J. Physiol. (1980), **298**, *pp.*71-78
With 1 *text-figure*
Printed in Great Britain

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

BY J. A. J. H. CRITCHLEY, P. ELLIS AND A. UNGAR

From the Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ

(*Received 3 May 1979*)

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 hexamethomium 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.

0022-3751/80/6440-0189 $07.50

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^{-1}.

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^{-1} kg body wt^{-1}. In the cat, the ratio was 1:1 with a mean total output of 35 pmol min^{-1} kg body wt^{-1}. In a further three dogs under pentobarbitone anaesthesia the ratio was again 1:4, with a mean total output of 48 ± 8 pmol min^{-1} kg body wt^{-1}.

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^{-1} kg^{-1}. In six chemoreceptor tests the mean total catecholamine output rose from 48 ± 8 to 80 ± 41 pmol min^{-1} kg^{-1}. The ratio of noradrenaline:adrenaline at rest was $0·25 \pm 0·3$, during baroreceptor tests was $0·22 \pm 0·03$ and during chemoreceptor tests was $0·20 \pm 0·03$.

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			Artenal pressure (mmHg)	Left adrenal venous effluent		
	P (mmHg)	P_{O_2} (kPa)	P_{CO_2} (kPa)		NA (pmol $min^{-1} kg^{-1}$)	A (pmol $min^{-1} kg^{-1}$)	NA %
				Dogs ($n = 5$)			
Control	142 ± 2	24 ± 3	5·4 ± 0·3	96 ± 13	14 ± 2	65 ± 5	19 ± 1
Baroreceptor test ($n = 36$)	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 ($n = 23$)	142 ± 2	4·4 ± 0·6	7·7 ± 0·7	179	27	138	—
Increments	—	—	—	83 ± 9	13 ± 2*	73 ± 17*	20 ± 3
				Cats ($n = 7$)			
Control	150 ± 2	28 ± 3	3·6 ± 0·1	81 ± 5	18 ± 3	17 ± 2	46 ± 4
Baroreceptor test ($n = 14$)	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 ($n = 10$)	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.

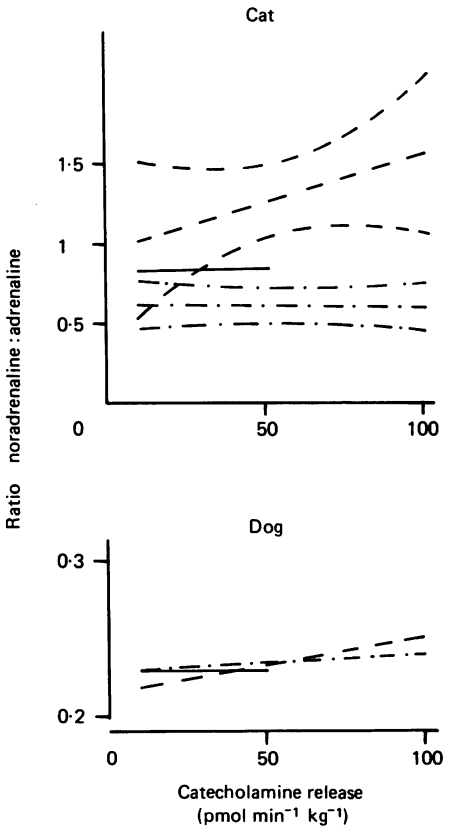

Fig. 1. The relationship between total catecholamine release and the ratio of noradrenaline to adrenaline. Regression lines of noradrenaline:adrenaline on total release. Continuous lines represent resting release, dashed lines release during baroreceptor tests, and dashed-dotted lines release during 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 ± 4 pmol min^{-1} kg^{-1}. This rose by 38 ± 17 pmol min^{-1} kg^{-1} during baroreceptor tests and by 22 ± 11 pmol min^{-1} kg^{-1} during chemoreceptor tests. After administration of hexamethonium bromide the resting output

was 14 ± 3 pmol min^{-1} kg^{-1}. The output now rose by $0{\cdot}05 \pm 2$ pmol min^{-1} kg^{-1} during baroreceptor tests, and by 41 ± 21 pmol min^{-1} kg^{-1} 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 ± 63 pmol min^{-1} kg^{-1}, and that of adrenaline by 712 ± 190 pmol min^{-1} kg^{-1}, 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 muscarnic 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.

We are grateful for the expert technical assistance of Mr P. H. Whelpdale. P. Ellis is an M.R.C. Training Scholar.

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**, 12–13*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*.

DE SCHAEPDRYVER, 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 muscanine 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. Pharmak.* **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). Uber das sympathicomimetische pressonschie Prinzip des Harns (Urosympathin). *Arch. exp. Path. Pharmak.* **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.