A novel histamine receptor subtype, histamine H3 receptor, mediates inhibition of peripheral autonomic neurotransmission. The present study was designed to examine vascular effects of histamine H3 receptor by using a selective histamine H3 receptor agonist, R-(−)-α-methylhistamine (α-methylhistamine), in rat mesenteric resistance arteries. The isolated mesenteric vascular beds were perfused with Krebs solution and perfusion pressure was measured. Active tone was produced by perfusion of Krebs solution containing 7 μM methoxamine. In preparations with intact endothelium, perfusion of α-methylhistamine (1—100 μM) for 1 min produced a concentration-dependent vasodilation. The maximum vasodilation at the highest concentration was approximately 45%. This vasodilation was abolished by endothelium removal and attenuated by histamine H1 receptor antagonists, thioperamide and clobenpropit, but not by chlorpheniramine (histamine H1 receptor antagonist) and cimeidine (histamine H2 receptor antagonist). Nω-nitro-l-arginine methyl ester (l-NAME, nitric oxide (NO) synthase inhibitor), indomethacin (cyclooxygenase inhibitor) and tetraethylammonium (nonselective K+ channel blocker) and high KCl (30 mM) significantly inhibited α-methylhistamine-induced endothelium-dependent vasodilation. These findings suggest that α-methylhistamine induces endothelium-dependent vasodilation mainly via endothelium histamine H3 receptors. It is also suggested that activation of histamine H3 receptors in the endothelium releases mainly NO and partially prostaglandin I2 and endothelium-derived hyperpolarizing factors to induce endothelium-dependent vasodilation.

Key words  histamine H3 receptor; endothelium-dependent vasodilation; rat mesenteric artery; endothelium-derived relaxing factor; endothelium-derived hyperpolarizing factor

The histamine H3 receptor, a novel histamine receptor subtype, has been identified in rat brain cortical slices and shown to inhibit histamine release1,2 in the brain and several peripheral tissues.3,4 Inhibitory presynaptic histamine H3 receptors have also been found on non-histaminergic nerves including cholinergic,5 adrenergic6,7 and non-adrenergic non-cholinergic nerves.8 Characterization of histamine H3 receptor-mediated responses has been greatly facilitated by the development of selective histamine H3 receptor agonists such as R-(−)-α-methylhistamine (α-methylhistamine),2,9,10 and antagonists such as thioperamide9,9 and clobenpropit.11,12 Various physiological responses to α-methylhistamine, a potent and selective histamine H3 receptor agonist, have been shown to be reduced by the histamine H3 receptor antagonists thioperamide and clobenpropit, but not by histamine H1 or H2 receptor antagonists.13,14 It also has been reported that intravenous administration of α-methylhistamine causes a decrease in systemic blood pressure in the rat and that responses are blocked by histamine H3 receptor antagonists, but not by histamine H1 or H2 receptor antagonists.14 However, the mechanisms of histamine H3 receptor-mediated vascular relaxant effects induced by α-methylhistamine remain unclear.

It is widely accepted that the endothelium at the luminal surface of blood vessels is an important regulator of vascular tone via release of various endothelium-derived endogenous substances, such as relaxing factors (EDRFs) and contracting factors (EDCFs).15 EDVRs and EDCFs include nitric oxide (NO), prostaglandin I2 (PGI2), endothelium-derived hyperpolarizing factors (EDHFs) and endothelin, prostaglandin F2α, thromboxane A2, respectively. Evidence has accumulated that NO, PGI2 and/or EDHFs play a prominent role in the tone control of the endothelium contact arteries.19—23 Therefore, the purpose of the present study was to investigate the vascular responses mediated by histamine H3 receptors using α-methylhistamine and the underlying mechanisms in the mesenteric vascular bed of the rat.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 220—350 g were used in the present study. The animals were given food and water ad libitum. They were housed in the Animal Research Center of Okayama University at a controlled ambient temperature of 22±2°C with 50±10% relative humidity and with a 12-h light/12-h dark cycle (lights on at 8:00 a.m.). This study was carried out in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center, Japanese Government Animal Protection and Management Law (No. 105), and Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6). Every effort was made to minimize the number of animals used and their suffering.

Perfusion of Mesenteric Vascular Beds The animals were anesthetized with pentobarbital-Na (50 mg/kg, intraperitoneally) and mesenteric vascular beds were isolated and prepared for perfusion as described previously.24 The su-
perior mesenteric artery was cannulated and flushed gently with Krebs–Ringer bicarbonate solution (Krebs solution) to eliminate blood from the vascular bed. After removal of the entire intestine and associated vascular bed, the mesenteric vascular bed was separated from the intestine by cutting close to the intestinal wall. Only the four main arterial branches from the superior mesenteric trunk running to the terminal ileum were perfused. All other branches of the superior mesenteric artery were tied off. The isolated mesenteric vascular bed was placed in a water-jacketed organ bath maintained at 37 °C, perfused with a modified (see below) Krebs solution at a constant flow rate of 5 ml/min with a peristaltic pump (model AC-2120, ATTO, Tokyo, Japan) and superfused with the same solution at a rate of 0.5 ml/min to prevent drying. The Krebs solution was bubbled with a mixture of 95% O₂–5% CO₂ before passage through a warming coil maintained at 37 °C. The modified Krebs solution was of the following composition (mm): NaCl 119.0, KCl 4.7, CaCl₂ 2.4, MgSO₄ 1.2, NaHCO₃ 25.0, KH₂PO₄ 1.2, disodium ethylenediaminetetraacetic acid (EDTA) 0.03 and dextrose 11.1 (pH 7.4). Changes in the perfusion pressure were measured with a pressure transducer (model TP-400T, Nihon Kohden, Tokyo, Japan) and recorded using a pen recorder (model U-228, Nippon Denshiki Kagaku, Tokyo, Japan).

Chemical Removal of Vascular Endothelium To remove the vascular endothelium, preparations with resting tone were perfused with a 1.80 mg/ml solution of sodium deoxycholate (Sigma Aldrich Japan Co., Tokyo, Japan) in saline for 30 s. This caused a transient increase (20—30 mmHg) in perfusion pressure. Then, the preparations were rinsed with sodium deoxycholate-free Krebs solution for 40 min. After the preparations were contracted by perfusion with Krebs solution containing methoxamine (a selective α₁-adrenoceptor agonist, 2 μM, Nihon Shinyaku, Kyoto, Japan), chemical removal of the endothelium was assessed by the lack of a relaxant effect induced by a bolus injection of 1 nmol acetylcholine (Daichi Pharmaceutical, Tokyo, Japan), which was injected directly into the perfusate proximal to the arterial cannula with an injection pump (model 975, Harvard Apparatus, Holliston, MA, U.S.A.). Volume was 100 μl for 12 s.

Experimental Protocols Isolated mesenteric vascular beds with intact endothelium were perfused with Krebs solution and the perfusion pressure was increased by continuous perfusion of methoxamine (7 μM). After the elevated perfusion pressure stabilized, Krebs solution containing methoxamine and α-methylhistamine (Sigma Aldrich) at a concentration of 1, 3, 10, 30 or 100 μM was perfused for 1 min. To assess the mechanism underlying the vascular effect, α-methylhistamine perfusion was carried out during perfusion of Krebs solution containing methoxamine and a histamine H₁ receptor antagonist thioperamide (1 μM, Sigma Aldrich) (pA₂=8.96) or clobenpropit (1 μM, Sigma Aldrich) (pA₂=10.10). A histamine H₁ receptor antagonist (chlorpheniramine; 1 μM, Sigma Aldrich) (pA₂=8.25) or a histamine H₃ receptor antagonist (cimetidine; 1 μM, Sigma Aldrich) (pA₂=6.65) Each antagonist was pretreated for 30 min before the first perfusion of α-methylhistamine.

To assess the influence of the endothelium, the α-methylhistamine-induced vascular responses were examined in preparations denuded with sodium deoxycholate. After sodium deoxycholate perfusion, the active tone of the preparation was produced by perfusion with Krebs solution containing methoxamine (2 μM). After confirming successful removal of the endothelium by the lack of a relaxant effect after 1 nmol acetylcholine injection, α-methylhistamine perfusions were performed.

In another series of experiments, the vascular responses to α-methylhistamine in preparations with intact endothelium were examined in the presence of a NO synthase inhibitor, Nω-nitro-l-arginine methyl ester (1-NAME, 100 μM, Sigma Aldrich), a cyclooxygenase inhibitor, indomethacin (1 μM, Sigma Aldrich), high KCl (30 mM, Wako Pure Chemical Ind., Ltd., Osaka, Japan) or a nonselective K⁺-channel blocker, tetrathylammonium (5 mM, Sigma Aldrich), Krebs solution containing methoxamine (2—7 μM), each inhibitor and α-methylhistamine at a concentration was perfused for 1 min. The concentration of each inhibitor was employed according to previous reports described by Jin H. et al. and Jin X. et al. At the end of each experiment, the preparation was perfused with 100 μM papaverine (Dainippon-Sumitomo Pharmaceutical, Osaka, Japan) to cause complete relaxation. Vasodilator activity was expressed as a percentage of the perfusion pressure at maximum relaxation induced by papaverine. All drugs, except sodium deoxycholate, were dissolved in distilled water and diluted with Krebs solution containing 2—7 μM methoxamine, then perfused or injected directly. Sodium deoxycholate was dissolved in 0.9% saline.

Statistical Analysis Data are presented as the mean±S.E.M. Statistical analysis was performed using Student’s unpaired between two groups and one-way analysis of variance followed by Tukey’s test among multiple groups. A value of p<0.05 was considered statistically significant.

RESULTS Vasodilator Responses Induced by Perfusion of α-Methylhistamine In the perfused mesenteric vascular bed with intact endothelium contracted by 7 μM methoxamine, a bolus injection of acetylcholine caused a sharp decrease in perfusion pressure due to vasodilation (Fig. 1A). The vasodilator response to acetylcholine has been shown to be endothelium-dependent and mediated by EDRFs including endothelial NO, prostanoids and endothelial-derived hyperpolarizing factors (EDHFs). As shown in Fig. 1A and Fig. 2, perfusion of α-methylhistamine for 1 min at concentrations of 1 to 100 μM produced a concentration-dependent decrease in perfusion pressure due to vasodilation. The α-methylhistamine-induced vasodilation was transient and disappeared within 5 min. The maximum response induced by the highest concentration (100 μM) of α-methylhistamine was 44.6±6.8% (Fig. 2).

Effects of Endothelium Removal on the α-Methylhistamine-Induced Vasodilator Responses Treatment of the perfused mesenteric vascular bed with sodium deoxycholate abolished the acetylcholine-induced vasodilation, indicating that the response was endothelium-dependent and confirmed successful removal of the endothelium (Fig. 1B). In this preparation, α-methylhistamine did not induce the concentration-dependent vasodilation (Fig. 1B, Fig. 2).

Effects of Thioperamide or Clobenpropit on the α-
Methylhistamine-Induced Vasodilator Responses In the preparations with intact endothelium, vasodilation in response to α-methylhistamine at concentrations of 1 to 30 μM was abolished in the presence of the histamine H₃ receptor antagonist, thioperamide (1 μM) or clobenpropit (1 μM) (Fig. 3). Each antagonist significantly inhibited, but did not abolish, the response to the highest concentration (100 μM), as shown in Fig. 3.

Effects of Chlorpheniramine and Cimetidine on the α-Methylhistamine-Induced Vasodilator Responses As shown in Fig. 4, in the preparation with intact endothelium, the histamine H₁ receptor antagonist chlorpheniramine (1 μM) significantly inhibited vasodilator response to α-methylhistamine at the highest concentration of 100 μM, but not low and moderate concentrations of 1 to 30 μM. However, the histamine H₂ receptor antagonist cimetidine (1 μM) did not affect the α-methylhistamine-induced vasodilation at any concentrations.
Effects of L-NAME and Indomethacin on the α-Methylhistamine-Induced Vasodilator Responses

In preparations with intact endothelium, L-NAME (100 μM) or indomethacin (1 μM) significantly attenuated, but did not abolish, the vasodilation in response to α-methylhistamine (Fig. 5). Combined perfusion of L-NAME (100 μM) and indomethacin (1 μM) abolished the α-methylhistamine-induced vasodilation (Fig. 5).

Effect of High KCl and Tetraethylammonium on the α-Methylhistamine-Induced Vasodilator Responses

In preparations with intact endothelium, high KCl (30 mM) almost abolished vasodilation in response to perfusion of α-methylhistamine (Fig. 6). The nonselective K⁺/H₃-channel inhibitor tetraethylammonium (5 mM) significantly attenuated, but did not abolish, the α-methylhistamine-induced vasodilation (Fig. 6).

DISCUSSION

The present study demonstrated that perfusion of α-methylhistamine, a selective histamine H₃ receptor agonist, produced concentration-dependent vasodilator responses in the rat mesenteric resistance artery. The α-methylhistamine-induced vasodilation was eliminated by endothelium removal, indicating that the vasodilation is endothelium-dependent. Furthermore, the histamine H₃ receptor antagonists thioperamide and clobenpropit, significantly inhibited the α-methylhistamine-induced vasodilation. In contrast, the histamine H₂ receptor antagonist, cimetidine, had no effect on the vasodilator responses to α-methylhistamine. Data indicate the mean±S.E.M. ∗∗p<0.01, compared with the control α-methylhistamine response (Tukey’s test).

Histamine H₃ receptor subtypes have been shown in neuronal terminals, both in the brain and in peripheral tissues. Histamine H₃ receptor-mediated vasodilation has also been reported in the endothelium-dependent rabbit mid-
dible cerebral artery,\textsuperscript{19,20} the hindlimb vascular bed\textsuperscript{22}\textsuperscript{22} and the mesenteric vascular bed of the cat.\textsuperscript{23} Taken together, the present findings suggest that $\alpha$-methylhistamine induces endothelium-dependent vasodilation, which is mediated by histamine $H_1$ receptors located on the endothelium of the rat mesenteric resistance artery. However, since the histamine $H_1$ receptor antagonist significantly inhibited the response to the highest concentration of $\alpha$-methylhistamine, $\alpha$-methylhistamine at higher concentrations of more than 100 $\mu$M has histamine $H_1$ receptor-agonistic activity. Similar results have been reported by Hegde et al.\textsuperscript{14} regarding $\alpha$-methylhistamine.

It is widely accepted that the endothelium at the luminal surface of blood vessels is an important regulator of vascular tone \textit{via} release of various endothelium-derived endogenous substances.\textsuperscript{27,31} Endothelial cells release different kinds of EDRFs\textsuperscript{15,16} including NO, PGI$_2$ and EDHFs. Vasodilator responses to $\alpha$-methylhistamine have been reported to be mediated by NO and PGI$_2$ release in the isolated cerebral artery of the rabbit,\textsuperscript{19,20} the aorta of the rat,\textsuperscript{21} the hindlimb vascular bed\textsuperscript{22} and the mesenteric vascular bed of the cat.\textsuperscript{23} Additionally, involvement of EDHFs in $\alpha$-methylhistamine-induced vasodilatation, which is associated with activation of the K$^+$-channel, has been shown in the hindlimb vascular bed of the cat.\textsuperscript{23}

The present study demonstrated that vasodilator responses to $\alpha$-methylhistamine were markedly attenuated by the NO synthase inhibitor $l$-NAME, suggesting that the release of NO from the endothelium plays a significant role in mediating vasodilator responses to this histamine $H_1$ receptor-selective agonist in the endothelium. These findings are in line with the observations of other reports.\textsuperscript{20,23} Additionally, the present study showed that indomethacin caused partial inhibition of vasodilator responses to $\alpha$-methylhistamine, which was smaller than that of $l$-NAME, implicating that $\alpha$-methylhistamine induces the release of relaxing factors, probably PGI$_2$, a metabolite of arachidonic acid, \textit{via} cyclooxygenase in the endothelium of the rat mesenteric resistance artery. A similar finding has been reported by Ea Kim et al.\textsuperscript{19,20} In their reports, the $H_1$ receptors have been shown to relax rabbit middle cerebral artery \textit{via} an endothelium-dependent mechanism involving both nitric oxide and prostanoid release. However, simultaneous treatment with $l$-NAME and indomethacin in the vascular mesenteric bed with an intact endothelium did not abolish the $\alpha$-methylhistamine-induced endothelium-dependent vasodilatation. Therefore, it is likely that other endothelium-derived relaxing factors besides NO and PGI$_2$ are involved in the histamine $H_1$ receptor-mediated vasodilatation.

Evidence has accumulated that NO and PGI$_2$ of EDRFs play a prominent role in the tone control of large conduit arteries, whereas EDHFs plays a major role in the small resistance arteries in response to vasoactive substances or physical stimuli.\textsuperscript{28,32,33} EDHFs, which has been described as an endothelium-derived non-NO and non-PGI$_2$ factor, induces hyperpolarization of vascular smooth muscle cells by opening K$^+$ channels.\textsuperscript{15,34,35} Jin et al.\textsuperscript{18} reported that endothelium-dependent vasodilatation in response to acetylcholine and Ca$^{2+}$-ionophore (A23187) in the rat mesenteric vascular bed was not inhibited by $l$-NAME and indomethacin, but was blunted by K$^+$-channel inhibition by high KCl and tetraethylammonium. Therefore, it is very likely that EDHFs is mainly responsible for the endothelium-dependent vasodilation in the rat mesenteric vascular bed, which is composed of many resistance arteries. Jin et al.\textsuperscript{26} demonstrated that histamine induces histamine $H_1$ receptor-mediated endothelium-dependent vasodilatation and histamine $H_2$ receptor-mediated endothelium-independent vasodilatation in the rat mesenteric vascular bed. Additionally, this report showed that the histamine-induced endothelium-dependent vasodilatation was resistant to $l$-NAME and indomethacin, suggesting that EDHF$s$ are responsible for histamine $H_1$ receptor-mediated vasodilatation. However, in the present study, the $\alpha$-methylhistamine-induced endothelium-dependent vasodilatation was markedly attenuated by elimination of NO and PGI$_2$. Therefore, stimulation of histamine $H_2$ receptors on the endothelium of the rat mesenteric arteries releases EDRFs, including NO and PGI$_2$, while activation of histamine $H_1$ receptors and muscarinic acetylcholine receptors releases mainly EDHFs. The histamine $H_2$ receptor-mediated vasodilator response in the rat mesenteric arteries is smaller than that of the vasodilator response mediated by histamine $H_1$ receptors or muscarinic acetylcholine receptors. The weak potency of the histamine $H_2$ receptor-mediated response seems to be due to a lower activity of EDHF$s$ release in the mesenteric artery.

In the present study, the $\alpha$-methylhistamine-induced endothelium-dependent vasodilatation was significantly inhibited, but not abolished, by a nonselective K$^+$-channel blocker, tetraethylammonium, suggesting that EDHFs are involved in the vasodilatation. However, this seems to be mainly due to a histamine $H_1$ receptor-mediated response, since $\alpha$-methylhistamine has only weak histamine $H_2$ receptor agonistic action. In contrast, nonselective K$^+$-channel blockade by a 30 mM high KCl medium abolished the $\alpha$-methylhistamine-induced vasodilatation. These findings suggest that the $\alpha$-methylhistamine-induced endothelium-dependent vasodilatation is an EDHFs-mediated response, which is associated with activation of K$^+$-channel involvement and is in agreement with the report of Champion and Kadowitz.\textsuperscript{22} However, it seems likely that abolishment of the $\alpha$-methylhistamine-induced response under high KCl conditions, which induce cell membrane depolarization, may result from blockade of EDRFs release.

In conclusion, the present findings suggest that $\alpha$-methylhistamine induces endothelium-dependent vasodilatation \textit{via} histamine $H_1$ receptors on the vascular endothelium of the rat mesenteric resistance artery. It is also suggested that histamine $H_3$ receptor-mediated vasodilator effects are mainly mediated by NO of EDRFs and partially by PGI$_2$ and EDHFs.

REFERENCES