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Muscarinic and nicotinic mechanisms in the responses of the adrenal medulla of the dog and cat to reflex stimuli and to cholinomimetic drugs

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1 In isolated perfused adrenal glands of the cat, muscarinic and nicotinic agonists selectively released adrenaline and noradrenaline respectively.

2 In isolated perfused adrenal glands of the dog, the output of adrenaline and noradrenaline remained in a fixed ratio at rest and when stimulated by muscarinic or by nicotinic agonists.

3 In the anaesthetized dog, a combination of muscarinic and nicotinic antagonists was needed to block reflex responses of the adrenal medulla. A nicotinic antagonist was more effective in blocking the baroreceptor reflex response than the chemoreceptor reflex response.

Introduction

Feldberg *et al.* (1934) found that the response of the adrenal medulla of the cat to acetyl choline, and to splanchnic nerve stimulation, could be abolished by a combination of nicotine and atropine, but not by either alone. This was the first evidence that both nicotinic and muscarinic transmission may be involved in the control of adrenal medullary secretion.

Douglas & Poisner (1965), again in the cat, found that drugs which act at muscarinic receptors selectively affect the release of adrenaline, while drugs which act at nicotinic receptors selectively affect the release of noradrenaline from the adrenal gland.

The possibility of separate physiological roles for nicotinic and muscarinic receptors was confirmed in the cat by Critchley *et al.* (1980), who showed that carotid baroreceptor and chemoreceptor activity selectively regulates adrenal release of noradrenaline and adrenaline respectively, and that the former is abolished by hexamethonium at a dose that does not inhibit the latter. In the dog, however, baroreceptor and chemoreceptor activity regulated the total output of catecholamines, but not the ratio of adrenaline to noradrenaline.

The experiments described in this paper were done to see whether the species difference found by Critchley *et al.* (1975, 1980) reflects different roles for nicotinic and muscarinic receptors in the adrenal medullae of dogs and cats, considered both from the point of view of central connections, and of the selective control of noradrenaline and adrenaline release.

Methods

Estimation of catecholamines

The concentrations of adrenaline and noradrenaline in plasma and perfusate were estimated fluorometrically, as described by Critchley *et al.* (1980). For plasma the estimation was performed after extraction on ion-exchange columns, but for perfusate no preliminary extraction was used. Plasma catecholamine concentrations were in the range 5–50 nM.

Replicate estimates of plasma containing 5 nM adrenaline or noradrenaline gave a standard deviation of 0.5 nM. The recovery of standards from plasma was 90%. During reflex responses arterial catecholamine concentrations did not rise above 2 × plasma blank, and were ignored.

Isolated perfused adrenal glands

Dogs and cats were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v. and 40 mg kg^{-1}, i.p. respectively) and heparinized. Both adrenolumbar veins were cannulated, the adrenal veins tied, and the glands excised, leaving their arterial orifices open. Each gland was flushed with a heparinized solution, and suspended by its cannula from a Langendorff heart perfusion apparatus, perfused at 37°C with an oxygenated modified Locke solution (concentration mM: Na^+ 154, K^+ 5.6, Ca^{2+} 2.2, Cl^- 164, HCO_3^- 1.8,

HPO_4^{2-} 2.15, $H_2PO_4^-$ 0.85 and glucose 10) at a constant flow of 2 ml min^{-1}. The effluent from the arterial orifices was collected for 30 s periods in glass tubes in a Unicam AC60 autoanalyser, which both served as a fraction collector and delivered the metered reagents for the fluorometric assay of catecholamines.

Anaesthesia, respiration and temperature

Mongrel dogs of either sex, weighing 11–25 kg were anaesthetized with an i.v. injection of chloralose (55 mg kg^{-1}) and urethane (550 mg kg^{-1}). 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 Pa_{CO_2} at 5 kPa and Pa_{O_2} above 20 kPa, measured from regular arterial blood samples on a Radiometer BMS3 analyser. A molar solution of sodium bicarbonate was injected when necessary to restore the plasma pH to 7.4. Body temperature was held near to 37°C by a heating pad controlled from a rectal thermistor probe. Before the abdomen was opened, indomethacin (5 mg kg^{-1}) was injected to prevent the progressive fall in blood pressure that results from handling of the abdominal viscera (Ellis & Ungar, 1981). Heparin (500 iu kg^{-1}) was injected after the completion of surgery.

Carotid perfusion

Both common carotid arteries were cannulated in both directions, and blood from one was perfused into both towards the head, by means of a Watson Marlow MHRE pump. Both superior thyroid, internal carotid and external carotid arteries were ligated, together with any other branches between the point of cannulation and the origins of the lingual arteries. Both lingual arteries were cannulated, and blood from them returned through silicon rubber tubing to a saphenous vein. The resistance of this shunt was adjusted with a screw clamp. A pressure tranducer was connected to the perfusion circuit and linked through a servo amplifier to the roller pump so that any given mean perfusion pressure could be held constant. Mean arterial pressure was recorded from a common carotid artery.

Reflex tests

Baroreceptor tests were performed by lowering the carotid sinus pressure from 130 to 90 mmHg for 1 min, while holding the P_{O_2} of carotid blood above 20 kPa. Chemoreceptor tests were performed and evaluated as described by Henderson & Ungar (1978). They consisted of a lowering of the P_{O_2} of the blood perfusing the carotid bifurcations, for 1 min by infusing into it a solution of sodium dithionite (Critchley & Ungar, 1975). During the chemoreceptor tests, carotid sinus pressure was held at 130 mmHg. The rate of infusion of dithionite was set so as to yield reflex responses roughly matching those to baroreceptor tests.

Both vagosympathetic trunks were divided in the neck to prevent secondary reflex responses from thoracic receptors. Under these conditions, adrenal medullary responses to both tests can be abolished by denervating either the carotid bifurcations or the adrenal glands (Critchley *et al.*, 1982).

Collection of adrenal venous blood

The abdomen was opened by a mid-line incision, the left adrenolumbar vein 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.

Drugs

The following drugs were used: acetyl-β-methyl choline chloride (BDH); atropine sulphate (BDH); chloralose (BDH); heparin (Evans); hexamethonium bromide (Koch-Light); *m*-hydroxy-phenylpropyl-trimethyl ammonium iodide (Dr R.B. Barlow); hyoscine methyl-bromide (Sigma); sodium dithionite (BDH); urethane (BDH).

Results

Isolated perfused adrenal glands

The resting rates of release of catecholamines from the isolated perfused adrenal glands of cats and dogs were of the same order of magnitude, but the ratio of noradrenaline to adrenaline was consistently higher in the resting effluent of feline glands than in that of canine glands (Table 1).

The results of infusing the selective nicotinic agonist *m*-hydroxy-phenylpropyl-trimethyl ammonium iodide (HPPTMA) (Barlow & Franks, 1971), and the selective muscarinic agonist acetyl-β-methyl choline chloride (MCh), are shown in Table 1. Both agonists stimulated the secretion of catecholamines from the adrenal glands of both species. In preliminary experiments we found that both agonists evoked dose-dependent responses over the range of concentrations 10^{-8}M–10^{-5}M, and a concentration of 10^{-6}M was used in all tests shown in Table 1.

The selectivity of the agonists was tested by adding blocking drugs to the perfusate. Hexamethonium (3×10^{-4}M) abolished the response to HPPTMA (10^{-5}M), but did not inhibit the response to MCh.

Table 1 Output of adrenaline (Ad) and noradrenaline (NA) (nmol min^{-1}) from the isolated perfused adrenal glands of dogs and cats: resting output and incremental release in response to 2 min periods of perfusion with perfusate containing acetyl β-methylcholine chloride (MCh, 10^{-6} M) or *m*-hydroxyphenylpropyl-trimethyl ammonium iodide (HPPTMA, 10^{-6} M)

	Resting catecholamine output			*Incremental catecholamine release*		
Drug	*NA*	*Ad*	*%Ad*	*NA*	*Ad*	*%Ad*
			Dogs			
Mch ($n = 10$)	0.42 ± 0.10	1.33 ± 0.35	76	1.19 ± 0.06	3.06 ± 0.12	72
HPPTMA ($n = 12$)	0.43 ± 0.13	1.59 ± 0.28	79	3.15 ± 0.75	9.17 ± 2.1	74
			Cats			
Mch ($n = 6$)	0.38 ± 0.02	0.56 ± 0.06	59	0.35 ± 0.06	1.84 ± 0.11	84
HPPTMA ($n = 12$)	0.41 ± 0.03	0.65 ± 0.05	61	1.67 ± 0.40	1.05 ± 0.16	38

Atropine (3×10^{-5}M) abolished the response to MCh (5×10^{-5}M), but did not inhibit the response to HPPTMA.

In the cat our results confirm those of Douglas & Poisner (1965) in showing that nicotinic agonists preferentially release noradrenaline while muscarinic agonists preferentially release adrenaline. In the dog, however, the relative composition of the incremental release stimulated by both drugs remained similar to that in the resting release.

Reflex experiments

The mean resting rates of release of catecholamines from the left adrenal glands of anaesthetized dogs, and the responses to baroreceptor and chemoreceptor tests are shown in Table 2. For these experiments *in vivo*, total catecholamine output (pmol min^{-1} kg^{-1}) refers to the sum of the outputs of adrenaline and noradrenaline, separately estimated. The fraction of adrenaline in the mixture did not vary, in resting or evoked release, outside the range of 70–80%.

Table 2 also shows the effects on these responses, in six dogs of hexamethonium, which selectively blocks nicotinic transmission, and in another six dogs of hyoscine methyl-bromide, which selectively blocks peripheral muscarinic transmission. Hexamethonium gave significantly greater inhibition of the baroreceptor reflex than of the chemoreceptor reflex in every dog ($P < 0.05$). Hyoscine gave greater inhibition, in all but

Table 2 Responses of anaesthetized dogs to baroreceptor tests (Baro) and chemoreceptor tests (Chemo)

	Carotid pressure (mmHg)		*Oxygen tension* (mmHg)		*MAP* (mmHg)	*Total CA output* (pmol $min^{-1}kg^{-1}$)		*% inhibn.*
	Control	Test	Control	Test		Control	Increment	
Control								
Baro	130	90	>150	-	71 ± 11	65 ± 21	186 ± 35	
Chemo	130	-	147 ± 19	28 ± 8	71 ± 11	64 ± 21	173 ± 52	
After hexamethonium								
Baro	130	90	>150	-	62 ± 6	16 ± 5	30 ± 16	83[a]
Chemo	130	-	169 ± 26	26 ± 5	62 ± 6	13 ± 6	56 ± 16	67[a]
Control								
Baro	130	90	>150	-	74 ± 13	44 ± 4	182 ± 29	
Chemo	130	-	180 ± 21	30 ± 3	74 ± 13	43 ± 4	188 ± 41	
After hyoscine								
Baro	130	90	>150	-	80 ± 9	18 ± 4	89 ± 29	51[b]
Chemo	130	-	171 ± 20	29 ± 5	80 ± 9	20 ± 5	64 ± 25	65[b]

The table shows changes in total catecholamine (CA) output before and after administration of hexamethonium (10 mg kg^{-1}) in 6 dogs and hyoscine methyl-bromide (10 mg kg^{-1}) in another 6 dogs. Mean arterial pressure (MAP) was measured immediately before each test.

[a]Significantly different ($P < 0.05$) from control value and from corresponding baroreceptor or chemoreceptor test.

[b]Significantly different ($P < 0.05$) from control value.

one dog, of the chemoreceptor reflex than of the baroreceptor reflex, but these differences were small and not statistically significant. In four dogs, a combination of the doses of the two antagonists abolished the reflex responses to both baroreceptor and chemoreceptor tests. Doubling the dose of hexamethonium or of hyoscine alone did not significantly increase the degree of block.

Discussion

We have previously shown that the cat differs from the dog in its ability to respond to different sensory stimuli by selectively releasing adrenaline or noradrenaline from its adrenal medullae. The dog responds to these stimuli by releasing both catecholamines in a fixed ratio (Critchley *et al.*, 1980). We have now presented evidence that the species difference extends to pharmacological responses. We have confirmed the observation of Douglas & Poisner (1965) that, in the isolated perfused, adrenal gland of the cat, muscarinic and nicotinic agonists preferentially release adrenaline and noradrenaline respectively. The canine gland, however, responds to the same drugs by releasing both catecholamines in a fixed ratio.

When we look at the transmission of reflex responses by the adrenal medulla, the pattern is more complicated. In the cat we have observed that hexamethonium at a dose that abolishes the baroreceptor reflex response of the gland, does not inhibit the chemoreceptor reflex response (Critchley *et al.*, 1980). In the dog, our present results show the same trend, although the difference is less clear cut.

Antagonism with hyocine gave a partial inhibition of more than 50% of both reflex responses, while the same doses of hexamethonium and hyoscine given together abolished both. This finding is consistent with the observation of Feldberg *et al.* (1934) that a combination of muscarinic and nicotinic block was needed to abolish adrenal secretory responses to acetylcholine.

The dose of hyoscine needed to inhibit reflex responses was much higher than would be needed to block parasympathetic reflexes. Also, the affinity constant when atropine antagonized the response to MCh in canine isolated glands was $6.4 \times 10^6 M^{-1}$, two orders of magnitude lower than its affinity constant for guinea-pig ileum (Henderson & Ungar, 1977). We do not think that lack of selectivity is responsible, since hexamethonium did not inhibit responses to MCh that were blocked by atropine.

There is a striking similarity between the present results and those of Henderson & Ungar (1978) where reflex vasoconstriction in the hind limb was examined. In both studies a nicotinic antagonist blocked all but a small, hyoscine-sensitive, component of the baroreceptor reflex, whereas neither antagonist alone could effectively block the chemoreceptor reflex. The simplest explanation of these findings would be that the baroreceptor and chemoreceptor reflexes have separate motor pathways right down to the synapse on the ganglion or chromaffin cell. The baroreceptor pathway would be predominantly nicotinic while the chemoreceptor had more equal proportions of the two receptors. We are not aware of any anatomical evidence that would support such an explanation, and have suggested an alternative based on differences in temporal patterns of firing of preganglionic neurones (Ungar & Phillips, 1983).

The cat seems to be unique among species that have been studied, in releasing noradrenaline and adrenaline selectively both in response to different sensory stimuli and to nicotinic or muscarinic agonists. This selectivity is only partial, both in our experiments and in those of Douglas & Poisner (1965). What intrigues us most about these results, is that in the cat the selectivity of nicotinic and muscarinic transmission for baroreceptor and chemoreceptor reflexes is much more complete than their selectivity for the control of noradrenaline and adrenaline release respectively. Furthermore, the reflex selectivity is still apparent in the dog, but does not extend to separate control of adrenaline and noradrenaline release.

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