Br. J. Pharmac. (1980), **68**, 651–661

PHARMACOLOGICAL ANALYSIS OF SALIVARY AND BLOOD FLOW RESPONSES TO HISTAMINE OF THE SUBMANDIBULAR GLAND OF THE DOG

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1 The submandibular gland *in situ* was perfused with blood through the glandular artery at constant pressure in anaesthetized dogs. Drugs were administered intra-arterially.

2 Histamine produced both salivation and an increase in blood flow, each response having an early and a late component.

3 Marked tachyphylaxis to histamine developed in both of the salivary responses but only in the late blood flow response to histamine.

4 The early and late salivary responses were abolished and the late blood flow response was diminished by infusion of tetrodotoxin in doses that abolished the salivary and blood flow responses to electrical stimulation of the chorda-lingual nerve.

5 The whole salivary response to histamine was abolished by infusion of (−)-hyoscyamine in doses that greatly antagonized the salivary and blood flow responses to acetylcholine, whereas the blood flow responses to histamine were scarcely modified. These doses of (−)-hyoscyamine abolished the salivary response to chorda-lingual nerve stimulation but left the blood flow response to it unaffected.

6 The salivary and blood flow responses to histamine were unaffected by infusion of hexamethonium in doses that almost abolished the salivary and blood flow responses to chorda-lingual nerve stimulation.

7 The whole salivary response to histamine was abolished and the late blood response to histamine was partially inhibited by the histamine H_1-receptor antagonist, mepyramine, but not by the histamine H_2-receptor antagonist, metiamide.

8 The early blood flow response to histamine was antagonized by both mepyramine and metiamide but mepyramine was far more effective than metiamide.

9 These results led to the following conclusions: (1) the whole salivary response and a part of the late blood flow response to histamine are due entirely to excitation of parasympathetic postganglionic neurones; (2) neuronal histamine receptors involved are exclusively of the H_1-type; (3) histamine has no direct stimulant action on the glandular cells; (4) the early blood flow response and the remaining part of the late blood flow response to histamine result from the direct action on vascular smooth muscle in the glandular vascular bed; (5) vascular histamine receptors consist of H_1- and H_2-receptors.

Introduction

It is known that in cats and dogs, histamine causes salivation from and vasodilatation in the submandibular gland (Emmelin, 1966). Dale & Laidlaw (1910) first demonstrated that in cats and dogs salivation from the submandibular gland elicited by intravenous injection of histamine was abolished by atropine, and pointed out the weak pilocarpine-like (i.e., muscarinic) action of histamine. Afterwards, Gibbs & McClanahan (1937) observed that salivation from the cat submandibular gland elicited by intra-arterial injection of histamine was potentiated by physostigmine and abolished by atropine. As a result of such observations, Gibbs & McClanahan (1937) suggested that histamine possibly either stimulates liberation of acetylcholine from the chorda-lingual nerve or interferes with the normal brake mechanism for liberation of acetylcholine therefrom. Nevertheless, the effect of intra-arterial histamine in eliciting salivation from the

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submandibular gland is assumed to be exerted to a great extent on the glandular cells (Emmelin, 1966), although the results obtained by Gibbs & McClanahan (1937) are in part interpreted by Emmelin (1966) in terms of the ganglionic stimulant action of histamine.

It seems highly likely that histamine causes salivation by its ganglionic stimulant action, since the submandibular gland has numerous parasympathetic ganglia and histamine is capable of stimulating the superior cervical ganglion via receptors distinct from nicotinic or muscarinic receptors (Trendelenburg, 1954). However, such a possibility has not been subjected to rigorous pharmacological examination. A further complication lies in the fact that in addition to the secretory effect on the glandular cells, histamine has been shown to expel saliva from the acini and ducts probably by contracting the myoepithelial cells of the submandibular gland, the effect being called a motor effect (MacKay, 1927; Babkin & MacKay, 1931). However, it is uncertain whether the motor effect of histamine is exerted directly on the myoepithelial cells or indirectly by stimulation of autonomic nerve fibres there.

As to the vasodilator effect of histamine on the submandibular gland, no pharmacological analysis in a modern sense has been done. As elsewhere, histamine is supposed to exert a direct vasodilator effect on the vascular bed of the submandibular gland. In addition to this, a contribution of reactive vasodilatation to the vasodilator effect of histamine is also suspected (Emmelin, 1966). In view of the foregoing, we designed the present experiments to elucidate the mode of action of histamine in eliciting salivation from and vasodilatation of the submandibular gland of the dog. We also investigated the type of histamine receptors involved in the salivary and blood flow responses to histamine.

Methods

Experiments were performed on 49 dogs of either sex, weighing 10 to 25 kg. The animals were anaesthetized with pentobarbitone sodium initially at a dose of 30 mg/kg intravenously and maintained on hourly supplementary doses of 4 to 5 mg/kg intravenously. In each dog on either the right or the left side, the duct of the submandibular gland, the chorda-lingual nerve and the external carotid, maxillary, facial and glandular arteries were exposed. The duct was cannulated with polyvinyl tubing and the saliva passed to a water-filled bottle to replace water which overflowed on to an electronic drop counter (Data-Graph, HT 21). The volume of one drop was approximately 15 μl. After the animal had been given heparin sodium, 500 units/kg intravenously, the external carotid and maxillary arteries were ligated and a polyethylene cannula was introduced into the facial artery through a cut made in the cranial end of the external carotid artery. Then, the facial artery was ligated just distally to the origin of the glandular artery. Small muscular branches were all ligated. Blood from the femoral artery was delivered to the cannula placed in the facial artery by means of a peristaltic pump (Harvard Apparatus, Model 1210). A Starling pneumatic resistance was placed distally to the pump to shunt a fraction of blood to the femoral vein. Thus, the glandular vascular bed was perfused selectively with blood at constant pressure. Perfusion pressure was set initially to approximate the mean systemic blood pressure and kept constant throughout the experiment. Perfusion pressure was monitored at the side arm of the perfusion circuit and systemic blood pressure at the external carotid artery with pressure transducers (Nihon Kohden, MPU-0.5). Blood flow through the glandular artery was measured with an electromagnetic flow meter (Nihon Kohden, MF-46), a flow probe of which was placed just proximally to the cannula introduced into the facial artery. The sympathetic nerve to each gland was left intact but the chorda-lingual nerve was cut in all experiments and its distal stump was stimulated electrically. Stimulus parameters were 6 V, 0.1 ms, 10 Hz and for 30 s, and fixed throughout the experiments. Details of the preparation have been given previously (Satoh, Takeuchi & Hashimoto, 1972).

The drugs used were acetylcholine chloride (Daiichi), histamine dihydrochloride (Wako), hexamethonium bromide (Yamanouchi), (−)-hyoscyamine sulphate (Alps Yakuhin), mepyramine maleate (pyrilamine maleate, Merck Sharp & Dohme), metiamide (Smith Kline & French) and tetrodotoxin (Sankyo; each ampoule contains 1 mg of tetrodotoxin and 5 mg of sodium citrate). All drugs except for metiamide were dissolved in 0.9% w/v NaCl solution (saline). Metiamide (244 mg) was first dissolved in 1.1 ml of 1 N HCl solution, and then 2 ml of 0.1 N NaOH solution was added, and finally the volume was adjusted to 10 ml with saline. Agonist solutions in a volume of 10 or 30 μl were injected (in 4 s) by the use of individual microsyringes into the perfusion circuit close to the glandular artery. Antagonist solutions were infused intra-arterially at a rate of 0.1 or 0.2 ml/min by the use of an infusion pump (Harvard Apparatus, Model 600-900).

Generally, both salivary and blood flow responses to histamine were biphasic and were termed the early and the late response (Figure 1). Dose-response curves were determined for each response in the following way. The early salivary and blood flow responses were distinct: dose-response curves for the early salivary response were constructed from the amount of saliva produced during the early salivary response

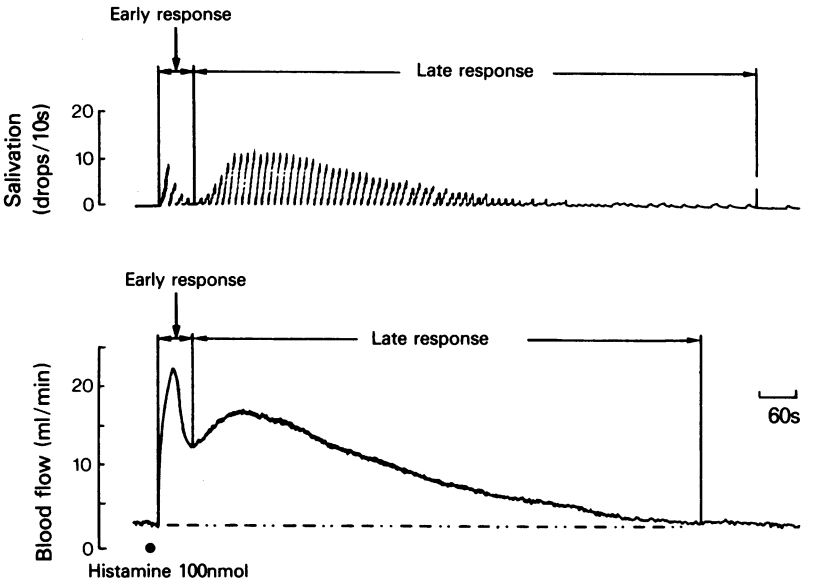

Figure 1 Biphasic salivary and blood flow responses of the submandibular gland of a dog to intra-arterial injection of 100 nmol of histamine. One drop of saliva had a volume of approximately 15 μl.

and those for the early blood flow response were expressed as its peak values. Dose-response curves for the late salivary response were also expressed as the amount of saliva produced during the late salivary response which occurred with a latent period of about 1 min after the injection. Dose-response curves for the late blood flow response were expressed as blood flow exceeding the basal blood flow at 2.8 min after the injection, whether the late blood flow responses were discernible or not. This time interval was chosen because in those 14 responses of 10 glands to 3 to 100 nmol of histamine which showed a distinct secondary peak increase in blood flow, the mean time to a secondary peak increase in blood flow was 2.8 ± 0.2 min.

Values in the text are arithmetic means ± s.e. (unless otherwise stated). The difference between mean values was analysed by Student's t test and judged to be significant when P values were less than 0.05. Parallelism of dose-response curves was analysed by the use of analysis of covariance techniques described by Snedecor & Cochran (1967).

Results

Basal values of main parameters under resting conditions

The spontaneous salivary output from 49 glands was 3.9 ± 0.5 drops/10 min (5.9 ± 0.8 μl/min). In these glands the mean glandular artery blood flow was 5.0 ± 0.3 ml/min at constant perfusion pressure of 117 ± 11 (s.d.) mmHg. The retrograde pressure of the glandular artery measured by clamping the tubing just proximal to the side arm to the pressure transducer was 65 ± 3 mmHg. The mean systemic pressure of the dog was 121 ± 2 mmHg.

Salivary and blood flow responses to histamine of the submandibular gland

Single injections of histamine (0.1 to 100 nmol) into the glandular artery produced salivation in 10 of 11 submandibular glands and increased blood flow in all 11 glands. Both salivary and blood flow responses were monophasic to low doses (0.1 to 1 nmol) of histamine and biphasic to high doses (3 to 100 nmol). Figure 1 shows simultaneous biphasic salivary and blood flow responses to 100 nmol of histamine in a typical gland. The early salivary response to histamine occurred immediately after the injection and ceased within about 1 min when the late salivary response developed slowly. Likewise, the blood flow responses developed with a similar time-course. With low doses (0.1 to 1 nmol) of histamine only the early salivary response occurred in most glands, but, in some only the late salivary response occurred. By contrast, the early blood flow response prevailed

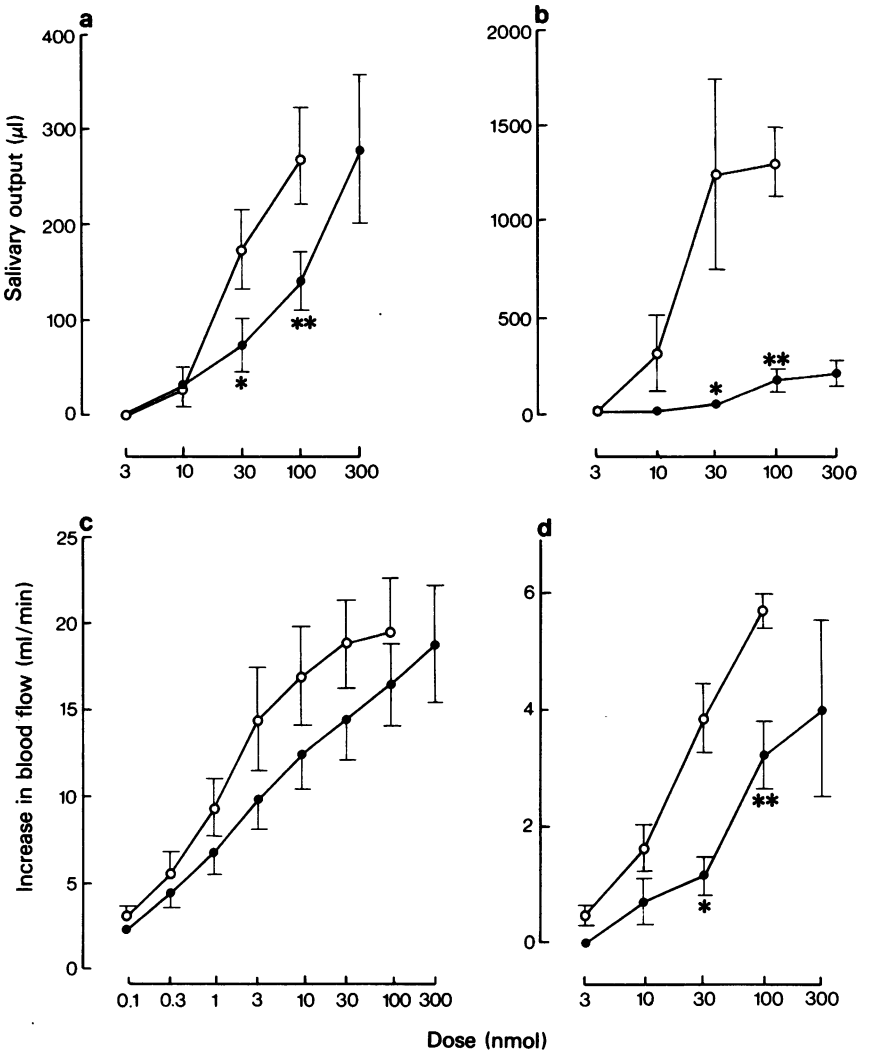

Figure 2 Mean dose-response curves for the early (a) and the late (b) salivary response and for the early (c) and the late (d) blood flow response to histamine: (○) denotes the first and (●) the second dose-response curves. The early salivary response is the amount of saliva produced during the first phase of the salivary response which ceased within about 1 min after the injection of histamine. The late salivary response is the amount of saliva produced during the second phase of the salivary response to histamine. The early blood flow response to histamine is the first peak increase in blood flow. The late blood flow response is the blood flow exceeding the basal flow at 2.8 min after the injection of histamine. Each point represents the mean of 5 values from 5 glands. Vertical bars show s.e. mean. $*P < 0.05$ and $**P < 0.01$ compared with corresponding values of the first dose-response curves.

when low doses were given and it accompanied the late blood flow response with high doses.

Tachyphylaxis of the early and the late salivary and the late blood flow response to histamine

To investigate tachyphylaxis to the action of histamine, in 5 submandibular glands a second dose-response curve was started about 30 min after the final response of the first dose-response curve had subsided. In the second dose-response curve, the early salivary response was significantly reduced and the late salivary response was suppressed almost completely (Figure 2). However, the early blood flow re-

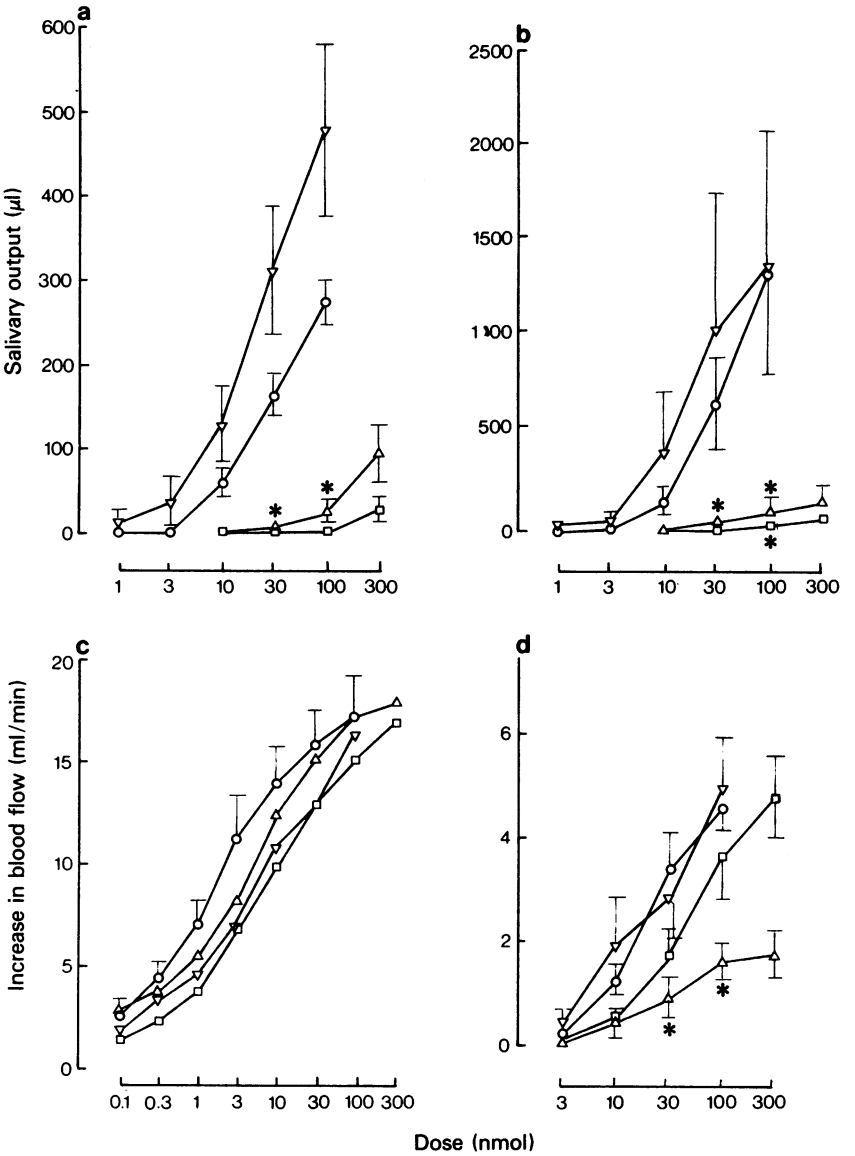

Figure 3 Mean dose-response curves for the early (a) and the late (b) salivary response and for the early (c) and the late (d) blood flow response to histamine obtained as control (○, $n = 11$), during infusion of tetrodotoxin (0.5 ± 0.2 (s.d.) nmol/min) (△, $n = 5$), during infusion of (−)-hyoscyamine (27.5 ± 6.1 (s.d.) nmol/min) (□, $n = 6$) and during infusion of hexamethonium (2.9 ± 1.8 (s.d.) µmol/min) (▽, $n = 6$). Vertical bars show s.e. mean. In (c) standard errors of the control dose-response curve only are shown to avoid confusion. $*P < 0.05$ compared with corresponding control curves to histamine.

sponse was not significantly altered although there was a shift of the late blood flow-response curve to the right (Figure 2). In short, marked tachyphylaxis developed in both the early and the late salivary response and also the late blood flow response to histamine. Consequently in the experiments described in the following sections a single dose-response curve to histamine was determined in each animal either following the administration of a given antagonist, or in the absence of interfering drugs (such as in the 11 submandibular glands which served as controls).

As described previously (Taira & Satoh, 1974;

Taira, Narimatsu & Satoh, 1975), acetylcholine (0.03 to 30 nmol) injected intra-arterially produced salivation and an increase in blood flow. No tachyphylaxis developed in the salivary and blood flow responses to acetylcholine.

Effects of tetrodotoxin on salivary and blood flow responses to histamine

In 5 submandibular glands tetrodotoxin was infused into the glandular artery at a rate of 0.5 ± 0.2 (s.d.) nmol/min. Before the infusion, increases in saliva and blood flow in response to submaximum electrical stimulation of the chorda-lingual nerve were 990 ± 86 μl and 10.8 ± 1.4 ml/min, respectively. A few minutes after the start of infusion the salivary and blood flow responses to chorda-lingual nerve stimulation with the same parameters were abolished. During the blockade of neural excitation thus attained, both the early and the late salivary response to histamine (1 to 300 nmol) were almost abolished (Figure 3). However, the early blood flow response to histamine (0.1 to 300 nmol) was unaffected by tetrodotoxin, although the late blood flow response to histamine (3 to 300 nmol) was greatly reduced (Figure 3).

Effects of (−)-hyoscyamine on salivary and blood flow responses to histamine

In 6 submandibular glands (−)-hyoscyamine was infused into the glandular artery at a rate of 27.5 ± 6.1 (s.d.) nmol/min. This drug abolished the salivary response (1373 ± 125 μl before the infusion) but spared the blood flow response (12.5 ± 1.2 ml/min against 10.2 ± 1.2 ml/min before the infusion) to chorda-lingual nerve stimulation in about 4 min after the start of infusion. From this time onwards the salivary and blood flow responses to acetylcholine (0.03 to 30 nmol) were greatly antagonized. Under these circumstances both the early and the late salivary response to histamine (1 to 300 nmol) were almost abolished (Figure 3), whereas the early and the late blood flow response to histamine (0.1 to 300 nmol) were not statistically different from those in control animals (Figure 3).

Effects of hexamethonium on salivary and blood flow responses to histamine

In 6 submandibular glands hexamethonium was infused into the glandular artery at a rate of 2.9 ± 1.8 (s.d.) μmol/min. Before the infusion, chorda-lingual nerve stimulation increased salivation to 1405 ± 118 μl and increased blood flow by 12.1 ± 1.9 ml/min. Within 10 min the salivary and blood flow responses to chorda-lingual nerve stimulation were almost abolished. During the period of ganglion blockade, the dose-response curves to histamine (1 to 100 nmol) for the early and the late salivary response were located slightly to the left of the control curves but differences between them were not significant (Figure 3). The early and the late blood flow response to histamine (0.1 to 100 nmol) were not significantly different from those in control animals (Figure 3).

As shown previously (Taira *et al.*, 1975) the dose-response curves for the salivary and blood flow responses to acetylcholine (0.03 to 30 nmol) obtained during infusion of hexamethonium were not significantly different from those before the infusion.

Effects of mepyramine and metiamide on salivary and blood flow responses to histamine

In 5 submandibular glands the selective histamine H_1-receptor antagonist, mepyramine, was infused into the glandular artery at a rate of 10 nmol/min. With this infusion, the basal blood flow and spontaneous salivary output were not altered. From about 15 min after the start of infusion onwards, both the early and the late salivary response to histamine were almost abolished (Figure 4). The dose-response curve for the late blood flow response obtained simultaneously was located to the right of that obtained from the 11 control glands. Both curves were parallel ($F_{1,44} = 0.86$) and the distance between them was about one log unit (Figure 4). The dose-response curve for the early blood flow response to histamine was, however, not significantly different from that of the 11 control glands (Figure 4). The salivary and blood flow responses to acetylcholine (0.03 to 30 nmol) and to chorda-lingual nerve stimulation were not affected by mepyramine.

The selective histamine H_2-receptor antagonist, metiamide, was infused intra-arterially at a rate of 100 nmol/min in 6 submandibular glands. The infusion of metiamide affected neither the basal blood flow nor the spontaneous salivary output. Test doses of agonists were injected from about 15 min after the start of infusion onwards. Metiamide (100 nmol/min) failed to affect the early and the late salivary response to histamine (Figure 4). The dose-response curves for the early and the late blood flow response to histamine appeared to be shifted to the right but were not significantly different from the respective curves obtained from the 11 control glands (Figure 4). Since with infusions of metiamide at rates higher than 100 nmol/min, the salivary and blood flow responses to chorda-lingual nerve stimulation and to acetylcholine (0.03 to 30 nmol) were often suppressed, the effects of higher doses than 100 nmol/min of metiamide on the effects of histamine were not examined.

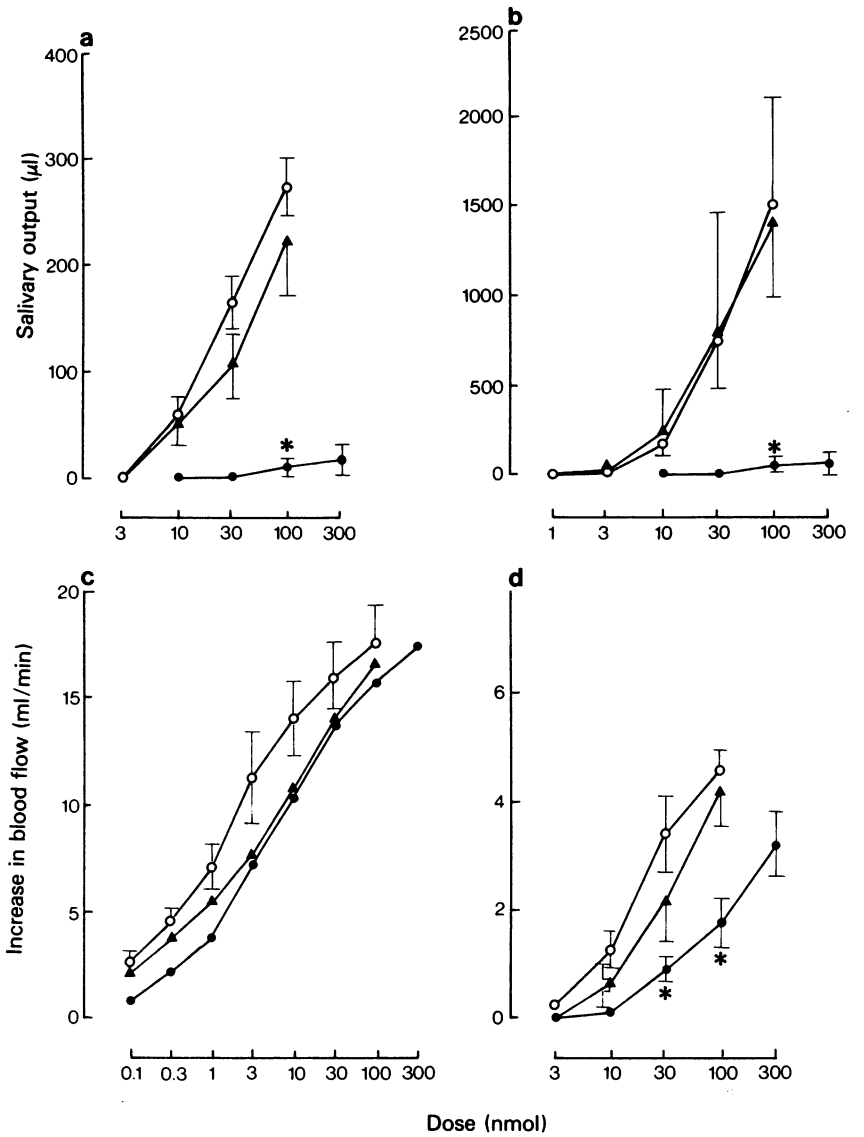

Figure 4 Mean dose-response curves for the early (a) and late (b) salivary and for the early (c) and the late (d) blood flow response to histamine obtained as control (○, $n = 11$), during infusion of mepyramine (10 nmol/min) (●, $n = 5$) and during infusion of metiamide (100 nmol/min) (▲, $n = 6$). The control dose-response curves to histamine are the same as those shown in Figure 3. Each point represents the mean value and vertical bars show s.e. mean. In (c) standard errors are shown of only the control dose-response curve to avoid confusion. *$P < 0.05$ compared with corresponding control values.

Antagonism by mepyramine and/or metiamide of the early blood response to histamine

In the experiments described in the preceding section, the mean dose-response curve for the early blood flow response to histamine in the presence of mepyramine or metiamide was not significantly different from the control curve obtained from other animals. Since no tachyphylaxis developed in the early blood flow response to histamine, the antagonism by mepyramine, metiamide or both was investigated in such a way that in the same gland the control dose-response

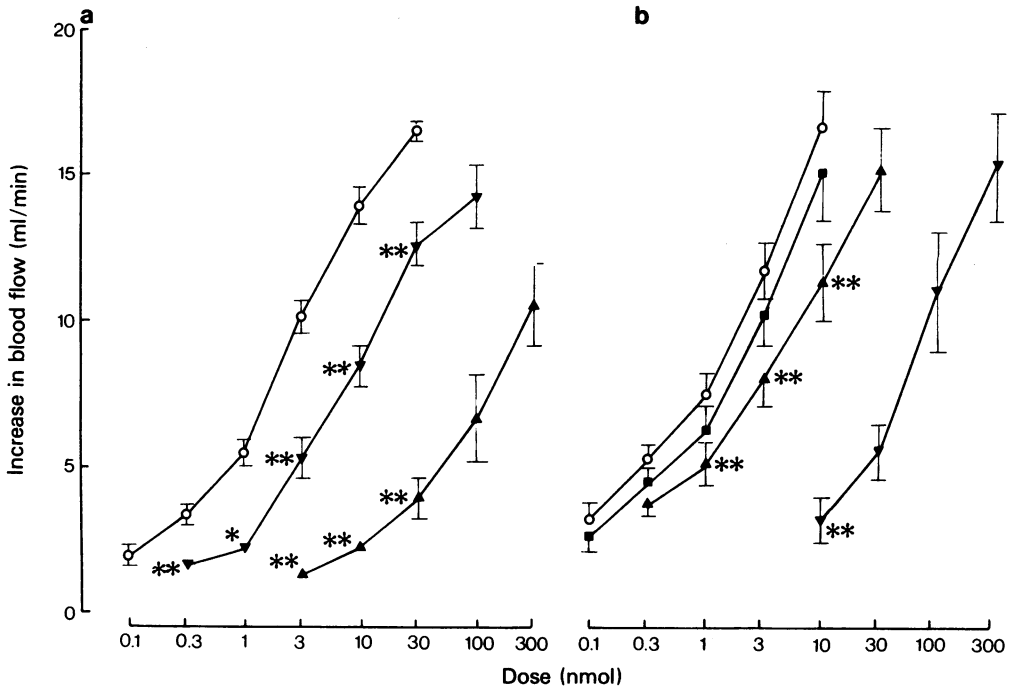

Figure 5 (a) Mean dose-response curves for the early blood flow response to histamine as control (before infusion) (○, $n = 5$), during infusion of mepyramine (▼, 10 nmol/min) and during infusion of mepyramine (10 nmol/min) plus metiamide (▲, 100 nmol/min). (b) Mean dose-response curves for the early blood flow response to histamine as control (before infusion) (○, $n = 5$), during infusion of metiamide (■, 10 nmol/min), during infusion of metiamide (▲, 100 nmol/min) and during infusion of metiamide (100 nmol/min) plus mepyramine (▼, 10 nmol/min). Each point represents the mean value and vertical bars show s.e. mean. $*P < 0.05$ and $**P < 0.01$ compared with corresponding control values.

curve was first determined and then the curves were obtained in the presence of each or both antagonists.

In 5 submandibular glands infusion of mepyramine at a rate of 10 nmol/min shifted the dose-response curve for the early blood flow response to histamine in a parallel fashion ($F_{1,36} = 0.91$) to the right by 0.7 log units (at the dose level increasing blood flow by 10 ml/min) (Figure 5a) and additional infusion of metiamide at a rate of 100 nmol/min to that of mepyramine (10 nmol/min) caused a parallel shift ($F_{1,36} = 1.03$) to the right of the curve by 1.9 log units (at the dose level increasing blood flow by 10 ml/min). Data are summarized in Figure 5a.

In 5 other submandibular glands metiamide alone was first infused at a rate of 10 nmol/min. With this infusion the dose-response curve for the early blood flow response to histamine was shifted only slightly to the right but in a parallel way ($F_{1,36} = 0.76$) (Figure 5b). When the rate of infusion of metiamide was raised to 100 nmol/min, the dose-response curve for the early blood flow response to histamine was shifted to the right in a parallel fashion ($F_{1,36} = 0.27$) by 0.5 log units (at the dose level increasing blood flow by 10 ml/min). Then, the infusion of metiamide at the rate of 100 nmol/min was combined with infusion of 10 nmol/min of mepyramine. With the combined infusion the dose-response curve for the early blood flow response to histamine was shifted in a parallel way ($F_{1,36} = 2.94$) to the right by 1.6 log units (at the dose level increasing blood flow by 10 ml/min). Data are summarized in Figure 5b.

In the two series of experiments described above, the displacement of the dose-response curves for the early blood flow response to histamine produced by a combination of mepyramine (10 nmol/min) and metiamide (100 nmol/min) were similar. Thus, comparison of the displacement of the curve by the individual antagonist obtained from different glands can be made. The displacement was greater by the low dose (10 nmol/min) of mepyramine than by the high dose (100 nmol/min) of metiamide (0.7 log units for mepyramine against 0.5 log units for metiamide). The displacement produced by mepyramine (10 nmol/min) plus metiamide (100 nmol/min) was greater than the

sum of the displacement produced by the individual antagonists (1.6 or 1.9 log units against 1.2 log units for the sum).

Discussion

In the present experiments histamine injected intra-arterially to the submandibular gland of the dog produced salivation from and an increase in blood flow through the gland. Both salivary and blood flow responses were biphasic; the early and the late response were discernible, as reported by Satoh *et al.* (1972). Since both the early and the late salivary response were abolished by tetrodotoxin, both of them are due entirely to excitation of neural elements in the gland. The whole salivary response was also susceptible to the blocking action of (−)-hyoscyamine. This implies that neural elements excited by histamine are parasympathetic cholinergic neurones in the gland. No part of the salivary response to histamine was affected by a dose of the nicotinic receptor blocking agent, hexamethonium, sufficient to block ganglionic transmission brought about by electrical stimulation of the chorda-lingual nerve. Thus, it seems reasonable to conclude that parasympathetic cholinergic neurones excited by histamine are postganglionic and that the receptors involved are distinct from nicotinic receptors. Thus, the mode of action of histamine in eliciting salivation elucidated so far by the present experiments is in line with that suggested by Gibbs & McClanahan (1937). Conversely, the present experiments failed to provide evidence for a direct secretory action of histamine on the glandular cells (Emmelin, 1966).

Histamine is known to have a motor effect, i.e., expulsion of saliva from the acini and ducts probably by contracting the myoepithelial cells of the salivary gland (MacKay, 1927; Babkin & MacKay, 1931). The present experiments were not designed to differentiate the motor effect of histamine from its secretory effect. Nevertheless, the short latency of the early salivary response to histamine together with the small amount of saliva produced during the response tempts us to speculate that the early salivary response might result from the motor effect. Even so, such a motor effect might be indirect, through excitation of parasympathetic nerve fibres innervating the myoepithelial cells, since the early salivary response was abolished by tetrodotoxin and (−)-hyoscyamine. In the dog salivary gland the myoepithelial cells are innervated by both parasympathetic and sympathetic nerve fibres and contract by excitation of either nerve (Emmelin, Garrett & Ohlin, 1969). The present experiments, however, failed to produce evidence showing the supposed direct action of histamine on the myoepithelial cells (MacKay, 1927; Babkin & MacKay, 1931) or the effect through excitation of sympathetic nerve fibres thereon.

The early blood flow response to histamine was not affected by tetrodotoxin, (−)-hyoscyamine or hexamethonium. Thus, it is reasonable to conclude that the early blood flow response is due to the direct stimulant action of histamine on vascular smooth muscle in the glandular vascular bed. The late blood flow response to histamine, unlike the early one, was susceptible to the blocking action of tetrodotoxin, although not abolished. The late blood flow response roughly paralleled the late salivary response in time course (Figure 1). Thus, it is conceivable that the tetrodotoxin-susceptible component of the late blood flow response to histamine, like the late salivary response, is due to the stimulant action of histamine on parasympathetic postganglionic neurones in the gland. The resistance to the blocking action of (−)-hyoscyamine of the tetrodotoxin-susceptible component of the late blood flow response is a similar phenomenon to the atropine-resistant vasodilatation of the salivary gland or tongue resulting from excitation of parasympathetic postganglionic neurones in the chorda-lingual nerves (Heidenhain, 1872; Taira *et al.*, 1975; Shimizu & Taira, 1978). The resistance to the blocking action by hexamethonium of the tetrodotoxin-susceptible component of the late blood flow response to histamine can be explained in a way similar to that in which the resistance to blockade by hexamethonium of the late salivary response is explained. The tetrodotoxin-resistant component of the late blood flow response to histamine is probably produced by the direct stimulant action of histamine on vascular smooth muscle in the glandular vascular bed. This component would be the end of the early blood flow response. Thus, in the dog submandibular gland, histamine is capable of eliciting vasodilatation by its direct action on vascular smooth muscle and by its stimulant action on parasympathetic postganglionic neurones. Emmelin (1966) suspected that the reactive vasodilatation contributed to the vasodilator action of histamine in the salivary gland. The present experiments provide experimental evidence for such a possibility.

In the present experiments, marked tachyphylaxis developed in the early and late salivary responses. Tachyphylaxis of the salivary responses to repeated injections of histamine has been described by several workers (Gibbs & McClanahan, 1937; Yonkman, Chess, Mathieson & Hansen, 1946; Emmelin, 1966). Trendelenburg (1954) demonstrated that in the superior cervical ganglion marked tachyphylaxis developed to the stimulant action of histamine. In view of the early and late salivary responses being due to the stimulant action of histamine on parasympathetic postganglionic neurones in the submandibular gland, tachyphylaxis would be inherent in the stimulant

action of histamine on neural elements. It was of interest that the reduction of the late blood flow response in the second dose-response curve and during the blockade of neural excitation by tetrodotoxin was to a similar extent. In the preceding paragraph a part of the late blood flow response to histamine is ascribed to the result of excitation of parasympathetic postganglionic neurones in the gland.

The whole salivary response and the tetrodotoxin-susceptible part of the late blood flow response to histamine were readily suppressed by the histamine H_1-receptor antagonist, mepyramine, but not by the histamine H_2-receptor antagonist, metiamide. These results indicate that histamine receptors on parasympathetic postganglionic neurones are exclusively of the H_1-type but not of the H_2-type. The present results on the effect of mepyramine on the salivary response to histamine are in line with those of Yonkman *et al.* (1946) who found in the submandibular gland of the cat, that salivation in response to histamine was antagonized by pyribenzamine, a histamine H_1-receptor antagonist. Of course, at this time it was uncertain whether histamine receptors involved in salivation are located on the glandular cells, the myoepithelial cells or the parasympathetic neurones, and subdivision of histamine receptors into two types had not been proposed. Trendelenburg (1954) demonstrated that in the cat superior cervical ganglion the stimulant action of histamine is antagonized specifically by mepyramine. Emmelin & Muren (1949) have shown that in the cat adrenal medulla the stimulant action of histamine is blocked by mepyramine. Thus, neuronal histamine receptors mediating the stimulant action of histamine are of the H_1-type.

As discussed in the preceding paragraph, the early blood flow response and a part of the late blood flow response are thought to result from the direct stimulant action of histamine on vascular smooth muscle of the gland and that part of the late blood flow response is considered to be the tail end of the early blood flow response. The dose-response curve for the early blood flow response to histamine was shifted to the right in a parallel fashion by either mepyramine or metiamide. However, the displacement of curves was greater with the low dose of mepyramine (10 nmol/min) than with the high dose of metiamide (100 nmol/min). The simultaneous infusion of mepyramine (10 nmol/min) and metiamide (100 nmol/min) produced a greater parallel displacement of the early dose-response curve to histamine than the sum of the displacement produced by the individual antagonist. Similar antagonism by histamine H_1- and H_2-receptor antagonists of the vasodilator (Black, Owen & Parsons, 1975; Flynn & Owen, 1975) or vasodepressor (Owen, 1975) effect of histamine has been described. Thus, histamine receptors in the glandular vascular bed are composed of H_1- and H_2-receptors as elsewhere.

The metiamide which we used was given to Professor K. Hashimoto in our department by Dr G.J. Durant, Research Institute of Smith Kline & French Laboratories Ltd., Hertfordshire. We are grateful to Division of Merck & Co., Inc, Merck Sharp & Dohme International, Rahway, N.J., U.S.A. for the supply of the mepyramine (pyrilamine).

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(Received February 14, 1979.
Revised May 23, 1979.)