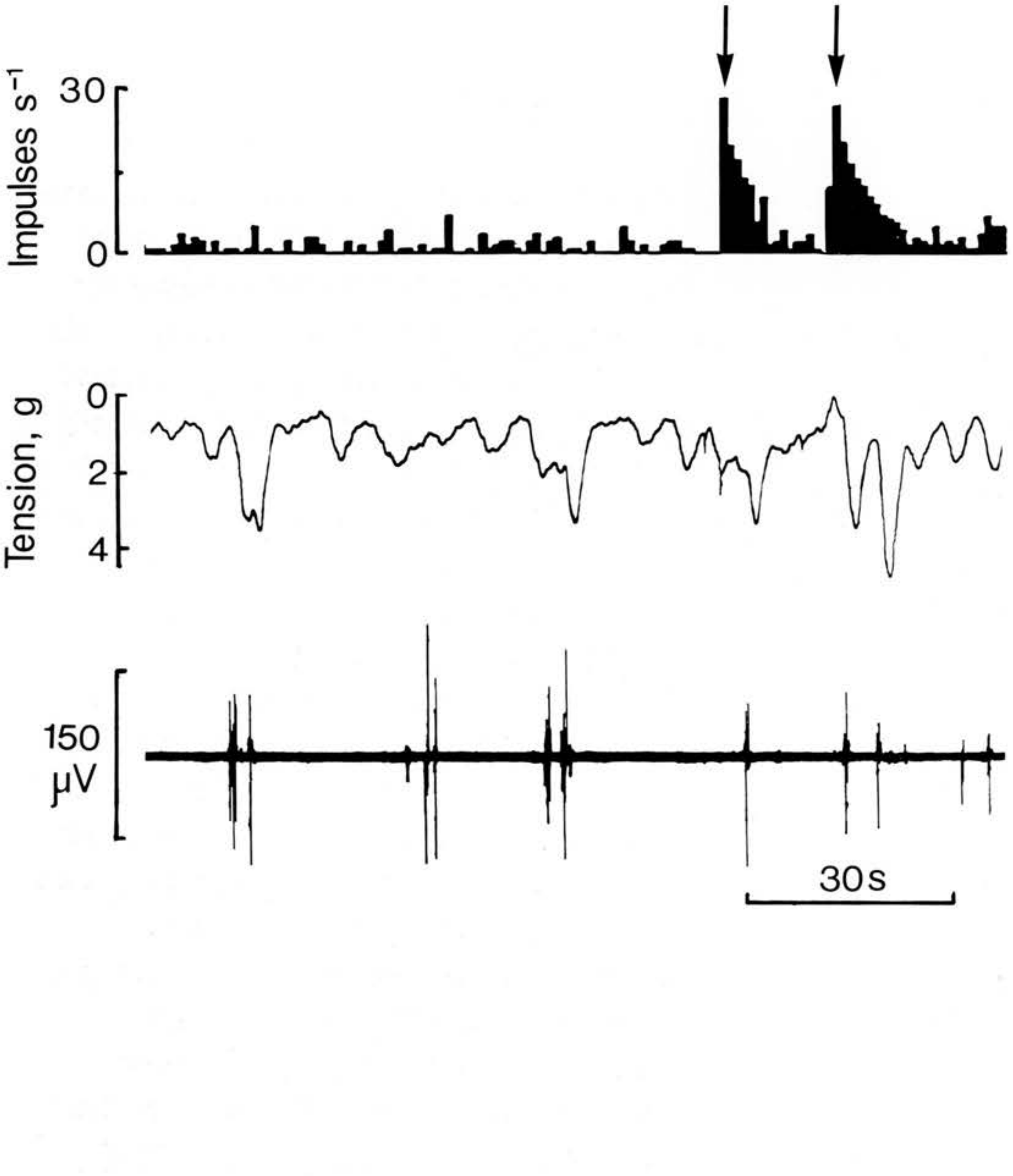
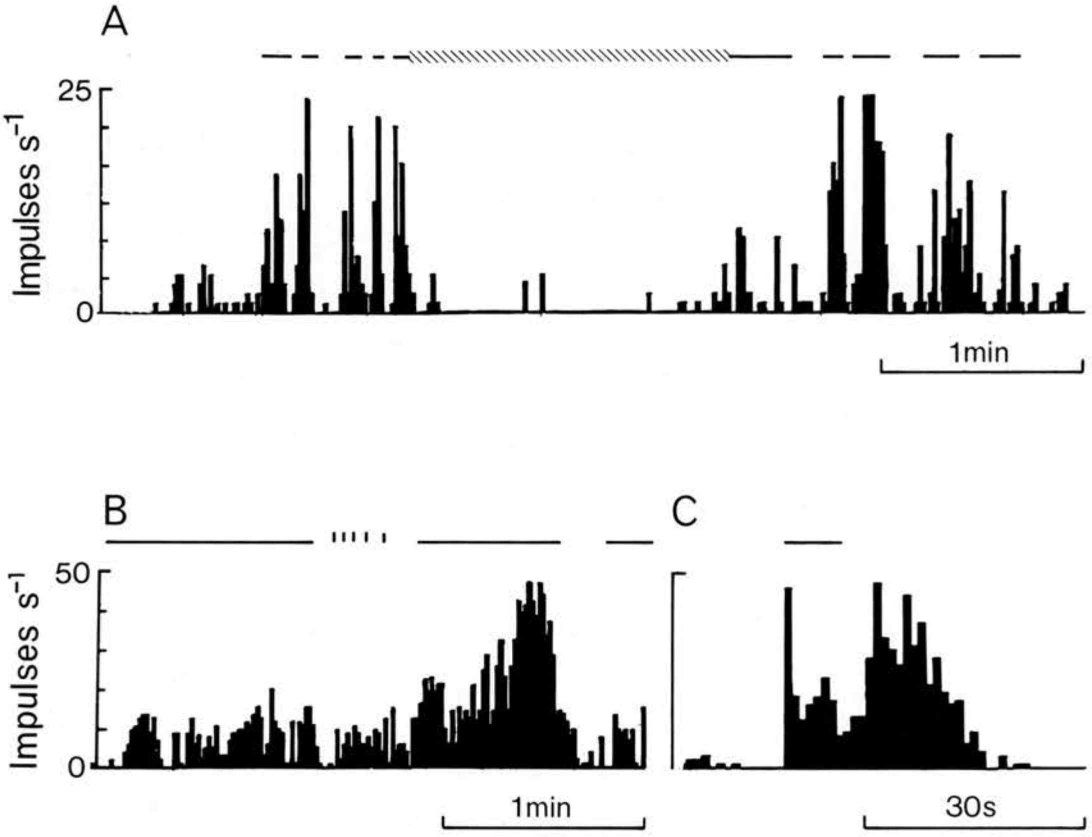

Impulses s^{-1}
30
0
Tension, g
0
2
4
150
µV
30 s

Figure 35 The responses of three spontaneously active mucosal mechanoreceptors. (A) Demonstrates the unstable response during repetitive application of a 25 g compression at each bar. The hatched bar indicates the time during which the receptive field was insensitive. (B) A unit which responded vigorously to brushing with cotton wool became insensitive to brushing (vertical bars) after a prolonged transmural compression (horizontal bars). Later the response returned, after which it again became refractory. (C) The application of a probe at the bar caused an early phasic discharge followed by an "after discharge" during which a depression developed in the receptive field.


A
25
0
Impulses s-1
1min
B
50
0
Impulses s-1
1min
C
30s

varied with prolonged periods of refractoriness in areas previously sensitive. The maximum frequency of the response (measured for a complete second) was 42.2 imp sec^{-1} (+/- 14.2 sd) and the minimum interspike interval measured was 22.6 ms (+/- 8.2 sd). On the basis of conduction velocity, two populations of afferent axons occurred : unmyelinated (9 units, 0.72 $msec^{-1}$ +/- 0.21 sd), and myelinated (4 units, 3.3 $msec^{-1}$ +/- 0.85 sd).

When the receptive field was mechanically stimulated the response was associated with emg and tension changes in the muscularis externa nearby. However, the spike train often outlasted the tension changes recorded (see figure 32). But unlike tension receptors, when tension and emg changes were induced with pentagastrin there was no response despite large changes in these parameters (see figure 33). Topical atropine and papaverine reduced or abolished the spontaneous and mechanically induced responses after a short period of excitation.

Spontaneously active units were also excited by KCl solutions. Topical applications of 5-HT, bradykinin and phenylbiguanide caused excitation by a rapid mechanism (<2 sec). They were also excited by close IA bradykinin (after a latency of 10 sec) but not 5-HT, nor phenylbiguanide by this route. The response to mucosally applied drugs was abolished by application of a few drops of mucosally applied atropine sulphate (100 mg ml^{-1}). The mechanical sensitivity was also abolished but the KCl response remained. A summary of mucosal receptor responses to drugs is given in table 8.

Table 8

Pharmacology of Mucosal Receptors

1. ALL GROUPS

Drug	Dose	Route	Response	Trials
lignocaine	few drops, 2%	topically	mechanical response vanishes within 10 sec, returns after 3 - 4 min with saline	n/n
pentagastrin	1 $\mu g\ Kg^{-1}$	close IA & jugular	no change	10/10
phenylbiguanide	20 - 100 μg	"	no change	10/10
veratrine	20 $\mu g\ Kg^{-1}$	"	no change	4/4
2. SILENT MUCOSAL RECEPTOR, SUB-GROUP 1				
acetylcholine	100 $\mu g\ ml^{-1}$ few drops	topically mucosa	induced activity	4/6
	1 - 10 μg	close IA	induced activity	5/5
atropine	2 $mg\ ml^{-1}$ flooded	mucosa	reduces mechanical sensitivity	4/6
bradykinin	1 - 25 μg	flooded mucosa	excitation with <1 sec latency	4/4
hexamethonium	100 $mg\ ml^{-1}$	flooded	reduced mechanical sensitivity	5/5
nor-adrenaline	100 $\mu g\ ml^{-1}$ few drops	mucosa	no change, excitation	5/10

Table 8 (continued)

2. SILENT MUCOSAL RECEPTOR, SUB-GROUP 1 (continued)

papaverine	100^{-6}M few drops	mucosa	first excited, then reduced sensitivity to mechanical stimuli	5/5
phenylbiguianide	20 μg	topically	excitation	3/3
prostaglandin $F_{2\alpha}$	5 mg ml^{-1} few drops	topically	excitation, no sensitisation to mechanical stimulation	6/6
	100 μg	close IA	no change	5/5
prostaglandin E_2	5 mg ml^{-1}	topical mucosa	no change	5/5

3. SILENT MUCOSAL RECEPTOR, SUB-GROUP 11

atropine	2 mg ml^{-1} flooded	mucosa	no alteration of mechanical sensitivity	8/8
hexamethonium	1 mg ml^{-1}	"	" " "	5/5
glucose saline	1 - 10%	close IA	no change	10/10
5-hydroxytryptamine	10 - 20 μg	close IA	no change	8/8
insulin	1 - 4 IU	close IA	no change	9/9
papaverine	10^{-6}M few drops	topically mucosa	no change	5/5

Table 8 (continued)

4. SPONTANEOUSLY ACTIVE MUCOSAL RECEPTOR

atropine	2 mg ml^{-1}	flooded mucosa	initial excitation, reduced mechanical sensitivity, KCl response remains	4/4
bradykinin	1 - 20 μg	"	excitation	5/5
CCK-8	2 μg	close IA	excitation (α)	3/4
5-hydroxytryptamine	10 - 20 μg	topically mucosa	excitation (β)	6/6
papaverine	10^{-6}M few drops	"	first excited, later desensitised to mechanical stimulus	6/6
phenylbiguanide	10 - 100 μg	"	excitation	5/7
prostaglandin E_2	20 μg	close IA	sensitised to mechanical probing	8/8

(α - see : figure 21B)

(β - see : Iggo, 1957a; Harding & Leek, 1972a; Leek, 1977)

DISCUSSION

These results concern a mucosal population of mechanoreceptors which were sensitive to chemicals in the lumen of the duodenum. All units were selected as a result of their mechanical sensitivity. This was because exhaustive tests on "silent", but otherwise healthy-looking strands always proved negative, and therefore, in this survey, chemoreceptors were not found. The receptors had many similarities with those discussed by Harding & Leek (1972a).

The mechanical stability provided by this preparation made it possible to demonstrate that most of the receptors were slowly adapting mechanoreceptors and it is interesting to speculate whether similar units described elsewhere as "rapidly adapting", might have been slowly adapting given these stable conditions (Iggo, 1957b; Mei, 1970b; Davison, 1972). The mechanical receptive field was also much smaller than those reported from less stable preparations.

The mechanosensitivity of the units agreed with measurements in the cat stomach (Davison, 1972). This low threshold makes them poor candidates for initiating extrinsic reflexes associated with excessive stretch, which has been suggested elsewhere (Iggo, 1957c; Clarke & Davison, 1978; Iggo & Leek, 1967a). The mechanical sensitivity of mucosal receptors is clearly suited to the detection of particulate fibrous matter which occurs naturally in ruminant chyme and this afferent information may be involved in reflex inhibition of gastric emptying mediated via extrinsic nerves. The stimulus may also cause an intrinsic reflex which directly modifies the peristalsis mechanism and produces the same effect (see Chapter 4, figure 26).

The presence of an "after-discharge" in some units during depression of the receptive field, particularly following brushing, has been seen before (Clarke & Davison, 1978), and suggests that the mechanical response of these units involves the local contraction of smooth muscle or elastin component. Some drugs are known to contract the muscularis mucosa. Walder (1953) in in vitro experiments with human muscularis mucosa from the stomach, demonstrated contractile responses to acetylcholine, and to adrenaline from some locations, while atropine reduced spontaneous contractions. The responses described here are similar to this activity and provides evidence for a presumptive location in muscularis mucosa. In addition, the response to mucosal application of papaverine, which abolished mechanical sensitivity, supports this idea. The spontaneously active units were similar to those suggested by Paintal (1957b) to be in the muscularis mucosa, on the inadequate criterion of villus movement induced by 30% saline. Distension-insensitive receptors in the cat are not inhibited by serosally applied atropine (Paintal, 1957b).

The close association of "spontaneous units" with emg records in the external muscle coat is interesting. Sporadic bursts were seen in some abomasal units by Harding & Leek (1972b). The inability to excite duodenal receptors with sustained slowly adapting responses by transmural electrical stimuli, transmural pressure and intravenous pentagastrin clearly distinguishes them from tension receptors, and suggests that the muscularis externa is an unlikely candidate for their location.

Some units were not excited by chemicals and a chemically-insensitive population of mucosal receptors might exist. Harding &

Titchen (1975) found acid-insensitive mucosal receptors in the cat oesophagus. It is also possible that receptors had lost their chemical sensitivity, as was found by Harding & Leek (1972a, 1972b).

The insensitivity of chemically active units to solutions of different osmolalities makes it difficult to support the presence of specific osmoreceptors in this location, and these results suggest that osmotic reflexes (Bell et al, cited above) involve an unidentified receptor population. The units are therefore dissimilar to the multi-modal receptors found by Harding & Leek (1972a) which may fulfil this role, because these have a degree of selective sensitivity. Two populations were found, one was excited by acids, KCl and by papaverine, and the other was excited by fatty acids and HCl, but not KCl and not papaverine. Mei (1978) has found that D-glucose receptors in the cat are not excited by KCl solutions (275 - 1100 mOsm Kg^{-1}). Glucose was ineffective in this survey, which suggests that there are species differences in chemical sensitivity of mucosal enteroceptors.

That some units were sensitive to KCl was unexpected. Potassium chloride was tested because it has often been used by gastroenterologists in reflex studies (Bell & Mostaghini, 1975; Barker, Cochrane, Corbett, Hunt & Roberts, 1974) to demonstrate possible osmoreceptors. However, the responses were not osmotic ones. It is possible that potassium ions are an important constituent of abomasal liquor after their release from digested cells. The level of K^+ ions in foods ranges from 25 - 125 m Eq Kg^{-1} (Diem & Lentner, 1970) and these values may possibly be reached in chyme after cellulysis. Concentrations in the rabbit duodenum are 39.0 +/- 9.7 sem (Alexander, 1965). It is also known that there is a net

K^+ flux from plasma to rumen (0.27 µEq cm^2 hr^{-1}) (Ferreira, Harrison & Keyens, 1964) and local potassium levels may therefore reach threshold for excitation. Elevated potassium depolarises all excitable tissue. It is improbable that the KCl excitation was due to changes in potassium conductance (Paintal, 1964), negative after potential (Sharma, 1967) or changes in sodium conductivity (Goodman & Gilman, 1975) because a non-selective response of all mucosal receptors would have occurred. It may act indirectly on gut smooth muscle possibly releasing acetylcholine (Daniel, 1968). These results suggest that KCl receptors may be associated with smooth muscle elements and that they are sensitive enough to be excited by physiological levels of potassium found in the abomasum (Hill, 1965). Their role in reflexes appears therefore to be one of negative feedback upon gastric emptying (Bell & Mostaghni, 1975) unconnected with osmolality.

Duodenal units were more sensitive to acetic, butanoic and propanoic acid than abomasal units (Leek, 1977, table 1). The chemical sensitivity to acetic and other acids was related to titratable acidity and molarity, and not pH or osmotic effects. The diffusion pathway was calculated as about 214 µm, from the assumptions of (1) the random walk model of diffusion ($x^2 = 2Dt$) (α), and (2) that 200 mM acetic acid at 13.5°C in aqueous phase (β) behaves the same as solutions of 25 - 150 mM acetic at 37°C in the duodenal mucosa. This figure closely agrees with the calculation of Harding & Leek (1972a). The predicted latency for HCl ought to be

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(α) x = distance (cm); t = time (sec); D = diffusion coefficient (cm^2 sec^{-1}).

(β) Handbook of Chemistry and Physics, 35th Ed., Chemical Rubber Publishing Company, p. 2007.

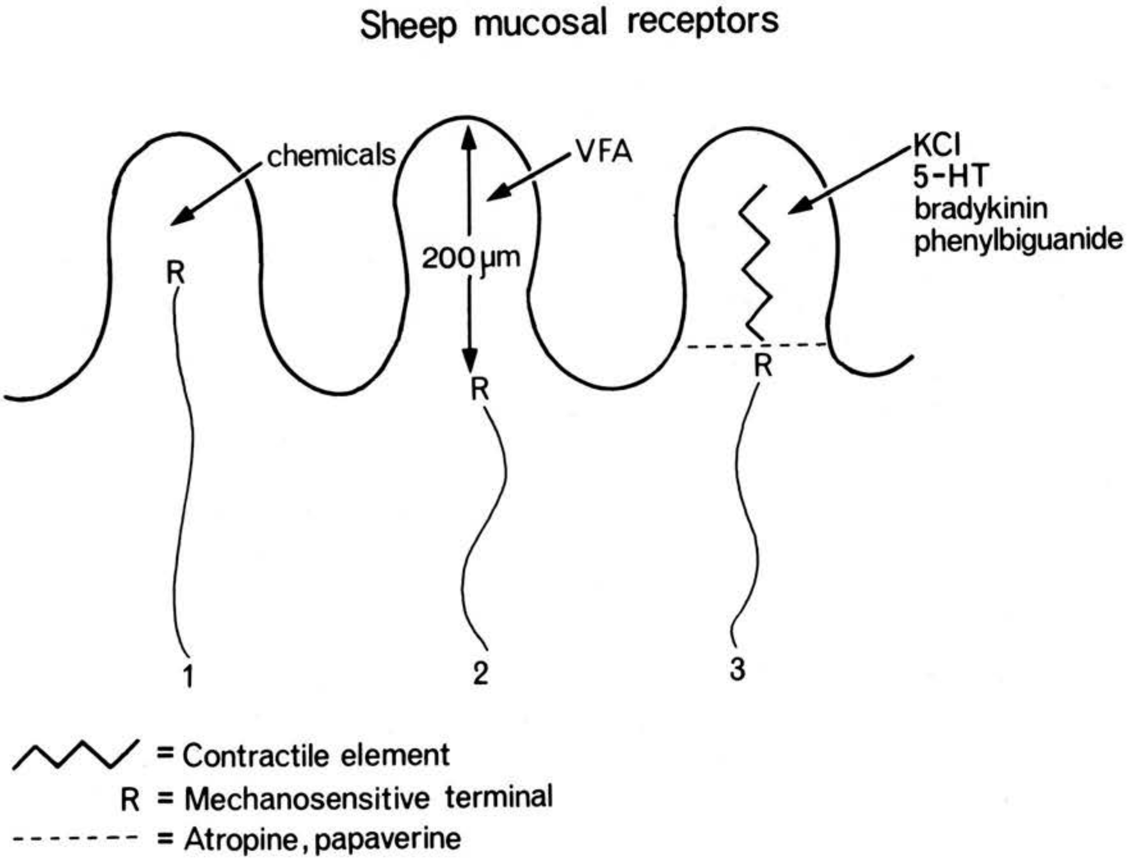
between three and seven seconds for the receptor site to be similarly located, but often the latency was greater than twenty seconds and only occasionally less than ten seconds, and then only on the first application. Often thereafter the receptor was refractory for long periods. The response is clearly different from the abomasum of sheep because Leek (1977) reports 96 units with a mean latency of 1.5 sec. It is possible that acid-sensitive receptors may be "turned off" for 5 - 15 min in pigs (Roze et al, 1977, figure 3). How this chemical desensitisation does not affect the mechanical sensitivity, which remains, has not been decided. It is possible that the secretory response provoked by acid in the duodenum, which was a feature of the duodenal preparation, physically opposes the diffusion of acid ions, or chemically buffers their effect. This may explain why more clear-cut latency measurements are found in the non-secretory reticulo-rumen (Harding & Leek, 1972a). The published results in the abomasal pyloric region do not support this suggestion (Leek, 1977), so presumably little buffering occurs there.

The pharmacological responses found here are for the most part unreported. There is agreement with the negative response to systemic acetylcholine (Paintal, 1957b; Iggo, 1966a), to close IA 5-HT (Iggo, 1966a; Harding & Leek, unpublished, see Leek, 1977), and phenylbiguanide (Iggo, 1966a). These reponses clearly distinguish mucosal receptors from the cat "distension insensitive" units (Paintal, 1954b) and the glucose receptors (Mei, 1978).

Spontaneously active mucosal receptors were excited by mucosally applied 5-HT, and the negative response to close IA 5-HT was not expected. 5-hydroxytryptamine is involved in the control of alimentary motility, participating as a preganglionic transmitter in inhibitory pathways in the stomach and is in high concentration in the mucosa (Kottegoda, 1970). It may be the transmitter in some neurones responsible for the non-adrenergic, non-cholinergic excitatory response of smooth muscle (Burnstock, 1979). Bulbring & Lin (1958) found that when 5-HT is introduced into the lumen of the intestine there is a stimulation of peristalsis which they attributed to stimulation of chemoreceptors and sensitisation of mechanoreceptors in the mucosa which may trigger the peristaltic reflex. Phenylbiguanide may have a similar action to 5-HT when applied to the mucosa, but 5-HT applied to the serosa inhibits peristalsis (Bulbring & Lin, 1958). However, the mucous membrane, muscularis mucosa and submucosa are not necessary for the peristalsis reflex (Ginzel, 1959). These 5-HT responses therefore support the view that superficial mucosal receptors are independent of the muscularis externa, and some are probably associated with the muscularis mucosa, and may be the same population involved in peristalsis (see Leek, 1977, p 167).

A summary model of sheep duodenal mucosal mechanoreceptors is presented in figure 36. There are two "silent" classes. Sub-group I (1) is excited by many chemicals, including KCl. Sub-group II (2) is excited by VFA's after a diffusion delay. The spontaneously active receptors (3) are excited by KCl and drugs which cause smooth muscle contraction, including CCK-8, and they have a characteristic after discharge when brushed. The mechanical sensitivity is lost when treated with atropine and papaverine. Mechanoreceptors are usually slowly adapting to probing, but rapidly adapting receptors are also found. A chemically-insensitive receptor may also exist.

Figure 36


Sheep mucosal receptors
chemicals
VFA
KCl
5-HT
bradykinin
phenylbiguanide
200 μm
R
R
R
1
2
3
= Contractile element
R = Mechanosensitive terminal
------- = Atropine, papaverine

The functional role of rumen epithelial receptors and tension receptors has been suggested by the work of Leek & Harding (1975). They propose that volatile fatty acid sensitive receptors provide a tonically active afferent input to gastric centres which reflexly inhibits reticulo-rumen motility (Leek, 1977). In contrast, tonically active tension receptors play a reflex excitatory role, except at high thresholds. Under the experimental conditions reported here, it was only possible to demonstrate mucosal receptor discharges which adapted to silence when repetitively stimulated with acids. These results do not therefore support the concept of tonically active chemoreceptors from the sheep duodenum. It seems more likely that duodenal hormones are involved in these prolonged responses. It is also possible that the tension receptor excitation by mucosally applied acids is important (see Chapter 4).

CHAPTER 6

OMENTAL RECEPTORS IN THE SHEEP AND MISCELLANEOUS FINDINGS IN THE RABBIT AND SHEEP, INCLUDING SOME HISTOLOGY OF THE MESENTERIC AND HEPATODUODENAL NERVES

INTRODUCTION

The activity of slowly adapting mechanoreceptors in the omentum has been described for a number of species (Iggo, 1957c; Crousillat et al, 1970; Ranieri et al, 1973; Floyd & Morrison, 1974b; Floyd, Hick & Morrison, 1976b). Mechanoreceptors are also found in the mesentery (Morrison, 1973; Floyd & Morrison, 1974a; Floyd et al, 1976a). Units usually have multiple punctate receptive fields. They are mostly associated with arterial bifurcations, although this is not always the case (Floyd et al, 1976). In the lesser omentum, they may function as gastric stretch receptors (Iggo, 1957c).

This section includes a description of omental receptors in the sheep. Also, in both the rabbit and sheep, some fibres prepared for afferent recording, did not have receptive fields in the gut, but had activity which appeared to be efferent in character. The possibility of recording from axon collaterals of myenteric neurones or antidromic cross-excitation is discussed. In addition, the histology of the hepatoduodenal nerve of sheep and mesenteric nerves of the rabbit is presented, and an analysis of the diameters of unmyelinated axon profiles is made.

METHODS

The experimental design for electrophysiological recording has already been discussed.

Photomicrographs were studied of the three terminal branches of the hepatoduodenal nerve in the sheep and primary, secondary and tertiary branches of the rabbit mesenteric nerve to the duodenum.

Isolated portions of nerve were fixed in 2.25 M glutaraldehyde in 0.1 M Na cacodylate buffer, post-fixed in 1% osmium tetroxide in the same buffer, and embedded in araldite. 0.5 μm sections were stained with Toluidine blue (Drury & Wallington, 1976) and viewed with a light microscope. From selected areas ultra-thin (90 - 100 nm) sections were made and stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (Phillips EM 400) and photographed.

The size of unmyelinated profiles was measured from electron-micrographs enlarged x 1650 to x 27,000 times, using a vernier caliper device and peripheral interface programme (Maxwell, 1979). The assumption was made that the unmyelinated axon is a symmetrical cylinder and that the minimim transverse cross-sectional diameter was the true diameter. Where profiles were distorted, or sections were obviously oblique, they were not used. The method of counting was to cover photographs with transparent paper, and each time a count was made the sheet was marked with a water-soluble pen.

RESULTS

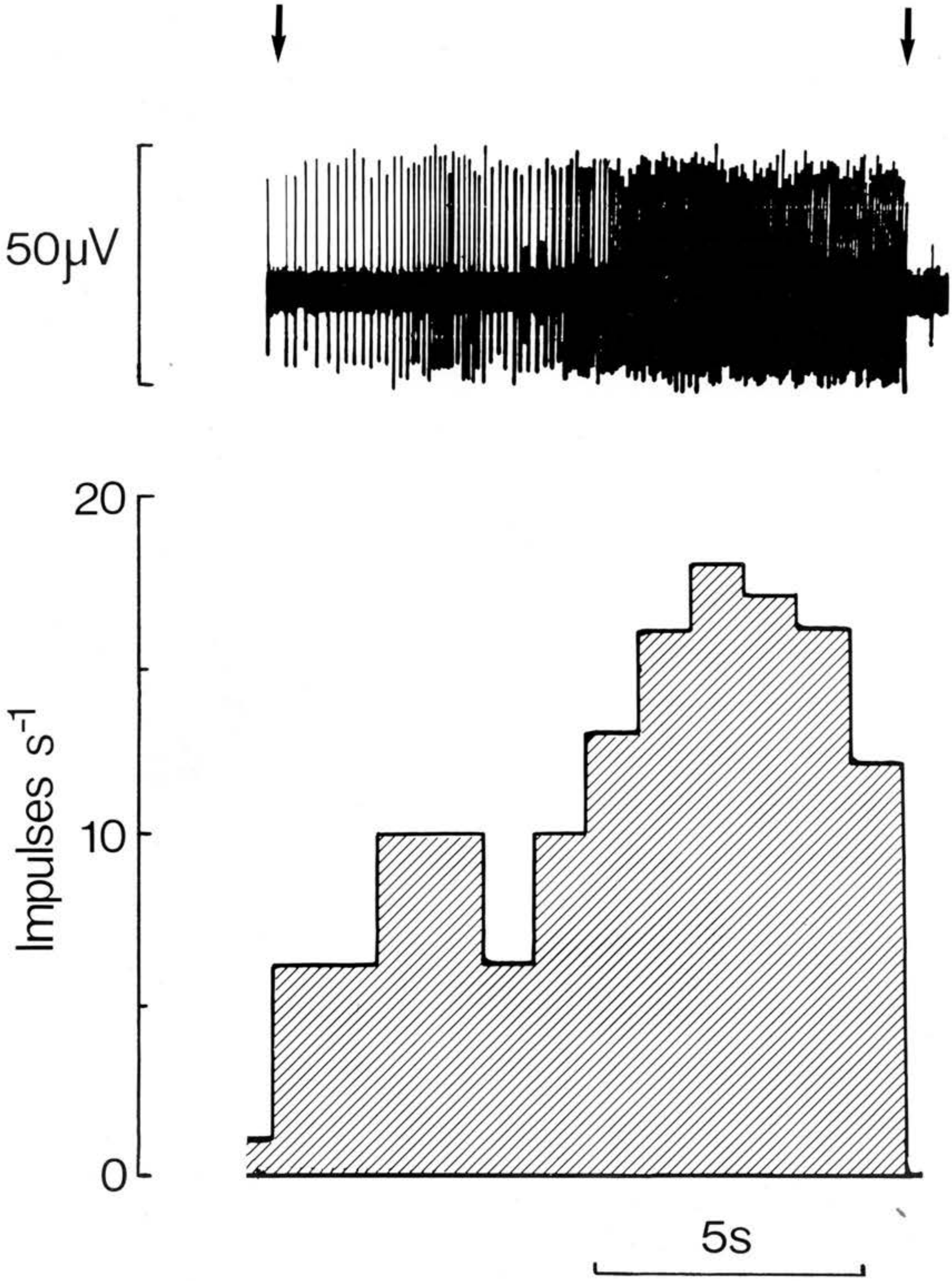
ELECTROPHYSIOLOGY

Omental Units

A total of nine omental units were studied. In one experiment a peripheral branch of the hepatoduodenal nerve was examined, which was rather more ventral in the dissection pool than usual. All the multi-unit afferent activity, and later single unit activity, was from mechanoreceptors in the lesser omentum, 5 centimeters from the pyloric sphincter. During other experiments omental units were found together with fibres containing tension receptors.

Omental units were mechanically sensitive and were not excited by local application of chemicals, thermal stimuli or drugs. They had no resting discharge unless the mechanical field was distorted by movement in the muscle of the pylorus. Units were typically slowly adapting to maintained tension or stretch of the mesentery, although in two units the impulse activity appeared more rapidly adapting than was generally the case. A characteristic response is shown in figure 37. The impulse frequency built up <u>in crescendo</u>, giving the appearance of a "reversed" spike train. An early phasic and later adapting response was not found. The discharge continued for one minute while the stimulus was maintained. Spontaneous activity (2 - 3 impulses per second), could always be abolished by off-loading the omentum from the duodenum, so that loop movement no longer distorted the omentum. There was no vascular rhythm. Neither intravenous pentagastrin (which produced vigorous peristalsis), nor electrical transmural excitation had any effect on the unit when distortion of the omentum was prevented. Topical application of nor-adrenaline

Figure 37 A sheep omental unit with a low frequency background discharge. The response to twisting of the lesser omentum denoted by the arrows was an increase in activity to a maximum rate 19 imp sec^{-1}, minimum ISI 50 ms. The response was slowly adapting during maintained stretch. Conduction velocity 0.86 $msec^{-1}$ ($39^{o}C$).


50µV
20
10
0
Impulses s⁻¹
5s

(1/10,000), systemic phenylbiguanide (200 μg) and veratrine (200 μg) and close IA papaverine (2 x 10^{-4}M) were without effect.

The receptive field appeared to be associated with the fat layer between the parietal and visceral sheet of the lesser omentum, because the response was maintained when the parietal sheet was excised. The visceral sheet was not easily available for examination so this location was not excluded. Receptive fields were associated with blood vessels and were easily excited electrically. The mechanical threshold to stretch was approximately 200 mg using sutured cotton thread attached to an isometric force transducer. Perpendicular probing did not excite the receptor unless stretching occurred. In most units the receptive field was a single spot 0.2 → 1 cm^2, but one unit had two discrete fields each 10 mm^2 in area and 1.2 cm apart. One receptive field in this unit was more rapidly adapting than the other.

The peak frequencies during mechanical stimulation were characteristically lower than other duodenal enteroceptors found in this study (25 imp sec^{-1}), with minimum interspike intervals of 23 ms. The conduction velocity indicated that most afferent axons were unmyelinated (0.86, 0.5, 0.75 $msec^{-1}$). One unit was probably myelinated (7.3 $msec^{-1}$).

"BURST" UNITS IN THE SHEEP

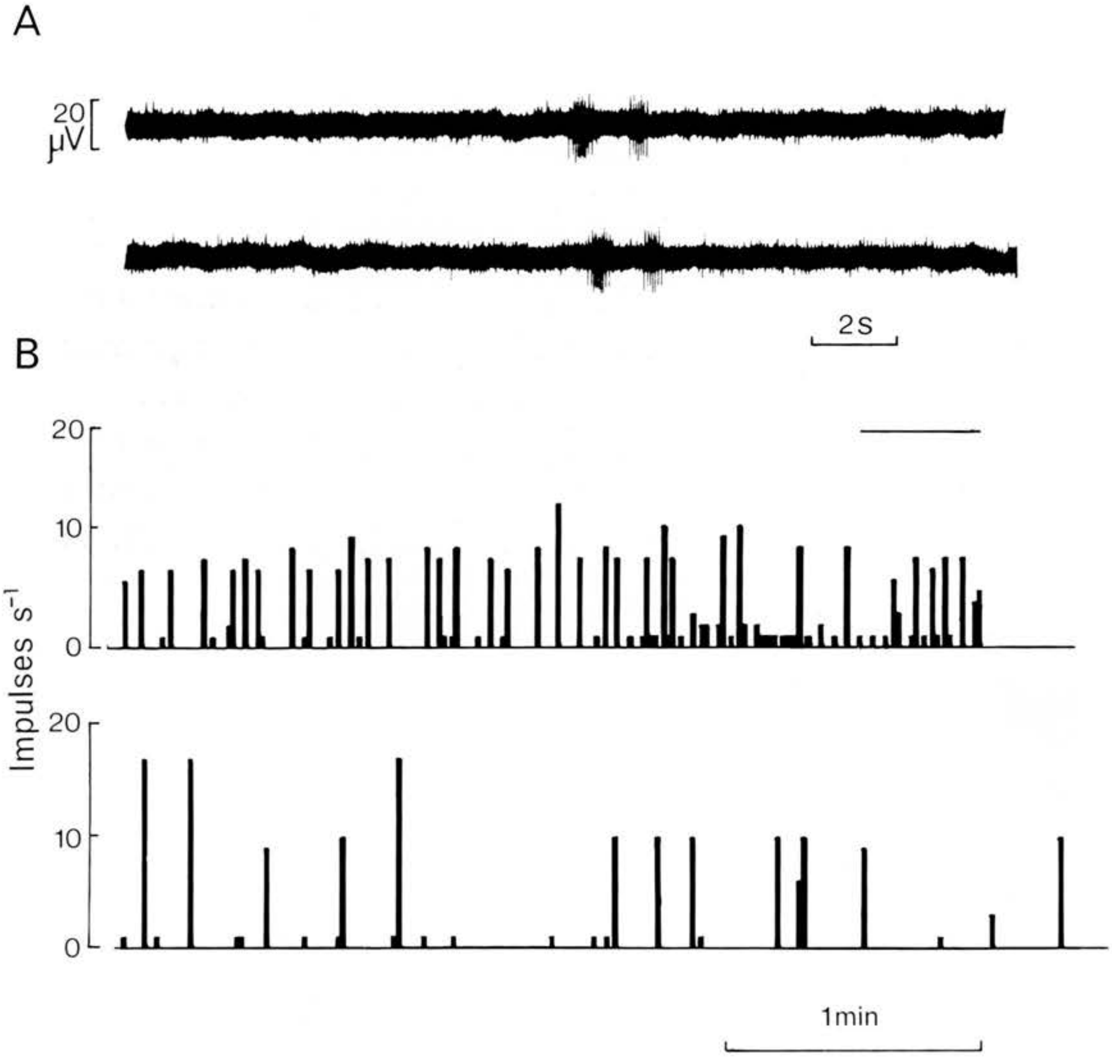
Eleven afferent-prepared units in the sheep had discharge patterns which could not be changed by mechanical, chemicals or thermal stimulation. The discharge occurred in short bursts and was of two different types :

(a) One type of activity was characterised by short high frequency bursts of 5 - 11 impulses with variable interburst intervals from 5 - 20 seconds. During the burst the interspike intervals was 8 msec and was constant. The interburst interval lengthened and the number of impulses per burst increased by 50% after stimulation of the right splanchnic nerve (see figure 38). There were four units of this type which could not be excited by direct electrical probing. In one unit the number of impulses per burst increased from 2/4 to 8/10 after manipulation of the lesser omentum and the discharge was in phase with inspiration. This activity was abolished during the pressor response induced by nor-adrenaline.

(b) Seven other units did not have this pattern of discharge. The background discharge was 1 - 5 imp sec^{-1}, and raised to 7 - 18 imp sec^{-1} during bursts which coincided with observed peristalsis in the isolated duodenal bulb. Units were not excited by sustained pressure, intravenous pentagastrin, close IA acetylcholine (50 µg) or 5-HT (200 µg). One unit increased in activity during cyanosis of the loop when maintenance doses of anaesthetic caused a temporary apnoea. One unit had increased activity after flooding of the mucosa with 100 mM L^{-1} acetic acid in saline (398 mOsm Kg^{-1}) and a further unit was excited by mucosal application of KCl (299 mOsm Kg^{-1}).

Electrical exploration of the mucosa and transmural excitation was ineffective in four units tried. Two units were excited electrically and the conduction velocities were 0.24 msec^{-1} (31^{o}C), and 0.52 msec^{-1}.

<u>Figure 38</u> (A) The impulse activity of a "burst" unit from a sheep with 5 - 11 impulses per burst and minimum ISI 5 msec. (B) The continuous record of a unit whose activity was not affected by mechanical probing or chemical perfusion, which was inhibited by electrical excitation of the splanchnic nerve at the bar (5 Hz, 5 volt, 0.5 msec).

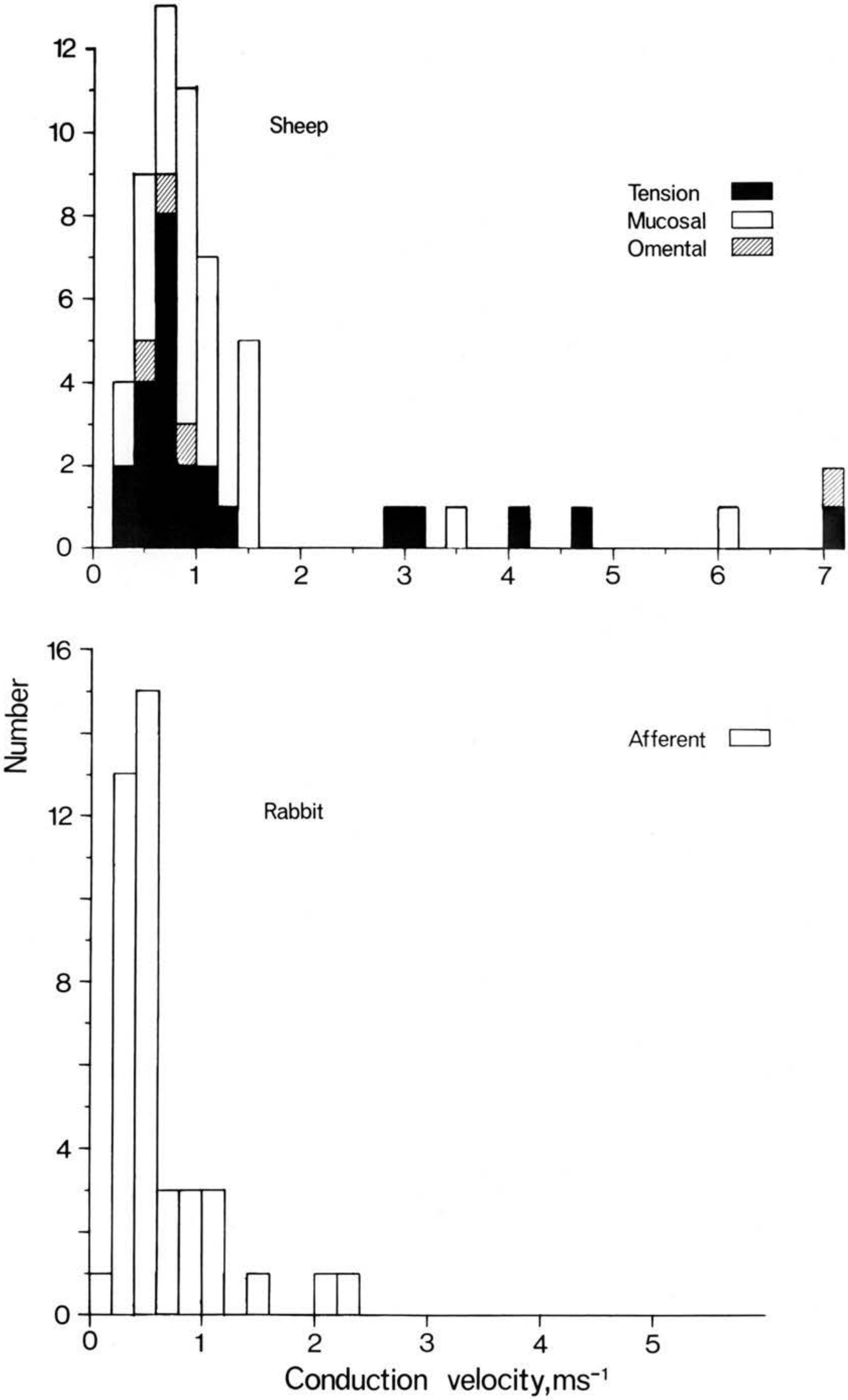

A
20 µV
2s
B
20
10
0
Impulses s⁻¹
20
10
0
1min

"BURST" UNITS IN THE RABBIT

In three experiments nerves were prepared for afferent recordings and on all occasions mechanosensitive multi-unit activity was isolated. However, after continued dissection, isolating single mechanoreceptor units in some strands, other strands had discharges with patterns similar to descriptions of splanchnic efferent activity: some discharges were synchronous with the cardiac cycle (Cohen & Gootman, 1970) and others had respiratory rhythms (Adrian, Bronk & Phillips, 1932). The central connection of the nerve had apparently been interrupted. No change in discharge pattern occurred during natural stimulation of the loop, or electrical excitation of the splanchnic nerve. Units were excited by electrical excitation in the pool near the mesenteric attachment. In all cases the demonstration of efferent-like activity occurred when the blood pressure was below a mean value of 65 mm Hg, or the pool temperature was 34^{0}C or below.

Distribution histograms of the conduction velocities of the single unit population isolated in this study are illustrated in figure 39.

Figure 39 Frequency histograms of functionally identified single afferent units from the duodenal nerves of the sheep and rabbit. The sheep population consists of 59 afferent units. The rabbit population contains 40 afferent units.


Sheep
Tension ■
Mucosal □
Omental ▨
12
10
8
6
4
2
0
0
1
2
3
4
5
6
7
Number
16
12
8
4
0
Rabbit
Afferent □
0
1
2
3
4
5
Conduction velocity, ms^{-1}

HISTOLOGY

In general, there was more endoneurial collagen in sheep nerves which were fasciculated, than the rabbit mesenteric nerves, where endoneurial collagen was much sparser and also whorled.

Rabbit

The mesenteric nerve had an exclusively unmyelinated population. From measurements of sections of axonal profiles three categories of mesenteric nerve were apparent. They were :

1. 23% were small nerves with small axons (mean 0.483 μm); 2. 46% of nerves contained only large axons (mean 1.092 μm); and 3. 31% of nerves had mixed populations (mean 0.755 μm). See figure 40.

Sheep

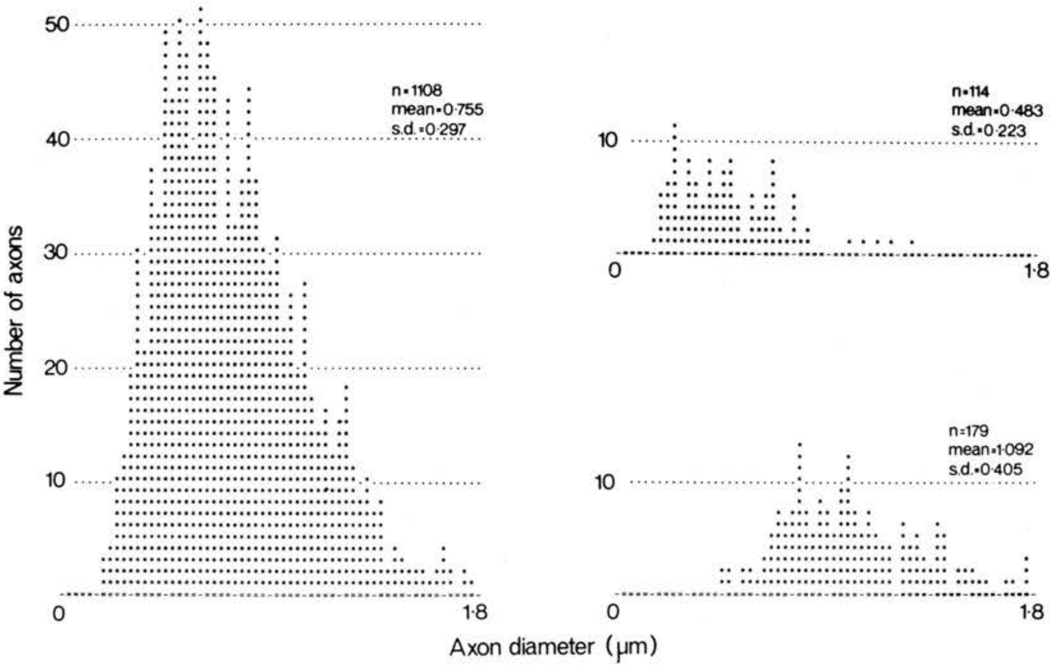
In the hepatoduodenal nerve, there was a mixed population of myelinated and unmyelinated axons. All nerves studied contained a range of cross-section diameters of unmyelinated axon profiles. A distribution histogram is shown in figure 41.

Figure 40 Analysis of unmyelinated axon profiles in rabbit mesenteric nerves. Left the mixed population, range 0.18 µm - 1.86 µm. Upper right small population, range 0.17 µm - 1.26 µm. Lower right large population, range 0.46 µm - 2.79 µm. Diameters greater than 1.80 µm are not displayed.

Each histogram was tested against the theoretical normal distribution :
$f(x) = 1/(sd.\surd(2\pi))\ .\ \exp -\frac{1}{2}((X - \overline{X})/sd)^2$, with
χ^2 test : $\chi^2 = \Sigma(O - E)^2/E$.
The mixed population was not normally distributed.
The small population was normally distributed
($\chi^2 = 19.5133$; $p < 0.05$).
The large population was normally distributed
($\chi^2 = 23.5011$; $p < 0.01$).
The small and large populations were tested with Student's t test and were significantly different ($p < 0.001$).

Below

Electron micrographs of rabbit mesenteric nerves : (A) primary, (B) secondary branch. Note the absence of myelinated axons.


Number of axons
50
40
30
20
10
0
1·8
n=1108
mean=0·755
s.d.=0·297
n=114
mean=0·483
s.d.=0·223
10
0
1·8
n=179
mean=1·092
s.d.=0·405
10
0
1·8
Axon diameter (µm)

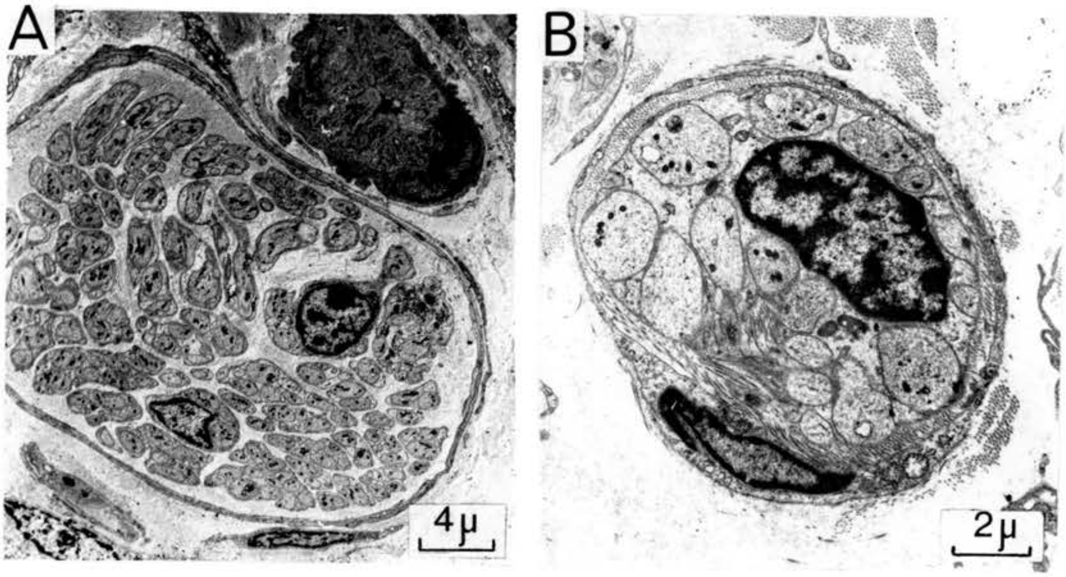
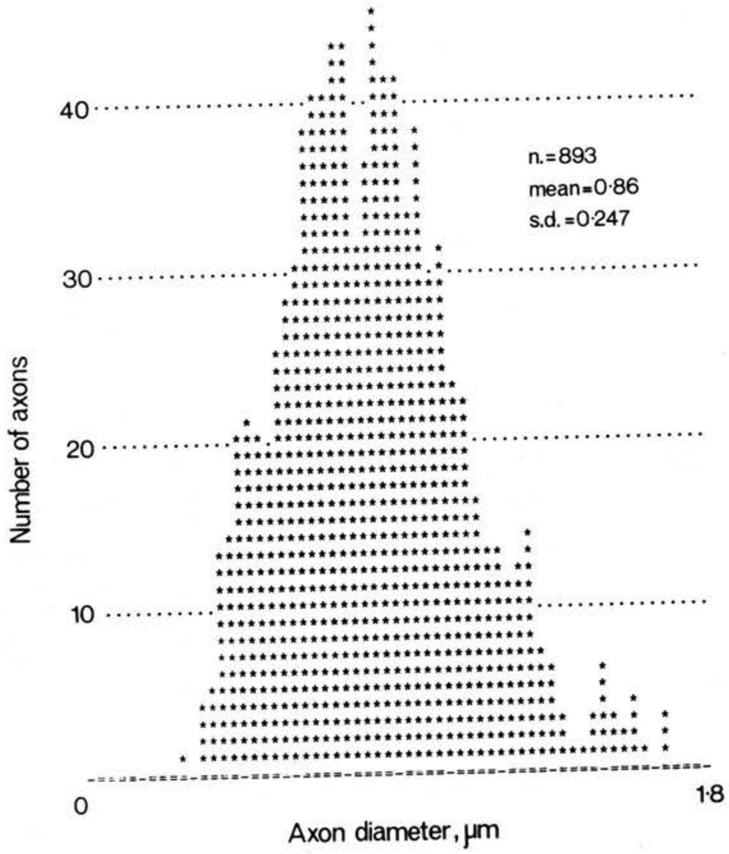
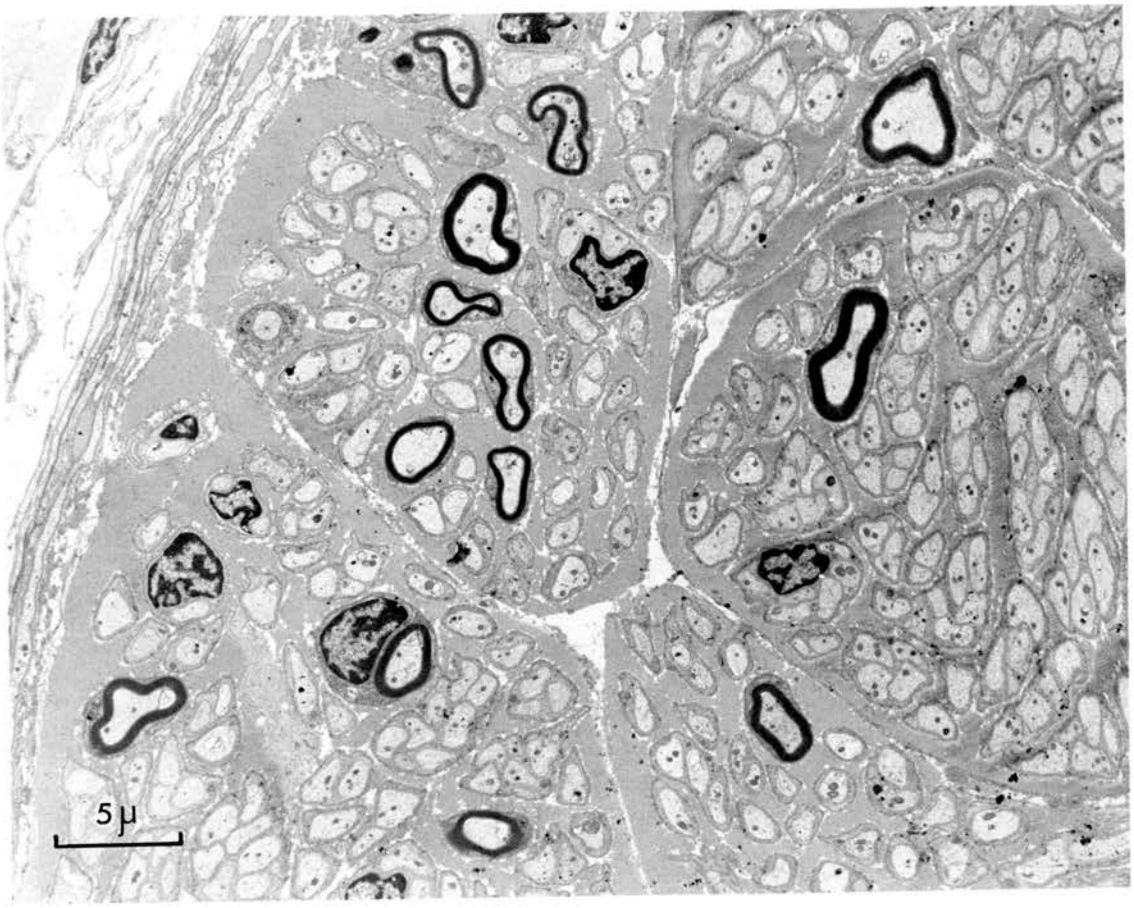

A
4µ
B
2µ

Figure 41 Above Histogram of the diameter of unmyelinated axon profiles in the sheep hepatoduodenal nerve. The population ranges from 0.28 μm - 1.71 μm. The histogram is not normally distributed. Below Electronmicrograph of the hepatoduodenal nerve of the sheep. Note the fasciculation, endoneurial collagen, myelinated axons and the large number of singleton-containing Schwann cells.


40
30
20
10
0
1·8
Number of axons
Axon diameter, μm
n.=893
mean=0·86
s.d.=0·247


5 μ

Discussion

ELECTROPHYSIOLOGY

Some interesting points arise from these incidental findings. The exclusive isolation of omental units from a single nerve bundle suggests that in its peripheral branches there may occasionally be viscerotopic isolation of functionally different units.

The observation that one omental unit contained two separate receptive fields is in agreement with the findings of Floyd & Morrison (1974b). Although it was impossible to obtain a slowly adapting response from one afferent unit with two endings, this may have been due to experimental difficulties, and does not necessarily indicate that two different receptors were associated with a single afferent axon. Both were stretch receptors, one adapted within one second, the other receptor maintained its activity for more than 10 seconds.

These results agree with the findings that omental units are insensitive to phenylbiguanide (Iggo, 1957c). The pharmacological sensitivity of omental units is clearly different from tension receptors which are also excited by veratrine (Paintal, 1957a, 1964). It seems unlikely that omental units fulfil an unambiguous "in parallel" function although they do seem to monitor changes in movement and stretch in the pyloric area. Receptors in this preparation were distinct from the duodenum and were not well placed to monitor changes in duodenal length. The slowly adapting discharge suggests that the receptor is associated with a relatively in-elastic element of the omentum.

The activity of "burst" units was a curiosity. Because mechanical stimulation caused tension receptor excitation in strands of the same parent bundle, this confirmed that the nerve preparations were afferent in direction. If these atypical units were sensitive receptors from the walls of the blood vessels (an explanation most favoured at the time of the experiment) then they were not excited by discrete mechanical probing or local venous and arterial occlusion (see : Gammon & Bronk, 1935; Andrews et al, 1972). The high frequency bursting discharge is similar to the pattern associated with the steady "burst type unit" found by extracellular records from myenteric plexus neurones (Wood, 1979, figure 1A). These "burst type units" are not mechanically sensitive and may be endogenous oscillators (Wood, 1979). It is therefore possible that activity may come from axon collaterals of myenteric neurones (Wood, 1976; Wood & Mayer, 1978). We have no information about the characteristics of such neurones in the sheep.

In the rabbit preparation the activity observed may be the result of antidromic cross-excitation from splanchnic efferents, which have been demonstrated to be active at low blood pressures (Dontas, 1955). It is possible that autonomic and afferent axons share common Schwann cell sheaths. The barrage of splanchnic activity may cause cross-excitation in the afferent axons and consequently antidromic activity might have resulted. It is generally assumed that there is no significant interaction between adjacent fibres, and that an impulse cannot jump from one fibre to another under natural conditions and that C-fibres bound in the same sheath behave independently. This is normally the case, but occasionally large waves of potential, much larger than can be attributed to single unit activity, can be recorded from visceral nerves and may be related to the phenomenon of impulse synchronisation because of axonal cross-excitation (Iggo, 1974). Local currents entering adjacent inactive fibres can become supra-threshold when their excitability is raised by de-calcification (Bures, Petran & Zachar, 1967). No large waves of activity were seen in multi-unit preparations, because the pre-amplifier filter was usually set at 80 Hz. In all other respects this investigation confirmed the independent activity of axons.

Ephaptic transmission may not necessarily be a phenomenon associated with low temperatures nor necessarily contingent upon the direction of propagation of the impulse in the exciting fibre (Rasminsky, 1980). It may be associated with a compression injury and the possibility therefore also exists that compression and stretch of the mesentery or omentum during preparation of the isolated bath may have caused this phenomenon to appear.

An alternative explanation is possible. Bueno, Garcia-Villar & Ruckebusch (1977) have recently demonstrated a high frequency burst (18 Hz) of spikes from intraluminally placed emg electrodes from dog colon. They suggest that activity originates from the muscularis mucosa. The characteristic activity (see their figure 2 and compare figure 38) is very similar. This location seems unlikely, however, because units could not be excited electrically from the mucosa which was possible with the mucosal receptors described above in Chapter 5.

HISTOLOGY

The histological composition of mesenteric nerves varies between different species. In the cat they contain both myelinated and unmyelinated axons (Schofield, 1960; Bessou & Perl, 1966); the rabbit has exclusively unmyelinated axons (Ross, 1958) and the rat has both (Schofield, 1960; Clothier et al, 1975), but none are greater than 2 μm diameter. The exclusively unmyelinated population of axons in the rabbit mesenteric nerves was confirmed (Ross, 1958). Three populations of nerve each with different axonal cross-sectional diameters occurred but the present method was insufficient to determine whether this indicates axonal collateral branching or short axonal looping in the mesentery, to form the basis of local reflexes (Andrews et al, 1972), but does not exclude their possibility. Neither does the method distinguish between afferent or efferent axons.

Habel (1956) and Iggo (1956a) have examined terminal branches of the vagus and other abdominal nerves in sheep and goats. They found both myelinated and unmyelinated axons with up to 30% unmyelinated axons. Only the largest myelinated axons (4 - 12 μm) innervate the reticulum, cardia and oesophageal groove. In the hepatoduodenal nerves no large myelinated axons were found (see figure 41) and a larger population of unmyelinated axons was present, being 95% of the total sample.

If it is assumed that the measurements of axon profile diameters and the single afferent units were made from the same population, then the conversion factor compares well with those values previously discussed (Gasser, 1955; Bessou & Perl, 1966). The conversion factor was from 0.82 - 1.26.

GENERAL DISCUSSION

This electrophysiological study has enabled many of the experimental aims to be accomplished. The recording technique allowed the isolation of single unit activity from a precisely located area of the duodenum and thus avoided the tedious dissection of large central nerve trunks. The dissection technique has the major disadvantage of interrupting the projecting pathway and so this information is lost. It is therefore impossible from this technique alone to decide whether projecting afferent fibres are involved in "axon reflexes" or "long reflexes" and we can only conclude from other evidence the putative projection and functional significance of such axons.

A number of receptor types was found in both the rabbit and sheep under conditions which allowed natural receptive field stimulation and, in the sheep, close intra-arterial drug administration. The single unit analysis demonstrated that small nerve bundles close to the duodenum usually contained a number of axons of enteroceptors with sensitivity to different modalities, some of which were chemosensitive. Amongst these chemosensitive receptors were distinct populations of fibres which were selectively sensitive to some chemicals. It was found that mechanoreceptors in the muscularis externa were also sensitised by chemicals and thermal stimuli applied to the mucosa and that the chemical sensitivity was the result of local reflexes. The presence of large numbers of mechanoreceptors in the muscularis externa suggests that there are serious limitations of interpreting multi-unit investigations of mesenteric nerves. For this, single unit investigations are required together with concurrent tension and emg measurements.

THE CONCEPT OF SELECTIVE SENSITIVITY

These results suggest that the concept of selective sensitivity of sensory receptors may not be applicable to all types of alimentary enteroceptor. Some receptors in the duodenal mucosa of sheep had a wide range of responses to chemical stimuli, although some were most sensitive to KCl and others to acetic acid, and all units were mechanically sensitive. No evidence was found for putative osmoreceptors or thermoreceptors. The multi-modal receptors did not respond to osmotic changes. Therefore sheep duodenal osmotic reflexes do not involve the enteroceptor population with projecting afferent axons found here. It is difficult to resolve these findings with those of Harding & Leek (1972a) which suggest otherwise.

No evidence was found for a receptor class responding only to high-threshold mechanical stimulation, although the adapted discharge of tension receptors in the sheep duodenum responsed linearly to transmural compression and, to this stimulus, some receptors were more sensitive than others. It seems very improbable that the low-threshold mucosal mechanoreceptors also function as high-threshold "tension"receptors (see below, page 168).

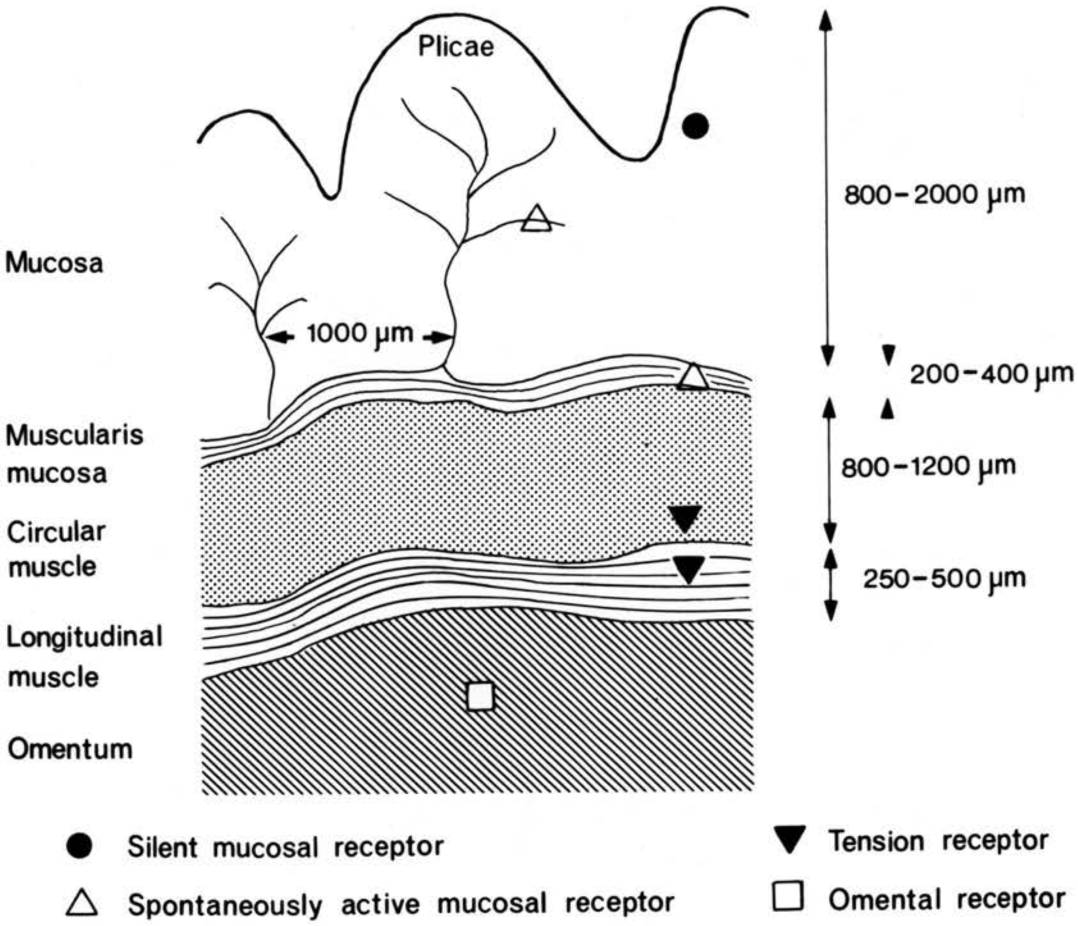
DUODENAL ENTEROCEPTORS - FUNCTIONAL MODELS

It is now possible to propose a model for the histological location of sheep duodenal enteroceptors in the manner schematically represented in figure 42.

The demonstration of spontaneously active units suggests that some mucosal receptors are associated with contractile elements, and

Figure 42

Suggested location of sheep duodenal enteroceptors



others are not. The modulation of this activity by topically applied drugs, which cause contraction and relaxation of the muscularis mucosa, suggests that tension receptors may be associated with this muscle. It has not been possible to confirm this directly with emg or mechanical records, but the "after discharge" and depression in the surface mucosa support this suggestion. These results therefore lend support to the concept that putative muscularis mucosal mechanoreceptors may be radially arranged "in series" receptors (Paintal, 1957b). It would be interesting to combine records of these units with electromyography using methods similar to those of Bueno et al (1977) to test this suggestion.

Support was found for the concept that chemoreceptors for acetic acid are located approximately 200 μm below the mucosal surface (Harding & Leek, 1972a). The response to hydrochloric acid suggested that in the duodenum changes in diffusion barrier (possibly buffer secretion and mucin production) modify the chemical sensitivity, without affecting the mechanical sensitivity. That chemoreceptors soon became refractory to repetitive challenges was annoying experimentally but may have some physiological significance, suggesting that in the duodenum "tonic inputs" from individual chemoreceptors to controlling centres may not occur. Support was found for the concept that duodenal receptors respond to titratable acidity (Hunt & Knox, 1972) and that it is inappropriate to describe them as "pH receptors" (Iggo, 1957b).

The responses of duodenal tension receptors to some drugs, transmural electrical excitation and mechanical compression in sheep, together with the evidence of emg and tension changes, suggest that they are located in longitudinal muscle, although it has not been possible to exclude the circular muscle or the myenteric plexus. In the rabbit these receptors may have been associated with circular muscle. The presence of continuing phasic activity after longitudinal duodenotomy suggests that the complete annular segment is not required for their activation.

Support was found for the concept proposed by Leek (1972a, and elsewhere) that tension receptors may be indirectly excited by intrinsic reflexes, because mechanical and chemical stimulation of the mucosa altered tension receptor activity, with a short latency. It was also found that mechanical stimulation of the mucosa may inhibit tension receptor discharges. This effect may either be a direct neural inhibition of afferent discharge (see : models proposed by Hirst & McKirdy, 1975; Wood, 1976) or involve indirect neural inhibition of muscle contraction.

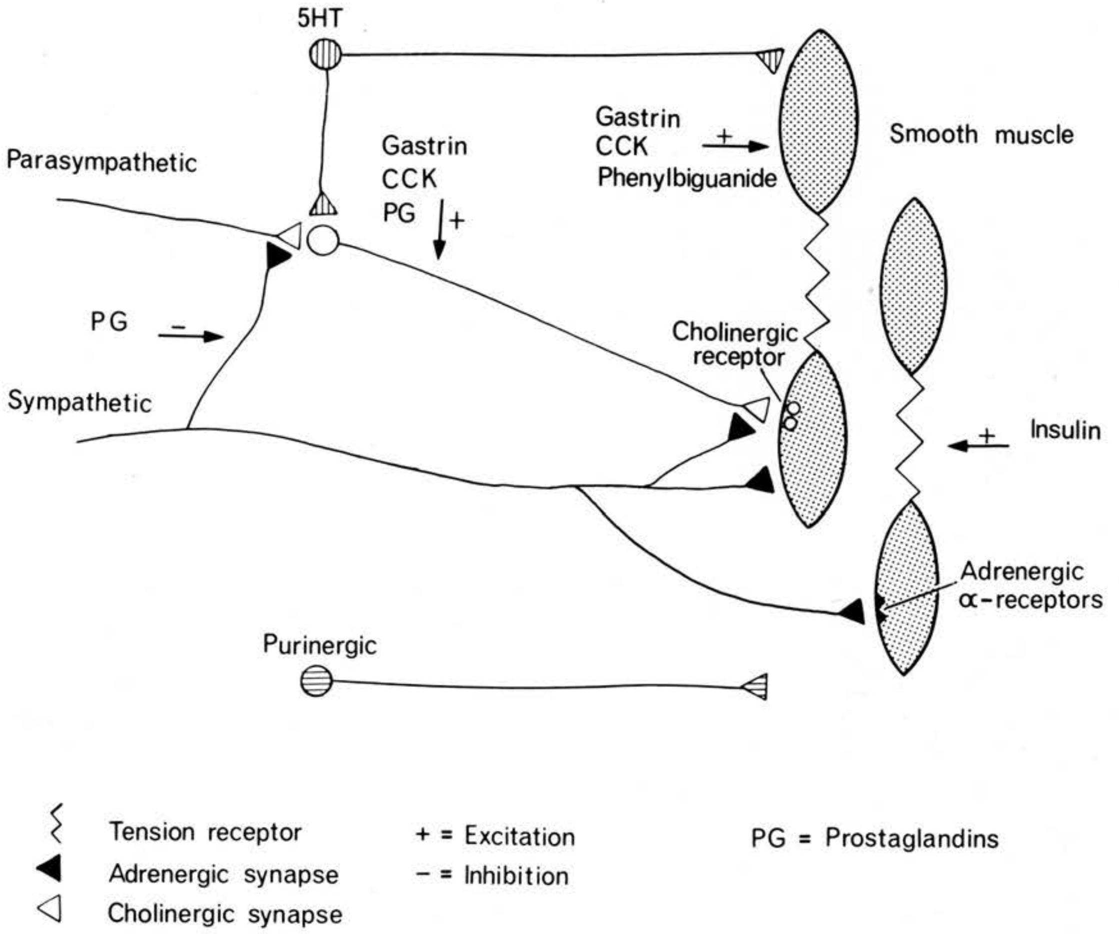
In addition, sensitive mechanoreceptors were found in the lesser omentum whose behaviour did not support the concept that they might act as "in parallel" stretch receptors.

THE PHARMACOLOGY OF DUODENAL ENTEROCEPTORS

These results support the observation that many systemically and locally perfused drugs alter the activity of alimentary enteroceptors, and now a summary diagram concerning sheep tension receptors is presented (see figure 43). No evidence was found for the suggestion made by Paintal (1954a, 1954c, 1964) that phenylbiguanide excites the receptor endings directly. Also, in the "silent" mucosal receptors no change was seen following close IA application of this drug and receptors were excited only when contraction occurred

Figure 43

Mode of action of drugs on sheep tension receptors



in the muscularis externa. Also, omental units were not excited by phenybiguanide. Similarly, the results suggest that 5-HT excited tension receptors indirectly by causing smooth muscle to contract. Neither 5-HT nor phenylbiguanide excited "silent" mucosal receptors although spontaneously active mucosal receptors were excited by both drugs by an atropine-sensitive mechanism (see figure 36). Tension receptors were excited by hormone analogues and it seems probable that endogenous hormones may play an important role in modulating post-prandial motor profiles in the duodenum. Neuro-modulation, defined as the release of transmitter from nerves by the action of neurohormonal agents on prejunctional receptors, is a relatively recent concept of physiological importance (Burnstock, 1979). It is an exciting prospect that this concept may be extended to include sensory as well as efferent neurones.

VISCERAL NOCICEPTORS?

No evidence has been found in this study for a separate population of sensory receptors which respond only to potentially damaging stimuli. However, sensitive mechanoreceptors were excited by drugs which may be involved with painful sensations.

The drugs bradykinin (Potter et al, 1962), acetylcholine (Braun et al, 1960) and 5-HT (Guzman et al, 1962) are profoundly algogenic. There are also some electrophysiological reports that these drugs excite visceral receptors and unmyelinated units in the somatosensory systems : acetylcholine (Gammon & Bronk, 1935; Gernandt & Zotterman, 1946; Brown & Gray, 1948; Iggo, 1957b); acetylcholine and histamine (Fjallbrant & Iggo, 1961); bradykinin with and without 5-HT

(Fjallbrant & Iggo, 1961; Floyd et al, 1977b) possibly by an indirect route; and 5-HT (Paintal, 1953, 1954b; Douglas & Ritchie, 1957) possibly at the same site as phenylbiguanide (Daniel, 1968). It is therefore probable that the endogenous release of 5-HT, bradykinin and acetylcholine sensitise enteroceptors and play a role in reflex behaviour. The present results suggest that these substances have an indirect effect on tension receptors and a direct atropine-sensitive effect on receptors associated with the muscularis mucosa.

Potassium chloride may act as an algogenic substance when applied to blister bases and injected close intra-arterially (Keele & Armstrong, 1964). It is possible that the physiological integrity of the mucosa was altered by the preparation which resulted in the discovery of KCl receptors. But this seems unlikely because receptors insensitive to KCl were found nearby in the same preparation. The receptors were sensitive to low levels of potassium which were demonstrated to exist in chyme and were therefore not nociceptors.

An incidental finding was that local injury to the mucosa, which resulted in peticheal haemorrhage caused a high-frequency discharge in spontaneously active sheep mucosal receptors. This "injury discharge" lasted for up to 30 sec. It is possible that this response was due to local potassium ion release from injured cells. Topically applied fresh blood did not excite mucosal receptors.

A COMPARISON OF SOMATOSENSORY AND VISCERAL RECEPTOR SENSITIVITY

Alimentary receptors do not form a functionally homogeneous group. The modality differences are related to either the histological location or receptor differences. We have no firm histological evidence for the structure of alimentary enteroceptors with afferent C-fibres, therefore it is not known how much the physiological heterogeneity is a consequence of the receptor environment, rather than a specific difference in the receptor ending itself. With this in mind, it is possible to compare the sensitivity of alimentary enteroceptors with non-alimentary receptors with C-fibre axons for, in the somatosensory system, afferent units with C-fibre axons are also heterogeneous (Iggo, 1958b). There are some obvious similarities.

Mucosal mechanoreceptors have receptive fields and thresholds similar to sensitive skin mechanoreceptors, whose receptive field is 2.3 x 1 - 2 mm, and threshold 25 mg - 5.0 g (Iggo, 1960). Both receptor types adapt to silence and have reduced responsiveness to repetitive stimuli (Iggo, 1959b, 1960), and there is a characteristic low frequency "after discharge" lasting 10 seconds after stroking (Zotterman, 1939; Iggo, 1958b, 1959b, 1960, 1966b; Bessou, Burgess, Perl & Taylor, 1971), which persists while small restorative movement of the skin occur (Iggo, 1974). But unlike sensitive skin mechanoreceptors no change was seen in sheep duodenal mucosal receptors when rapid changes of temperature were applied (Iggo, 1959b).

Receptors is skeletal muscle with C-fibre afferent axons have some properties similar to the tension receptors, which are excited by thresholds 5 - 50 g, and have receptive fields less than 5 mm^2 (Iggo, 1961). Also, skeletal muscle receptors respond to warming above 41^{o}C and cooling below 25^{o}C with a persistent discharge, which is not unlike the responses found in sheep tension receptors. A summary of sheep duodenal receptor characteristics is found in table 9.

Table 9 The Characteristics of Sheep Duodenal Enteroceptors. A Summary

SHEEP

enteroceptor	conduction velocity $m sec^{-1}$	receptive field mm^2	perpendicular threshold g tension	resting frequency imp sec^{-1}	minimum natural interspike interval ms	maximum frequency imp sec^{-1}	minimum interspike interval ms
tension	(a) 0.68 +/- 0.24 (b) 4.53 +/- 1.69	92 +/- 93	6.12 +/- 2.9	phasic 7.86 +/- 4.1	91.4 +/- 57.8	38.9 +/- 17.5	24.6 +/- 11.3
silent mucosal	0.90 +/- 0.33	1 - 5.5	0.132	usually zero 1 - 3	-	28.2 +/- 11.05	29.6 +/- 20.2
spontaneous mucosal	0.72 +/- 0.21	1	0.75 +/- 0.76	phasic 1 - 5 < 19	150	42.2 +/- 14.2 (variable sensitivity)	22.6 +/- 8.2
omental	(a) 0.70 +/- 0.14 (b) 7.3	20 - 100	0.2	none	-	25	23

(m +/- s.d.)

FUNCTIONAL ANATOMY

One of the difficulties of recording at the extremes of the peripheral nervous system is that little can be concluded about the size of the "afferent unit". With the exceptions of one sheep omental and one mucosal unit, all units had single receptive fields, therefore, whether central convergence of axon collaterals occurs cannot be judged from this work alone. The receptive field areas were small and similar in size to C-fibre afferent units in the somatosensory system. It is clear from other work (Niijima, 1961; Floyd & Morrison, 1974c) that many splanchnic afferent units have multiple receptive fields, often in different areas of the gut. Also, rumen epithelial receptors in the vagus nerve have considerable branching (Leek, 1977). Therefore some afferent units have a large number of receptive fields. In converging afferent axons the final coded message entering the CNS must be modified by the number of receptors simultaneously activated. Presumably impulse collision and peripheral resetting occur. This arrangement may form the basis for axon reflexes in the alimentary canal.

In the toad it has been suggested that receptors attached to the same axon may give rise to rapidly adapting and others to slowly adapting responses (Niijima, 1960, 1961). This phenomenon was also found in a single sheep omental unit but was considered to be due to the difficulty of providing the same stimulus to different areas of a moveable tissue, and did not necessarily imply that different receptors were attached to the same axon.

These electrophysiological results provide some additional information about the functional anatomy of the visceral afferent system :

1. Nerve fibres within a peripheral nerve bundle close to a viscus may be modality specific. In some cases the afferent projection may be monomodal; in sheep some omental units appeared to occupy a single bundle in which no other receptor type was found. This has previously been suggested by Sharma & Nasset (1962) who found that some multi-unit records from mesenteric nerves had no chemosensitivity, and who concluded that there was separation of vagal nerves "with stretch and tension" modalities and sympathetic nerves "with chemosensitive units".

2. There is probably a viscerotopic organisation of afferent axons in the gut. All fibres isolated were attached to precise areas of the preparation, for example, some nerve bundles supplied only omentum and others the muscularis externa. There is other evidence for viscerotopic organisation in the periphery of the visceral afferent system. In the toad stomach vagal afferent units innervate the cardiac region and splanchnic afferent units innervate the corpus (Niijima, 1962). Rawson & Quick (1972) found that ipselateral splanchnotomy abolished thermal reflexes from that side only; and in the cat anus, rectum and other sites, mucosal receptors have both ipselateral and contralateral projections (Clifton et al, 1976). Clarke & Davison (1978) found no evidence for viscerotopic organisation of alimentary units while traversing the cervical vagus, although Mei (1970a) found that the nodose ganglion is organised, and Harding & Leek (1973) found viscerotopical organisation of interneurones with "afferent-like activity" in the gastric centre of the medulla oblongata.

DUODENAL RECEPTORS, THEIR ROLE IN FEEDING

These results demonstrate that sensitive enteroceptors are present in the duodenum of sheep with projecting afferent axons which monitor the mechanical and chemical environment, and allow us to conclude that some of the so-called "reflex" phenomena described in the literature (pp 8 - 29) may involve these afferent nerves. The receptor thresholds found in electrophysiological studies were similar to thresholds found to invoke reflex changes in the anaesthetised rabbit and to modulate gastric emptying in ruminants. Further, it is interesting to speculate about the possible role of these visceral afferent receptors, and the central integration of their activity. Information is accumulating that the duodenum, liver and rumen are involved in the control of food intake (Forbes, 1980).

There is evidence from feeding trials for the role of duodenal afferent nerves in feeding behaviour. Snowdon (1970) with vagotomised rats, demonstrated that rapid gastric emptying causes premature satiety and results in small and frequent meals. To what extent the duodenum is de-sensitised by this procedure is not known. Novin (1976) reports that duodenal perfusion with isotonic glucose suppresses food intake in ad-libitum fed, but not food-deprived rabbits, whereas hepatic portal perfusion of concentrated glucose solutions is ineffective in ad-libitum fed, but effective in deprived rabbits. This role of intraluminal glucose in the duodenal control of food intake is vagus dependent.

The ventromedial hypothalamic (VMH) and lateral hypothalamic (LH) areas contain important chemoreceptors and integrators, and probably account for the control of food intake over the long term (Novin, 1976;

Powley, Weingarten & Opsahl, 1977) because vagotomy, sympathectomy and gastrectomy do not abolish the ability to regulate body weight and food intake. The activity from viscera may be involved in the short term regulation of food intake, and the liver and duodenum appear to be most important in this control (Novin, 1976). Stephens (1980) has shown that a three minute intraduodenal, but not intragastric, perfusion of 15% glucose (250 ml) suppresses eating 15 - 20 minutes after the end of infusion in duodenal-cannulated piglets. The control meal lasted 30 - 40 minutes. Unlike the rabbit experiments, hepatic portal perfusion has no effect on meal size (Stephens & Baldwin, 1974). These long latencies do not exclude the involvement of hormones in the responses.

Volatile fatty acids may be more important than glucose in ruminants. Intraportal, but not systemic, influsion of propionate (threshold 1.2 mM min^{-1}) causes a reduction of feeding in sheep, by a mechanism which depends upon afferent nerves from the liver to the hepatic plexus (Anil & Forbes, 1980). The existence of chemoreceptors sensitive to volatile fatty acid in the ruminant stomach is postulated to account for the depression of food intake following the injection of acetic acid into the rumen (Baile & Pfander, 1966). This may form the basis of a peripheral chemoreceptor mechanism for regulating food intake in the ruminant (Leek, 1972a).

The peptide hormones CCK and gastrin may have a sensory as well as a motor role to play in the gut. Immunohistochemical techniques have shown that CCK and gastrin-containing neurones are most numerous in the myenteric and submucous plexus of the colon where they surround, and appear to innervate, non-immunoreactive ganglion cell bodies (Larsson & Rehfeld, 1979). Gastrin and CCK-containing neurones are also numerous in the coeliac-superior mesenteric plexus (Larsson & Rehfeld, 1979). Whether these neurones are sensory or motor is not known. It is possible that they play a role in sensory mechanisms. Ligation and transport-blocking techniques demonstrate the accumulation of substance P, somatostatin, vasoactive intestinal polypeptide and CCK-like peptides on the alimentary side of vagus and splanchnic nerves (Lundberg et al, 1978). This is interesting because low doses of CCK injected intraperitoneally interrupt feeding and induce satiety in laboratory rats (Gibbs, Young & Smith, 1973; Nemeroff, Osbahr, Bissette, Jahnke, Lipson & Prange, 1978). Presumably exogenous peptides act both peripherally and centrally and either excite or mimic the effect of increased sensory activity in alimentary receptors and induce behavioural responses by exciting the satiety centres. The central distribution of gastrointestinal peptides is extensive (Hokfelt et al, 1980).

In the present study only insulin appeared to excite receptors independently of mechanical change. This may be by a direct excitation of the receptor membrane, and although tension changes always coincided with the administration of other peptide hormones, it is possible that receptor sensitisation also occurred. Mei (1978) has also observed the excitation of enteroceptors by insulin and considers that hypoglycaemia is the stimulus. Mei (1978) used much larger doses of insulin (10 - 20 IU) than the present investigation. It was not known whether the levels produced were higher than physiological levels which are 4 - 160 m IU/L in the human circulation (Diem & Lentner, 1970).

Paintal (1953) first demonstrated that there is increased activity in cat gastric tension receptors when stimulated with intra-arterial glucose. Paintal (1953, 1954b) seems to have been the first investigator to attempt to correlate known alimentary afferent activity with sensation. He suggested that the activity of vagal afferent units from gastric stretch receptors might be inversely related to "hunger pangs", although he was incorrect in assuming that there is reduced activity of these units during contraction (Iggo, 1957a). His demonstration that stretch receptors are excited by intra-arterially injected glucose is interesting but has received little attention. It indicates the possible basis for a mechanism where there is peripheral interaction between energy status and afferent activity of alimentary mechanoreceptors.

The evidence that tension receptor activity increased when tension and emg activity was greatest in the gut, makes it possible to conceive a unifying hypothesis about their possible role in the peripheral control of food intake. The emg evidence, developed by Ruckebusch (Ruckebusch, Grivel & Santini, 1968), can now be reinterpreted to indicate the level of afferent activity reaching the CNS from tension receptors. Bueno & Ruckebusch (1976) have shown that increased emg activity in dog jejunum occurs with intravenous glucose, and in sheep intestine occurs with intravenous volatile fatty acid. Bueno & Ruckebusch (1976) show that the emg pattern becomes a continuous spiking one, similar to fed dogs, when insulin, D-glucose and amino acids (L-leucine and L-arginine) are administered to dogs, and when insulin and VFA's are given to sheep. They conclude that endogenous insulin levels are important in the control of jejunal motor profiles. This hormone is more important than gastrin and vagotomy in sheep (Bueno & Ruckebusch, 1975). Gastrin is important in dogs where it changes a fasted MMC to the fed pattern

(Weisbrodt, Copeland, Kearley, Moore & Johnson, 1974) and vagotomy alters the non-fasted emg pattern possibly via a gastrin-release mechanism, in dogs (Weisbrodt, Copeland, Moore, Kearley & Johnson, 1975). These results are similar to the present findings in which hormones were applied both to the mucosa and systemically.

In addition, both acid solutions applied to the mucosa and light mucosal mechanical stimulation modified tension receptor activity. These findings make it difficult to understand single unit observations that chemicals do not alter tension receptor activity, but agree with those of Zamiatina (1957) for mixed populations. There are possibly species differences in the sensitivity of mechanoreceptors in the muscularis externa because, unlike the sheep tension receptors, those in the rabbit were insensitive to intraluminal acid. In addition, glucose and hypertonic saline caused excitation in the rabbit units but not those in the sheep.

Single unit evidence has been provided for an intrinsic mechanism which modifies tension receptor activity (see figure 26). Ruckebusch & Bueno (1975) have shown that digestive bulk affects electromyographic activity by an intrinsic mechanism which is not affected by vagotomy or splanchnotomy. This supports the suggestion that there may be a peripheral mechanism which alters the sensitivity of tension receptors because mucosal receptor excitation may, by an intrinsic reflex, inhibit the muscularis externa and reduce the activity of tension receptors. This hypothesis may also explain the underlying mechanism for the "eructation inhibition" reflex in ruminants; caecal emptying of gas in herbivora and the mechanism which distinguishes flatus in the lower bowel. Atmospheric gases did not excite mucosal receptors, which responded with low mechanical threshold to particulate material.

The apparently ambiguous afferent input from gastric tension receptors is discussed by Leek (1972a): during active contraction of the empty stomach there is a feeling of "hunger pangs" and emptiness, whereas with a distended stomach, with presumably vigorous tension receptor activity, there is a sensation of "fullness". This conflict may be resolved centrally (Leek, 1972a). The evidence provided here suggests that the excitation of sensitive mucosal receptors is one mechanism whereby this ambiguity may be resolved, the mechanism not necessarily involving long reflex "high threshold" loops, but occurring peripherally in the intrinsic plexus. The assumption that mucosal receptors behave as high threshold receptors is based on the observation that distended balloons excite afferent activity (Leek & Harding, 1975). This technique, in the absence of stable mechanical conditions, is likely to expose the slowly adapting nature of the mucosal receptors because of the better contact between balloon and mucosa. In the natural condition an empty, contracting stomach would probably not excite mucosal receptors, whereas the presence of digesta would do so.

The sensitivity of tension receptors therefore involves the circulating energy-containing moiety (glucose or VFA's) appropriate to the species in question, the circulating levels of insulin and other hormones, and the contents of the gut itself.

The chemical sensitivity of afferent units from mucosal receptors has also been demonstrated in this work. Their central and peripheral activity must also play an important role in the control of appetite, and evidence for this has been provided from reflex studies by Harding & Leek (1972c) who found that chemical excitation of the abomasum influences central nervous activity.

DUODENUM IN THE CONTROL OF GASTRIC EMPTYING - AN OVERVIEW

It is generally held that the duodenum plays an important role in regulating gastric emptying (Edwards & Rowlands, 1968). Evidence has been presented which supports the role of the duodenum as a reflexogenic and as a reflex target organ. The stomach and duodenum can communicate myogenically as well as by axon reflexes, extrinsic ganglionic reflexes and long reflexes. The neural means of communication can influence the intrinsic myogenic oscillations in membrane potential which are the basis of the motor profile of the two regions. The rate of BER of the stomach and duodenum are widely different and often behave independently of one another. However a phase-locking can occur after feeding and an ordered gastric emptying results. The pattern of gastric emptying is exponential in character (Hunt & Knox, 1968a; Bell & Razig, 1973a). The exponential emptying of the stomach can be modified by extrinsic reflexes initiated from duodenal sensory receptors, and these may be either excitatory or, more usually, inhibitory on gastric motility and secretion. The myogenic and neurogenic control are influenced by the gastro-intestinal hormones released post-prandially. These may alter the release of neural transmitters in the gut plexus, directly affect muscle excitability, and also sensitise sensory receptors to external stimuli. The duodenal mucosal receptors and post-prandially released duodenal hormones, may play a role in pre-absorptive satiety and influence ingestive behaviour.

FUTURE INVESTIGATIONS

Any future analysis of the projecting afferent axons from the gut must consider : that at several levels of the gut, there is a complicated neural network which allows interaction of activity not dissimilar to the lateral inhibition and facilitation in the somatosensory nervous system; that some units may have a degree of selective sensitivity, but others may not; that some mechanoreceptors are sensitised by hormones and temperature; that there is possibly species variation in the response of chemoreceptors which have been selected to respond to the appropriate environmental conditions, so that those found in carnivora may respond to glucose, and those of the ruminant to volatile fatty acids; that the secretory response of some tissues may affect the sensitivity of chemoreceptors; and that repetitive mechanical stimulation may cause reflexes which seriously disturb the physiological status of the preparation and that a correct choice of species is necessary to demonstrate physiological events.

The preparation of the sheep duodenum may be utilised to investigate further aspects of duodenal sensory and reflex mechanisms :

1. Quantitative aspects of distribution of enteroceptors in the tissue. With the exception of Pacinian corpuscles, there is no good evidence for the histology of duodenal sensory receptors and their location. It might be possible to stain either receptor endings or cell bodies of enteroceptors located peripherally, by the technique of retrograde transport of horse radish peroxidase, after exposing the cut nerves to HRP solutions. The mechanical stability of the preparations would make this attempt feasible.

2. The central projection of afferent axons could be investigated by both : (a) electrophysiological recording in gastric medullary centres, and (b) histologically by retrograde projection measurements to nodose ganglia, splanchnic nerve and centrally, as well as to local preganglia. Such an histological technique would require long survival times and probably chronic preparations. The information gained might allow more meaningful interpretation of duodenal afferent activity in the control of gastric function and appetite control.

3. The difficulty of interpreting projection pathways might be overcome by utilising microelectrode techniques. This would be a difficult exercise because of the small axonal size. The addition of agar gels would add further stability to the preparation for such an attempt. The locations of the cell bodies as a result of an histological study might suggest a suitable recording site.

4. The demonstration that unmyelinated axons can be recorded as single afferent units close to the duodenum is encouraging for the use of similar techniques to provide information about efferent activity. Experiments including active and passive mechanical changes, as well as chemical stimulation may be rewarding. The continuation of the ventral vagus is easily available for electrical recording and an analysis of its efferent activity while stimulating abomasum or duodenum would be an interesting development of this study.

5. The sheep duodenal preparation is suitable for a detailed pharmacological study of functionally identified enteroceptors with unmyelinated axons. It would be necessary to combine such a study

with an in vitro analysis of drug action on isolated strips of muscularis mucosa, longitudinal and circular muscle. An in-vitro preparation would have the advantage of overcoming the reluctance to utilise blocking drugs during acute electrophysiological investigations because of their unknown and prolonged effects.

6. The physiological role of the KCl-sensitive duodenal mucosal receptors deserves further investigation. For this, chronically fistulated sheep and calves might be used, together with selective neurotomy and/or mucosal anaesthesia, while studying abomasal emptying.

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