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GUNNING VICTORIA JUBILEE ESSAY

Submitted for the CHRISTISON prize for pharmacology.



Reflex and neuroendocrine release of adrenaline and  
noradrenaline from the adrenal glands of cats and dogs.

Peter Ellis

( B.Sc. University of Edinburgh 1978 )



This essay is a report of the experiments conducted by myself in conjunction with my supervisor, Dr. A. Ungar, within the Department of Pharmacology, University of Edinburgh, during the last three years.

The first set of experiments is published in the Journal of Physiology and I present them here unchanged to act as an introduction and outline of methods used.

In conjunction with the reflex study in the whole animal, we also conducted a study using isolated perfused adrenal glands from cats and dogs to investigate the preferential release of adrenaline and noradrenaline as first suggested by Douglas and Poisner (1965).

#### Isolated gland study

Adrenal glands are removed after cannulating the adreno-lumbar vein towards the gland and ligating the adrenal vein between the gland and the vena cava. The gland is then flushed with heparinised Locke's solution to remove any blood present and then retrogradely perfused at 2 ml/min with either oxygenated Locke's solution (at 37°C and pH 7.4) or with the appropriate drug made up in Locke's solution. The effluent passing from the arterial vessels of the gland either went to waste or was collected as 30 second samples (1 ml) which were assayed for adrenaline and noradrenaline using the tri-hydroxyindole method of Vendalsu as mentioned previously.

The drugs used in this study were nicotine, m-hydroxyphenyl-propyltrimethyl ammonium (HPPTMA), a specific nicotinic agonist, acetyl- $\beta$ -methyl choline (Methacholine), a specific muscarinic agonist and the nicotinic and muscarinic antagonists, hexamethonium bromide and -hyoscine methyl bromide respectively.

The isolated gland study confirmed the difference in the proportion of adrenaline and noradrenaline present in the resting adrenomedullary secretion of the cat and the dog. In the cat, the resting output of noradrenaline represents about 40% of the total catecholamine secretion. - This figure is in agreement with that found in the adrenal venous blood of pentobarbitone anaesthetized cats. In the dog a much lower percentage of noradrenaline (20-25%) was present in the resting output. This is again in agreement with our results for adrenal venous blood and is also similar to that reported in the perfusate from isolated canine glands by Vogt, Robinson and Tsujimoto and Hishikawa.

Both cat and dog adrenal glands showed a marked increase in

adrenomedullary secretion of catecholamines following a 2-minute infusion of either nicotine ( $10^{-5}$ m), HPPTMA ( $10^{-6}$ m) or acetyl- $\beta$ -methyl choline ( $10^{-6}$ m). However, during nicotinic drug infusions to cat adrenal glands, the incremental secretion contained a higher percentage of noradrenaline than at rest; (This change in noradrenaline percentage is significant when using the specific agonist, HPPTMA but is not significant when nicotine is used), whereas, the muscarinic agonist, acetyl- $\beta$ -methyl choline, produced an incremental secretion containing a significantly lower percentage of noradrenaline than at rest.

The drug-evoked secretion from dog adrenal glands, whether nicotinic or muscarinic, did not differ in composition from that seen at rest.

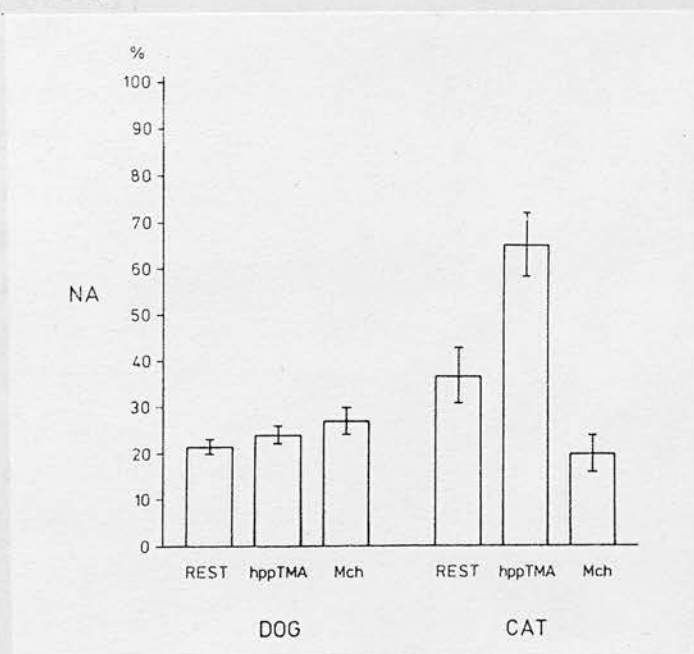


Figure 1: Percentage noradrenaline in the incremental release evoked by HPPTMA and methacholine. (Bars represent standard errors.)

The nicotinic antagonist, hexamethonium bromide ( $3 \times 10^{-4}$ m), abolished the release in response to HPPTMA but did not abolish the release in response to acetyl- $\beta$ -methyl choline; whereas, the muscarinic antagonist, atropine ( $3 \times 10^{-5}$ m), abolished the release in response to acetyl- $\beta$ -methyl choline but did not abolish the release in response to HPPTMA.

From the results obtained (figure 1) a species difference in

cholinergic receptor population is apparent between the chromaffin cells of the cat and the dog. In the cat a preferential release of noradrenaline occurs in response to nicotinic agonists, whereas the muscarinic agonist, acetyl-B-methyl choline, causes a preferential release of adrenaline. We must conclude from these observations that within the cat adrenal medulla both types of chromaffin cell possess cholinergic receptors, with the nicotinic receptors located predominantly on the noradrenaline containing cells and the muscarinic receptors located predominantly on the adrenaline containing cells. In the dog neither nicotinic nor muscarinic agonists evoked any preferential release of either catecholamine. The simplest explanation of this is that both chromaffin cell types possess both classes of cholinergic receptor.

The preferential catecholamine release in the cat produced by cholinergic agonists mimics that produced by vasomotor reflexes. (see figure 2) This, in conjunction with hexamethonium bromide selectively inhibiting the baroreceptor reflex release of catecholamines in the cat, is good evidence for the existence of two separate pathways to the cat adrenal medulla mediating baroreceptor and chemoreceptor reflexes. ie. Baroreceptor reflexes are nicotinicly mediated and preferentially release noradrenaline, whereas chemoreceptor reflexes are muscarinicly mediated and preferentially release adrenaline.

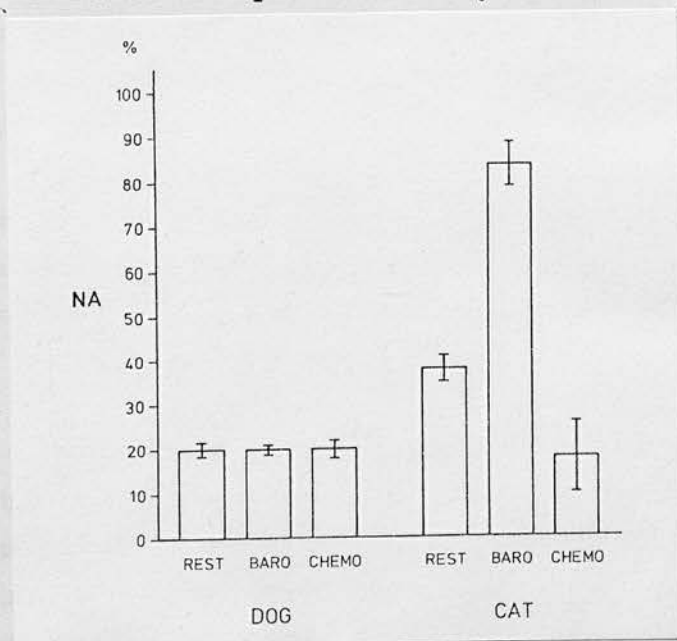


Figure 2: Percentage noradrenaline in the incremental release evoked by baroreceptor and chemoreceptor reflexes. (Bars represent standard errors.)

Dog study - Effects of cholinergic antagonists on reflex catecholamine release.

There is no evidence to support or contradict this theory of two separate pathways existing in the dog. To test the hypothesis in the dog we performed baroreceptor and chemoreceptor tests before and after the i.v. administration of either hexamethonium bromide (10mg/kg) or -hyoscine methyl bromide (10mg/kg), a selective muscarinic antagonist.

As expected, no change occurred in the ratio of adrenaline: noradrenaline throughout the experiments, but, hexamethonium bromide did consistently inhibit the catecholamine release in response to baroreceptor tests more than the release in response to chemoreceptor tests, whereas -hyoscine methyl bromide inhibited the release in response to chemoreceptor tests more than the release in response to baroreceptor tests.

In the presence of both antagonists no release occurred in response to either baroreceptor or chemoreceptor reflexes.

These results indicate that transmission at the chromaffin cell, during the baroreceptor reflex, is mediated predominantly by nicotinic receptor activation with a subsidiary muscarinic pathway and vice versa for the chemoreceptor reflex. Therefore, a parallel nicotinic and non-nicotinic pathway mediating baroreceptor and chemoreceptor reflexes does exist to dog as well as cat chromaffin cells.

Thus, the two pathways could be the same in both cat and dog with a different receptor distribution on the chromaffin cells accounting for the selective release of catecholamines occurring in the cat and not in the dog.

The simplest explanation of two pathways to the adrenal medulla and the sympathetic ganglion is the existence of two separate nerves, one nicotinic and one muscarinic. Although this is a possible explanation there is no anatomical or electrophysiological evidence to support it. Another possibility, conforming to the existence of only one nerve, could be that

differing firing patterns within the nerve could relay different reflexes and stimulate different receptors. ie. Long EPSP's, of the muscarinic type, could summate with rapid, non-synchronous firing, whereas the short, nicotinic, EPSP's could summate with synchronous firing. The type of summation occurring could selectively trigger either nicotinic or muscarinic receptors. To investigate this hypothesis would obviously require the additional skills and equipment of an electrophysiologist which we do not have available. Until a suitable investigation can be made this must remain only a hypothesis.

Dog study - Investigating possible humoral release.

All experiments discussed have investigated the response of the adrenal medulla to only short hypoxic stimuli, localised to the carotid bifurcations, lasting one minute. We always found that the release of catecholamines began and ended sharply with the beginning and end of the stimulus, as one would expect of a reflex response mediated by the autonomic nervous system. We observed, however, that if the stimulus was prolonged to ten minutes, the release of catecholamines outlasted the stimulus by at least a further thirty minutes, although both the adrenal glands and the central nervous system remained perfused with well oxygenated blood throughout the experiment.

A secretory response which outlasts the stimulus that evoked it, by more than a few minutes, is more likely to be mediated by a humoral than a neural mechanism. The crucial test of such a hypothesis is to divide the motor nerves supplying the effector organ.

This was done by cutting the left splanchnic nerve 30mm from the adrenal gland and dissecting in an arc around the gland the retroperitoneal connective tissue to interrupt any additional sympathetic fibres, particularly around the arteries supplying the gland. All chemoreceptor tests in this study were performed as previously described except that the stimulus applied (hypoxia) was of less intensity and lasted either 10 or 20 minutes as opposed to only one minute.

In dogs with innervated adrenal glands baroreceptor tests always produced an increase in catecholamine output and 10 minute chemoreceptor tests produced an instant release of catecholamines with the release reaching a peak after the end of the stimulus. (see figure 3a).

In dogs with denervated left adrenal glands baroreceptor tests followed 10 minutes later by 20 minute chemoreceptor tests were performed. The output of catecholamines did not rise during baroreceptor tests, although the usual reflex rise in systemic blood pressure was seen. This confirmed the total denervation of the gland. All the denervated glands responded to the chemoreceptor tests with a rise in catecholamine output, but in contrast to the response of innervated glands a rise was not seen during the first 5 minutes of a test. (see figure 3b).

We thus appear to be dealing with a reflex which has a neural sensory pathway, since it is abolished by carotid sinus nerve block (see methods in publication), but a circulating humoral component in its motor pathway. It was necessary to exclude the possibility that this response, like tachycardia and vasodilation, could be a secondary neural or chemical consequence of the increased pulmonary ventilation evoked by carotid body hypoxia (Daly and Scott, 1963). This possibility is excluded by our experiments since the lungs were ventilated at a constant rate and the vagi were cut.

We took as a working hypothesis the involvement of the pituitary-adrenocortical axis since we know that carotid chemoreceptor stimulation releases corticotrophin from the anterior lobe of the pituitary gland, and thus corticosteroids from the adrenal cortex. (Anichkov et al, 1960; Marotta, 1972) The conversion of noradrenaline to adrenaline in the mammalian chromaffin cell depends on the induction of the enzyme phenylethanolamine-N-methyl transferase (PNMT) by the high concentration of corticosteroids that reaches the adrenal medulla through direct portal channels from the cortex. Little is known, however, of the influence of corticosteroids upon the release, as distinct from the synthesis, of medullary

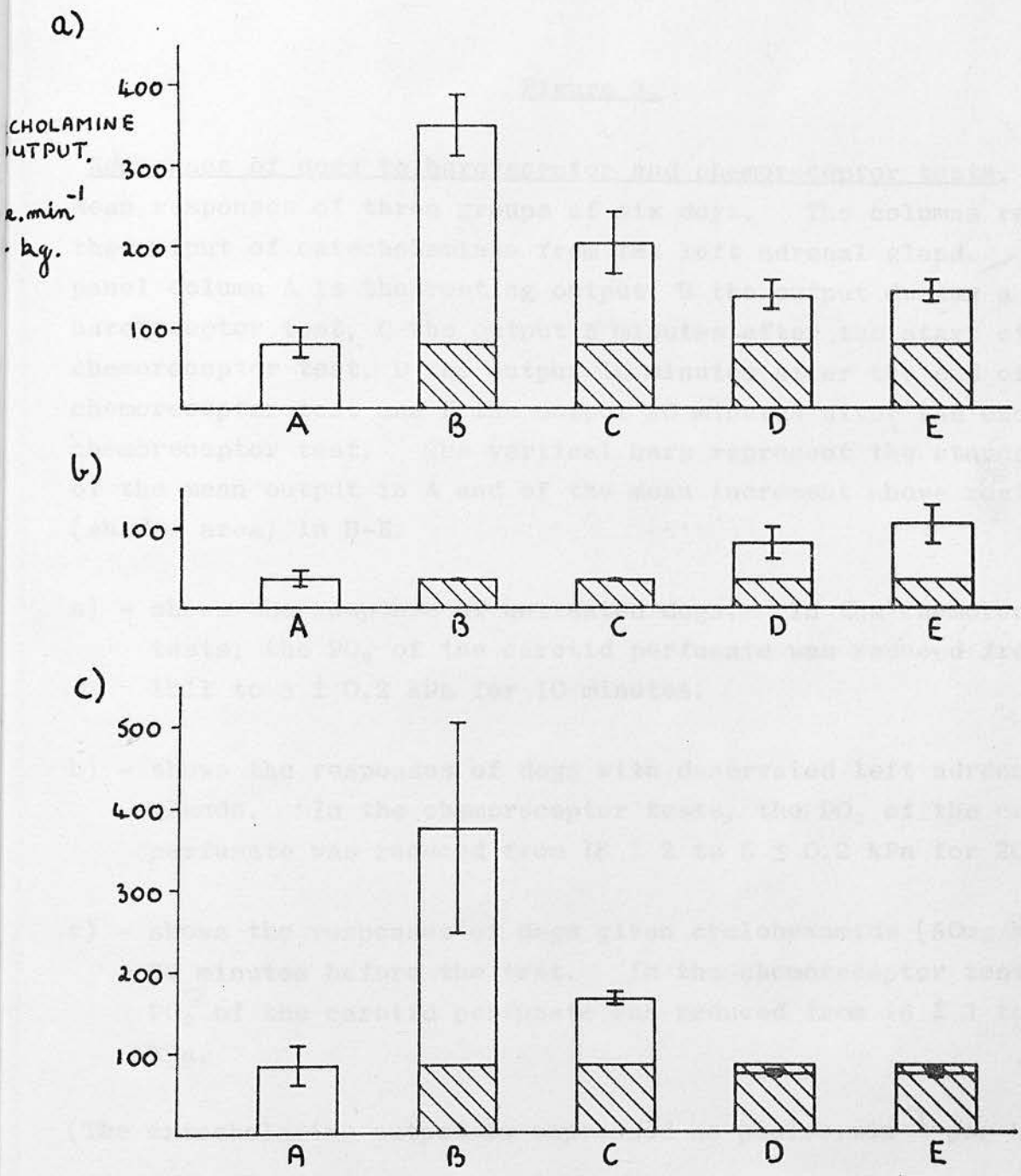


Figure 3.

Legend on sheet 9b.

Figure 3.Responses of dogs to baroreceptor and chemoreceptor tests.

Mean responses of three groups of six dogs. The columns represent the output of catecholamines from the left adrenal gland. In each panel column A is the resting output, B the output during a baroreceptor test, C the output 5 minutes after the start of a chemoreceptor test, D the output 10 minutes after the end of a chemoreceptor test and E the output 20 minutes after the end of a chemoreceptor test. The vertical bars represent the standard error of the mean output in A and of the mean increment above resting level (shaded area) in B-E.

- a) - shows the response of untreated dogs. In the chemoreceptor tests, the  $PO_2$  of the carotid perfusate was reduced from  $15 \pm 1$  to  $5 \pm 0.2$  kPa for 10 minutes.
- b) - shows the responses of dogs with denervated left adrenal glands. In the chemoreceptor tests, the  $PO_2$  of the carotid perfusate was reduced from  $18 \pm 2$  to  $5 \pm 0.2$  kPa for 20 minutes.
- c) - shows the responses of dogs given cyclohexamide (50mg/kg) 20 minutes before the test. In the chemoreceptor tests, the  $PO_2$  of the carotid perfusate was reduced from  $16 \pm 3$  to  $4 \pm 0.3$  kPa.

(The catecholamine output is expressed as  $\text{pmoles} \cdot \text{min}^{-1}$  per kg. )

catecholamines. We tested this hypothesis by devising experiments to answer three questions:-

- 1) Does inhibition of the release of adrenal corticosteroids affect the release of catecholamines in response to carotid hypoxia, and if so, does it also affect other reflex responses of the adrenal medulla?

Cyclohexamide is a drug which inhibits the release of corticosteroids in response to corticotrophin. We performed tests before and after the i.v. injection of cyclohexamide (50mg/kg) in three innervated dogs. In all dogs the resting output of catecholamines fell. The immediate release of catecholamines during both chemoreceptor and baroreceptor tests was not impaired by cyclohexamide. In all three dogs, however, the sustained release in response to the chemoreceptor tests was abolished by cyclohexamide. (see figure 3c)

These results support the idea of two components to the chemoreceptor response, of which the late component is abolished by cyclohexamide.

- 2) Do corticosteroids affect the release of catecholamines, and if so, is this action also blocked by the inhibition of release of corticosteroids?

In seven isolated perfused adrenal glands hydrocortisone was added to each perfusate at concentrations of 30, 50 and 100 $\mu$ g/ml for 5-minute periods. Hydrocortisone was found to release catecholamines in a dose-dependent manner, the time course of the release being similar to that of the late component of the response to chemoreceptor tests.

Cyclohexamide did not alter the release of catecholamines in response to hydrocortisone.

- 3) Does exogenous corticotrophin affect the release of catecholamines, and if so, is this action also blocked by the inhibition of the release of corticosteroids?

In four dogs corticotrophin (25 $\mu$ g) was infused intravenously. The corticotrophin increased the output of catecholamines, with

a similar time course of release to that shown by prolonged chemoreceptor tests and hydrocortisone-stimulated release in isolated glands.

In three dogs corticotrophin was infused 50 minutes after the injection of cyclohexamide (50mg/kg). The release was almost completely blocked.

We conclude from our results that there are two components to the response of the adrenal medulla to arterial chemoreceptor stimulation. The rapid component requires an intact nerve supply to the adrenal gland, but is independent of adrenocortical function. The delayed component, on the other hand, requires an intact pituitary-adrenocortical axis, but is independent of the motor nerves to the gland.

In these experiments we have found that moderate hypoxia, with carotid arterial  $PO_2$  between 6.0 and 6.5 kPa was sufficient to evoke the release of catecholamines. In previous experiments we found that a lower carotid arterial  $PO_2$  is needed to evoke the immediate, presumably neural, release. This suggests that the humoral mechanism may have a lower hypoxic threshold than the neural mechanism, and that it could thus have the greater physiological importance.

#### Dog study - Effects of indomethacin.

A problem in studying adreno-medullary reflexes is the massive fall in blood pressure following laparotomy and handling of the viscera. Terragno et al (1977) reported that laparotomy causes release of prostaglandins and that indomethacin, given after laparotomy, restores the blood pressure. We gave indomethacin (5mg/kg) in divided doses to dogs before and after laparotomy. This enabled us to maintain a mean blood pressure around 100mmHg for up to three hours, whereas without indomethacin it was difficult to maintain a blood pressure of 70mmHg.

In contrast to Feuerstein et al's (1979) results in the cat, which reported that indomethacin increases the resting and stimulated release of catecholamines from the adrenal gland. We found indomethacin did not affect the amounts of adrenaline or noradrenaline released at rest or in response to chemo-

receptor or baroreceptor tests. This is confirmed by our studies using isolated canine adrenal glands in which indomethacin did not affect the release stimulated by nicotine, and prostaglandin  $E_2$ , which is reported to inhibit release from feline adrenal glands, is in fact a weak releaser of catecholamines from canine adrenal glands. However, indomethacin did reduce both the resting adrenal blood flow and the increase in blood flow during the reflex release of catecholamines

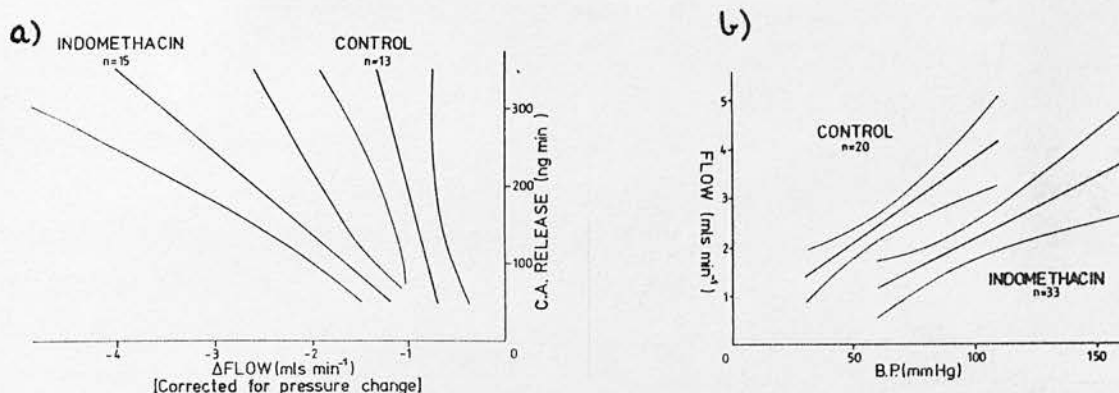


Figure 4: a) Resting pressure flow curves for left adrenal gland with and without indomethacin.

b) Regression of change in adrenal flow (corrected for pressure from figure a)) on catecholamine release. All lines show 95% confidence limits.

The depression of the resting pressure flow curve (seen figure 4a) suggests the involvement of prostaglandins in maintaining adrenal blood flow. Figure 4b) was derived using these curves to correct the flow changes accompanying the reflex rise in blood pressure. The results in figure 4b) after indomethacin imply a marked vasoconstriction associated with the release of catecholamines which is not seen without indomethacin. This suggests that prostaglandins are released with catecholamines and maintain adrenal blood flow by opposing their constricting action. This may also explain why platelet aggregation due to high concentrations of catecholamines does not occur in adrenal veins.

References:- in addition to those already listed in the publication as follows.

Critchley, J.A.J.H., Henderson, C.G., Moffat, L.E.F., Ungar, A., Waite, J. & West, Christine P. (1975) The release of catecholamines from perfused canine adrenal glands by corticosteroids. J. Physiol. 254, 30-31P.

Critchley, J.A.J.H., Tibenham, J. Isobel, Ungar, A., Waite, J. & West, Christine P. (1975) The effects of nicotinic and muscarinic agonist drugs on the release of catecholamines from the isolated perfused adrenal glands of the dog. Br. J. Pharmacol. 54, 259P.

Critchley, J.A.J.H. & Ungar, A. (1974) Do the anterior pituitary and adrenal cortex participate in the reflex response of the adrenal medulla to arterial hypoxia? J. Physiol. 239, 16-17P.

Daly, M. de B. & Scott, M. J. (1963) The cardiovascular responses to stimulation of the carotid body chemoreceptors in the dog. J. Physiol. 165, 179-197.

Feldberg, W.B., Minz, B. & Tsudzimura, H. (1934) The mechanism of the nervous discharge of adrenaline. J. Physiol. 81, 286-304.

Feuerstein, W., Feuerstein, G. & Gutman, Y. (1979) Endogenous prostaglandins modulate adrenal catecholamine secretion. Eur. J. Pharmacol. 58, 489-492.

Garren, L.D., Ney, R.L. & Davis, W.W. (1965) Studies on the role of protein synthesis in the regulation of corticosterone production by adrenocorticotrophic hormone in vivo. Proc. natn. Acad. Sci. U.S.A. 53, 1443-1450.

Marotta, S.F. (1972) Comparative effects of hypoxia, adrenocorticotrophin and methylcholine on adrenocortical secretory rates. Proc. Soc. Exp. Biol. Med. 141, 923-927.

Robinson, R.L. (1967) Stimulation of the catecholamine output of the isolated, perfused adrenal gland of the dog by angiotensin and bradykinin. J. Pharmac. Exp. Ther. 156, 252-257.

Terragno, N.A., Terragno, D.A. & McGiff, J.C. (1977) Contribution of prostaglandins to the renal circulation in conscious, anaesthetized and laparotomized dogs. Circ.Res. 40, 590-595.

Tsujimoto, A. & Nishikawa, T. (1975) Further evidence for nicotinic and muscarinic receptors and their interaction in dog adrenal medulla. Eur.J.Pharmacol. 34, 337-344.

Vogt, M. (1965) Release of medullary amines from the isolated, perfused adrenal gland of the dog. Br.J.Pharmacol. 24, 561-565.

Wurtman, R.J., Pohorecky, L.A. & Baliga, B.S. (1972) Adrenocortical control of the biosynthesis of epinephrine and proteins in the adrenal medulla. Pharmacol.Rev. 24, 411-426.

## THE REFLEX RELEASE OF ADRENALINE AND NORADRENALINE FROM THE ADRENAL GLANDS OF CATS AND DOGS

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### SUMMARY

1. We have studied the release of noradrenaline and adrenaline from the adrenal glands of dogs and cats in response to the lowering of carotid sinus pressure (baroreceptor tests) and to the perfusion of the vascularly isolated carotid bifurcations with hypoxic blood (chemoreceptor tests).

2. In cats, the resting output of catecholamines had a ratio of noradrenaline to adrenaline of 1:1. The ratio in the incremental release during baroreceptor tests rose to 3:1, and during chemoreceptor tests it fell to 1:6.

3. In dogs, the ratio of noradrenaline to adrenaline at rest was 1:4. The ratio did not change over a wide range of outputs during baroreceptor tests, chemoreceptor tests and splanchnic nerve stimulation.

4. The release of catecholamines in response to baroreceptor tests in the cat was abolished by hexamethonium bromide at doses that did not diminish the response to chemoreceptor tests.

### INTRODUCTION

In both the cat and the dog the adrenal glands release a mixture of adrenaline and noradrenaline into the bloodstream (Holtz *et al.* 1947; Bulbring & Burn, 1949). Many authors have argued for and against the independent control of the release of the two catecholamines. If one discounts work based on estimates of catecholamines in peripheral blood, where noradrenaline circulates from sympathetic adrenergic endings as well as from the adrenal glands, most of the evidence in favour of independent control comes from work on the cat (Von Euler & Folkow, 1953; Folkow & Von Euler, 1954; Düner, 1953), while most of the evidence against comes from work on the dog (Malmejac, 1964).

No direct comparison has been made between the two species, using similar techniques of stimulation, collection and assay. We therefore set out to apply to cats and dogs reflex stimuli that should, on the basis of previous work, preferentially release adrenaline or noradrenaline, and to assay both in the venous effluent of an adrenal gland.

Anichkov, Malyghina, Poskalenko & Ryzhenkov (1960) compared the effect of carotid occlusion with that of cyanide injection in cats. They found that the former produced a relatively greater vasoconstriction in a denervated hind limb, while the latter produced relatively greater contraction of a denervated nictitating membrane.

These results suggest that the baroreceptor reflex may preferentially control noradrenaline release, and the chemoreceptor reflex preferentially control adrenaline release. We have therefore used baroreceptor and chemoreceptor stimuli to test the adrenal gland for independent release of adrenaline and noradrenaline.

A preliminary account of part of this work has been published (Critchley, Ungar & Welburn, 1973).

#### METHODS

*Anaesthesia.* Dogs were anaesthetized with an i.v. injection of chloralose (55 mg/kg) and urethane (550 mg/kg) or of sodium pentobarbitone (30 mg/kg) (see Discussion). Cats were anaesthetized with an i.p. injection of sodium pentobarbitone (40 mg/kg). In both species anaesthesia was maintained by continuous i.v. infusion of the anaesthetic agent at a rate of about one tenth of the initial dose per hour, adjusted so as just to suppress the paw withdrawal reflex.

*Respiration, acid base balance and temperature control.* The trachea was cannulated and connected to a Starling 'Ideal' pump. The lungs were ventilated with a metered oxygen-nitrogen mixture so as to hold  $P_{a,CO_2}$  at 5 kPa in dogs and at 4 kPa in cats, and  $P_{a,O_2}$  above 20 kPa in both species, measured from frequent arterial blood samples on a Radiometer BMS 3 analyser. A molar solution of sodium bicarbonate was injected after each sample to hold the arterial plasma pH at 7.4. Body temperature was held near to 37 °C by a heating pad linked to a rectal thermistor probe.

*Carotid perfusion.* Both common carotid arteries were cannulated both ways and blood from one of them was perfused into both towards the head, by a Watson Marlow MHRE pump. Both superior thyroid, internal carotid and external carotid arteries were ligated, and any other branches between the point of cannulation and the origins of the lingual arteries. Only the lingual arteries were left open to maintain an adequate flow through the system and thus allow changes in blood composition to affect the carotid bodies rapidly.

A pressure transducer was connected to the perfusion circuit. The signal was passed through a servo amplifier to the perfusion pump so that perfusion pressure could be set and held constant.

*Stimulation of reflexes.* Tests were performed, and the method evaluated as described by Henderson & Ungar (1978). Baroreceptor tests consisted of a lowering of carotid perfusion pressure from a constant resting level, while the  $P_{a,O_2}$  of the perfusing blood was held high. Chemoreceptor tests consisted of a lowering of the  $P_{a,O_2}$  of the perfusing blood, at constant perfusion pressure, while infusing into it a 1 M solution of sodium dithionite at a rate of about 150 mg/min (Critchley & Ungar, 1975). The duration of each test was 60 s in dogs and 120 s in cats.

The systemic arterial blood gas tension did not change whilst sodium dithionite was infused into the carotid circuit. In two dogs the application of lignocaine to both carotid sinus nerves completely abolished the vascular and respiratory responses to baroreceptor and chemoreceptor tests.

Both vagosympathetic trunks were cut in the neck in order to abolish secondary reflexes from thoracic receptors.

*Collection of adrenal venous blood.* In the dog, the left adrenolumbar vein was cannulated towards the gland, and the venous outflow collected into refrigerated heparinized tubes after ligation of the adrenal vein.

In the cat, cannulation of the adrenolumbar vein creates too great a back pressure on the gland. We therefore made a closed sac of the main vein into which the left adrenal gland drained: either the left renal vein or the inferior vena cava. The sac was bypassed by a silicone rubber tube. Adrenal venous blood was collected from the sac by a double-lumen cannula, the dead space of the sac being washed through by perfusing 10% sucrose solution at 4 ml min<sup>-1</sup>.

*Estimation of catecholamines.* Adrenal venous blood was collected in centrifuge tubes containing measured volumes of 19% sucrose solution with EDTA 5 g/l and immediately centrifuged at 4 °C. The use of sucrose instead of salt was found to reduce the centrifugation time. The volumes of supernatant and of packed cells were recorded.

The samples were loaded onto Amberlite CG120 columns (mesh 100–200, length 20 mm,

diameter 2.5 mm). They were rinsed with 20 ml EDTA solution, 1 g/l, 2.5 ml phosphate buffer pH 6.5 and finally with 5 ml water. They were eluted with 4 ml *m*-hydrochloric acid and stored at 0 °C.

Trihydroxyindole derivatives were prepared by the method of Vendsalü (1960) with minor modifications (Critchley, 1976). This method gave an index of discrimination between adrenaline and noradrenaline of 8. Replicate estimates of plasma containing 5 nmol catecholamine/l gave a standard deviation of 0.5 nmol/l. The recovery of standards in plasma was 90%.

*Analysis of results.* The statistical significance of results was assessed by the paired *t* test.

*Drugs.* Chloralose and urethane (B.D.H. chemicals); sodium pentobarbitone (Abbott) and hexamethonium bromide (Koch Light).

## RESULTS

*Resting levels.* The mean outputs of noradrenaline and adrenaline from the left adrenal glands of five dogs under chloralose (eighty-three samples) and seven cats (thirty samples) at rest are shown in Table 1. In the dog, noradrenaline and adrenaline were released with a ratio of about 1:4, and a mean total output of 77 pmol min<sup>-1</sup> kg body wt<sup>-1</sup>. In the cat, the ratio was 1:1 with a mean total output of 35 pmol min<sup>-1</sup> kg body wt<sup>-1</sup>. In a further three dogs under pentobarbitone anaesthesia the ratio was again 1:4, with a mean total output of 48 ± 8 pmol min<sup>-1</sup> kg body wt<sup>-1</sup>.

*Baroreceptor and chemoreceptor tests.* The results are shown in Table 1. Baroreceptor and chemoreceptor tests in both species gave two to threefold increases in catecholamine output. In the dog, there was no change in the ratio of noradrenaline to adrenaline during either of the reflex responses. In the cat, on the other hand, the ratio of noradrenaline to adrenaline rose from 1:1 to 3:1 in the increment over control output during baroreceptor tests, and fell from 1:1 to 1:6 in the increment over control output during chemoreceptor tests. These changes are statistically significant (*P* < 0.01).

*The effect of magnitude of response on the ratio of catecholamines released.* In both dogs and cats we investigated the effect of varying the intensity of chemoreceptor and baroreceptor tests on the ratio of noradrenaline to adrenaline released. The results are shown in Fig. 1 in the form of a regression analysis of ratio on total release. Over a more than fivefold range of rate of release in each group, the ratio in cats rose significantly above the resting ratio during baroreceptor tests, and fell significantly below it during chemoreceptor tests. In dogs the ratio remained fixed throughout the ranges of both stimuli.

*Tests on dogs under pentobarbitone anaesthesia.* In view of the possibility that the selective release of noradrenaline and adrenaline could be due to anaesthetic rather than species differences, we carried out baroreceptor and chemoreceptor tests on five dogs under pentobarbitone anaesthesia. In six baroreceptor tests, in which the carotid sinus pressure was lowered from 120 to 90 mmHg, the mean total catecholamine output rose from 48 ± 8 to 105 ± 15 pmol min<sup>-1</sup> kg<sup>-1</sup>. In six chemoreceptor tests the mean total catecholamine output rose from 48 ± 8 to 80 ± 41 pmol min<sup>-1</sup> kg<sup>-1</sup>. The ratio of noradrenaline:adrenaline at rest was 0.25 ± 0.3, during baroreceptor tests was 0.22 ± 0.03 and during chemoreceptor tests was 0.20 ± 0.03.

Thus qualitatively the reflex responses of dogs under chloralose and under pentobarbitone were similar. Quantitatively the adrenal medulla was more responsive to baroreceptor tests under pentobarbitone than under chloralose. The responses to

TABLE 1. Outputs of noradrenaline (NA) and adrenaline (A) from the left adrenal glands of dogs and cats, at rest and during baroreceptor and chemoreceptor tests. \* indicates data significantly different from the corresponding controls ( $P < 0.01$ )

	Carotid perfusate			Arterial pressure (mmHg)	Left adrenal venous effluent		
	P (mmHg)	P <sub>O<sub>2</sub></sub> (kPa)	P <sub>CO<sub>2</sub></sub> (kPa)		NA (pmol min <sup>-1</sup> kg <sup>-1</sup> )	A (pmol min <sup>-1</sup> kg <sup>-1</sup> )	NA %
Control	142 ± 2	24 ± 3	5.4 ± 0.3	96 ± 13	14 ± 2	65 ± 5	19 ± 1
Baroreceptor test	84 ± 2	24 ± 3	5.4 ± 0.3	135	39	171	—
Increments	—	—	—	39 ± 14	25 ± 4*	106 ± 22*	19 ± 2
Control	142 ± 2	24 ± 3	5.4 ± 0.3	96 ± 13	14 ± 2	65 ± 5	19 ± 1
Chemoreceptor test	142 ± 2	4.4 ± 0.6	7.7 ± 0.7	179	27	138	—
Increments	—	—	—	83 ± 9	13 ± 2*	73 ± 17*	20 ± 3
Control	150 ± 2	28 ± 3	3.6 ± 0.1	81 ± 5	18 ± 3	17 ± 2	46 ± 4
Baroreceptor test	84 ± 4	28 ± 3	3.6 ± 0.1	140	37	26	—
Increments	—	—	—	59 ± 8	19 ± 4*	9 ± 2*	74 ± 6*
Control	149 ± 1	35 ± 6	3.9 ± 0.3	84 ± 5	18 ± 3	17 ± 2	46 ± 4
Chemoreceptor test	149 ± 1	5 ± 2	9.2 ± 1.5	164	48	89	—
Increments	—	—	—	83 ± 10	30 ± 17*	72 ± 3*	16 ± 5*

chemoreceptor tests, however, were substantially but variably inhibited by pentobarbitone. Except in one dog, lowering of the carotid perfusate  $P_{O_2}$  to between 4 and 5 kPa did not release catecholamines, although strong respiratory and vascular responses were obtained with stimuli of this intensity, but release was obtained in chemoreceptor tests where the  $P_{O_2}$  fell to 3 kPa.

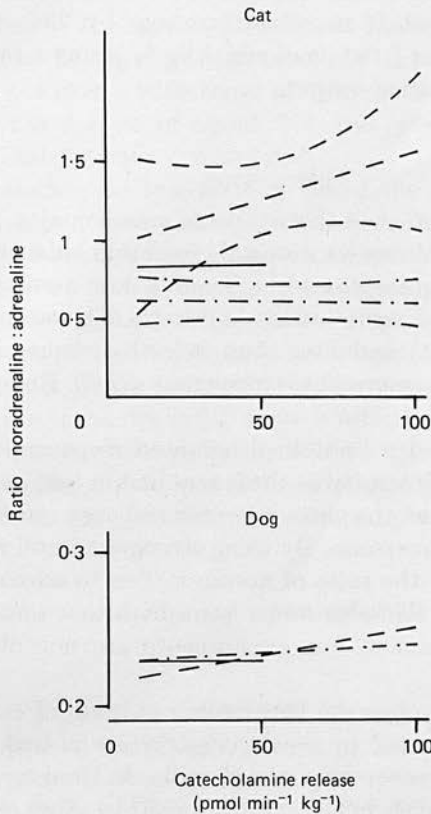


Fig. 1. The relationship between total catecholamine release and the ratio of noradrenaline to adrenaline. Regression lines of noradrenaline:adrenaline on total release. Continuous lines represent resting release, dashed lines release during baroreceptor tests, and dashed-dotted lines release during chemoreceptor tests. The upper graph represents results on five cats, with fifteen resting levels, nine chemoreceptor tests and twelve baroreceptor tests. The lower graph represents results in five dogs with thirty resting levels, fourteen chemoreceptor tests and fourteen baroreceptor tests. For each line the range of total release is greater than 5:1. The lines for chemoreceptor tests and baroreceptor tests in cats show 95% confidence limits. Note that the scale of the ordinate for dogs is 10 times that for cats.

*The effect of hexamethonium bromide on baroreceptor and chemoreceptor tests.* In three cats, we carried out baroreceptor and chemoreceptor tests before and after i.v. injection of hexamethonium bromide (2 mg/kg body wt). The mean resting output of catecholamines was  $36 \pm 4$  pmol min<sup>-1</sup> kg<sup>-1</sup>. This rose by  $38 \pm 17$  pmol min<sup>-1</sup> kg<sup>-1</sup> during baroreceptor tests and by  $22 \pm 11$  pmol min<sup>-1</sup> kg<sup>-1</sup> during chemoreceptor tests. After administration of hexamethonium bromide the resting output

was  $14 \pm 3$  pmol min<sup>-1</sup> kg<sup>-1</sup>. The output now rose by  $0.05 \pm 2$  pmol min<sup>-1</sup> kg<sup>-1</sup> during baroreceptor tests, and by  $41 \pm 21$  pmol min<sup>-1</sup> kg<sup>-1</sup> during chemoreceptor tests. The response to chemoreceptor tests was thus undiminished by a dose of hexamethonium bromide that abolished the response to baroreceptor tests.

*Electrical stimulation of the greater splanchnic nerve.* In three dogs fifteen tests of electrical stimulation of the greater splanchnic nerve were performed. During stimulation, the mean output of noradrenaline rose by  $224 \pm 63$  pmol min<sup>-1</sup> kg<sup>-1</sup>, and that of adrenaline by  $712 \pm 190$  pmol min<sup>-1</sup> kg<sup>-1</sup>, giving a ratio not significantly different from that in the resting output.

#### DISCUSSION

*Anaesthesia.* The choice of anaesthetic agents was a major problem. We found that in cats under pentobarbitone we were able to obtain balanced adrenal responses to chemoreceptor and baroreceptor tests, from a low resting level. Cats under chloralose have high resting outputs with a high ratio of noradrenaline to adrenaline (Kaindl & Von Euler, 1951) and also show selective depression of baroreceptor responses in contrast to chemoreceptor responses (Neil, Redwood & Schweitzer, 1949).

In dogs on the other hand we obtained balanced responses under chloralose to stimuli of the same order of intensity as those required in cats under pentobarbitone. In dogs under pentobarbitone, the chemoreceptor response was strongly inhibited in relation to the baroreceptor response. By using stronger stimuli we were nevertheless able to exclude any shift in the ratio of noradrenaline to adrenaline in the adrenal effluent of dogs with either stimulus under pentobarbitone anaesthesia. The differences between dogs and cats in our experiments are not due to differences in anaesthesia.

*Resting levels and size of responses.* Our resting outputs of catecholamines in the dog are similar to those reported by previous workers who took similar precautions to avoid excessive blood loss or hypoxia (Rapela & Houssay, 1952; De Schaepdryver, 1959). Our resting levels in the cat are similar to those reported by Feurstein & Gutman (1971) for cats under pentobarbitone anaesthesia.

The size of our reflex responses is similar to that obtained by De Schaepdryver (1959) with carotid occlusion, but far smaller than those found by other workers using more massive stimuli such as haemorrhage and asphyxia (Rapela & Houssay, 1952). The responses to electrical stimulation show that our preparations are capable of maximal outputs of catecholamines far greater than their reflex responses to discrete sensory stimuli.

*Selective release of noradrenaline and adrenaline.* Our results in the cat provide direct confirmation for the conclusion of Anichkov *et al.* (1960) that the arterial baroreceptors selectively control noradrenaline output from the adrenal glands while the arterial chemoreceptors selectively control adrenaline output. Since we are dealing with concentrations of catecholamines in the adrenal venous effluent, there is no question of our results being confused by noradrenaline circulating from peripheral sympathetic endings.

In the dog we have found no evidence for selective control of the release of

noradrenaline or of adrenaline from the adrenal medulla. Our results are compatible with those of previous workers on the dog (Malmejac, 1964; De Schaepdryver, 1959; Wurtman, Casper, Pohorecky & Bartler, 1968) who failed to find evidence for selective release from the adrenal medulla in response to physiological stimuli.

Having studied the reflex release of catecholamines in substantially similar preparations in dogs and cats, we support the view that the controversy on selective release can be resolved by the species difference in the control of the adrenal medulla between dogs and cats. In the dog the ratio of noradrenaline to adrenaline does not deviate from about 1:4 over a wide range of outputs. In the cat, on the other hand, the resting output has a ratio of about 1:1, but when release is stimulated the ratio can swing at least between 1:6 and 3:1.

*The effect of hexamethonium bromide.* We found the release of catecholamines in response to baroreceptor stimulation in the cat to be abolished by hexamethonium bromide at a dose that did not diminish similar responses to chemoreceptor stimulation. This finding may be relevant to the observation of Douglas & Poisner (1965), in isolated cat adrenal glands, that noradrenaline is preferentially released by nicotinic agonist drugs and adrenaline by muscarinic agonists. It also matches the findings of Henderson & Ungar (1978) that reflex vasoconstriction in the hind limb of the dog in response to baroreceptor tests is selectively inhibited by hexamethonium bromide, and that to chemoreceptor tests by hyoscine methyl bromide. There thus appear to be parallel nicotinic and non-nicotinic pathways both to chromaffin cells and to sympathetic ganglia mediating baroreceptor and chemoreceptor reflexes respectively.

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#### REFERENCES

- ANICHKOV, S. V., MALYGHINA, E. I., POSKALENKO, A. N. & RYZHENKOV, V. E. (1960). Reflexes from carotid bodies upon the adrenals. *Archs int. Pharmacodyn. Ther.* **129**, 156-165.
- BULBRING, E. & BURN, J. H. (1949). Liberation of noradrenaline from the suprarenal gland. *Br. J. Pharmac.* **4**, 202-208.
- CRITCHLEY, J. A. J. H. (1976). Reflex and neuroendocrine mechanisms of catecholamine release from the adrenal medulla. PhD Thesis, Edinburgh University, Edinburgh.
- CRITCHLEY, J. A. J. H. & UNGAR, A. (1975). A chemical method of lowering the  $P_{O_2}$  of blood in experimental studies of arterial chemoreceptor reflexes. *J. Physiol.* **244**, 12-13P.
- CRITCHLEY, J. A. J. H., UNGAR, A. & WELBURN, P. J. (1973). The release of adrenaline and noradrenaline by the adrenal glands of cats and dogs in reflexes arising from the carotid chemoreceptors and baroreceptors. *J. Physiol.* **234**, 111-112P.
- DE SCHAEPPDRYVER, A. F. (1959). Physio-pharmacological effects on suprarenal secretion of adrenaline and noradrenaline in dogs. *Archs int. Pharmacodyn. Ther.* **121**, 222-244.
- DOUGLAS, W. W. & POISNER, A. M. (1965). Preferential release of adrenaline from the adrenal medulla by muscarinic and pilocarpine. *Nature, Lond.* **208**, 1102-1103.
- DÜNER, H. (1953). The influence of the blood glucose level on the secretion of adrenaline and noradrenaline from the suprarenal. *Acta physiol. scand.* **28**, Suppl. 102.
- EULER, U. S. VON & FOLKOW, B. (1953). Einfluss verschiedener afferenter Nervenreize auf die Zusammensetzung des Nebennierenmarkinkretes bei der Katze. *Arch. exp. Path. Pharmac.* **219**, 242-247.
- FEUERSTEIN, G. & GUTMAN, Y. (1971). Preferential secretion of adrenaline or noradrenaline by the cat adrenal *in vitro* in response to different stimuli. *Br. J. Pharmac.* **43**, 764-775.

- FOLKOW, B. & EULER, U. S. VON. (1954). Selective activation of noradrenaline and adrenaline producing cells in the cat's adrenal gland by hypothalamic stimulation. *Circulation Res.* **2**, 191-195.
- HENDERSON, C. G. & UNGAR, A. (1978). Effect of cholinergic antagonists on sympathetic ganglionic transmission of vasomotor reflexes from the carotid baroreceptors and chemoreceptors of the dog. *J. Physiol.* **277**, 379-385.
- HOLTZ, P., CREDNER, K. & KRONEBERG, G. (1947). Über das sympathicomimetische pressionschie Prinzip des Harns (Urosympathin). *Arch. exp. Path. Pharmacol.* **204**, 228-243.
- KAINDL, F. & EULER, U. S. VON. (1961). Liberation of noradrenaline and adrenaline from the suprarenals of the cat during carotid occlusion. *Am. J. Physiol.* **166**, 284-288.
- MALMEJAC, J. (1964). Activity of the adrenal medulla and its regulation. *Physiol. Rev.* **44**, 186-218.
- NEIL, E., REDWOOD, C. R. M. & SCHWEITZER, A. (1949). Pressor responses to electrical stimulation of the carotid sinus nerve in cats. *J. Physiol.* **109**, 259-271.
- RAPELA, C. E. & HOUSSAY, B. A. (1952). Adrenalina y noradrenalina de la sangre suprarenal del perro durante la asfixia. *Rev. Soc. Argent. Biol.* **28**, 7-11.
- VENDSALÜ, A. (1960). Studies on adrenaline and noradrenaline in human plasma. *Acta physiol scand.* **49**, suppl. 173.
- WURTMAN, R. J., CASPER, A., POHORECKY, C. A. & BARTLER, F. C. (1968). Impaired secretion of epinephrine in response to insulin among hypophysectomized dogs. *Proc. natn. Acad. Sci. U.S.A.* **61**, 522-528.