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Histamine receptor subtypes mediating hyperpolarization in the isolated, perfused rat mesenteric pre-arteriolar bed

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Abstract

Histamine is a general dilator of rat blood vessels. We investigated the relative contribution of receptor subtypes to the rat mesenteric dilator responses initiated by histamine and related agonists. Histamine initiated dose, and endothelium-dependent, dilation of constricted mesenteric beds with an ED_{50} of 0.4 ± 0.1 nmol. The ED_{50} was increased 10-fold by 0.1 μM chlorpheniramine (a histamine H_1-receptor selective antagonist). Histamine H_2 receptor blockade with tiotidine (0.1 μM) slightly decreased, while thioperamide (1 μM), a selective histamine H_3 receptor antagonist, did not block histamine-induced dilation. Mesenteric bed dilation initiated by histamine H_2 receptor selective agonists, amthamine and dimaprit, were antagonized markedly by tiotidine. However, the dilation initiated by the putative histamine H_3 receptor selective agonists, *R*(−)- or *S*(+)-α-methylhistamine and imetit were not affected by thioperamide (1 μM). Histamine H_2- and H_3-receptor mediated dilator effects were endothelium-independent and were blocked by either excess (80 mM) extracellular K^+, or 1 mM tetrabutylammonium (a non-selective K^+ channel blocker), as well as by 1 μM dequalinium, a non-peptide blocker of the small conductance Ca^{2+}-activated (SK_{Ca}) K^+ channels. We conclude that (i) histamine H_1 receptor subtype predominantly mediates endothelium-dependent dilator effect of histamine, and (ii) vascular hyperpolarization through opening of K^+ channels (SK_{Ca}) mediate the dilator responses to histamine H_2 receptor (amthamine and dimaprit) and the putative histamine H_3 receptor (*R*(−)-α-methylhistamine and imetit) agonists. © 1998 Elsevier Science B.V.

Keywords: Perfused mesenteric bed; Histamine receptor subtype; Vascular hyperpolarization

1. Introduction

Histamine is a general dilator of rat blood vessels, and until recently, this effect was thought to be mediated directly or indirectly through the activation of histamine H_1 and/or H_2 receptors. Histamine elicits endothelium-dependent relaxations of conduit elastic and muscular peripheral blood vessels such as the aorta, common carotid, and superior mesenteric arteries through activation of histamine H_1 receptors (Van De Voorde and Leusen, 1984; Carrier et al., 1984; Krstic et al., 1988). It also initiates endothelium-independent relaxation of the rat femoral artery through histamine H_2 receptor activation (Krstic et al., 1991). The histamine H_3 receptor subtype, thought to be located on histaminergic neuronal terminals in the central nervous system, also exists on presynaptic sites of the cardiovascular system (Ishikawa and Sperelakis, 1987) and on sympathetic neurones innervating resistance vessels of the pithed rat (Malinowska and Schlicker, 1993).

The mesenteric vascular bed of the rat is a good candidate for all of the three histamine receptor subtypes: H_1 and H_2, respectively, in association with endothelium and smooth muscle, and H_3, in association with the dense adrenergic (Furness and Marshall, 1974) and sensory (Wharton et al., 1986) innervations of the vascular bed. Thus, the first objective of the current investigation was to test the hypothesis that the vasodilator response initiated by histamine in the rat perfused mesenteric bed represents a summation of the effect of the three receptor subtypes rather than of a particular subtype. In addition to histamine (a nonselective agonist), we used the subtype-selective agonists and antagonists: amthamine and dimaprit (histamine H_2 receptor selective agonists), tiotidine (histamine H_2 receptor selective antagonist); *R*(−)-α-methyl his-

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tamine and imetit (histamine H_3 receptor selective agonists), and thioperamide (histamine H_3 receptor selective antagonist), and chlorpheniramine (histamine H_1 receptor selective antagonist) as investigational tools. We have previously established that histamine dilates the perfused rat mesenteric bed predominantly through hyperpolarization of the vascular smooth muscle cells (Adeagbo and Triggle, 1993). In that study, histamine-mediated vasodilation was not significantly attenuated by nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, but was profoundly blocked by K^+ channel antagonists, and also by procedures such as high extracellular K^+ that counteracts hyperpolarization. Thus, a second objective of the present study was to determine whether hyperpolarization contributes to the dilator effects initiated by histamine H_2- and H_3-receptor selective agonists, and to characterize with selective antagonists, the K^+ channel type(s) involved in the hyperpolarization mechanism. Selective K^+ channel blockers used include: glibenclamide (K_{ATP}), dendrotoxin (K_V), penitrem A (BK_{Ca}—a large conductance Ca^{2+}-activated K^+ channel blocker), and dequalinium (SK_{Ca}—a non-peptide, small conductance Ca^{2+}-activated K^+ channel blocker).

An abstract of the manuscript has been presented at the Pharmacology '97 meeting, San Diego, CA. (Oriowo and Adeagbo, 1997).

2. Methods

2.1. Perfused mesenteric vascular bed

Male Sprague–Dawley rats (250–350 g) were used in these studies. Following pentobarbital (60 mg/kg) anesthesia, the abdominal cavity of individual rats was opened, and the superior mesenteric artery was cannulated through an incision at its confluence with the dorsal aorta. The entire mesenteric vascular bed was then flushed with heparinized physiological salt solution (PSS) and trimmed off the small intestinal borders. It was then transferred to a warmed chamber where it was perfused with carbogen (95% O_2/5% CO_2)-saturated PSS maintained at 37°C. The perfusion was at a constant rate of 5 ml/min using a Masterplex peristaltic pump. Changes in perfusion pressure were recorded through Statham pressure transducers coupled to a Grass polygraph recorder (model 7H).

The composition of our PSS in mM is as follows: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 12.5, Glucose 11.1. High K^+ (80 mM) PSS was prepared by substituting an equimolar amount of K^+ for Na^+ ions. The pH of all PSS was 7.4 after saturation with 95% O_2/5% CO_2 gas mixture. Tissues were routinely allowed to equilibrate for 1 h before the start of all experiments.

2.2. Experimental protocol

Three series of experiments were performed. The first series was conducted to determine the effects of histamine receptor subtype-selective antagonists chlorpheniramine (H_1), tiotidine (H_2) or thioperamide (H_3) on the dilator responses initiated by histamine. For this purpose, the perfusion pressure of the vascular beds was increased by a continuous infusion of the α_1-selective adrenoceptor agonist cirazoline (1 μM). Dose-response curves to histamine were constructed in the absence, and in the presence of each antagonist, or a combination of any two of them. An antagonist under study was usually added to the perfusion medium 30 min prior to re-establishing histamine dose-response curve. Only one antagonist concentration was employed in our study: thioperamide (1 μM), chlorpheniramine and tiotidine (0.1 μM).

In the second series of experiments, we investigated the effects of histamine H_2 receptor selective (amthamine and dimaprit) and histamine H_3 receptor selective ($R(-)$-, $S(+)$-α-methylhistamine and imetit) agonists on cirazoline-preconstricted vascular beds. As in series one above, dose-response curves were constructed to the agonist under test in the absence, and in the presence of 0.1 μM tiotidine (histamine H_2 receptor selective antagonist), or in the case of α-methylhistamine ($R(-)$ or $S(+)$) and imetit, in the presence of 1 μM thioperamide alone and in combination with tetrodotoxin, a Na^+ channel blocker. We also assessed the role of endothelium on the effects of these compounds. In assessing the role of endothelium, we compared dose-response curves to agonists on endothelium-intact, with those obtained on endothelium-denuded vascular beds. Endothelium was denuded from the vascular beds by an infusion of distilled water for 5 min.

Experiments in the third series were conducted to determine the influence of high (80 mM) K^+ PSS perfusion, and of K^+ channel blockers (tetrabutylammonium, dendrotoxin, dequalinium and penitrem A), on the vasodilator responses initiated by the histamine H_2 (amthamine and dimaprit) and histamine H_3 receptor ($R(-)$ and $S(+)$ analogs of α-methylhistamine and imetit) selective agonists tested in series two above. First, we recorded responses of mesenteric beds to these agonists during perfusion with normal PSS. The perfusion medium was then changed to 80 mM K^+ PSS, and after equilibration tonus had been attained, responses to agonists were repeated. In cases where the influence of K^+ channel blockers were examined, the dilator responses to dimaprit or (R)-α-methylhistamine were performed before, and during infusion of the blockers: tetrabutylammonium, 1 mM; dendrotoxin or penitrem A, 100 nM; and dequalinium, 1 μM.

2.3. Drugs

The following drugs were purchased from Research Biochemicals Int. (Natick, MA): cirazoline, dimaprit dihy-

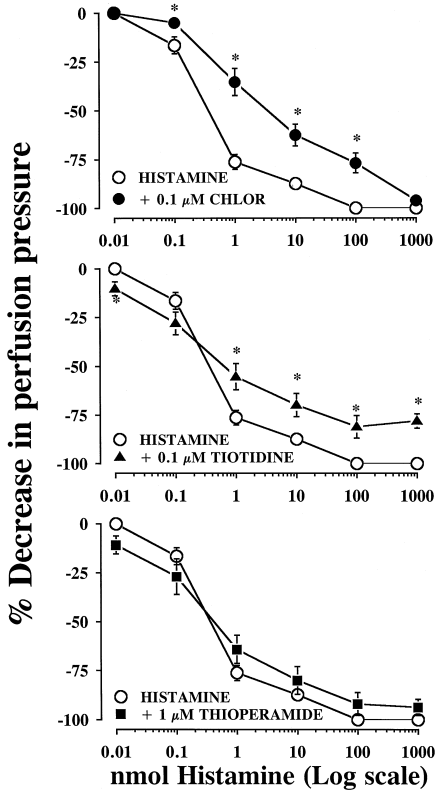

Fig. 1. Antagonism of histamine-mediated dilation by chlorpheniramine (CHLOR, upper panel), tiotidine (middle panel) and thioperamide (bottom panel) in isolated, perfused rat mesenteric vascular bed. Sustained vascular tone was maintained with a continuous infusion of 1 μM cirazoline. Each data point represents the mean ± S.E.M. of eight experiments, $* P < 0.05$.

drochloride, imetit hydrochloride, thioperamide and chlorpheniramine as maleate salts. Amthamine dihydrobromide, $R(-)$-α-methylhistamine, $S(+)$-α-methylhistamine as dihydrobromide salts, dequalinium and tiotidine were purchased from Tocris Cookson (St. Louis, MO). Histamine dihydrochloride, tetrabutylammonium iodide and tetrodotoxin were obtained from Sigma (St. Louis, MO). Dendrotoxin, glibenclamide and penitrem A were purchased from Biomol Res. Lab. (Plymouth Meeting, PA). Tiotidine, glibenclamide and penitrem A were dissolved in dimethylsulfoxide (DMSO), the stock solutions of all other compounds were made in distilled water.

2.4. Data analysis

Changes in perfusion pressure were expressed as percentage of the pressure before the administration of a vasodilator agent. Where appropriate, the K_B of an antagonist was calculated from the expression: $K_B = [B]/\text{dose ratio} - 1$, where dose ratio refers to the concentration of agonist required to elicit 50% of the maximal response (EC_{50}) in the presence of a concentration $[B]$, of the antagonist. Values are expressed as mean ± S.E.M., and differences between the mean values were compared using Student's *t*-test. The difference between means were considered significant when $P < 0.05$.

3. Results

3.1. Histamine-mediated vasodilation and effects of receptor subtype-selective antagonists

Histamine (0.01–100 nmol) initiated dose and endothelium-dependent decrease in perfusion pressure of pre-constricted mesenteric beds with ED_{50} of 0.4 ± 0.1 nmol. Histamine dose-response curve was shifted rightwards by chlorpheniramine (0.1 μM); the $-\log K_B$ (pA_2) for the antagonism was calculated to be 8.71 ± 0.30 ($n = 8$). The vasodilator responses to histamine was weakly antagonized by tiotidine, a histamine H_2 receptor antagonist, at 0.1 μM, while thioperamide, a histamine H_3 receptor selective antagonist, at 1 μM, was without effect (Fig. 1). A combination of chlorpheniramine (0.1 μM) with either tiotidine (0.1 μM), or thioperamide (1 μM) was not more effective than chlorpheniramine alone in antagonizing histamine. However, chlorpheniramine produced an additive antagonism of histamine dilator responses in vascular beds that were previously treated with tiotidine (see Fig. 2).

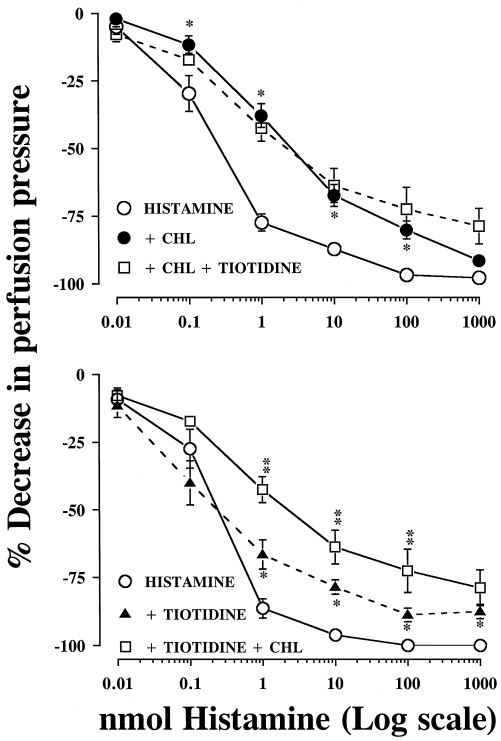

Fig. 2. Influence of chlorpheniramine (CHL) or tiotidine, and their combination on the vasodilation initiated by histamine in perfused, cirazoline-preconstricted mesenteric bed. In the upper panel, chlorpheniramine treatment preceded tiotidine, whereas, tiotidine preceded chlorpheniramine application in the bottom panel. Each data point on the graphs represents the mean ± S.E.M., $n = 6$; $*$ or $**$ $P < 0.05$.

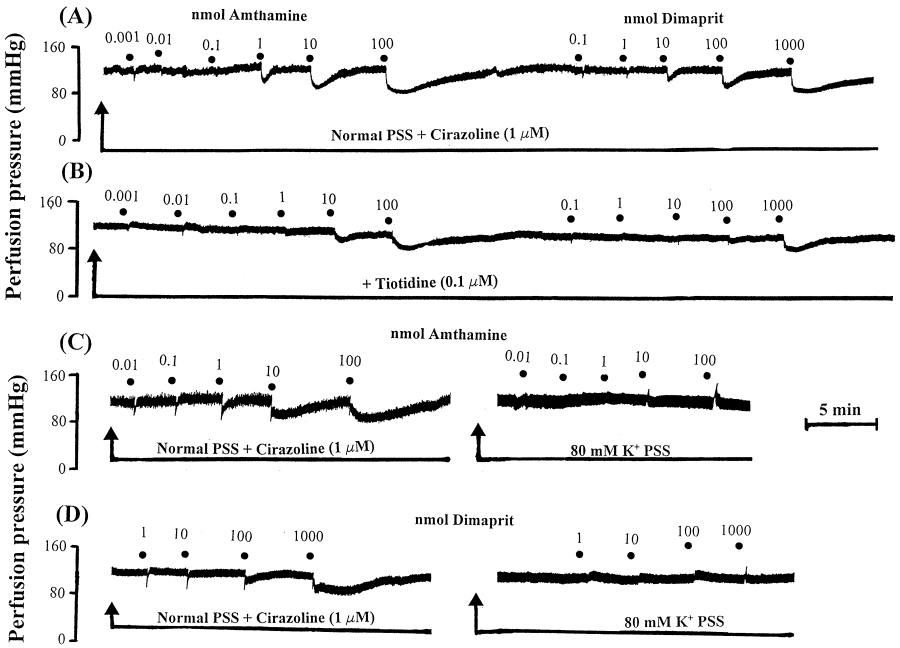

Fig. 3. Representative tracings of the effects of the dilator responses initiated by amthamine and dimaprit (A), and its antagonism by 0.1 μM tiotidine (B) in cirazoline-preconstricted vascular beds. In panels C and D, respectively, the dilator responses initiated by amthamine and dimaprit in normal PSS-perfused beds were compared with those obtained in 80 mM K^+ PSS perfused beds.

3.2. Effects of histamine H_2- and histamine H_3-receptor selective agonists

The histamine H_2 receptor selective agonists amthamine (0.01–100 nmol) and dimaprit (1–100 nmol) initiated dose-related dilator responses. Typical responses of perfused mesenteric bed to doses of amthamine and dimaprit are shown in Fig. 3. Tiotidine (0.1 μM) antagonized the dilator responses of the vascular beds, and produced a significant rightward shift of amthamine and dimaprit dose-response curves with $-\log K_B$ (calculated) values of 9.08 ± 0.19 ($n = 5$) and 9.11 ± 0.20 ($n = 5$), respectively.

Two putative histamine H_3-receptor selective agonists, *R*-(−)-α-methylhistamine (0.1–100 nmol) and imetit, as well as the *S*(+) isomer of α-methylhistamine (1–100 nmol) also initiated dose-dependent dilator responses. Thioperamide (a histamine H_3 receptor selective antagonist) at 1 μM, alone or in combination with tetrodotoxin (1 μM) failed to antagonize the responses to the agonists (Fig. 4). The responses to these agonists, as with those to

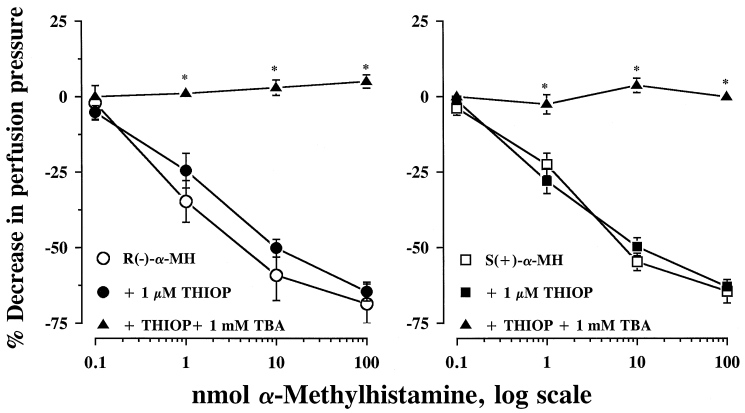

Fig. 4. The influence of thioperamide (THIOP) and tetrabutylammonium (TBA) on the vasodilator responses initiated by *R*(−)-, left panel, and *S*(+)-, right panel, enantiomers of α-methylhistamine in perfused, cirazoline preconstricted mesenteric beds. Each data point on the graphs represents the mean ± S.E.M., $n = 5$; $* P < 0.05$.

Table 1
Influence of endothelium on the vasodilator responses to histamine and related agonists in perfused rat mesenteric bed

Compounds (nmol)	% Decrease in perfusion pressure	
	Endo-intact	Endo-denuded
Histamine (10)	−75.2±3.0	−6.8±4.2[a]
Amthamine (30)	−72.0±3.2	−70.2±5.0
Dimaprit (30)	−65.8±6.7	−69.5±9.7
Imetit (100)	−52.9±2.3	−54.0±2.0
R(−)-α-methylhistamine (100)	−70.6±3.0	−68.0±2.6
S(+)-α-methylhistamine (100)	−73.0±5.1	−70.8±4.7

Data are means±S.E.M. ($n=6$).
[a] $P<0.05$ from corresponding endo-intact value.

amthamine and dimaprit, were not altered by endothelial denudation (Table 1). Chlorpheniramine (0.1 μM) also did not antagonize any of the histamine H_2- or H_3-receptor selective agonists.

3.3. Effect of high K^+ PSS perfusion and K^+ channel blockers on agonist-induced vasodilation

The dilator responses induced by histamine H_2 receptor agonists (see Fig. 3), as well as by histamine H_3 receptor agonists (data not shown), were abolished in vascular beds perfused with high (80 mM) K^+ depolarizing PSS. Responses initiated by histamine in high (80 mM) K^+ PSS were markedly reduced, and slower in time-course compared to those obtained in normal PSS.

Tetrabutylammonium (1 mM), a non-selective K^+ channel antagonist alone, as with dequalinium (1 μM), produced a significant reduction in perfusion pressure. In the case of tetrabutylammonium, pressure returned to the pre-application level within 30 min. However, in the presence of dequalinium the concentration of cirazoline had to be titrated to adjust the perfusion pressure to pre-inhibitor level. Penitrem A (100 nM), glibenclamide (10 μM) and dendrotoxin did not cause significant alteration in the perfusion pressure. Tetrabutylammonium (1 mM) abolished amthamine- and dimaprit-induced dilator responses (Table 2), and also abolished the dilator responses to *R*(−)-, and *S*(+)-, analogs of α-methylhistamine that were insensitive to thioperamide and tetrodotoxin (Fig. 4). Dequalinium (1 μM), a SK_{Ca} blocker significantly attenuated dimaprit or *R*(−)-α-methylhistamine induced dilator responses on its own or after prior treatment with penitrem A (a BK_{Ca} blocker), or glibenclamide (a K_{ATP} blocker), see Fig. 5. However, neither penitrem A (100 nM) nor glibenclamide (10 μM), applied individually, was effective at blocking these responses. Tetrabutylammonium, as well as ouabain (50 μM), significantly reduced histamine-induced dilator responses. However, ouabain did not affect responses initiated by amthamine, *R*(−)-α-methylhistamine (see Table 2).

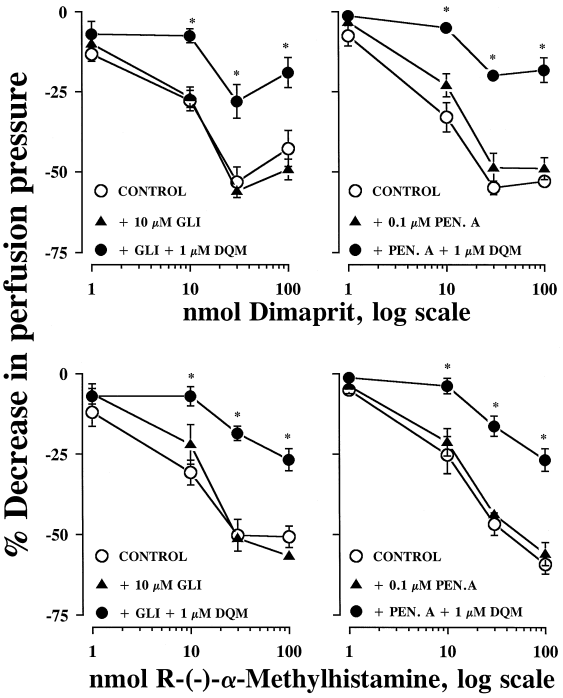

Fig. 5. Effects of dequalinium (DQM, 1 μM) on vasodilation initiated by dimaprit (upper panels), and *R*(−)-α-methylhistamine (bottom panels) in perfused mesenteric beds. Glibenclamide, GLI, 10 μM, (left panels), and penitrem A, PEN. A, 0.1 μM (right panels) were, respectively, not effective as blockers. Each data point on the graphs represents the mean±S.E.M., $n=5$; * $P<0.05$.

4. Discussion

Histamine receptors are characterized into three subtypes: H_1, H_2 and H_3. Depending on the vessel type and

Table 2
Influence of ouabain and tetrabutylammonium (TBA) on the vasodilator responses to histamine and related agonists in perfused rat mesenteric bed

Compounds	% Change in perfusion pressure			
	Control	+Ouabain (50 μM)	Control	+TBA (1 mM)
Histamine	−68.6±2.1	−54.0±1.8[a]	−72.0±2.7	−8.4±1.6[a]
Amthamine	−56.8±2.0	−54.2±2.2	−60.4±3.0	+10.8±1.2[b]
Dimaprit	−46.2±2.0	−50.2±4.2	−48.4±2.1	+5.2±0.8[b]
R(−)-α-methylhistamine	−65.7±3.3	−60.0±7.8	−68.0±2.7	+5.0±2.2[b]

Data represent the means±S.E.M. ($n=5$).
[ab] Denote statistically significant differences from control ($P<0.05$).

the animal species in question, direct or indirect vasodilator effects of histamine can be mediated through any of these receptor subtypes. Our present study demonstrates the existence of multiple histamine receptor subtypes, all of which mediate dilation of perfused rat mesenteric vascular bed. Based on the use of specific antagonists, we have found that histamine H_1 receptor subtype predominantly accounts for the dilator effects of histamine. However, our results with subtype-specific agonists unequivocally demonstrates the presence of histamine H_2 receptors, and an atypical histamine H_3, or another yet uncharacterized receptor subtype, which also mediate endothelium-independent hyperpolarization of perfused rat mesenteric vascular bed.

Histamine produced endothelium-dependent relaxations of perfused rat mesenteric bed. The vasodilator responses were attenuated potently ($-\log K_B = 8.71 \pm 0.30$) by chlorpheniramine, a histamine H_1 receptor selective antagonist. Tiotidine, a histamine H_2 receptor selective antagonist (Yellin et al., 1979; Hill, 1990), antagonized histamine vasodilation to a much smaller extent, while thioperamide, a histamine H_3 receptor selective antagonist (Arrang et al., 1987) was without effect. These results are consistent with histamine H_1 receptors being the predominant subtype that mediates dilation to histamine. Histamine relaxes various isolated blood vessels of the rat, including the aorta (Van De Voorde and Leusen, 1982, 1983; Carrier et al., 1984), common carotid, renal and superior mesenteric arteries (Krstic et al., 1988, 1989). In all of these vessels, the vasorelaxant effect of histamine was attributed to activation of histamine H_1 receptor subtype.

The concept of dual (now multiple) histamine receptors mediating depressor responses is an old one. The depressor responses to histamine in anesthesized cats and dogs involve both histamine H_1 and H_2 receptors (Black et al., 1975; Powell and Brody, 1976). These authors found that only a combination of histamine H_1 and H_2 receptor antagonists abolished the depressor effects of histamine in cats and dogs. In the gracilis muscle, histamine-induced vasodilation was attenuated by mepyramine (a histamine H_1 receptor selective antagonist) but not by burimamide (a histamine H_2 receptor selective antagonist), a combination of the two antagonists still abolished the response to histamine (Powell and Brody, 1976). This observation was interpreted as suggesting that there is a predominance of histamine H_1 receptors in the gracilis muscle and that histamine H_2 receptor-mediated effect of histamine could only be unmasked in the presence of histamine H_1 receptor blockade. In our study, the blockade of histamine dilation produced by chlorpheniramine (a histamine H_1 receptor blocker) was not increased by its combination with tiotidine (a histamine H_2 receptor blocker) or thioperamide (a histamine H_3 receptor blocker). However, in experiments where histamine H_2 receptor blockade with tiotidine preceded chlorpheniramine treatment, the dilator effect of histamine was additively antagonized by the combination. Thus, there seems to be a histamine H_2 receptor population contributing in a secondary fashion to histamine vasodilation. Our observation that the histamine H_2 receptor selective agonists, amthamine (Eriks et al., 1992; Poli et al., 1993) and dimaprit (Parsons et al., 1977; Hill, 1990) initiated dilator responses that were susceptible to antagonism by tiotidine confirms the existence of this receptor subtype in mesenteric vascular preparation. However, a non-competitive (E_{max} was depressed) antagonism by tiotidine would suggest a small population of histamine H_2 receptors. This is consistent with the failure of tiotidine to affect histamine-induced dilation singly or in combination with chlorpheniramine.

Another important question of our study is whether histamine H_3 receptor subtypes are present in mesenteric vascular bed, and what signal of activity they mediate. Histamine H_3-receptor subtype are found in neuronal terminals, both in the brain and in peripheral tissues (Arrang et al., 1983; Ishikawa and Sperelakis, 1987; Barnes and Ichinose, 1989). Histamine H_3 receptor mediated vasodilation has also been reported in the rabbit middle cerebral artery (Ea-Kim and Oudart, 1988; Ea-Kim et al., 1992). Histamine H_3 receptors can be selectively activated by compounds such as $R(-)$-α-methylhistamine (Arrang et al., 1985, 1987) and imetit (Garbarg et al., 1992), and can be potently and selectively antagonized by thioperamide (Arrang et al., 1985, 1987). In the present study, $R(-)$-α-methylhistamine and imetit produced reproducible vasodilation. However, the presence of histamine H_3 receptors in this vascular bed seems unlikely. (1) The dilator responses initiated by these putative selective agonists were not antagonized by thioperamide, a selective antagonist of histamine H_3 receptors. The concentration of thioperamide used in our study was several-fold higher than its reported K_i value (see Hill, 1990). (2) Compound $S(+)$-α-methylhistamine, a relatively inactive analog of the selective agonist, $R(-)$-α-methylhistamine, should exhibit strikingly weaker agonistic effect (Hill, 1990). In our study however, the $R(-)$-, and the $S(+)$-, enantiomers of α-methylhistamine exhibited indistinguishable vasodilatory properties of the mesenteric bed. It is thus doubtful whether the receptor subtype that mediates the dilator responses initiated by $R(-)\alpha$-methylhistamine and imetit in this vascular bed is H_3. It is either histamine H_3 receptor subtype with atypical properties, or another, yet uncharacterized subtype entirely. Tetrodotoxin, a Na^+ channel blocker, had no effect on the dilator responses initiated by $R(-)\alpha$-methylhistamine and imetit. The dilator effects of these compounds is therefore either entirely postjunctional, or it is coupled to a prejunctional tetrodoxin-insensitive mechanism. Postjunctionally located histamine H_3 receptors have been demonstrated in guinea pig trachea (Cardell and Edvinsson, 1994; Ea-Kim and Oudart, 1988; Ea-Kim et al., 1992).

Vasodilation initiated by the histamine H_2 (amthamine and dimaprit) and the putative histamine H_3 ($R(-)$-α-

methylhistamine and imetit) receptor agonists used in our study were not affected by endothelial denudation. This suggests that these receptors mediating the effects are located on the vascular smooth muscle cells. Histamine H_2 receptor mediated, endothelium-independent relaxations have also been demonstrated in the rat femoral artery (Krstic et al., 1991). Thus, the mechanism responsible for their dilator effect most likely resides in the vascular smooth muscle cells. The dilator responses to all four compounds were abolished in vascular beds perfused with 80 mM K^+ depolarizing PSS. These observations indicate that the mechanism of vasodilator action of these compounds relates to smooth muscle cell hyperpolarization. Activation of Na^+/K^+-ATPase and transmembrane K^+ channels are the two most important ways of initiating hyperpolarization in cells. Therefore, we examined the effect of blockers of these processes on the vasodilation initiated by histamine H_2 and H_3 receptor agonists. Ouabain did not block dilation initiated by any of the agonists suggesting that the transmembrane sodium pump did not mediate their effects. On the other hand, tetrabutylammonium, a non-selective K^+ channel blocker, abolished the vasodilator responses to the agonists.

Numerous potassium channels have been reported. They can be grouped into ATP-dependent (K_{ATP}), calcium-dependent (K_{Ca}), receptor-coupled and voltage-dependent (K_V) K^+ channels. There are three types of K_{Ca}: high-conductance (BK_{Ca}), intermediate conductance (IK_{Ca}) and small conductance (SK_{Ca}) channels. Vasodilation initiated by histamine H_2 and putative histamine H_3 receptor agonists in the present study was blocked by dequalinium, a selective non-peptide blocker of SK_{Ca} channels (Dunn, 1994). The responses were not altered by the indole alkaloid, penitrem A, an irreversible BK_{Ca} blocker which, at 10 nM, produced 100% block of [^{125}I]charybdotoxin to BK_{Ca} in bovine aortic smooth muscle sarcolemmal membranes (Knaus et al., 1994). Glibenclamide and dendrotoxin, selective antagonists of K_{ATP} and K_V, respectively, also did not alter the vasodilator responses.

The opening of SK_{Ca} channels mediate smooth muscle hyperpolarization in rat (Adeagbo and Triggle, 1993) and rabbit (Murphy and Brayden, 1995; Parsons et al., 1996) mesenteric arteries. This channel type underlies the maintained hyperpolarization that follows action potentials in many neurones, including those of the sympathetic ganglia (Pennefather et al., 1985; Kawai and Watanabe, 1986), and also the inhibitory action of α_1-adrenoceptor activation on intestinal smooth muscles. Thus, dequalinium may conceivably block SK_{Ca} on smooth muscle cells and/or on tetrodotoxin-insensitive neuronal terminals. However, since both the dense adrenergic and sensory innervation of the rat mesenteric vascular bed are tetrodotoxin-sensitive (Kawasaki et al., 1988), we deduce from our study that dequalinium blocked the hyperpolarizing effects of histamine H_2 and H_3 receptor agonists through SK_{Ca} channels located on vascular smooth muscle cells.

In summary, our study has demonstrated the existence of multiple histamine receptor subtypes in the perfused rat mesenteric pre-arteriolar bed. All of the receptor subtypes mediate vasodilation. Endothelium-dependent vasodilation in response to histamine was antagonized by chlorpheniramine, and to a much smaller extent by tiotidine. Thioperamide, alone or in combination with tetrodotoxin, did not block histamine mediated vasodilation. The dilator responses to amthamine and dimaprit (histamine H_2 receptor agonists), and to *R*(−)-α-methylhistamine and imetit (putative histamine H_3 receptor agonists) are endothelium-independent, and was totally blocked during perfusion with 80 mM K^+ PSS, and by tetrabutylammonium. Responses to these agonists were also blocked by dequalinium, a non-peptide blocker of SK_{Ca}. We conclude that (1) histamine H_1 receptor subtype predominantly mediates endothelium-dependent dilator effect of histamine. (2) A small population of histamine H_2 receptors, coupled to vascular smooth muscle cell SK_{Ca} channels, and mediating hyperpolarization is present in the rat mesenteric pre-arteriolar bed, and contributes to the overall dilation of the perfused rat mesenteric vascular bed. (3) *R*-(−)-α-methylhistamine and imetit vasodilates through opening of vascular smooth muscle cell SK_{Ca} channels, it, however, remains to be determined if such responses are coupled to a histamine H_3, or to another, yet uncharacterized receptor subtype.

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