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With 4 text-figures

THE ROLE OF THE PITUITARY–ADRENOCORTICAL AXIS IN REFLEX RESPONSES OF THE ADRENAL MEDULLA OF THE DOG

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SUMMARY

1. The release of catecholamines from the adrenal medulla, in response to carotid body hypoxia, may outlast the stimulus by more than 30 min.

2. After denervation of the adrenal gland the immediate release of catecholamines in response to carotid hypoxia is abolished, but the prolonged release remains.

3. The prolonged release of catecholamines is abolished by cycloheximide.

4. Both corticotrophin *in vivo* and hydrocortisone in the isolated perfused adrenal gland release adrenomedullary catecholamines.

5. It is concluded that a component of the response of the adrenal medulla to carotid body hypoxia is mediated by corticotrophin and corticosteroid release.

INTRODUCTION

In previous work from this laboratory we studied the response of the adrenal medulla to short hypoxic stimuli, localized to the carotid bifurcations, lasting 30–60 sec (Critchley, Ungar & Welburn, 1973; Critchley, 1976; Critchley, Ellis & Ungar, 1980). 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 10 min, the release of catecholamines outlasted the stimulus by at least a further 30 min, although both the adrenal glands and the central nervous system remained perfused with well-oxygenated blood throughout the experiment.

Carotid chemoreceptor stimulation releases corticotrophin from the anterior lobe of the pituitary gland, and thus corticosteroids from the adrenal cortex (Anichkov, Malyghina, Poskalenko & Ryzenkov, 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 (Wurtman, Pohorecky & Baliga, 1972). Little is known, however, of the influence of corticosteroids upon the release, as distinct from the synthesis, of medullary catecholamines.

We have now investigated the possibility that the sustained release of catecholamines in response to transient carotid hypoxia could be mediated by the pituitary–adrenocortical axis.

Preliminary reports of parts of this work have been published (Critchley & Ungar, 1974; Critchley, Henderson, Moffatt, Ungar, Waite & West, 1975).

METHODS

Anaesthesia, respiration, acid–base balance and temperature control

Dogs were anaesthetized with an I.V. injection of chloralose (55 mg/kg) and urethane (550 mg/kg). Anaesthesia was maintained by a continuous I.V. infusion of the anaesthetic mixture adjusted so as just to suppress the paw withdrawal reflex. 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 and P_{a,O_2} above 20 kPa, measured from frequent arterial blood samples on a Radiometer BMS3 analyser. A molar solution of sodium bicarbonate was injected when necessary to restore arterial plasma pH to 7·4. Body temperature was held near to 37 °C by a heating pad controlled from a rectal thermistor probe. Heparin (500 i.u./kg) was injected after the completion of surgery. Dextran was infused if necessary to prevent the mean systemic arterial pressure from falling below 75 mmHg.

Carotid perfusion

Both common carotid arteries were cannulated both ways, and blood from one was perfused into both arteries towards the head by a Watson Marlow MHRE pump. The superior thyroid, internal carotid and external carotid arteries and any other branches between the point of cannulation and the origins of the lingual arteries, were ligated. The lingual arteries were left open to maintain an adequate flow through the system and thus allow changes in the perfusing blood to affect the carotid bodies quickly, but were occluded to test the vascular isolation of the carotid bifurcations. A pressure transducer was connected to the perfusion circuit and linked through a servo amplifier to the perfusion pump so that perfusion pressure could be held constant, or varied at will.

Stimulation of reflexes

Chemoreceptor tests were performed and evaluated as described by Henderson and Ungar (1978). They consisted of a lowering of the P_{O_2} of the perfusing blood by infusing into it a solution of sodium dithionite (Critchley & Ungar, 1975). Carotid sinus pressure was held during chemoreceptor tests at 150 or 140 mmHg. Baroreceptor tests were performed by lowering the carotid perfusion pressure by 40 mmHg for 60 sec.

Both vagosympathetic trunks were divided in the neck to abolish secondary reflexes from thoracic receptors. In two dogs the application of lignocaine (2 % solution) to both carotid sinus nerves abolished the release of catecholamines, as well as the respiratory and cardiovascular responses to chemoreceptor and baroreceptor tests.

Collection of adrenal venous blood and estimation of catecholamines

The left adrenolumbar vein was cannulated towards the gland, and the adrenal vein ligated between the gland and the vena cava. The outflow was collected for timed periods in cooled tubes. The concentrations of adrenaline and noradrenaline in the plasma were estimated fluorometrically, after extraction on ion exchange columns, as described by Critchley *et al* (1980). By catecholamine concentration, throughout this paper, we mean the sum of the concentrations of adrenaline and noradrenaline, separately estimated. The fraction of adrenaline in the mixture did not vary in resting or evoked releases outside the range of 75–80 %. Replicate estimates of plasma containing 5 nM-catecholamine gave a standard deviation (S.D.) of 0·5 nM. The recovery of standards from plasma was 90 %.

Arterial concentrations of catecholamines during reflex responses did not exceed 2 × plasma blanks.

Denervation of the left adrenal gland

The left splanchnic nerve was identified and cut about 30 mm from the adrenal gland. The retroperitoneal connective tissue was dissected in an arc around the gland to interrupt additional sympathetic fibres, particularly around the arteries supplying the gland. The abolition of the

adrenal response to baroreceptor tests, while the pressor response remained, was taken as evidence of complete denervation.

Isolated adrenal glands

Dogs, used for unrelated acute experiments, were anaesthetized and heparinized as above. Each adrenolumbar vein was cannulated towards the gland, the adrenal vein tied between the gland and the vena cava, and the gland excised leaving its arterial orifices open. The gland was flushed through with Locke's solution (Na^+, 156 mM; K^+, 5·6 mM; Ca^{2+}, 4·3 mM; Cl^-, 164 mM; HCO_3^- 1·8 mM; glucose 5 mM) and suspended by its cannula from a Langendorff isolated heart perfusion apparatus, perfused with Locke's solution at 37 °C at a constant flow of 2 ml./min.

The effluent from the arterial orifices was collected for 30 sec periods in glass tubes in a Unicam AC60 autoanalyser, which delivered the reagents for catecholamine assay, without the preliminary extraction as for plasma.

Drugs

Chloralose, (BDH); corticotrophin, (Ciba); cycloheximide, (Sigma); heparin, (Evans); hydrocortisone, (Glaxo); urethane, (BDH).

RESULTS

Resting output of catecholamines in dogs with innervated adrenal glands

In twenty-two dogs the mean resting output of catecholamines from the left adrenal gland was 57 p-mole/min . kg, with a S.D. of 20. In those dogs which did not undergo irreversible procedures, such as denervation, the resting levels remained stable over the duration of the experiment; over a period of 90 min the S.D. of the output did not exceed 25% of the resting output.

Chemoreceptor tests in dogs with innervated adrenal glands

In each of six dogs a baroreceptor test, lasting 1 min, and 10 min later a chemoreceptor test, of 10 min duration, were performed. The mean output of catecholamines in the course of these tests is shown in the top panel of Fig. 1. In each test a rapid response was followed by a sustained output of catecholamines which outlasted the stimulus by 20–60 min. During these tests the mean systemic blood pressure rose from 73 ± 8 to 105 ± 20 mmHg.

Chemoreceptor tests in dogs with denervated adrenal glands

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 by a neural mechanism. The crucial test of such a hypothesis is to divide the motor nerves supplying the effector organ.

In six dogs with denervated left adrenal glands, baroreceptor tests lasting 1 min were performed to confirm the total denervation of the gland. Ten minutes later chemoreceptor tests, lasting 20 min, were performed. The mean results are shown in the middle panel of Fig. 1. The output of catecholamines did not rise during the baroreceptor test, although the usual reflex rise in systemic blood pressure was seen. All the denervated glands responded to 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 min of a test. The time course of a single response is shown in Fig. 2.

Chemoreceptor tests in dogs with fully isolated carotid bifurcations

In three dogs the lingual arteries were cannulated, completing the vascular isolation of the carotid bifurcations, and blood was returned through a resistance to a femoral vein. Two of the dogs had denervated left adrenal glands. They gave no response in baroreceptor test nor in the early phase of chemoreceptor tests, but

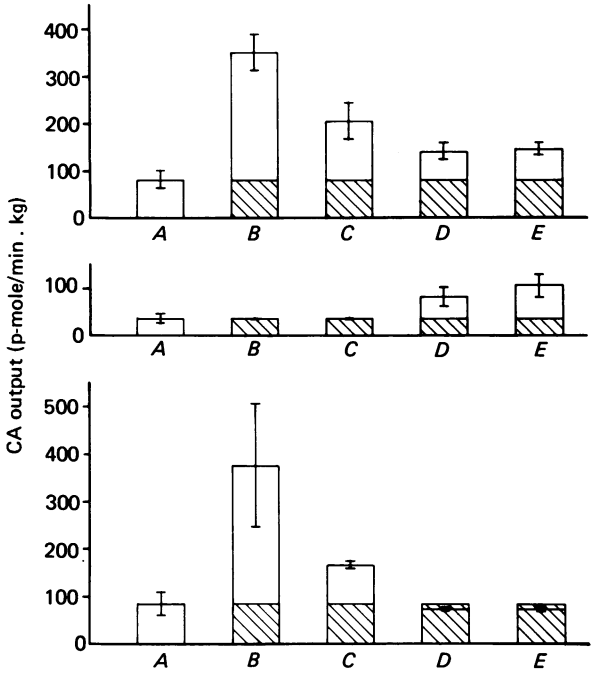

Fig. 1. Responses of dogs to baroreceptor and chemoreceptor tests. Mean responses of three groups of six dogs. The columns represent the output of catecholamines from the left adrenal gland (CA output). In each panel column *A* is the resting output, *B* the output during a baroreceptor test, *C* the output 5 min after the start of a chemoreceptor test, *D* the output 10 min after the end of a chemoreceptor test and *E* the output 20 min after the end of a chemoreceptor test. The vertical bars represent the S.E. of the mean output in *A* and of the mean increment above resting level (hatched) in *B–E*. The top panel shows the response of untreated dogs. In the chemoreceptor tests, the P_{O_2} of the carotid perfusate was reduced from 15 ± 1 to $5 \pm 0{\cdot}2$ kPa for 10 min. The middle panel shows the responses of dogs with denervated left adrenal glands. In the chemoreceptor tests, the P_{O_2} of the carotid perfusate was reduced from 18 ± 2 to $5 \pm 0{\cdot}2$ kPa for 20 min. The bottom panel shows the responses of dogs given cycloheximide 50 mg/kg, 20 min before the test. In the chemoreceptor tests, the P_{O_2} of the carotid perfusate was reduced from 16 ± 3 to $4 \pm 0{\cdot}3$ kPa.

catecholamine output rose to 3× and 4× the resting output 20 min after the end of the chemoreceptor stimulus. The third dog, whose adrenal innervation was intact, showed a 3× increase in output in a baroreceptor test, and 4× increases in output both during and 20 min after the chemoreceptor test.

The patterns of these responses are identical with those of dogs having open lingual arteries, and thus exclude the possibility that any part of the responses could be mediated by structures reached directly by lingual arterial blood.

The effect of cycloheximide on the responses of innervated glands to chemoreceptor and baroreceptor tests

Cycloheximide is a drug which inhibits the release of corticosteroids in response to corticotrophin (Garren, Ney & Davis, 1965). We performed tests before and after the I.V. injection of cycloheximide (50 mg/kg) in six dogs. The results are shown in the bottom panel of Fig. 1. The immediate release of catecholamines during both

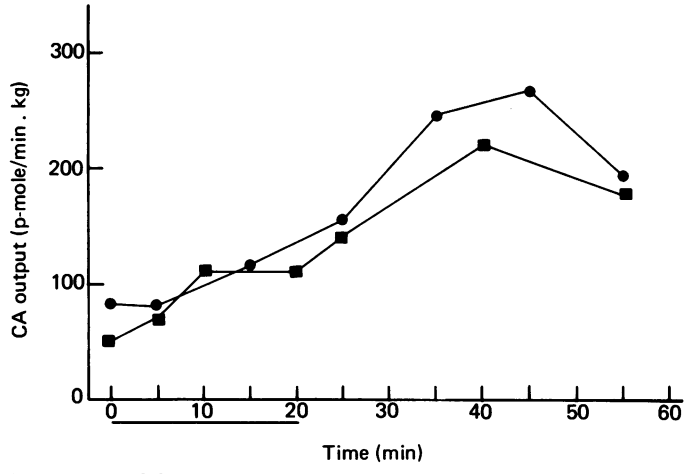

Fig. 2. The time course of the responses to chemoreceptor tests and to corticotrophin. Time course of the output of catecholamines (CA output) from the denervated left adrenal glands of two dogs. Circles indicate the response to a chemoreceptor test lasting 20 min and squares the response to an infusion of corticotrophin 25 μg over 20 min.

chemoreceptor and baroreceptor tests was not impaired by cycloheximide. In all six dogs, however, the sustained release in response to the chemoreceptor tests was abolished by cycloheximide.

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

The effect of exogenous corticotrophin on adrenomedullary catecholamine output in vivo

In six dogs corticotrophin (25 μg) was infused intravenously. The mean results are shown in Fig. 3 and the time course of a single response in Fig. 2. In two of these dogs regular baroreceptor tests were performed before and after the administration of corticotrophin. The release of catecholamines during baroreceptor stimulation was potentiated 3–4-fold at the peak of the response to corticotrophin.

Corticotrophin increased the output of catecholamines with a similar time course to that shown by prolonged chemoreceptor tests. At the peak of the action of corticotrophin the response to baroreceptor tests was potentiated.

In three dogs corticotrophin (25 μg) was infused 50 min after the injection of cycloheximide (50 mg/kg). The mean output of catecholamines rose from 21 ± 12 p-mole/min.kg by a mean of 13 ± 5 p-mole/min.kg. This can be compared with

the rise in dogs untreated with cycloheximide, from a mean of 86 ± 16 p-mole/min . kg by a mean of 216 ± 76 p-mole/min . kg.

The release of catecholamines by hydrocortisone from isolated perfused adrenal glands

Seven isolated adrenal glands were perfused. Hydrocortisone was added to each perfusate at concentrations of 30, 50 and 100 μg/ml. for 5 min periods. The effect on the catecholamine output into the effluent is shown in Fig 4 *A*. These concentrations

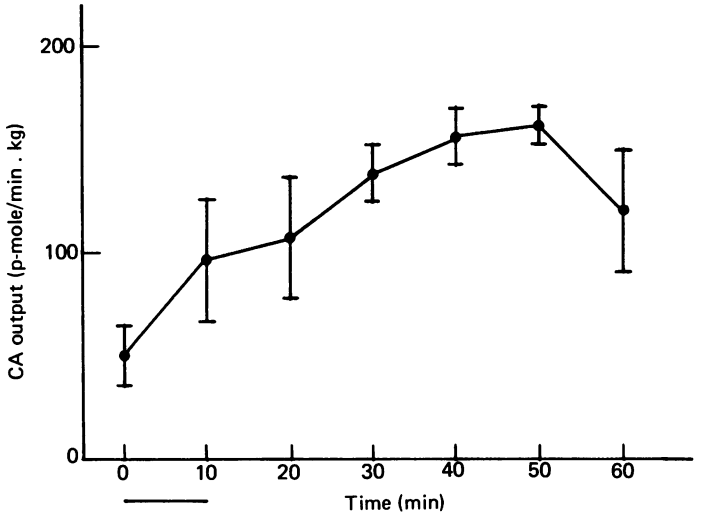

Fig. 3. The response of the adrenal medulla to corticotrophin. Mean output of catecholamines from the left adrenal glands of six dogs (CA output). The bar indicates the intravenous infusion of 25 μg corticotrophin. Vertical bars represent S.E.

of corticosteroid are within the physiological range for adrenal portal blood, if one assumes that the adrenal blood flow was of the order of 1 % of the cardiac output.

The time course of the response to the middle dose of hydrocortisone is shown in Fig. 4*B*. The time of onset of the release of catecholamines is similar to that of the late component of the response to chemoreceptor tests, and to that of the response to corticotrophin.

Three isolated adrenal glands were each perfused for 10 min with Locke's solution containing cycloheximide (1 mg/ml.), a concentration far higher than that circulating in the experiments *in vivo*. There was no change in the output of catecholamines.

DISCUSSION

We have previously reported (Critchley *et al.* 1980; Critchley, 1976) that in baroreceptor and chemoreceptor tests lasting 1 min the output of catecholamines from the left adrenal gland rose to between 2 × and 5 × the resting output. The output invariably returned to or to below the resting level within 2 min after baroreceptor tests, and within 5 min after chemoreceptor tests. The last figure is certainly an

overestimate, because of the time taken to wash hypoxic blood out of the perfusion system.

In the present experiments we used chemoreceptor tests lasting 10 or 20 min, and the response was always sustained for 20–60 min after the end of the stimulus. Although the hypoxic stimulus was milder than that used in the short tests, the peak

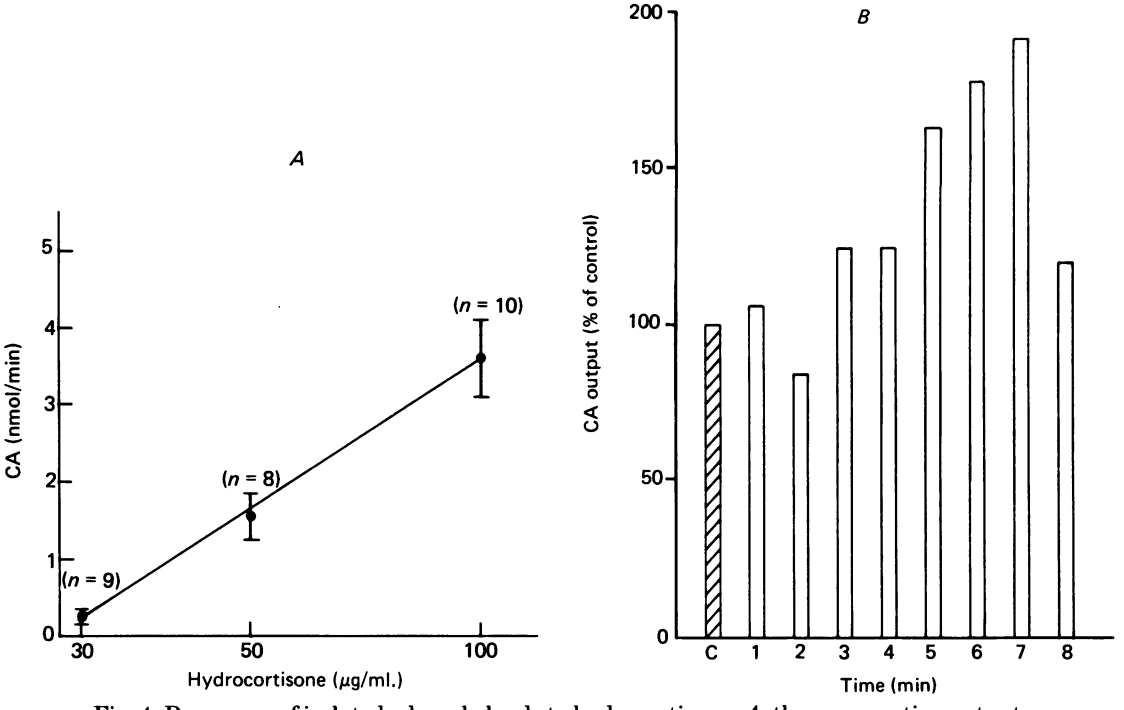

Fig. 4. Responses of isolated adrenal glands to hydrocortisone. *A*, the mean resting output of catecholamines (CA) from seven canine glands perfused with Locke's solution at 2 ml./min and 37 °C was 2·6 ± 1·0 n-mole/min. The graph shows the incremented outputs when the glands were exposed to three concentrations of hydrocortisone in the perfusate. The vertical bars indicate standard deviations and the numbers of tests are shown in brackets. *B*, the mean time course of the normalized output of catecholamines (CA output) in response to the middle concentration of hydrocortisone shown in *A*, added to the perfusate for 5 min as indicated by the horizontal bar.

output of catecholamines was greater. We were able to separate the response into a fast and a slow phase. The fast phase, together with the baroreceptor response, was abolished by denervation of the adrenal gland. The slow phase was abolished by cycloheximide, and corresponded in its time course with the response to exogenous corticotrophin.

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.

We were surprised to find no previous investigation of the direct effect of adrenocortical function on the release of adrenal catecholamines, although their action on the synthesis of adrenaline has been studied in detail. Wurtman, Casper, Pohorecky & Bartter (1968) studied the release of adrenomedullary catecholamines in dogs in response to insulin-hypoglycaemia. They compared the responses of normal dogs

TABLE 1. Insulin hypoglycaemia in normal dogs, hypophysectomized dogs, and hypophysectomized dogs treated with corticotrophin

	Resting output (p-mole/min . kg)			Peak response to insulin (p-mole/min . kg)		
	Noradrenaline	Adrenaline	Total	Noradrenaline	Adrenaline	Total
Normal	8·6	40·5	49·1	37·8	172·3	210·1
Hypophysectomized	14·0	22·7	36·7	51·3	84·2	135·5
Hypophysectomized + corticotrophin	16·7	38·9	55·6	174·4	401·8	576·2

Data recalculated from the results of Wurtman *et al.* (1968).

Resting output and peak stimulated output of adrenaline and noradrenaline from the adrenal glands of five dogs.

with those of hypophysectomized dogs, and of hypophysectomized dogs treated with corticotrophin. Their results were interpreted entirely with regard to changes induced by corticosteroids in the synthesis of adrenaline, and are expressed as outputs and percentages of adrenaline in adrenal venous plasma. In Table 1 we have recalculated their data to show resting and evoked outputs of adrenaline and noradrenaline. It is evident that corticotrophin increased the absolute output of noradrenaline, as well as that of adrenaline, in the resting state and particularly during insulin hypoglycaemia. Their results do not seem compatible with an action of corticosteroids solely on adrenaline synthesis. They are more simply explained by an action on the release of catecholamines, and suggest both direct release by steroids and the potentiation of the effect of hypoglycaemia on the output of both amines. This action of corticotrophin resembles the potentiation of the baroreceptor reflex that we have seen. There could thus be two parallel actions of corticosteroids, one on synthesis and the other on release. A further, and simpler, possibility, which seems to us to be compatible with all the work reviewed by Wurtman *et al.* (1972), is that induction of PNMT could be secondary to release of adrenaline, as happens with dopamine β-hydroxylase and other synthetic enzymes. If in fact steroids do not act directly on release, but only by the induction of synthesis, then it follows both from our results and from those of Wurtman *et al.* (1968) that enhanced synthesis and overflow of noradrenaline must be as important a part of the response as that of adrenaline.

In these experiments we have found that moderate hypoxia, with carotid arterial P_{O_2} between 6·0 and 6·5 kPa was sufficient to evoke the release of catecholomines. In previous experiments we found that a lower cartoid arterial P_{O_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.

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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. Thér.* **129**, 156–165.

CRITCHLEY, J. A. J. H. (1976). Reflex and neuroendocrine mechanisms of catecholamine release from the adrenal medulla. Ph. D Thesis, Edinburgh University, Edinburgh.

CRITCHLEY, J. A. J. H., ELLIS, P. & UNGAR, A. (1980). The reflex release of adrenaline and noradrenaline from the adrenal glands of cats and dogs. *J. Physiol.* **298**, 71–78.

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**, 30–31*P*.

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**, 16–17*P*.

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**, 12–13*P*.

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**, 111–112*P*.

HENDERSON, C. G. & UNGAR, A. (1978). Effect of cholinergic antagonistics on sympathetic ganglionic transmission of vasomotor reflexes from the carotid baroreceptors and chemoreceptors of the dog. *J. Physiol.* **277**, 379–385.

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.

WURTMAN, R. J., CASPER, A., POHORECKY, L. A. & BARTTER, F. C. (1968). Impaired secretion of epinephrine in response to insulin among hypophysectomized dogs. *Proc. natn. Acad. Sci. U.S.A.* **61**, 522–528.

WURTMAN, R. J., POHORECKY, L. A. & BALIGA, B. S. (1972). Adrenocortical control of the biosynthesis of epinephrine and proteins in the adrenal medulla. *Pharmac. Rev.* **24**, 411–426.