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PHARMACOLOGICAL ANALYSIS OF HISTAMINE RECEPTORS IN MUSCULATURE AND VASCULATURE OF THE DOG TRACHEA *in situ*

N. HIMORI & N. TAIRA

Department of Pharmacology, Tohoku University School of Medicine, Sendai 980, Japan

1 The role of histamine H_1- and H_2-receptors in the musculature and vasculature of the dog trachea was investigated in the blood-perfused trachea *in situ*.

2 Histamine and acetylcholine caused increases in blood flow (tracheal vasodilatation) and in intraluminal pressure (tracheal constriction) in a dose-dependent manner. Histamine was almost equipotent to acetylcholine in causing tracheal vasodilatation but was about 30 times less potent in causing tracheal constriction.

3 The histamine H_2-receptor agonist, dimaprit, caused a dose-dependent increase in tracheal blood flow but failed to cause tracheal constriction.

4 The tracheal constriction produced by histamine was inhibited strongly by diphenhydramine but not modified by metiamide. The tracheal vasodilatation produced by histamine was antagonized by both diphenhydramine and metiamide; diphenhydramine was more effective than metiamide.

5 It is concluded that in the tracheal musculature, histamine receptors are exclusively of the H_1-type and mediate constriction, whereas in the tracheal vasculature, both histamine H_1- and H_2-receptors mediate vasodilatation but histamine H_1-receptors are predominant.

Introduction

The receptors involved in the responses of many tissues or organs to histamine have been divided into H_1- and H_2-receptors. Histamine H_1-receptors, as defined by Ash & Schild (1966), mediate constrictions of the guinea-pig ileum and tracheobronchi, whereas stimulation of histamine H_2-receptors (see also Black, Duncan, Durant, Ganellin & Parsons, 1972) causes gastric acid secretion, increases guinea-pig heart rate and inhibits the rat uterus. The guinea-pig bronchus is very sensitive to histamine (Dale & Laidlaw, 1910) and the bronchoconstriction is readily antagonized by the histamine H_1-receptor antagonists, diphenhydramine or mepyramine (Herxheimer, 1956; Arunlakshana & Schild, 1959; Holgate & Warner, 1960). On the other hand, Maengwyn-Davies (1968) observed that in the cat isolated tracheal chain, histamine causes a relaxation which is antagonized partly by mepyramine and partly by the β-adrenoceptor antagonist, pronethalol. Eyre (1973) reported that histamine-induced relaxation of the sheep bronchus is antagonized by burimamide, the H_2-receptor antagonist (Black *et al.*, 1972) but not by mepyramine, and that relaxation of the cat trachea in response to histamine is effectively abolished by both histamine H_1- and H_2-receptor antagonists. In the dog, histamine produces bronchoconstriction, a substantial part of which has been ascribed to a reflex *via* the vagus (de Letona, de la Mata & Aviado, 1961; Wasserman, 1975). However, no detailed pharmacological analysis has been made of the mode of action of histamine in the dog trachea which contracts in response to histamine (Akçasu, 1959). Furthermore, no information is available about the type of histamine receptors involved in the histamine-induced bronchial or tracheal constriction of the dog. In vascular beds, histamine produces a variety of responses depending upon animal species and organs (Owen, 1977). In the dog, histamine dilates coronary, femoral, mesenteric, renal, vesical, saphenous and splenic arterial beds (see discussion by Satoh & Hashimoto, 1973), but not the pulmonary vessels (Dale & Laidlaw, 1910). In most vascular beds of the dog histamine H_1- and H_2-receptors are involved in vasodilatation in response to histamine (Owen, 1977).

In view of these observations we designed the present experiments to investigate the role of histamine H_1- and H_2-receptors and their relative predominance in the tracheal musculature and vasculature of the dog by the use of the blood-perfused trachea *in situ* (Himori & Taira, 1976).

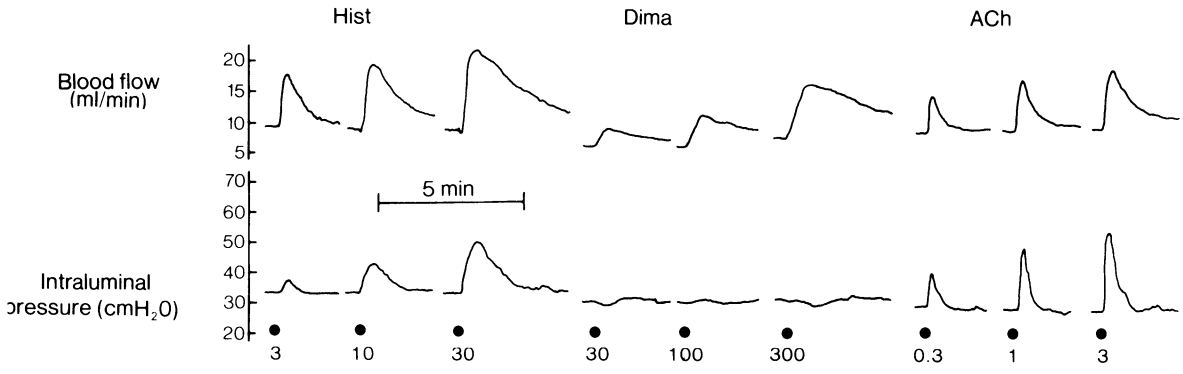

Figure 1 Responses of the tracheal vasculature (blood flow) and musculature (intraluminal pressure) to intra-arterial histamine (Hist), dimaprit (Dima) and acetylcholine (ACh) in dogs. All doses in μg.

Methods

Experiments were performed on 32 adult mongrel dogs of either sex, weighing 11.5 to 16 kg. Anaesthesia was induced by a single intravenous injection of pentobarbitone sodium 30 mg/kg and maintained with hourly supplemental intravenous doses of approx. 5 mg/kg. The upper cervical region was incised in the midline and muscular, pharyngeal and cricothyroid branches of the cranial thyroid arteries were ligated. The upper trachea was perfused with blood led from the right femoral artery through the cannulated cranial thyroid arteries which run down along the trachea on both sides supplying the tracheal musculature. A peristaltic pump (Harvard Apparatus, Model 1215) and a Starling pneumatic resistance were used for constant pressure perfusion. The perfusion pressure was adjusted initially to approximate the mean systemic blood pressure. Prior to the perfusion, heparin sodium 500 units/kg intravenously was given initially and 100 units/kg intravenously in addition at hourly intervals. Blood flow through the perfused area was measured with an electromagnetic flowmeter (Nihon Kohden, MF-46-3). Responses of the tracheal musculature were measured with a pressure transducer (Nihon Kohden, MPU-0.1) as changes in intraluminal pressure of a water-filled cuff of a tracheal tube introduced into the trachea via the mouth. The resting intraluminal pressure was adjusted to 25 to 35 cmH_2O. Details of the preparation have been described previously (Himori & Taira, 1976).

The drugs used in this study were acetylcholine chloride (Daiichi), atropine sulphate (Merck), dimaprit (Smith Kline & French), diphenhydramine hydrochloride (Kowa), histamine dihydrochloride (Wako), metiamide (Smith Kline & French), phentolamine methanesulphonate (Ciba) and (±)-propranolol hydrochloride (ICI). All drugs except 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 by the addition of saline. All drug solutions were diluted with saline to the desired concentrations. Agonist solutions in a volume of 30 or 100 μl were injected intra-arterially (in 4 s) by the use of microsyringes. Diphenhydramine solutions were injected intra-arterially in a volume of 10 to 100 μl (in 4 s) and metiamide solutions in a volume of 41 to 410 μl (in 4 to 15 s). Doses of all drugs except diphenhydramine and metiamide refer to their salts and those of the latter two antagonists to their bases.

Values in the text are arithmetic means ± s.e. (unless stated otherwise). Significance of differences between mean values was evaluated by P values which were calculated by Student's t test. The difference was judged to be significant when P values were less than 0.05.

Results

The average blood flow through the tracheal vascular bed of the 32 dogs was 8.7 ± 1.1 ml/min at the average perfusion pressure of 126 ± 6 (s.d.) mmHg, approximating previous values (Himori & Taira, 1976; 1977). The average resting intraluminal pressure of the trachea was 22 ± 4 cmH_2O (n = 32).

Effects of histamine, dimaprit and acetylcholine on tracheal blood flow and intraluminal pressure

Single intra-arterial injections of histamine (0.3 to 30 μg) and acetylcholine (0.3 to 10 μg) produced increases in blood flow through the tracheal vascular bed (tracheal vasodilatation) and in intraluminal pressure of the trachea (tracheal constriction) in a dose-dependent manner. Typical experiments are shown in

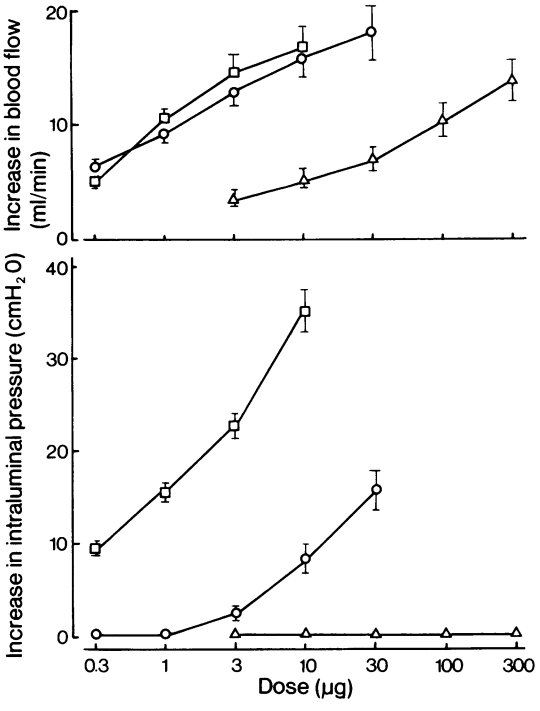

Figure 2 Dose-response curves for change in blood flow and in intraluminal pressure of the dog trachea to intra-arterial histamine (○, $n = 13$), dimaprit (△, $n = 4$) and acetylcholine (□, $n = 11$). Each point represents the mean value and vertical bars show s.e. mean.

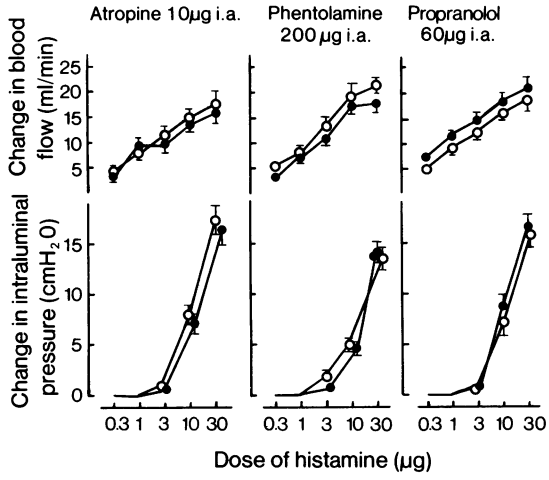

Figure 3 Dose-response curves for change in blood flow (upper panel) and in intraluminal pressure (lower panel) of the dog trachea to intra-arterial histamine before (○) and after (●) a single injection of atropine (10 μg i.a.), phentolamine (200 μg i.a.) or propranolol (60 μg i.a.). Each point represents the mean of 4 observations (phentolamine, propranolol) or 5 observations (atropine) on 7 animals. Vertical bars show s.e. mean.

Figure 1 and dose-response curves for peak increases in blood flow and tracheal intraluminal pressure are presented in Figure 2. Dimaprit, the highly specific histamine H_2-receptor agonist (Parsons, Owen, Ganellin & Durant, 1977), (3 to 300 μg) caused a dose-dependent increase in blood flow through the tracheal vascular bed, but failed to produce tracheal constriction (Figures 1 and 2). As clearly seen in Figures 1 and 2, the dose-response curves to the three agonists for increase in blood flow were parallel, and in causing vasodilatation histamine and acetylcholine were almost equipotent while dimaprit was about 100 times less potent than histamine on a weight basis. By contrast, in causing tracheal constriction histamine was about 30 times less potent than acetylcholine. Histamine 30 μg intra-arterially caused a fall in systemic blood pressure of about 25 mmHg, but the tracheal constrictor response to histamine always preceded the fall in systemic blood pressure.

Failure of atropine, phentolamine and propranolol to modify tracheal vasodilatation and tracheal constriction in response to histamine

In previous experiments (Himori & Taira, 1976) 10 μg of atropine, 200 μg of phentolamine and 60 μg of propranolol (all i.a.) effectively suppressed responses of both the tracheal vasculature and musculature to acetylcholine (tracheal vasodilatation-tracheal constriction), to noradrenaline in the presence of β-blockade (tracheal vasoconstriction-tracheal constriction) and to isoprenaline (tracheal vasodilatation-tracheal relaxation), respectively. In these doses, each antagonist failed to modify significantly the vasodilator and tracheal constrictor responses to histamine, as shown in Figure 3 although propranolol enhanced the vasodilator responses to histamine.

Effects of diphenhydramine and metiamide on tracheal vasodilatation and tracheal constriction in response to histamine

Diphenhydramine is known to have a local anaesthetic action about 3 times that of procaine (Dutta, 1949). However, preliminary experiments showed that the antagonism as observed over a period of 20 to 60 min after a single injection of diphenhydramine

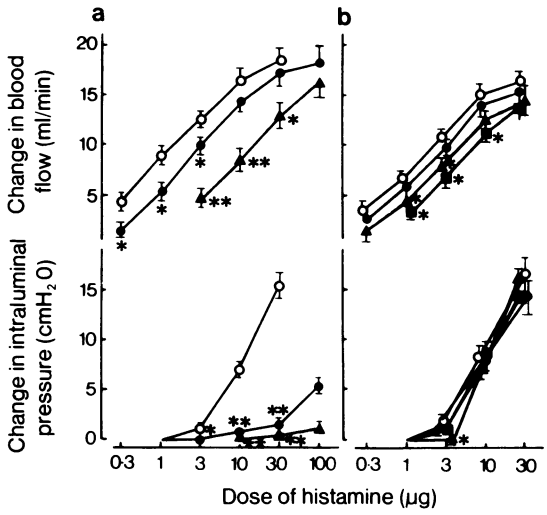

Figure 4 Effects of diphenhydramine (a) and metiamide (b) on the dose-response curves for histamine of the tracheal vasculature (blood flow) and tracheal musculature (intraluminal pressure). (a) Diphenhydramine, mg, i.a.: (○) no drug, $n = 8$; (●) 0.3, $n = 8$; (▲) 1, $n = 6$. (b) Metiamide, mg, i.a.: (○) no drug, $n = 7$; (●) 1, $n = 7$; (▲) 3, $n = 7$, (■) 10, $n = 4$. $^*P < 0.05$ and $^{**}P < 0.01$ against control values in corresponding doses.

(1 mg i.a.) was specific toward the tracheal vasodilator and tracheal constrictor responses to histamine. In this period diphenhydramine (1 mg i.a.) did not affect significantly the tracheal vasodilator and tracheal constrictor responses to acetylcholine. Therefore, the antagonism of histamine-induced responses by diphenhydramine as the histamine H_1-receptor antagonist was determined within this definite period. Unlike diphenhydramine, metiamide, the highly specific histamine H_2-receptor antagonist (Black, Duncan, Emmett, Ganellin, Hesselbo, Parsons & Wyllie, 1973), has no local anaesthetic action (Hind & Sutton, 1977). This was true in preliminary experiments on the blood-perfused trachea *in situ*; metiamide did not affect the tracheal vasodilator and tracheal constrictor responses to acetylcholine.

As shown in Figure 4a, diphenhydramine produced dose-dependent shifts of the dose-flow response curves for histamine without significantly affecting their slopes or maxima. The tracheal constriction induced by histamine was also inhibited significantly by diphenhydramine. However, it was impossible to determine whether the inhibition of the histamine-induced tracheal constriction by diphenhydramine would be overcome by increasing doses of histamine because of its intense hypotensive effect.

Metiamide produced a dose-dependent inhibition of the histamine-induced vasodilatation. However, the histamine-induced tracheal constriction was not inhibited by metiamide (Figure 4b).

During the blockade of histamine H_1-receptors by diphenhydramine (0.3 mg i.a.), a single injection of metiamide (3 mg i.a.) exerted further inhibition of the increase in blood flow in response to histamine. The amount of inhibition produced by the combination of the H_1- and H_2-receptor antagonists was roughly the sum of the amounts of inhibition caused by the individual antagonists, as shown in Table 1.

Diphenhydramine *per se* produced a dose-dependent increase in intraluminal pressure and at 1 mg intra-arterially caused an increase in intraluminal pressure amounting to 10 to 20 cmH_2O but this disappeared in about 5 min. Unlike diphenhydramine, metiamide had no effect on the resting tone of the trachea. Both diphenhydramine and metiamide caused a slight but clear increase in blood flow, but the increase wore off in a few minutes.

Discussion

In the present experiments histamine injected intra-arterially into the tracheal vascular bed produced vasodilatation and tracheal constriction. In causing vasodilatation, histamine and acetylcholine were

Table 1 Effects of intra-arterial diphenhydramine and metiamide on tracheal vasodilatation produced by intra-arterial histamine

Dose of histamine (μg)	n	*Increase in blood flow (ml/min) caused by histamine*				
		Control	*After injection of* *Diphenhydramine (0.3 mg)*	*Metiamide (3 mg)*	*Diphenhydramine + Metiamide (0.3 mg)*	*(3 mg)*
0.3	4	4.4 ± 0.6	2.1 ± 0.1*	2.9 ± 0.4	1.2 ± 0.2**	
1	6	8.9 ± 0.9	4.6 ± 0.3*	5.8 ± 0.6*	2.2 ± 0.3**	
3	6	13.8 ± 0.9	8.9 ± 0.6*	9.3 ± 1.0*	3.9 ± 0.6**	

n = Number of experiments. Values are mean ± s.e. mean. $^*P < 0.05$, $^{**}P < 0.01$ when compared with corresponding control values.

almost equipotent on a weight basis, whereas in causing tracheal constriction histamine was approximately 30 times less potent than acetylcholine on a weight basis. Thus, the tracheal musculature of the dog, unlike that of the guinea-pig trachea in which histamine and acetylcholine are almost equipotent (Takagi, Takayanagi & Fujie, 1958; Carlyle, 1963), is less sensitive to histamine than to acetylcholine. The low sensitivity of the dog tracheal musculature to histamine is rather surprising in view of the fact that histamine is a potent bronchoconstrictor in the dog when administered intravenously or into the bronchial and pulmonary artery (de Letona *et al.*, 1961; Wasserman, 1975). However, a reflex increase in bronchomotor tone *via* the vagus nerve has been shown to contribute to a substantial degree to the bronchoconstriction produced by histamine administered *via* the above routes (de Letona *et al.*, 1961; Wasserman, 1975). Histamine is an irritant or excitant of afferent nerve endings and is able to trigger reflexes (Mills, Sellick & Widdicombe, 1969; Wasserman, 1975). In the present experiments, the tracheal constriction produced by histamine was not inhibited by atropine in a dose that antagonized acetylcholine-induced tracheal constriction (Himori & Taira, 1976). This rules out possible involvement of a parasympathetic (cholinergic) component in the response to histamine. Thus, histamine injected into the tracheal vascular bed appears to fail to stimulate irritant receptors to trigger the vagal reflex. Alternatively there may be no irritant receptors responsive to histamine in the dog trachea. This may explain the low sensitivity or responsiveness of the tracheal musculature to histamine. The response of tracheal musculature to histamine was not modified by phentolamine, indicating no involvement of an α-adrenoceptor mechanism in the histamine-induced tracheal constriction. Moreover, the tracheal response to histamine was not affected by propranolol in a dose that effectively suppressed the responses to catecholamines (Himori & Taira, 1976). This rules out the possibility that the weak tracheal constrictor response to histamine might be due to the relaxant action of released catecholamines. It has been suggested that histamine relaxes the cat trachea by releasing catecholamines in addition to stimulation of histamine receptors of the tracheal musculature (Eyre, 1973). Thus, it is reasonable to conclude that the tracheal constriction caused by histamine is mediated exclusively by specific histamine receptors. In the present experiments the highly specific histamine H_2-receptor agonist, dimaprit (Parsons *et al.*, 1977) failed to cause tracheal constriction, and the histamine-induced tracheal constriction was antagonized by the histamine H_1-receptor antagonist, diphenhydramine but not by the highly specific histamine H_2-receptor antagonist, metiamide (Black *et al.*, 1973). Thus, in the dog trachea, as in the guinea-pig trachea, histamine receptors are exclusively of the H_1-type and mediate constriction.

The tracheal vasodilatation produced by histamine was modified by neither atropine nor propranolol, excluding possible involvement of parasympathetic (cholinergic) and β-adrenoceptor mechanisms in the response. Dimaprit produced tracheal vasodilatation, although it was about 100 times less potent than histamine on a weight basis. Furthermore, the dose-flow response curves for histamine were shifted to the right in a parallel way by both diphenhydramine and metiamide. The displacement of the curves was greater by diphenhydramine than by metiamide. The amount of inhibition of the histamine-induced tracheal vasodilatation produced by the combination of diphenhydramine and metiamide was roughly equal to the sum of the amount of inhibition caused by each antagonist. Thus, it is safe to conclude that in the tracheal vasculature of the dog both histamine H_1- and H_2-receptors mediate vasodilatation, although histamine H_1-receptors are predominant. The results are in line with the findings on most vascular beds of the dog (see Owen, 1977).

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