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VASODILATATION IN THE SUBMAXILLARY GLAND OF THE RABBIT

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SUMMARY

1. In the rabbit, in contrast to the cat and dog, the vasodilatation and the secretion in the submaxillary gland which accompany parasympathetic nerve stimulation are correspondingly sensitive to atropine block.

2. It is concluded that true vasodilator nerve fibres to the submaxillary gland exist in the chorda tympani nerve of the rabbit.

3. The vasodilatation which follows sympathetic vasoconstriction in the submaxillary gland of the rabbit is small and variable. The possibility that this after-dilatation is due to an adrenergic neurotransmitter agent acting on β-vascular receptors is discussed.

INTRODUCTION

Evidence was presented (Bhoola, Morley, Schachter & Smaje, 1965), in accord with the view that vasodilator nerve fibres are present in the parasympathetic nerve supply to the cat's submaxillary gland. It was concluded that salivary kallikrein does not play a significant role in the vasodilatation produced by stimulation of the chorda-lingual nerve. Since the submaxillary gland of the rabbit, like that of the cat and dog, contains a kinin-releasing enzyme, and since most experiments on vascular regulation in the submaxillary gland have been made on the cat and dog, we decided to extend this study to the rabbit. We found, to our surprise, that both the secretion *and* the vasodilatation, which result from chorda-lingual nerve stimulation in the rabbit, in contrast to what is seen in the cat and dog, are equally sensitive to atropine blockade.

Some of these results were presented to the Physiological Society (Morley, Schachter & Smaje, 1963).

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METHODS

Animals. Rabbits of different strains weighing 2·0–4·5 kg were anaesthetized with urethane (1·5 g/kg) injected intravenously.

Blood flow. The submaxillary gland was exposed and the blood flow through it measured using the *open* system described for the cat by Bhoola *et al.* (1965). Nerves were stimulated and close-arterial injections made as in the cat with minor variations. Thus, in some experiments, the chorda tympani rather than the chorda-lingual nerve was stimulated by placing the electrodes under both the chorda tympani nerve and the submaxillary duct near the hilus of the gland. Also, for close arterial injections, the external maxillary rather than the lingual artery, was cannulated retrogradely, rostral to the origin of the submaxillary artery. Solutions were injected in 0·5 ml. volumes in 20 sec.

Arterial blood pressure was recorded from the femoral artery with a mercury manometer.

Saliva was collected from a glass or polythene cannula placed in the papilla of Wharton's duct in the mouth, and the volume of secretion determined by measuring the distance the saliva moved along a horizontal graduated polyvinyl tube connected to the cannula (108 cm = 1 ml.).

Drugs and other materials. Saliva required for injection close arterially was collected by cannulating both submaxillary ducts and by stimulating the chorda-lingual nerve or by injecting pilocarpine (200 μg/kg I.V.). It was then dialysed against large volumes of 0·9 % NaCl at 4° C for 24 hr.

Noradrenaline, adrenaline and isoprenaline were used as the bitartrates and their weights expressed as base. Weights of other drugs are expressed as the salt. Atropine was used as sulphate, mepyramine as maleate, acetylcholine as chloride, pilocarpine as nitrate, hexamethonium and prostigmine as bromides, and dichlorisoprenaline (DCI), eserine, pronethalol ('Alderlin') and AT-3 (ethyl-fluoren-9-yl-2 iodethylamine) as hydrochlorides.

RESULTS

Parasympathetic nerve stimulation

Electrical stimulation of the chorda-lingual or chorda tympani nerve regularly produced a rapid increase in blood flow through the submaxillary gland, usually to about twice the basal rate (Fig. 1), although greater increases occurred occasionally. A brisk secretion of saliva accompanied the vasodilatation.

The effect of intravenous injections of various doses of atropine on the vasodilatation caused by parasympathetic nerve stimulation was recorded in twenty-three rabbits. The results (Table 1) demonstrate that the vasodilatation was usually blocked by atropine (25–250 μg/kg), in doses which are completely ineffective in blocking this effect in the cat or dog (cf. Burgen & Emmelin, 1961). The results of three such experiments are illustrated in Fig. 2. In all three experiments the atropine block was partially reversed by the intravenous injection of eserine or prostigmine.

Occasionally, complete block of vasodilatation required higher atropine dosage, but in all experiments in which salivary secretion or cardiac slowing produced by vagal stimulation was measured at the same time as vasodilatation, the sensitivity to atropine of these parasympathetic

effects closely paralleled that of vasodilatation (Table 1 and Fig. 3). In two experiments a cumulative dose of atropine totalling 2·0 and 2·1 mg/kg respectively were required before complete block of vasodilatation was achieved (Expts. 22 and 23, Table 1). In another experiment, each successive dose of atropine of 100 μg/kg up to a total dose of 1·9 mg/kg, blocked

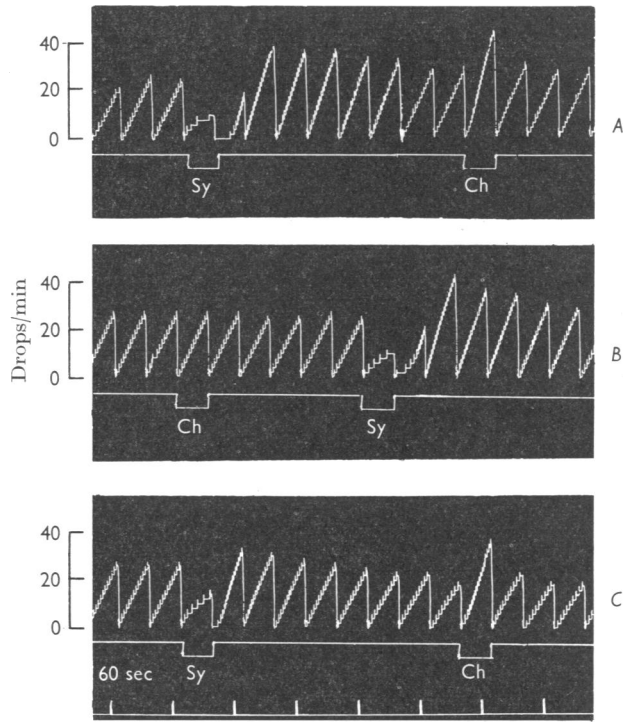

Fig. 1. Record of blood flow in submaxillary gland of rabbit (4·3 kg) showing vascular responses to chorda-lingual, Ch, and sympathetic, Sy, nerve stimulation. *A*, Control. *B*, 35 min after atropine (250 μg/kg I.V.). *C*, 6 min after eserine (250 μg/kg I.V.)

both chorda vasodilatation and vagal cardiac slowing for only 1–2 min after each injection (Expt. 11, Table 1). These observations suggest that the variation in sensitivity of chorda vasodilatation to atropine blockade in different experiments is due to the marked differences in the concentration of atropinase in the plasma of different rabbits (Sawin & Glick, 1943).

The vasodilator and secretory effects of chorda stimulation were blocked completely by hexamethonium (2·5 mg/kg, I.V.). This indicates that these effects are not due to the antidromic stimulation of sensory nerve fibres.

The injection of ACh close-arterially mimicked the vasodilator effects of

stimulation of the chorda-lingual nerve. Similar injections of dialysed rabbit saliva, however, were relatively ineffective in causing vasodilatation; this vasodilatation was slow in onset and protracted when it did occur.

In some rabbits a slight secretion of saliva occurred in the absence of nerve stimulation. The secretion was unaffected by doses of atropine which prevented secretion resulting from nerve stimulation. This observation has also been reported by Nordenfelt & Ohlin (1957).

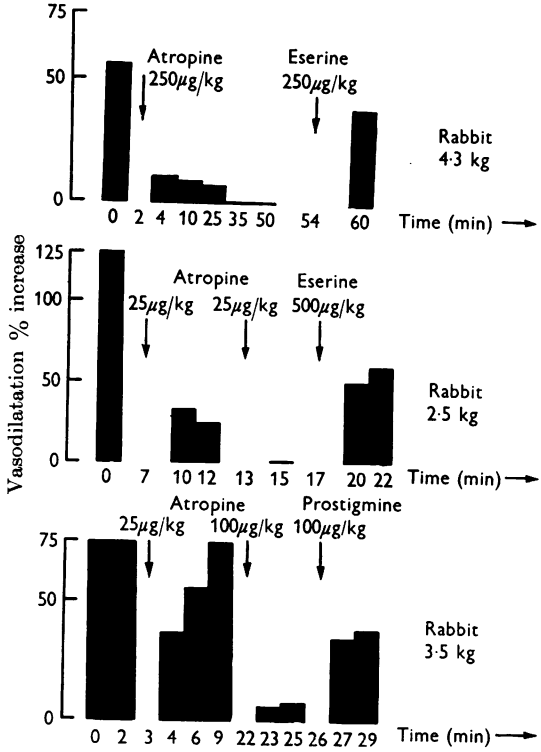

Fig. 2. Effect of intravenous injections of atropine and eserine (or prostigmine) on vasodilatation caused by stimulation of the chorda-lingual nerve for 30 sec. Three experiments. Time scale (non-linear) indicates duration of each experiment.

Sympathetic nerve stimulation

Electrical stimulation of the cervical sympathetic nerve always caused an immediate vasoconstriction in the submaxillary gland. The marked after-dilatation which follows this constriction in the cat was not so evident in the rabbit. In four of ten rabbits there was no vasodilatation following the constriction, but in the remainder, a small variable after-dilatation

occurred when nerve stimulation ceased (Fig. 1). Sympathetic stimulation never caused salivation.

The vascular effects produced by sympathetic stimulation were unaffected by atropine or eserine. The powerful vasoconstrictor effect was

TABLE 1. Effect of atropine on chorda vasodilatation and salivation in the rabbit submaxillary gland, and on vagal inhibition of the heart. (See text for details)

Expt.	Stimulation at Hilus (H), or Ch. ling N. (Ch)	Atropine (μg/kg, I.V.) 1st dose	Total dose required to block: Chorda vasodil.	Total dose required to block: Chorda saliv.	Total dose required to block: Vagal cardiac inhibition	Duration of block (min)*	Reversal by eserine (E) or by prostigmine (P)(μg/kg)
1	Ch	25	25	25	—	9	—
2	H	25	—	25	—	1–2	Yes (P, 100)
3	H	40	40	—	—	18	No (P, 100)
4	Ch	25	50	—	—	3†	Yes (E, 500)
5	H	50	50	50	—	17	—
6	H	50	50	—	—	12	—
7	Ch	50	50	—	—	8†	Yes (E, 250)
8	H	50	100	—	—	—	—
9	H	100	100	—	—	—	—
10	Ch	100	—	100	—	6†	Yes (P, 100)
11	H	100	100 1900	—	100 1900	1–2 20	Yes (P, 50)
12	Ch	25	125	125	—	3†	Yes (dilat.) (P, 100) No (saliv)
13	H	100	200	—	200	15	—
14	H	100	200	—	—	12	Yes (P, 100)
15	H	25	250	250	—	—	Yes (Saliv) (E, 300) No (dilat)
16	H	50	250	—	—	Not established	—
17	Ch	250	250	—	—	48	Yes (E, 250)
18	Ch	250	250	250	—	3 Rabbit died	—
19	H	25	—	350	—	8†	Yes (E, 200)
20	Ch	450	450	—	—	16†	Yes (E, 450)
21	Ch	50	850 (75 % reduction)	—	850 (Complete block)	—	—
22	H	50	2000	—	2000	10	—
23	H	100	2100	—	2100	14	—

* Criterion for block: chorda or vagal effects must be reduced more than 90 %.

† Period of block duration was terminated by injection of eserine or prostigmine.

—, not measured.

abolished in three experiments by the adrenergic α-blocker, compound AT-3 (500 μg/kg I.V.) (Graham, 1960) whereas the small and variable after-dilatation persisted. This after-dilatation, however, unlike that in the cat (Bhoola *et al.* 1965), was not converted to an immediate one when the vasoconstriction was abolished. It still occurred only after nerve stimulation had ceased. After injection of the β-adrenergic blocking drugs DCI

(2·5–5·0 mg/kg I.V.) or pronethalol (0·5–2·0 mg/kg I.V.), however, the vasoconstriction was protracted and any after-dilatation initially present failed to occur.

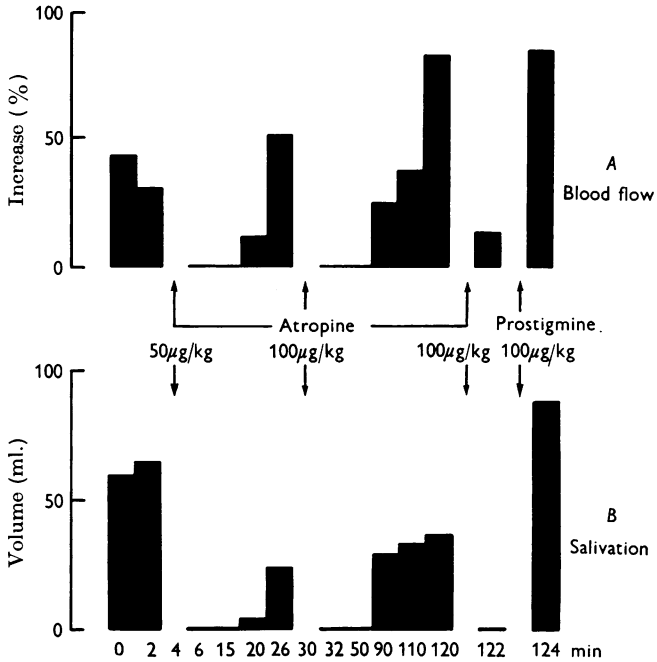

Fig. 3. Shows the parallel effects of intravenous injections of atropine and prostigmine on the vasodilatation (*A*), and salivation (*B*) in the submaxillary gland of a rabbit (4·7 kg) caused by stimulation of the chorda-lingual nerve for 30 sec. Time scale as in Fig. 2.

DISCUSSION

The fact that the salivary secretion in response to parasympathetic stimulation, but not the vasodilatation, is blocked by atropine (in the cat and dog) was the observation which led to the investigations of Hilton & Lewis (1955, 1956) and the conclusion that true vasodilator nerves to these glands do not exist. The conclusion that the vasodilatation is mediated by release of kallikrein as a result of an increase in metabolism of the secretory cell still does not explain why secretion but not increased metabolism is blocked by atropine. This view merely shifts the phenomenon of atropine-resistance from vascular receptors for ACh to metabolic, gland cell receptors for this compound. The mediation of functional dilatation by kallikrein in the cat's submaxillary gland has been questioned in earlier work (Bhoola *et al.* 1965; Beilenson, Schachter & Smaje, 1965), since the absence of the enzyme, kallikrein, or of its substrate, kininogen, did not

prevent the production of a normal, atropine-resistant vasodilatation in response to parasympathetic stimulation.

The present experiments in the rabbit, which demonstrate the parallel sensitivity of secretion and vasodilatation to atropine, are most rapidly explained by assuming that true vasodilator as well as secretory nerve fibres exist and that both are cholinergic. Our view is that this vasodilatation in the submaxillary gland of the cat and dog on the one hand, and of the rabbit on the other, simply represents extreme cases of the well-known variation in the sensitivity of cholinergic receptors to atropine (Ambache, 1955). Of interest in this connexion is the fact that in the sheep, the vasodilatation evoked by parasympathetic stimulation in the parotid gland is atropine-sensitive while that in the submaxillary gland is atropine-resistant (S. C. Beilenson, M. Schachter & L. H. Smaje, unpublished).

The after-dilatation in the rabbit submaxillary gland on sympathetic stimulation, unlike that in the cat, is small and variable. It was unaffected by doses of atropine which blocked parasympathetic secretion and vasodilatation. It was absent, however, after the injection of the adrenergic β-blockers pronethalol and DCI, whilst the initial vasoconstriction was unaffected or enhanced. The vasoconstriction, however, was completely abolished by the α-blocker, AT-3. The possibility arises, therefore, that both the sympathetic constrictor and dilator vascular effects in the rabbit are due to the catecholamines released from the sympathetic nerve fibres which act on both α- and β-vascular receptors with the former predominating. Not readily consistent with this view, however, is the fact that on no occasion after α-receptor blockade was the after-dilatation 'unmasked' to become an immediate dilatation.

The mediator of the sympathetic after-dilatation in the rabbit, in our opinion, remains uncertain. The smallness of this vasodilatation makes it doubtful that it is of physiological significance.

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