Br. J. Pharmac. (1985), **86**, 619–625

Pharmacological characterization of the histamine receptor in the isolated muscularis mucosae of the guinea-pig oesophagus

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1 To characterize the histamine receptors in the muscularis mucosae, the isotonic responsiveness of the isolated muscularis mucosae of the guinea-pig oesophagus to histamine receptor agonists and antagonists was examined *in vitro*.

2 Histamine (0.1–100 μM) produced a concentration-dependent contraction of the muscularis mucosae (EC_{50} = 1.6 ± 0.2 μM). The contractions were rapid in onset, sustained, reversible by washing and the preparation did not show tachyphylaxis.

3 2-Methylhistamine (2-MH), 2-pyridylethylamine (PEA) and 4-methylhistamine (4-MH) produced similar sustained contractions of the muscularis mucosae. The order of sensitivity was histamine > 2-MH > PEA > 4-MH. Impromidine (10–300 μM) and dimaprit (10–300 μM) caused no response in this tissue.

4 The contractile responses to histamine, 2-MH, and PEA were competitively antagonized by diphenhydramine, and the pA_2 values were almost the same (approximately 8.1). Cimetidine (100 μM) could not modify the contractile response to these agonists.

5 The contractile response to histamine was slightly inhibited by tetrodotoxin (0.3 μM), atropine (1 μM), indomethacin (0.1–3 μM) or aspirin (30–300 μM), and the EC_{50} value was increased about 2–6 times by these drugs.

6 When the preparation was incubated in Tyrode solution containing various calcium concentrations (0, 0.45, 0.9 and 1.8 mM), the concentration-response curve to histamine was shifted to the right and downward; the effect was inversely dependent on the calcium concentration, and in a calcium-free medium the response to histamine was abolished. Verapamil (1–10 μM) partially inhibited the contractile response to histamine.

7 The present results indicate that the contraction of the guinea-pig oesophageal muscularis mucosae to histamine is mediated mainly by a direct action on the smooth muscle and partly by indirect actions via the stimulation of either endogenous prostaglandin biosynthesis or intramural cholinergic nerves. The histamine receptors responsible for contractions of this tissue are probably mainly of the H_1-subtype with H_2-receptors having a negligible role.

Introduction

The muscularis mucosae, a thin band of smooth muscle located at the base of the gastrointestinal mucosa, has received very little attention when compared with the external muscle layers of the gut wall. However, the muscularis mucosae probably has a great influence on the absorptive and secretory functions of the mucosa (King *et al.*, 1922). Our recent studies have concentrated on the autonomic innervation and receptor system in the guinea-pig oesophageal muscularis mucosae. This tissue consists only of longitudinal smooth muscle, and is innervated chiefly by excitatory cholinergic nerves and very sparsely by inhibitory adrenergic nerves; there are no non-adrenergic, non-cholinergic nerves (Kamikawa & Shimo, 1979). The responsiveness of this tissue to catecholamines, acetylcholine, 5-hydroxytryptamine, tachykinins and opioid peptides is different from those of the external muscle of the guinea-pig ileum (Kamikawa *et al.*, 1982; 1985b; Kamikawa & Shimo, 1983a,b, 1984). The aims of the present study were to examine

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the responsiveness of the isolated muscularis mucosae of the guinea-pig oesophagus to histamine and its related drugs, and to clarify the receptor types involved.

Methods

Male guinea-pigs (300–500 g) were killed by stunning and bleeding, the oesophagus was excised. The oesophagus was divided into two parts at the entrance to thorax, upper cervical and lower thoracic part (Kamikawa & Shimo, 1979). Each oesophagus was pinned on a cork mat immersed in Tyrode solution. The outer striated muscle coat was cut longitudinally, and gently peeled away leaving an inner tube. The tube including longitudinal muscularis mucosae, about 15 mm long without a load, was immersed in a 10 ml organ bath filled with a modified Tyrode solution of the following composition (mM): NaCl 136.8, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, disodium ethylenediaminetetraacetic acid (EDTA: 2Na) 0.03, ascorbic acid 0.12 and glucose 5.56 (pH 7.4). This solution was bubbled with 5% CO_2 and 95% O_2 and maintained at 37°C. The preparation was suspended under a 0.5 g load and 60 min was allowed to elapse before experiments were started. During this equilibration period, the tissue was washed with fresh Tyrode solution every 15 min.

Responses of the longitudinal muscularis mucosae were isotonically recorded on a kymograph, magnification ×8 (Uchida, 1983). After the 60 min equilibration period, the preparation was maximally contracted with a single concentration of carbachol (10 μM) and after washout was allowed to equilibrate for 30 min. This was repeated until two successive contractions of approximately equal size had been obtained. The muscularis mucosae was then exposed to cumulatively increasing concentrations of histamine or its related agonists to obtain a full concentration-response curve. The concentrations of these agonists causing 50% of the carbachol (10 μM)-induced maximum contraction (EC_{50}) were determined from each curve. The dissociation constant (K_A) of histamine was estimated by the method described by Furchgott (1966). After obtaining the control concentration-response curve to histamine, the muscularis mucosae was treated with phenoxybenzamine (30 nM) for 20 min. After washing out the drug, the second concentration-response curve to histamine was obtained. The K_A value was calculated from plotting double reciprocals of the equieffective concentrations of histamine before and after phenoxybenzamine treatment.

In experiments where the role of calcium ions on the histamine-induced contraction was investigated, a concentration-response curve to histamine was obtained after a 30 min incubation of the tissue in Tyrode solution containing various concentrations of calcium (0, 0.45, 0.9 and 1.8 mM). In experiments to examine the inhibitory effects of various drugs on the contractile response to histamine, the muscularis mucosae was incubated with indomethacin or aspirin for 20 min, and with tetrodotoxin, atropine, verapamil, diphenhydramine or cimetidine for 10 min before the application of histamine. The pA_2 value of diphenhydramine against histamine agonists was estimated by plotting the log (EC_{50} ratio − 1) versus log concentrations of diphenhydramine (Arunlakshana & Schild, 1959).

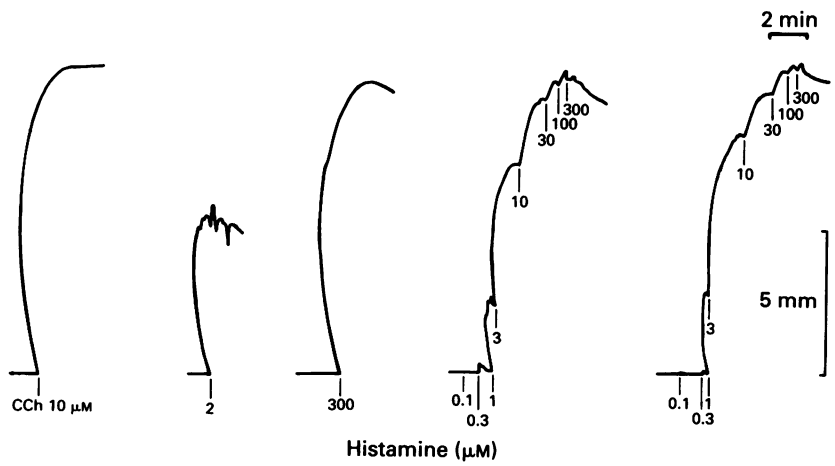

Figure 1 Typical tracing of contractions to carbachol (CCh, 10 μM) and histamine (0.1–300 μM) of the isolated muscularis mucosae of the guinea-pig thoracic oesophagus. Histamine was applied to this tissue with the single or cumulative dose techniques.

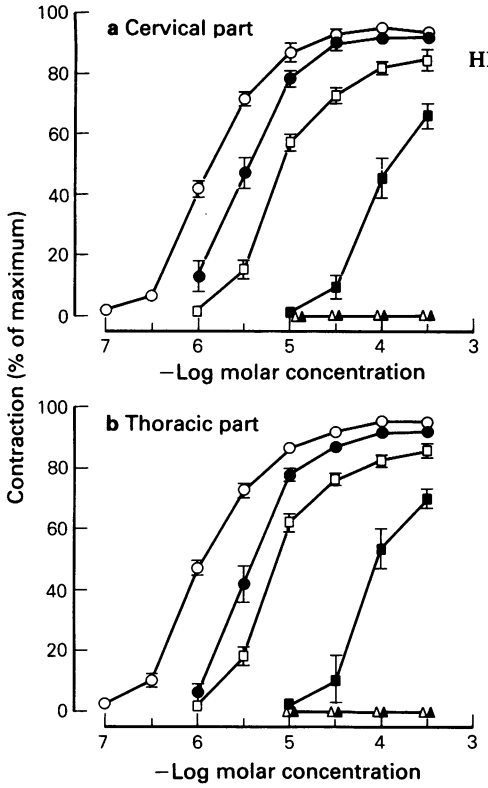

Figure 2 Cumulative concentration-response curves for the contractile responses to histamine (○), 2-methylhistamine (●), 2-pyridylethylamine (□), 4-methyl-histamine (■), impromidine (△) and dimaprit (▲) of the isolated muscularis mucosae of the guinea-pig cervical (a) and thoracic (b) oesophagus. The ordinate scales show the amplitude of contraction as a percentage of the maximum contraction induced by carbachol (10 μM). A concentration of the histamine receptor agonists greater than 300 μM could not be prepared from the commercial substances used here. Each point represents the mean response; vertical lines show s.e.mean. Numbers of observations are the same as those shown in Table 1.

The data obtained are expressed as mean ± s.e.mean. Each experimental group consisted of 6–10 preparations taken from different animals. Student's *t* test for paired or unpaired observations was used for statistical evaluation of the data. *P* values smaller than 0.05 were considered to be significant.

Drugs used were histamine dihydrochloride, atropine sulphate (Wako), 2-methylhistamine dihydrochloride (2-MH), 4-methylhistamine dihydrochloride (4-MH), 2-pyridylethylamine dihydrochloride (PEA), dimaprit dihydrochloride, impromidine trihydrochloride, cimetidine (Smith, Kline & French), indomethacin, tetrodotoxin (Sankyo), aspirin (Mitsui Toatu), diphenhydramine hydrochloride (Sigma), verapamil hydrochloride (Eisai) and phenoxybenzamine hydrochloride (Tokyo Kasei). To prepare the drug solutions, indomethacin and aspirin were dissolved in distilled water containing equimolar concentrations of Na_2CO_3 and diluted with 0.9% w/v NaCl solution (saline); all other drugs were dissolved in and diluted with normal saline. The molar concentrations of drugs described in this paper refer to the final bath concentrations.

Results

Responses to histamine and its related agonists

The muscularis mucosae isolated from guinea-pig cervical and thoracic oesophagus usually showed neither tone nor spontaneous activity in the organ bath. Histamine, above 0.1 μM, produced a contraction of the muscularis mucosae in both parts of oesophagus, in a concentration-dependent manner (Figures 1 and 2). The contractions were rapid in onset (2 min or less), sustained, reversible by washing and

Table 1 Concentrations of histamine receptor agonists causing 50% of the carbachol (10 μM)-induced maximal contraction (EC_{50}) in the isolated muscularis mucosae of the guinea-pig cervical and thoracic oesophagus

Agonist	*EC_{50} (μM)*	*Potency*	*Maximum responses*
		Cervical part	
Histamine	1.60 ± 0.15 (10)	1.00	95.98 ± 2.37
2-Methylhistamine	3.83 ± 0.72 (6)	0.42	93.20 ± 1.97
2-Pyridylethylamine	8.95 ± 0.69 (10)	0.18	85.12 ± 1.94
4-Methylhistamine	119.86 ± 31.95 (7)	0.01	66.63 ± 3.57
		Thoracic part	
Histamine	1.37 ± 0.13 (10)	1.00	95.91 ± 3.15
2-Methylhistamine	4.62 ± 0.86 (7)	0.30	93.23 ± 1.45
2-Pyridylethylamine	7.73 ± 0,70 (8)	0.18	86.19 ± 1.37
4-Methylhistamine	92.33 ± 20.29 (6)	0.01	69.55 ± 3.84

EC_{50} values shown are mean ± s.e.mean. Potency = EC_{50} of histamine/EC_{50} of each agonist. Maximum responses = the maximum contraction heights obtained by each of the agonists at the highest concentrations used, and are expressed as % of the carbachol (10 μM)-induced one. Numbers in parentheses show numbers of observations.

the preparation did not show tachyphylaxis. The concentration-response relationship in this tissue could be fully reproduced either by the single-dose technique or by the cumulative-dose technique (Figure 1). The EC_{50}s of histamine in cervical and thoracic parts of the oesophageal muscularis mucosae were 1.6 and 1.4 μM, respectively (Table 1). These were not significantly different. As shown in Table 1, the maximum contraction induced by histamine (100 μM) was slightly lower in magnitude than that induced by carbachol (10 μM). When histamine (0.1–100 μM) was applied to the tissue which had been maximally contracted with carbachol (10 μM), neither contraction nor relaxation was observed ($n = 5$). Among the several histamine receptor agonists tested, 2-methylhistamine (2-MH), 2-pyridylethylamine (PEA) and 4-methylhistamine (4-MH) also produced only a contraction of this tissue, but impromidine and dimaprit were virtually ineffective (Figure 2).

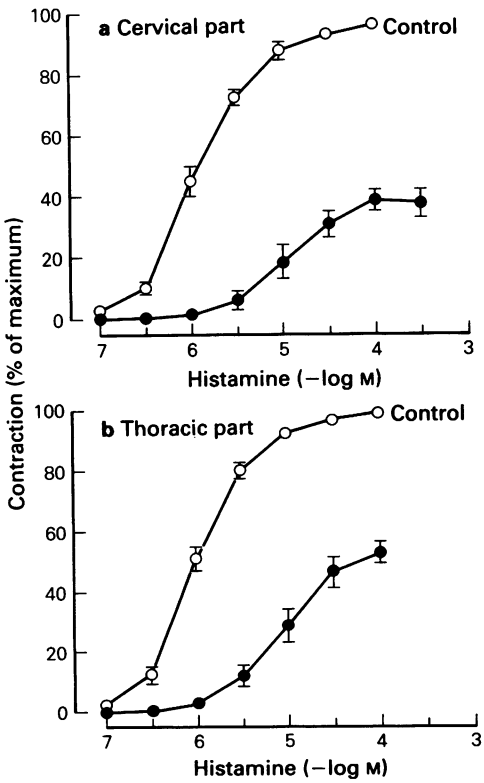

Figure 3 Cumulative concentration-response curves for the contractile response to histamine of the isolated muscularis mucosae of the guinea-pig cervical (a) and thoracic (b) oesophagus before (control, ○) and after (●) treatment of the tissue with phenoxybenzamine (30 nM) for 20 min. Each point represents the mean response from 8 (a) or 10 (b) observations; vertical lines show s.e.mean.

Table 2 The pA_2 values and Schild plot slopes for diphenhydramine against histamine receptor agonists-induced contractions of the isolated muscularis mucosae of the guinea-pig cervical and thoracic oesophagus

Agonist	pA_2	*Slope*
	Cervical part	
Histamine	8.11 ± 0.06 (10)	1.11 ± 0.03
2-Methylhistamine	8.11 ± 0.57 (7)	1.08 ± 0.02
2-Pyridylethylamine	8.13 ± 0.10 (10)	1.09 ± 0.05
	Thoracic part	
Histamine	8.12 ± 0.05 (10)	1.09 ± 0.03
2-Methylhistamine	7.96 ± 0.02 (6)	1.11 ± 0.02
2-Pyridylethylamine	8.09 ± 0.10 (8)	1.07 ± 0.08

The pA_2 values and slopes are mean ± s.e.mean. The pA_2 value and slope were determined from the method described by Arunlakshana & Schild (1959). Every slope for diphenhydramine was close to unity. The pA_2 value against 4-methylhistamine could not be estimated, since the full Schild plot was not composed because of its low potency and limitation of the available drug solution. Numbers in parentheses show numbers of observations.

However, as summarized in Table 1, EC_{50} values of these agonists were apparently higher than that of histamine. Again, the value for each agonist was not significantly different between cervical and thoracic parts of the oesophageal muscularis mucosae.

To examine for the presence of reserve receptors for histamine in this tissue, the K_A value of histamine was determined after partial irreversible blockade of the receptor, according to the method of Furchgott (1966). After pretreatment of this tissue with phenoxybenzamine (30 nM), the concentration-response curve to histamine was shifted to the right and downward (Figure 3). The effect of phenoxybenzamine was irreversible and even after 2 h the diminished response was not restored. The K_A value of histamine was estimated to be 21.8 ± 0.64 μM ($n = 8$) in the cervical part and 21.1 ± 0.51 μM ($n = 10$) in the thoracic part of the oesophageal muscularis mucosae, respectively. There was no significant difference between the cervical and thoracic part in the effect of phenoxybenzamine on the histamine response.

Effects of histamine receptor antagonists

The histamine H_1-receptor antagonist, diphenhydramine (0.03–1 μM), caused a parallel rightward shift of the concentration-response curve to histamine, 2-MH and PEA without any reduction of their

Table 3 The EC_{50} ratio of histamine before and after pretreatment, with various drugs, of the isolated muscularis mucosae of the guinea-pig cervical and thoracic oesophagus

		EC_{50} ratio (mean ± s.e.mean)	
Treatment		*Cervical part*	*Thoracic part*
Tetrodotoxin	0.3 μM	1.5 ± 0.1 (8)*	1.4 ± 0.1 (9)*
Atropine	1 μM	2.4 ± 0.3 (7)*	2.0 ± 0.2 (8)*
Indomethacin	0.1 μM	2.2 ± 0.3 (8)***	2.2 ± 0.1 (8)***
	1 μM	3.5 ± 0.4 (8)***	3.5 ± 0.6 (8)***
	3 μM	6.1 ± 1.2 (8)***	5.8 ± 0.9 (8)***
Aspirin	30 μM	2.0 ± 0.1 (8)***	2.5 ± 0.3 (8)***
	100 μM	3.1 ± 0.1 (8)***	3.5 ± 0.6 (8)***
	300 μM	4.3 ± 0.2 (8)***	4.6 ± 0.6 (8)***
Verapamil	1 μM	3.0 ± 0.3 (8)***	3.1 ± 0.3 (8)***
	10 μM	11.5 ± 2.7 (8)***	13.7 ± 3.6 (8)***

EC_{50} ratio = EC_{50} of histamine after treatment with drugs/EC_{50} of histamine before treatment. Numbers in parentheses show numbers of observations.
*$P < 0.05$, ***$P < 0.001$; compared with the values before drug treatment using the paired t test.

maximum responses. Transformation of the results into Schild plots gave a straight line with the slope near the theoretical value of 1.0 (Table 2). This indicates that the antagonism is competitive in nature. As summarized in Table 2, the pA_2 values for diphenhydramine were almost the same, approximately 8.1, against each agonist or in either part of the oesophagus. The concentration-response curve to 4-MH was also shifted to the right by the lowest concentration (0.03 μM) of diphenhydramine, giving an EC_{20} ratio of approximately 3.3 ($n = 7$). However, 0.1 μM diphenhydramine abolished the contraction to 4-MH (300 μM) and therefore a full Schild plot was not composed.

The histamine H_2-receptor antagonist, cimetidine (100 μM), did not modify the contractile responses to these histamine receptor agonists. The EC_{50} of histamine was unaffected by pretreatment with 100 μM cimetidine (EC_{50} ratio = 1.0–1.2; both parts of the oesophagus, $n = 8$).

Effects of various drugs on the histamine-induced contraction

The contractile response to histamine was slightly inhibited by tetrodotoxin (0.3 μM), atropine (1 μM), indomethacin (0.1–3 μM) or aspirin (30–300 μM), and the EC_{50} value was increased about 2–6 times by these drugs (Table 3). The inhibitory effects of indomethacin and aspirin were irreversible and the diminished concentration-response curve to histamine was not restored 2 h after washout of the drugs from the organ bath. Again, inhibitory effects of these drugs were almost similar in both the cervical and thoracic parts of the oesophageal muscularis mucosae (Table 3).

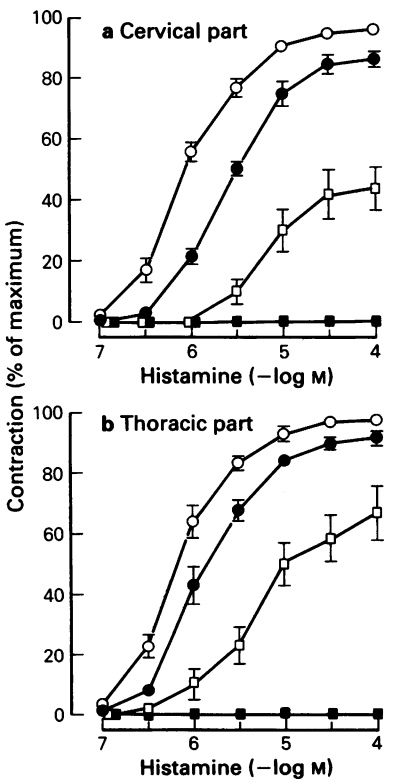

Figure 4 Cumulative concentration-response curves for the contractile response to histamine of the isolated muscularis mucosae of the guinea-pig cervical (a) and thoracic (b) oesophagus after 30 min incubation with Tyrode solution containing various calcium concentrations; (○) Ca^{2+} 1.8 mM, (●) Ca^{2+} 0.9 mM, (□) Ca^{2+} 0.45 mM and (■) Ca^{2+} 0 mM. The ordinate scales show the amplitude of contraction as a percentage of the maximum contraction induced by carbachol (10 μM) in the presence of 1.8 mM calcium. Each point represents the mean response from 8 observations; vertical lines show s.e.mean.

Role of calcium ions in the histamine-induced contraction

When the muscularis mucosae of both parts of the guinea-pig oesophagus was incubated in Tyrode solution containing various concentrations of calcium (0, 0.45, 0.9 and 1.8 mM) for 30 min, the concentration-response curve to histamine was shifted to the right and downward; the effect being inversely related to the calcium concentration, and in a calcium-free medium the response to histamine was abolished (Figure 4). The curve to histamine at any one calcium concentration was not significantly different between cervical and thoracic parts of the oesophagus.

The calcium antagonist, verapamil, slightly inhibited the contractile response to histamine and in the presence of 1 and 10 μM verapamil the EC_{50} value of histamine was increased by about 3 and 12–14 times, respectively, in both parts of the oesophagus (Table 3). The inhibition of the maximum response to histamine by verapamil was only 20–30%.

Discussion

Although many studies on the responsiveness to histamine of external smooth muscles of the alimentary tract have been published (Bertaccini, 1982), there are few accounts of the effect of histamine on the muscularis mucosae (Walder, 1953; Hughes, 1955; Bartlet, 1968; Bartlet & Hassan, 1968). The present investigation confirms that histamine produces a tonic contracture of the muscularis mucosae isolated from the guinea-pig oesophagus. The contractile response to histamine is mediated solely by H_1-receptors, not by H_2-receptors. This is supported by the present results showing the relative potencies of histamine agonists and the affinity constants for histamine and the H_1-receptor antagonist diphenhydramine (Table 2). The estimated K_A value of histamine and the pA_2 value of diphenhydramine are comparable with those obtained in the guinea-pig ileum where histamine interacts preferentially with H_1-receptors (Arunlakshana & Schild, 1959; Furchgott, 1966; Bertaccini, 1982). The K_A value of histamine was approximately 14 fold higher than the EC_{50} value. This difference suggests the presence of a significant reserve receptor in this tissue (Waud, 1968). 4-MH, which is known to be a relatively selective H_2-receptor agonist (Black *et al.*, 1972), might act as a weak H_1-receptor agonist in this tissue, since the contraction was abolished by a low concentration of diphenhydramine, but not by cimetidine. The fact that neither H_2-agonists nor H_2-antagonists affected the responsiveness of the muscularis mucosae suggests that the functional significance of H_2-receptors in this tissue is probably negligible. By comparing the responsiveness of the muscularis mucosae between cervical and thoracic parts of the guinea-pig oesophagus, it seems likely that excitatory histamine H_1-receptors are uniformly distributed throughout the oesophagus.

The histamine H_1-receptor-mediated contraction of this tissue seems to be elicited mainly by a direct action on longitudinal smooth muscles, but partly by indirect actions. The latter might involve the stimulation of intramural cholinergic nerves and of endogenous prostaglandin biosynthesis, since the response to histamine was slightly inhibited by tetrodotoxin, atropine, indomethacin or aspirin (Table 3). Previously, Bartlet (1968) found that atropine increased the dose-ratio for the histamine-induced contraction of the guinea-pig whole oesophagus by 1.8. Partial mediation by cholinergic nerves of the histamine-induced contraction has also been observed in some external muscles of the mammalian gut (Bertaccini, 1982). The release of prostaglandin-like substances by histamine and the modification of the histamine-induced contraction by cyclo-oxygenase inhibitors have already been observed in the guinea-pig tracheal (Grodzinska *et al.*, 1975; Orehek *et al.*, 1975) and ileal smooth muscle (Famaey *et al.*, 1977). Also in the present preparations, we have shown that the arachidonic acid (0.2–3 μM)-induced contraction was fully prevented by indomethacin or aspirin at the concentration used here (Uchida *et al.*, 1983; Kamikawa *et al.*, 1985a).

The contraction of muscularis mucosae to histamine might be elicited by the influx of extracellular calcium. This is supported by the findings that the response was fully dependent on the extracellular calcium concentrations and abolished in a calcium-free medium (Figure 4). There are at least two types of ion channel in the smooth muscle cell membrane that allow calcium to enter the cell, potential-dependent calcium channels (PDCs) and receptor-operated calcium channels (ROCs) (Bolton, 1979). The former open in association with membrane depolarization and action potentials, while the latter open by the agonist-receptor coupling and are independent of membrane potentials. Organic calcium antagonists such as verapamil can selectively compete with PDCs rather than the ROCs at a concentration less than 10 μM (Janis & Triggle, 1983). In fact, verapamil (3–10 μM) completely prevented the high potassium (60 mM)-induced tonic contracture of the oesophageal muscularis mucosae which is probably produced by membrane depolarization. Therefore, the present finding that the histamine-induced contraction was partially inhibited, but not abolished, by verapamil (Table 3) suggests that the response is elicited by opening both PDCs and ROCs. This contrasts with the finding, in the guinea-pig ileum, that verapamil (8.8 μM) completely abolished the histamine-induced contraction (Bury & Mashford, 1976). The difference may reflect

the relative extent to which calcium is mobilized by histamine through PDCs and ROCs in both smooth muscle preparations, and in the oesophageal muscularis mucosae histamine causes a calcium influx mainly though ROCs rather than PDCs. We have recently demonstrated that cholinomimetics also produce a contraction mainly by opening the ROCs in the oesophageal muscularis mucosae but in the ileal longitudinal muscle by opening the PDCs (Kamikawa *et al.*, 1985b).

In conclusion, the present experiments provide functional evidence for homogeneous populations of excitatory histamine H_1-receptors in the muscularis mucosae of the guinea-pig oesophagus. In addition, the H_1-receptors may be partly linked with the stimulation of endogenous prostaglandin biosynthesis or of intramural cholinergic nerves. The physiological significance of the histamine receptors located in the muscularis mucosae is currently under investigation.

We are grateful to Smith, Kline & French Research Ltd for the gifts of 2-methylhistamine, 4-methylhistamine, 2-pyridylethylamine, dimaprit, impromidine and cimetidine, and Eisai Co. for verapamil.

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(*Received March 4, 1985.*
Revised June 19, 1985.
Accepted July 15, 1985.)