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# EFFECT OF CHOLINERGIC ANTAGONISTS ON SYMPATHETIC GANGLIONIC TRANSMISSION OF VASOMOTOR REFLEXES FROM THE CAROTID BARORECEPTORS AND CHEMORECEPTORS OF THE DOG

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SUMMARY

1. In anaesthetized dogs the reflex vascular resistance changes in a perfused hind limb were studied following carotid baroreceptor or chemoreceptor stimulation.

2. The observed rises in resistance were sympathetically mediated and thus provided a means of studying the action of the cholinergic antagonists on the sympathetic ganglion transmission.

3. The reflex response to carotid baroreceptor stimulation produced by lowering the pressure in the carotid sinuses was abolished by hexamethonium bromide but not reduced by hyoscine methyl bromide.

4. The reflex response to carotid body chemoreceptor stimulation, by hypoxia, was not altered by hexamethonium bromide but was greatly reduced by the hyoscine methyl bromide. No reflex response was seen when both antagonists were present.

5. These results indicate that sympathetic ganglion synaptic transmission during the baroreceptor reflex is mediated by nicotinic receptor activation. The transmission evoked by chemoreceptor stimulation involves muscarinic receptors with a subsidiary nicotinic pathway. High doses of an antagonist were necessary to block the muscarinic component of transmission and this is discussed in relation to previous work.

6. No non-cholinergic transmission of the reflex responses was observed.

## INTRODUCTION

The existence of muscarinic receptors in sympathetic ganglia is well established. Residual responses to electrical stimulation of preganglionic sympathetic nerves, after the administration of nicotinic antagonist drugs, have been attributed to muscarinic transmission (Brown, 1967; Flacke & Gillis, 1968; Trendelenburg, 1966). Similarly the reflex responses to carotid occlusion (Steinberg & Hilton, 1966) and to raised intracranial pressure (Hilton & Steinberg, 1966) have components that resist blockade by nicotinic antagonists, the residue being blocked by atropine-like drugs. Chinn & Hilton (1976) stated that the cardiac responses to electrical stimulation of the sympathetic rami can be partly blocked by atropine and partly by chlorisondamine.

These observations are compatible with the theory that there are two populations of ganglionic synapses, having nicotinic and muscarinic post-junctional receptors respectively. An alternative explanation is that muscarinic receptors are present but do not have a direct role in transmission unless the main nicotinic receptors are blocked.

This project was provoked by the chance observation that hexamethonium bromide, at a dose that abolished the reflex rise in blood pressure in response to lowering the carotid sinus pressure, did not even diminish the rise in blood pressure to carotid body hypoxia. Both responses were abolished by guanethidine sulphate. These observations made a prima facie case for non-nicotinic transmission in the sympathetic ganglia of the chemoreceptor, but not of the baroreceptor, vasomotor reflex. We have therefore investigated this difference.

## METHODS

### *Anaesthesia*

Dogs weighing between 9 and 31 kg were anaesthetized by an I.V. injection of sodium pentobarbitone (30 mg/kg). Anaesthesia was maintained by further doses as necessary.

### *Respiration and heat 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 4 KPa and $P_{a,O_2}$ above 13 KPa, measured from frequent arterial blood samples on a Radiometer BMS3 analyser. A molar solution of sodium bicarbonate was injected I.V. after each arterial sample to hold the arterial pH at 7·4. Body temperature was held near to 37 °C by a heating pad linked to a rectal thermistor probe.

### *Hind-limb perfusion*

One femoral artery was cannulated at the level of the inguinal ligament, and its major collaterals were ligated. The hind limb was perfused with blood from the other femoral artery through a Watson Marlow MHRE pump. When the pump was stopped the perfusion pressure always fell to below 30% of the systemic pressure. The pump was set so that hind-limb perfusion pressure was always higher than systemic blood pressure. One hind limb was thus perfused at constant flow, and changes in perfusion pressure were taken as a measure of changes in vascular resistance in that limb.

### *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 perfusion system.

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 at will and held constant.

### *Recording of blood pressure*

Systemic blood pressure, hind limb and carotid perfusion pressures were recorded by means of Bell and Howell L222 pressure transducers, calibrated against a mercury manometer, on a Honeywell Visicorder 2206.

*Stimulation of reflexes*

Two kinds of tests were performed. 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 by infusing into it a 1 M solution of sodium dithionite, at a rate of about 150 mg/min (Critchley & Ungar, 1974), while holding the perfusion pressure constant. The systemic arterial blood gas tensions did not change whilst sodium dithionite was infused into the carotid circuit. In each experiment we adjusted the stimuli in the two kinds of tests to match the amplitudes of the reflex responses. The duration of each test was 30 sec.

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

*Drugs*

Drugs used were sodium pentobarbitone (Nembutal, Abbot or Sagatal, May and Baker), hexamethonium bromide (Koch-light, then recrystallized), (±) hyoscine methyl bromide (Upjohn), guanethidine sulphate (B.D.H.), sodium dithionite (B.D.H.) dextran with dextrose (Fisons and B.D.H.), and lignocaine (Astra Chemicals).

## RESULTS

*Evaluation of baroreceptor and chemoreceptor tests by carotid sinus nerve block*

In two dogs baroreceptor and chemoreceptor tests were performed before and after the application of 0·5 ml. 2% lignocaine solution to each carotid sinus nerve. The hind-limb perfusion pressure immediately rose from 165 and 135 mmHg to 257 and 270 mmHg respectively, and recovered within 10 min to 200 mmHg in both dogs. Similar rises occurred in the systemic blood pressure. The results are illustrated in Fig. 1*A* and *B*. Sinus nerve block totally abolished the vascular responses to both kinds of tests and also abolished the reflex changes in systemic blood pressure. In spontaneously breathing dogs the respiratory response to chemoreceptor tests was also abolished.

Both reflex responses had recovered after 1 hr.

*The action of hexamethonium bromide on reflex vasoconstriction*

In six dogs baroreceptor and chemoreceptor tests were performed before, and between 10 and 30 min after, the I.V. administration of the nicotinic antagonist hexamethonium bromide (1–2 mg/kg). The systemic blood pressure tended to fall after hexamethonium bromide but was restored by the injection of 5% dextran in 5% dextrose solution if necessary. There was a transient fall in hind-limb perfusion pressure which was corrected if necessary by increasing the flow. The mean systemic blood pressure was $90 \pm 4$ before and $78 \pm 5$ mmHg after hexamethonium bromide. The mean resting hind-limb perfusion pressure was $106 \pm 2$ before and $105 \pm 2$ mmHg after hexamethonium bromide.

The results are set out in Table 1(*a*) and illustrated in Fig. 1.

Hexamethonium bromide almost completely abolished the responses to baroreceptor tests, but did not significantly affect those to chemoreceptor tests.

*The action of hyoscine methyl bromide on reflex vasoconstriction*

In six dogs baroreceptor and chemoreceptor tests were performed before, and between 20 and 40 min after, the I.V. administration of the muscarinic antagonist

hyoscine methyl bromide (10 mg/kg). The mean systemic pressure was $107 \pm 3$ before and $104 \pm 4$ mmHg after the drug. The mean resting hind-limb perfusion pressure was $117 \pm 2$ before and $113 \pm 2$ mmHg after hyoscine methyl bromide.

The results are set out in Table 1(*b*) and illustrated in Fig. 1. Hyoscine methyl bromide substantially inhibited the responses to chemoreceptor tests ($P < 0{\cdot}01$), but had little or no effect on those to baroreceptor tests.

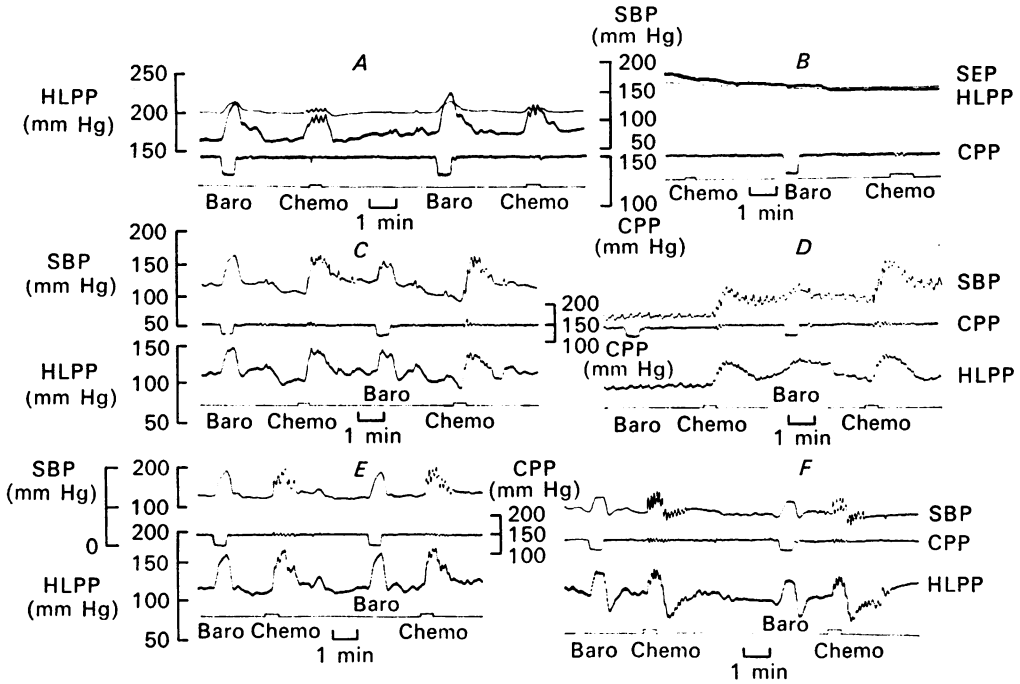


Fig. 1. Records from three experiments showing the reflex vasoconstriction following baroreceptor and chemoreceptor tests before and after drug administration. Sections *A*, *C* and *E* show the control vascular responses to both tests before drug administration. Section *B* shows the abolition of responses 15 min after carotid sinus nerve block. Section *D* shows the reflex vascular responses to both tests 20 min after I.V. administration of hexamethonium bromide (2 mg/kg). Section *F* shows the reflex vascular responses to both tests 30 min after I.V. administration of hyoscine methyl bromide (10 mg/kg). SBP, mean systemic blood pressure; CPP, carotid perfusion pressure; HLPP, hind-limb perfusion pressure.

*Combined action of hexamethonium bromide and hyoscine methyl bromide on reflex vasoconstriction*

In five dogs there was no residual response to baroreceptor tests or to chemoreceptor tests in the presence of both hexamethonium bromide (2 mg/kg) and hyoscine methyl bromide (10 mg/kg).

*The action of guanethidine sulphate on reflex vasoconstriction*

In two dogs the intravenous administration of guanethidine sulphate (10 mg/kg) abolished the changes in hind-limb perfusion pressure in response to both baroreceptor and chemoreceptor tests. In two further dogs the close arterial injection of

TABLE 1. The modification of the reflex vasoconstriction following intravenous administration of hexamethonium bromide or hyoscine methyl bromide respectively

CPP, carotid perfusion pressure; HLPP, hind-limb perfusion pressure

(*a*) Hexamethonium bromide

| | Before hexamethonium | | | | After hexamethonium | | | | Inhibition (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Baroreceptor test | | Chemoreceptor test | | Baroreceptor test | | Chemoreceptor test | | | |
| Dog no. | CPP fall (mmHg) | HLPP rise (mmHg) | Carotid $P_{O_2}$ (kPa) | HLPP rise (mmHg) | CPP fall (mmHg) | HLPP rise (mmHg) | Carotid $P_{O_2}$ (kPa) | HLPP rise (mmHg) | Baro-receptor tests | Chemo-receptor tests |
| 1 | 150 → 120 | 38 | 14·2 → 6·0 | 49 | 150 → 120 | 6 | 14·6 → 5·5 | 38 | 84 | 22 |
| 2 | 150 → 100 | 33 | 23·5 → 2·8 | 12 | 150 → 100 | 4 | 23·5 → 3·3 | 12 | 88 | 0 |
| 3 | 130 → 110 | 31 | 14·4 → 6·4 | 40 | 130 → 110 | 7 | 14·4 → 6·4 | 40 | 78 | 0 |
| 4*a* | 130 → 110 | 12 | 14·9 → 6·1 | 12 | 130 → 110 | 3 | 14·5 → 4·1 | 11 | 75 | 8 |
| 4*b* | 130 → 110 | 17 | 14·6 → 5·2 | 9 | 130 → 110 | 3 | 15·7 → 6·6 | 9 | 83 | 0 |
| 5 | 130 → 100 | 29 | 17·3 → 4·8 | 30 | 130 → 100 | 12 | 14·2 → 4·4 | 29 | 59 | 3 |
| 6 | 120 → 100 | 51 | 18·4 → 5·6 | 78 | 120 → 100 | 4 | 16·1 → 5·1 | 65 | 92 | 17 |
| | Means ± S.E. | 30 ± 3 ($n$ = 17) | | 34 ± 6 ($n$ = 15) | | 6 ± 1 ($n$ = 19) | | 31 ± 4 ($n$ = 16) | | |

(*b*) Hyoscine methyl bromide

| | Before hyoscine Me Br | | | | After hyoscine Me Br | | | | Inhibition (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Baroreceptor test | | Chemoreceptor test | | Baroreceptor test | | Chemoreceptor test | | | |
| Dog no. | CPP fall (mmHg) | HLPP rise (mmHg) | Carotid $P_{O_2}$ (kPa) | HLPP rise (mmHg) | CPP fall (mmHg) | HLPP rise (mmHg) | Carotid $P_{O_2}$ (kPa) | HLPP rise (mmHg) | Baro-receptor tests | Chemo-receptor tests |
| 7 | 150 → 120 | 55 | 20·9 → 6·1 | 47 | 150 → 120 | 55 | 24·5 → 6·2 | 33 | 0 | 30 |
| 8 | 150 → 120 | 18 | 19·7 → 5·3 | 19 | 150 → 120 | 21 | 18·1 → 5·3 | 7 | −17 | 64 |
| 9 | 150 → 120 | 47 | 12·8 → 5·1 | 57 | 150 → 120 | 38 | 16·5 → 5·7 | 29 | 19 | 49 |
| 10 | 150 → 130 | 53 | 24·0 → 8·0 | 58 | 150 → 130 | 46 | 18·2 → 7·3 | 28 | 14 | 52 |
| 11 | 150 → 130 | 24 | 25·0 → 6·4 | 46 | 150 → 130 | 26 | 24·6 → 6·4 | 38 | −8 | 17 |
| 12 | 150 → 120 | 44 | 19·4 → 5·5 | 32 | 150 → 120 | 41 | 20·3 → 4·3 | 20 | 6 | 37·5 |
| | Means ± S.E. | 45 ± 3 ($n$ = 21) | | 46 ± 3 ($n$ = 21) | | 39 ± 3 ($n$ = 23) | | 25 ± 2 ($n$ = 25) | | |

guanethidine sulphate (1·75 mg/kg) to the perfused hind limb abolished the hind-limb response to chemoreceptor stimulation without affecting the systemic response.

These experiments show that both reflex responses are mediated solely by the activation of sympathetic adrenergic fibres. The absence of a dilator response to chemoreceptor stimulation after guanethidine administration is consistent with the conclusion of Marshall (1977) that chemoreceptor-induced cholinergic vasodilatation, seen in the decerebrate animal, is not seen under barbiturate anaesthesia.

## DISCUSSION

### *Baroreceptor reflex*

As far as the carotid sinus baroreceptors are concerned, our results indicate that reflex vasoconstriction in the canine hind limb involves nicotinic transmission in the sympathetic ganglia to adrenergic vasomotor fibres. The reflex is abolished by moderate doses of hexamethonium bromide, and is unaffected by doses of hyoscine methyl bromide that lack nicotinic antagonist activity.

These results seem to conflict with those of previous workers, who have shown that the reflex response to carotid occlusion has a component resistant to blockade by nicotinic antagonists but sensitive to atropine (Steinberg & Hilton, 1966).

Carotid occlusion, however, gives a mixed baroreceptor and chemoreceptor reflex response unless high arterial oxygen tensions are maintained, since the carotid bodies are sensitive to ischaemia (Heymans & Neil, 1958).

### *Chemoreceptor reflex*

Our results for the carotid body chemoreceptors are harder to interpret. Reflex vasoconstriction in response to chemoreceptor stimulation is unaffected by doses of hexamethonium bromide that abolish a reflex response, of similar amplitude and time course, to lowering of carotid sinus pressure. In some experiments we gave doses of hexamethonium bromide five times those sufficient to block the baro-receptor response without impairing that to chemoreceptor stimulation. In the presence of hexamethonium bromide the chemoreceptor response is abolished by hyoscine methyl bromide. In the absence of nicotinic antagonism, however, we only obtained a partial blockade varying from dog to dog between 17 and 64%.

The interaction of the antagonists on the chemoreceptor reflex implies that the pathways of the reflex must involve a complex interaction of muscarinic and nicotinic transmission. The simplest explanation of our results is that the main pathway of the chemoreceptor vasomotor reflex is by non-nicotinic transmission to adrenergic post-ganglionic fibres, in the sympathetic ganglia, sensitive to high concentrations of muscarinic antagonists, and that a subsidiary nicotinic pathway can sustain partial transmission after muscarinic blockade.

A problem is the high dose of muscarinic antagonist needed to inhibit the chemo-receptor reflex. The doses that we used are 100 times greater than those needed to block vagal bradycardia. In preliminary experiments with atropine we were concerned about the low ratio of muscarinic to nicotinic blocking potency, and indeed whether we were dealing with a population of nicotinic receptors relatively resistant to blockade. With hyoscine methyl bromide we are on stronger ground since the ratio of muscarinic to nicotinic blocking potency is about 20 times that for atropine.

Experiments on isolated tissues showed hyoscine methyl bromide to be about five times more potent than atropine as an antagonist at the parasympathetic post-ganglionic junction but about four times less potent than atropine as a nicotinic antagonist. Additionally, being a quaternary amine, hyoscine methyl bromide lacks the actions of atropine or hyoscine on the central nervous system.

For the present experiment it was necessary to exclude an action of hyoscine methyl bromide on the sensory side of the chemoreceptor reflex. R. J. Docherty & D. S. McQueen (personal communication) found that hyoscine methyl bromide at the dosage used in our experiments did not alter the resting discharge in single afferent fibres from the carotid body or the increase in impulse frequency in response to cyanide, to acetylcholine, or to nitrogen breathing.

In one of our own experiments we tested the reflex effect of chemoreceptor tests on pulmonary ventilation in a spontaneously breathing dog. Neither the resting ventilation, as recorded by an integrated pneumotachogram, nor the increase during chemoreceptor tests, was affected by hyoscine methyl bromide. No deterioration in the ventilatory response to chemoreceptor tests was seen during the time course of the experiment.

The high doses of antagonist needed raises the possibility of heterogeneous muscarinic receptors. This would be compatible with our findings on the homologous muscarinic receptors of the canine adrenal medulla (Henderson & Ungar, 1977). We found that the affinity constants for three muscarinic antagonists, to the release of catecholamines by acetyl $\beta$ methylcholine from perfused adrenal glands, were of the order of 100 times lower than their corresponding affinity constants at parasympathetic post-ganglionic endings.

We are very grateful to Mr R. J. Docherty and Dr D. S. McQueen for their help, by investigating whether hyoscine methyl bromide had any action on the sensory side of the chemoreceptor reflex. We should also like to thank Mr I. T. Cameron for collaborating on some of the experiments and Mr P. H. Whelpdale for his invaluable technical assistance.

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