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Lafutidine Facilitates Calcitonin Gene-Related Peptide (CGRP) Nerve-Mediated Vasodilation via Vanilloid-1 Receptors in Rat Mesenteric Resistance Arteries

Tetsuhiro Sugiyama[1], Yukako Hatanaka[1], Yukiko Iwatani[1], Xin Jin[1], and Hiromu Kawasaki[1,*]

[1]*Department of Clinical Pharmaceutical Science, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan*

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Abstract. Lafutidine is a histamine H_2-receptor antagonist with gastric antisecretory and gastroprotective activity associated with activation of capsaicin-sensitive nerves. The present study examined the effect of lafutidine on neurotransmission of capsaicin-sensitive calcitonin gene-related peptide (CGRP)-containing vasodilator nerves (CGRPergic nerves) in rat mesenteric resistance arteries. Rat mesenteric vascular beds were perfused with Krebs solution and vascular endothelium was removed by 30-s perfusion with sodium deoxycholate. In preparations preconstricted by continuous perfusion of methoxamine (α_1 adrenoceptor agonist), perfusion of lafutidine (0.1 – 10 μM) concentration-dependently augmented vasodilation induced by the periarterial nerve stimulation (PNS, 1 Hz) without affecting vasodilation induced by exogenous CGRP (10 pmol) injection. Perfusion of famotidine (H_2-receptor antagonist, 1 – 100 μM) had no effect on either PNS-induced or CGRP-induced vasodilation. Perfusion of lafutidine concentration-dependently augmented vasodilation induced by a bolus injection of capsaicin (vanilloid-1 receptor agonist, 30 pmol). The presence of a vanilloid-1 receptor antagonist, ruthenium red (10 μM) or capsazepine (5 μM), abolished capsaicin-induced vasodilation and significantly decreased the PNS-induced vasodilation. The decreased PNS-induced vasodilation by ruthenium red or capsazepine was not affected by perfusion of lafutidine. These results suggest that lafutidine facilitates CGRP nerve-mediated vasodilation by modulating the function of presynaptic vanilloid-1 receptors located in CGRPergic nerves.

Keywords: lafutidine, calcitonin gene-related peptide (CGRP)ergic nerve-mediated vasodilation, vanilloid receptor-1, mesenteric resistance artery (rat)

Introduction

Lafutidine is a histamine H_2-receptor antagonist with gastric antisecretory (1) and gastroprotective activities (2). Lafutidine has been shown to increase the gastric mucosal blood flow (3) and gastric mucus secretion (4, 5) and accelerate epithelial restitution in rats (3). It has been reported that the gastroprotective effect of lafutidine was due to the activation of capsaicin-sensitive afferent nerves in the rat stomach (6).

It is widely accepted that peripheral vascular tone is mainly regulated by vascular adrenergic nerves. However, recent studies have revealed that the mesenteric resistance arteries are innervated not only by adrenergic nerves, but also by nonadrenergic noncholinergic (NANC) nerves (7, 8). Previously, we reported that periarterial nerve stimulation (PNS) in rat mesenteric arteries produces NANC neurogenic vasodilation (7), which is mediated by calcitonin gene-related peptide (CGRP), a potent vasodilator neurotransmitter (7). CGRP-containing vasodilator nerves (CGRPergic nerves) are sensitive to capsaicin, a vanilloid-1 receptor agonist, which induces the release of CGRP from primary sensory neurons (9).

However, the effect of lafutidine on neurotransmission of CGRPergic nerves in rat mesenteric resistance

*Corresponding author. kawasaki@pheasant.pharm.okayama-u.ac.jp
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arteries is still unclear. Therefore, the present study was designed to examine the effect of lafutidine on neurotransmission of CGRPergic nerves in rat mesenteric resistance arteries.

Materials and Methods

Animals

Male Wistar rats weighing 240 – 350 g were used in the present study. All animals were given food and water ad libitum. They were housed in the Animal Research Center 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. All experiments conformed to international guidelines on the ethical use of animals.

Perfusion of mesenteric vascular beds

The animals were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally) and the mesenteric vascular beds were isolated and prepared for perfusion as described previously (7, 10). The superior mesenteric artery was cannulated and flushed gently with a modified (see below) Krebs-Ringer bicarbonate solution (Krebs solution) to eliminate blood in 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 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 then placed in a water-jacked organ bath maintained at 37°C and perfused with Krebs solution at a constant flow rate of 5 mL/min with a peristaltic pump (model AC-2120; ATTO Co., Tokyo). The preparation was also 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% O_2 – 5% CO_2 before passage through a warming coil maintained at 37°C. The modified Krebs solution had the following composition: 119.0 mM NaCl, 4.7 mM KCl, 2.4 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 0.03 mM disodium EDTA, and 11.1 mM glucose (pH 7.4). Changes in the perfusion pressure were measured with a pressure transducer (model TP-400T; Nihon Kohden, Tokyo) and recorded using a pen recorder (model U-228; Nippon Denshi Kagaku, Tokyo).

Chemical removal of the vascular endothelium

To remove the vascular endothelium, preparations with resting tone were perfused with 1.80 mg/mL sodium deoxycholate (SD) in saline for 30 s as described previously (11, 12). Then, the preparations were rinsed with SD-free Krebs solution for 60 min. After the preparations were perfused with Krebs solution containing methoxamine to contract vascular tone and guanethidine to block adrenergic neurotransmission, chemical removal of the endothelium was assessed by the lack of a relaxant effect after a bolus injection of 1 nmol acetylcholine (ACh), which was injected directly into the perfusate proximal to the arterial cannula with an infusion pump (model 975; Harvard Apparatus, Holliston, MA, USA). Volumes were 100 μL for 12 s.

Periarterial nerve stimulation (PNS)

PNS was applied for 30 s using bipolar platinum ring electrodes placed around the superior mesenteric artery. Rectangular pulses of 1 ms and supramaximal voltage (50 V) were applied at 1 Hz using an electronic stimulator (model SEN 3301, Nihon Kohden).

Bolus injection of CGRP and capsaicin

CGRP (10 pmol) and capsaicin (30 pmol) were directly injected into the perfusate proximal to the arterial cannula with an infusion pump. A volume of 100 μL was injected for 10 s.

Experimental protocols

After responses to the first PNS (S_1) or CGRP injection (I_1) were obtained as the control, the Krebs solution containing methoxamine and guanethidine was switched to Krebs solution containing methoxamine and guanethidine with lafutidine (0.1 – 10 μM) or famotidine (1 – 100 μM), and then the PNS (S_{2-4}) or CGRP injections (I_{2-4}) were carried out. To assess the effect of lafutidine and famotidine on neurotransmission of CGRPergic nerves, changes in perfusion pressure in response to PNS or CGRP injection were expressed as the ratio between the vasodilation induced by S_{2-4} and S_1 or by I_{2-4} and I_1, respectively.

In another series of experiments, the effect of lafutidine on vasodilation induced by capsaicin [an agonist for transient receptor potential vanilloid-1 receptors (TRPV-1)] was examined. Since capsaicin has been shown to stimulate the primary sensory nerves with low concentrations but causes functional ablation of sensory nerves with high concentrations (13, 14), a

low concentration of 30 pmol was injected with the infusion pump over 10 s. After responses to the first (C_1) and second (C_2) bolus injection of capsaicin were obtained as the control, the Krebs solution containing methoxamine and guanethidine was switched to Krebs solution containing methoxamine, guanethidine and lafutidine (1 or 10 μM), and then the third (C_3) and fourth (C_4) injections of capsaicin were carried out. The effect of lafutidine on vasodilator response to capsaicin injection was expressed as the ratio between the vasodilation induced by C_{2-4} and C_1, respectively.

To assess the underlying mechanisms involved in lafutidine on neurotransmission of CGRPergic nerves, after responses to the first PNS (S_1) were obtained as the control, the Krebs solution containing methoxamine and guanethidine was switched to Krebs solution containing methoxamine, guanethidine, and 10 μM ruthenium red (a nonselective antagonist for vanilloid-1 receptors); methoxamine, guanethidine, and ruthenium red plus lafutidine (1 or 10 μM); methoxamine, guanethidine, and 5 μM capsazepine (a selective antagonist for vanilloid receptor-1); or methoxamine, guanethidine, and capsazepine plus lafutidine (1 or 10 μM). Thereafter, PNS (S_{2-4}) was carried out. To estimate the effects of the agents tested, vasodilator responses to PNS were expressed as the ratio between the vasodilation induced by S_{2-4} and S_1, respectively.

At the end of each experiment, 100 μM papaverine was perfused to produce complete relaxation. Vasodilation is expressed as the percentage perfusion pressure at maximum relaxation induced by papaverine.

Statistical analyses

Experimental results are each expressed as the mean ± S.E.M. Statistical analysis was performed by the Student's unpaired *t*-test and one-way analysis of variance followed by Tukey's test. A *P*-value less than 0.05 was considered significant.

Drugs

The following drugs were used: ACh chloride (Daiichi-Sankyo Pharmaceutical Co., Tokyo); capsaicin, capsazepine,famotidine, ruthenium red, and SD (Sigma Chemical Co., St. Louis, MO, USA); guanethidine sulfate (Tokyo Kasei, Tokyo); methoxamine hydrochloride (Nihon Shinyaku Co., Kyoto); papaverine hydrochloride (Dainippon Sumitomo Pharmaceutical Co., Osaka); rat CGRP (Peptide Institute, Osaka); and lafutidine (Taiho Pharmaceutical Co., Tokushima). All drugs, except for capsaicin and SD, were dissolved in

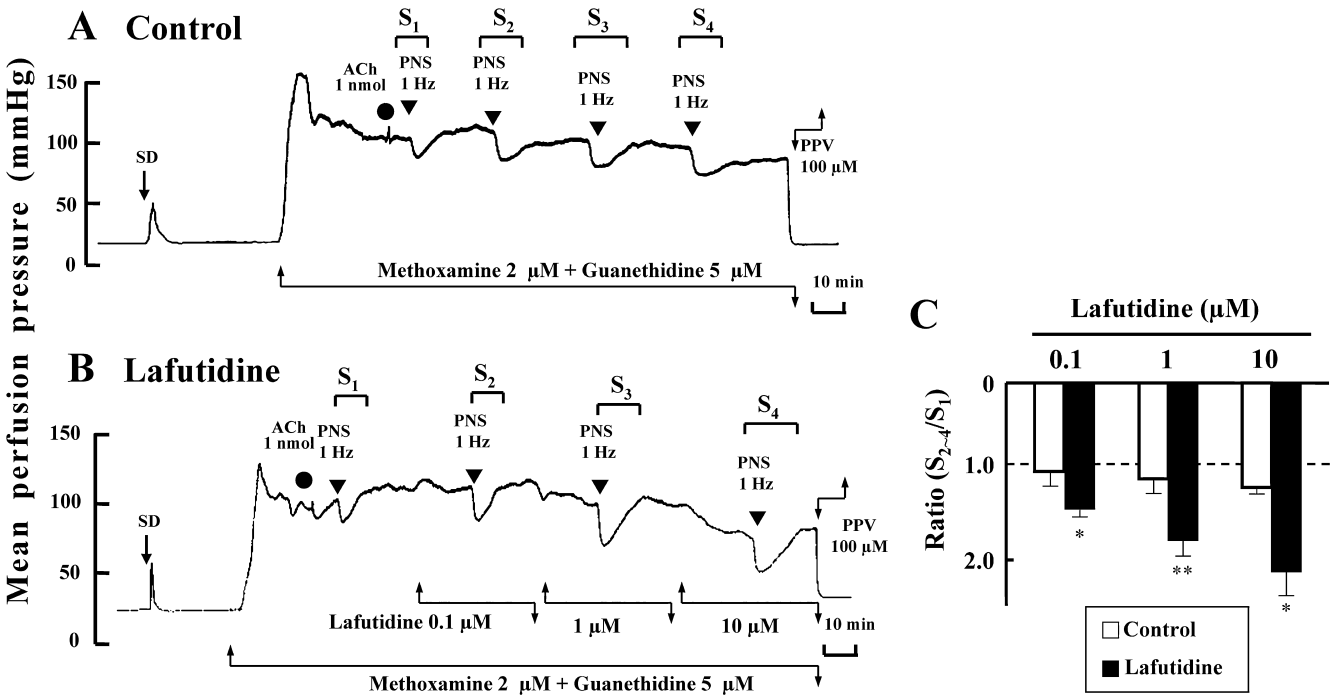

Fig. 1. Typical records (A and B) and bar graph (C) showing the effect of lafutidine (0.1 – 10 μM) on vasodilator responses to PNS in rat perfused mesenteric vascular beds without the endothelium and with active tone. SD, perfusion of sodium deoxycholate for 30 s; ACh, bolus injection of acetylcholine (1 nmol, solid circles); PNS, periarterial nerve stimulation (1 Hz, solid inverted triangles); PPV, perfusion of 100 μM papaverine. S_1, S_2, S_3, and S_4 indicate the 1st, 2nd, 3rd, and 4th PNS, respectively. Ordinates (C) indicate the ratio of S_1- and S_{2-4}-induced vasodilation. Values each represent the mean ± S.E.M. of five rats. **P*<0.05, ***P*<0.01 *vs* Control.

distilled water and diluted with Krebs solution containing 2 – 20 μM methoxamine and 5 μM guanethidine and then perfused or injected directly. Capsaicin was dissolved in 50% ethanol and diluted with Krebs solution (final alcohol concentration, 0.4 mg/mL). SD was dissolved in 0.9% saline.

Results

Effect of lafutidine on vasodilation induced by PNS

As shown in Fig. 1A, in preconstricted and perfused mesenteric vascular beds without the endothelium, PNS (1 Hz) induced a decrease in perfusion pressure due to vasodilation. Repeated PNS (S_{1-4}) caused reproducible vasodilator responses. In the control response, the ratios of S_2/S_1, S_3/S_1, and S_4/S_1 were and 1.10 ± 0.11, 1.21 ± 0.12, and 1.38 ± 0.08, respectively. There was no significant difference in the control response to PNS between S_1, S_2, S_3, and S_4. We previously reported that PNS-induced vasodilation is mediated by endogenous CGRP released by stimulation of CGRPergic nerves (7).

Figure 1 (B and C) shows the effect of lafutidine (0.1 – 10 μM) on vasodilator responses to PNS. Perfusion of lafutidine at a concentration of 10 μM caused a decrease in perfusion pressure ($48.8 \pm 2.1\%$, $n = 5$) (Fig. 1B). As shown in Fig. 1, the PNS induced-vasodilation was significantly augmented by lafutidine in a concentration-dependent manner.

Effect of lafutidine on vasodilation induced by a bolus injection of CGRP

A bolus injection of CGRP (10 pmol) into the perfusate also caused vasodilation (Fig. 2A). Repeated CGRP injection (I_{1-4}) caused reproducible vasodilator responses. The ratios of I_2/I_1, I_3/I_1, and I_4/I_1 were 1.03 ± 0.12, 1.23 ± 0.10, and 1.26 ± 0.10, respectively. There was no significant difference in control response to PNS and CGRP injection between I_1, I_2, I_3 and I_4.

Figure 2 (B and C) shows the effect of lafutidine (0.1 – 10 μM) on vasodilator responses to CGRP injection. Perfusion of lafutidine did not affect vasodilation induced by exogenous CGRP injection.

Effect of famotidine on vasodilation induced by PNS and CGRP injection

As shown in Fig. 3, famotidine at concentrations of

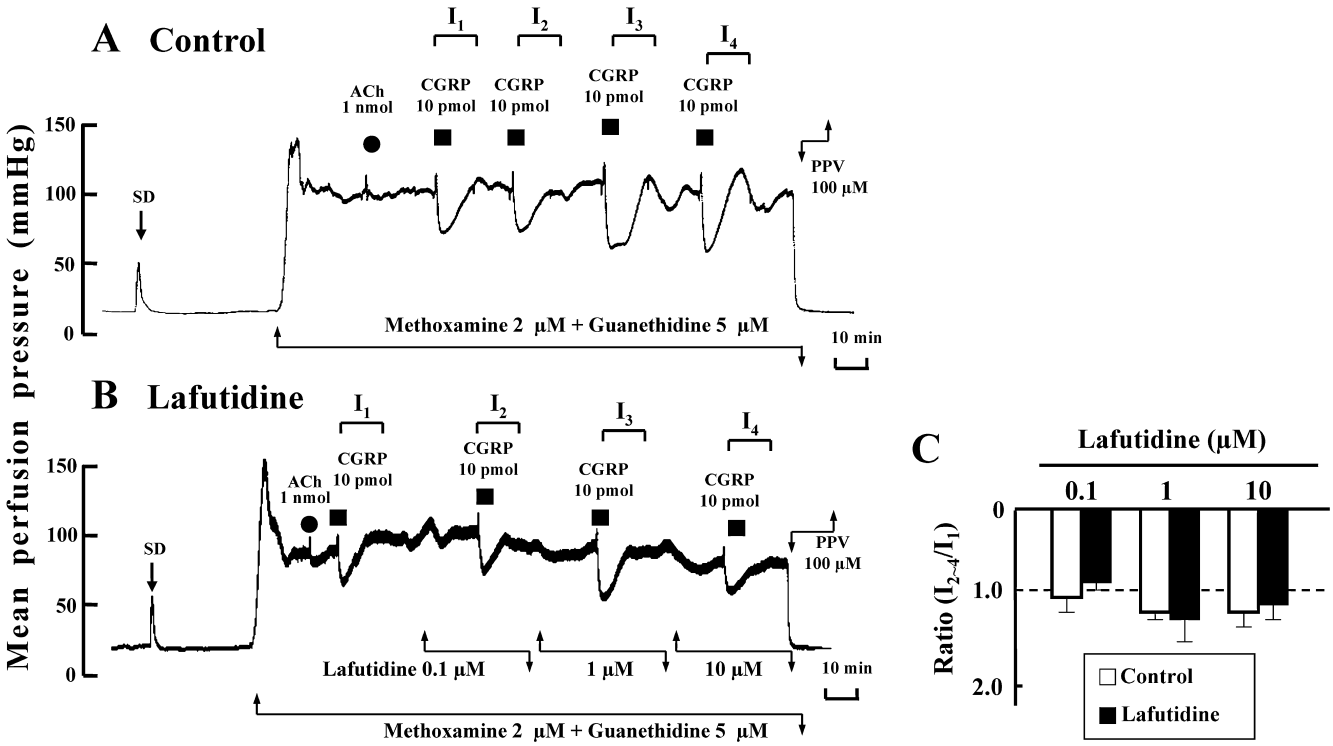

Fig. 2. Typical records (A and B) and bar graph (C) showing effect of lafutidine (0.1 – 10 μM) on vasodilator responses to bolus injections of CGRP in rat perfused mesenteric vascular beds without the endothelium and with active tone. SD, perfusion of sodium deoxycholate for 30 s; ACh, bolus injection of acetylcholine (1 nmol, solid circles); CGRP, bolus injection of CGRP (10 pmol, solid squares); PPV, perfusion of 100 μM papaverine. I_1, I_2, I_3, and I_4 indicate the 1st, 2nd, 3rd, and 4th injections of CGRP, respectively. Ordinates (C) indicate the ratio of I_1- and I_{2-4}-induced vasodilation. Values each represent the mean ± S.E.M. of five rats.

1 – 100 μM had no effect on either PNS-induced vasodilation or CGRP-induced vasodilation.

Effect of lafutidine on capsaicin-induced vasodilation

As shown in Fig. 4A, in preconstricted and perfused mesenteric vascular beds without the endothelium, a bolus injection of capsaicin (30 pmol) into the perfusate caused vasodilation. Repeated capsaicin injection (C_{1-4}) caused reproducible vasodilator responses (Fig. 4A). In the control response, the ratios of C_2/C_1, C_3/C_1, and C_4/C_1 were 1.11 ± 0.10, 1.26 ± 0.17, and 1.30 ± 0.11, respectively. There was no significant difference in control response to capsaicin injection between C_1, C_2, C_3, and C_4.

As shown in Fig. 4 (B and C), perfusion of lafutidine (0.1 – 10 μM) concentration-dependently augmented vasodilation induced by capsaicin injection. There was a significant difference between 10 μM lafutidine and the control (Fig. 4C).

Effects of ruthenium red and capsazepine on lafutidine-induced augmentation of PNS-induced vasodilation

The vanilloid receptor-1 antagonists, ruthenium red (10 μM) or capsazepine (5 μM), abolished vasodilation

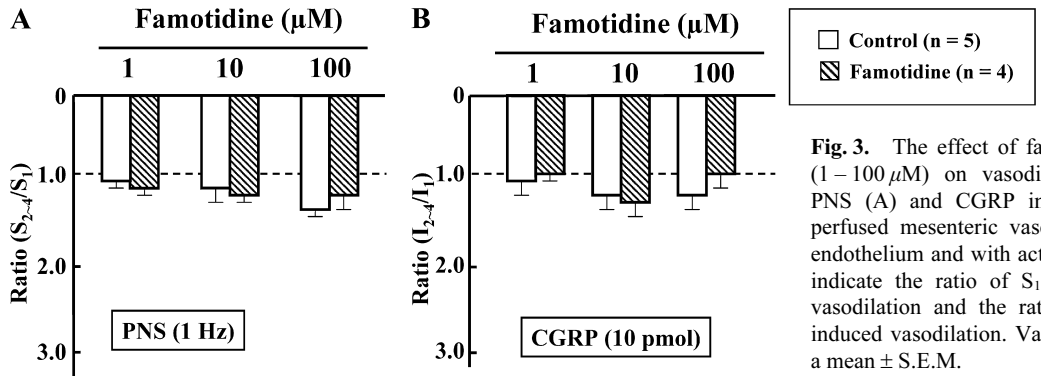

Fig. 3. The effect of famotidine perfusion (1 – 100 μM) on vasodilator responses to PNS (A) and CGRP injection (B) in rat perfused mesenteric vascular beds without endothelium and with active tone. Ordinates indicate the ratio of S_1- and S_{2-4}-induced vasodilation and the ratio of I_1- and I_{2-4}-induced vasodilation. Values each represent a mean ± S.E.M.

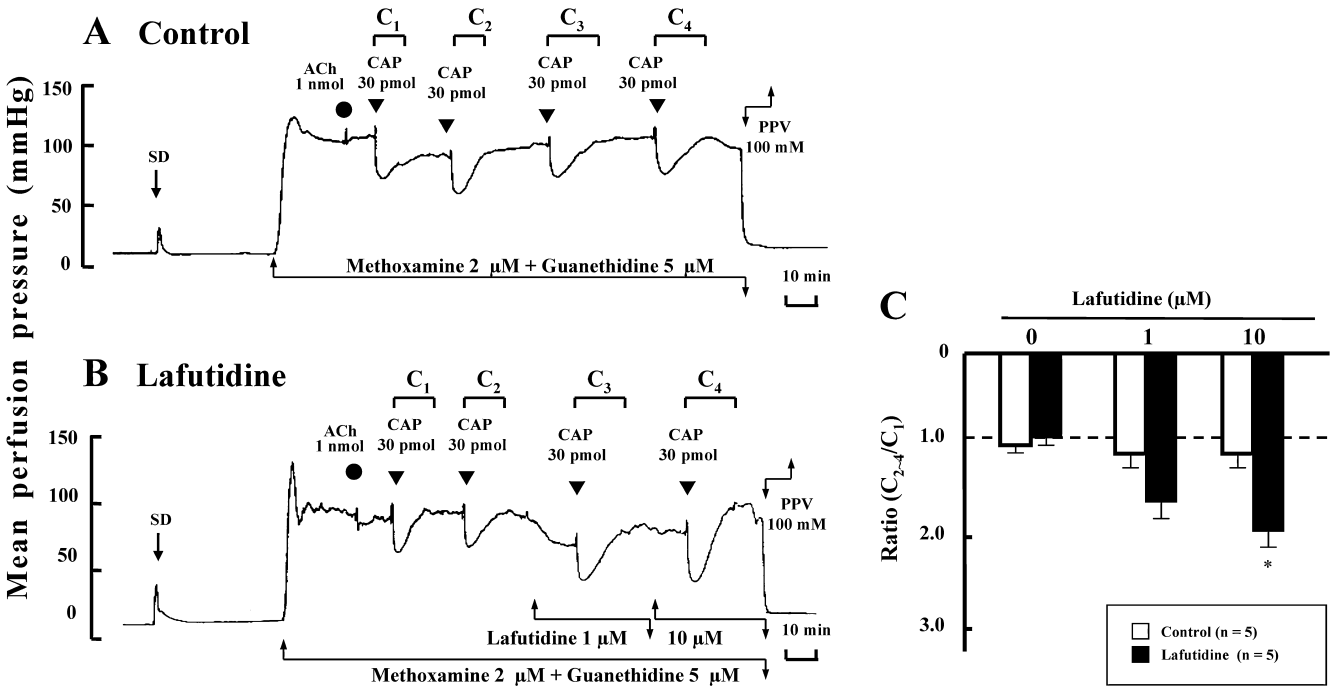

Fig. 4. Typical records (A and B) and bar graph (C) showing effect of lafutidine (1 and 10 μM) on vasodilator responses to capsaicin injection (30 pmol) in rat perfused mesenteric vascular beds without endothelium and with active tone. SD, perfusion of sodium deoxycholate for 30 s; ACh, bolus injection of acetylcholine (1 nmol, solid circles); CAP, bolus injection of capsaicin (solid inverted triangles); PPV, perfusion of 100 μM papaverine. C_1, C_2, C_3, and C_4 indicate the 1st, 2nd, 3rd, and 4th injections of capsaicin, respectively. Ordinate (C) indicates the ratio of C_1- and C_{2-4}-induced vasodilation. Values each represent a mean ± S.E.M. *P<0.05 *vs* Control.

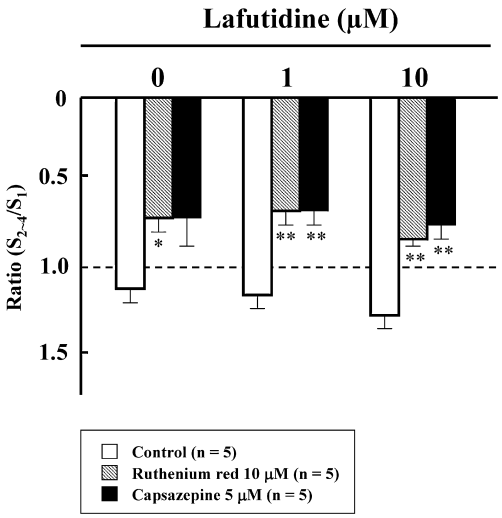

Fig. 5. Effects of ruthenium red and capsazepine on lafutidine (1 – 10 μM)–induced facilitation of vasodilator responses to PNS in rat perfused mesenteric vascular beds without endothelium and with active tone. Ordinates indicate the ratio of S_1- and S_{2-4}-induced vasodilation. Values each represent a mean ± S.E.M. **P*<0.05, ***P*<0.01 *vs* Control.

induced by a bolus injection of capsaicin (30 pmol): control (38.3 ± 4.1%) and ruthenium red (0.43 ± 0.2%), n = 5, *P*<0.01; capsazepine (0.32 ± 0.2%), n = 5, *P*<0.01. The presence of ruthenium red or capsazepine significantly inhibited the PNS-induced vasodilation (Fig. 5). However, neither agent affected the CGRP-induced vasodilation (data not shown). The inhibition of PNS-induced vasodilation by ruthenium red or capsazepine was not affected by perfusion of lafutidine (1 – 10 μM) (Fig. 5). There was no significant difference in the PNS-response between ruthenium red or capsazepine alone and the presence of lafutidine. Ruthenium red (10 μM) or capsazepine (5 μM) did not affect vasodilation induced by perfusion of lafutidine (10 μM): none (48.8 ± 2.1%) and ruthenium red (65.4 ± 7.6%), n = 5; capsazepine (54.3 ± 9.0%), n = 5.

Discussion

The present and previous studies (7) demonstrated that PNS of the perfused mesenteric vascular bed contracted by methoxamine, a selective α_1 adrenoceptor agonist, in the presence of guanethidine, an adrenergic neuron blocker, produces a frequency-dependent decrease in perfusion pressure due to vasodilation. This vasodilation is mediated by NANC nerves because the response was abolished by tetrodotoxin, but not by an anticholinergic drug (atropine) or a β-adrenoceptor antagonist (propranolol) (7). In addition, PNS of the perfused mesenteric vascular bed causes the tetrodotoxin-sensitive release of CGRP associated with the vasodilation (7). Moreover, the results obtained with PNS-induced NANC vasodilation with CGRP (8-37) (CGRP-receptor antagonist) (15) has confirmed that the NANC vasodilation is mediated by endogenous CGRP released from CGRP-containing vasodilator nerves (16). The present study demonstrated that, in rat mesenteric arteries without the endothelium and with active tone, lafutidine, a histamine H_2-receptor antagonist, augmented PNS-induced vasodilation in a concentration-dependent manner. However, lafutidine did not affect vasodilation induced by exogenous CGRP, which is mediated by postsynaptic CGRP receptors. Additionally, the histamine H_2-receptor antagonist famotidine had no effect on either PNS- or CGRP-induced vasodilation. Lafutidine has gastroprotective activities (2), which are associated with the activation of capsaicin-sensitive afferent nerves (CGRPergic nerves) in the rat stomach (6). Taken together, these results suggest that lafutidine presynaptically facilitates neurotransmission of CGRPergic nerves via a mechanism in which histamine H_2-receptors are not involved.

The vanilloid receptor-1 agonist capsaicin causes vasodilation in rat mesenteric resistance arteries when applied at low concentrations (17). Capsaicin-induced vasodilation has been shown to be mediated by CGRP released from CGRPergic nerves (18). An immunohistochemical study demonstrated that vanilloid-1 receptors are present in CGRP-like immunorectivities–containing nerves in the rat mesenteric artery (19). In the present study, perfusion of lafutidine concentration-dependently augmented capsaicin-induced vasodilation, which was abolished by ruthenium red, a non-selective antagonist for vanilloid receptor-1, and capsazepine, a selective vanilloid receptor-1 antagonist. These findings suggest that lafutidine modulates CGRPergic neurotransmission via vanilloid receptor-1 located in CGRPergic nerves. This notion is supported by a previous report that the gastroprotective effect of lafutidine resulted from the activation of capsaicin-sensitive afferent nerves in the rat stomach (6).

The present study demonstrated that the PNS-induced vasodilation in rat mesenteric arteries was markedly inhibited by ruthenium red and capsazepine. Similar findings have been shown by Eguchi et al. (19), who reported that capsazepine and ruthenium red suppressed PNS-induced vasodilation without affecting vasodilation induced by exogenous CGRP. The vanilloid receptor-1 is a nonselective cation channel with high permeability for Ca^{2+}. Since neurogenic release of CGRP from the CGRPergic nerves is Ca^{2+}-dependent and the vanilloid receptor-1 antagonists inhibited, but did not abolish the CGRPergic nerve–mediated vasodi-

lation, it seems likely that PNS induces activation of vanilloid receptor-1, which increases Ca^{2+} influx and induces CGRP release. In the present study, perfusion of lafutidine concentration-dependently augmented PNS and capsaicin-induced vasodilation without affecting vasodilation induced by exogenously applied CGRP. Additionally, the facilitatory effect of lafutidine on PNS-induced vasodilation was abolished in the presence of two vanilloid-1 receptor antagonists, ruthenium red and capsazepine, which also abolished capsaicin-induced vasodilation. However, lafutidine has been shown not to bind vanilloid-1 receptors (6). Additionally, the present findings showed that ruthenium red and capsazepine did not affect lafutidine-induced vasodilation. Taken together, these findings strongly suggest that lafutidine modulates the function of vanilloid-1 receptors to facilitate the release of CGRP from CGRPergic nerves.

In conclusion, the present study suggests that lafutidine facilitates neurotransmission of CGRPergic nerves in rat mesenteric resistance arteries. It is also suggested that lafutidine modulates the function of presynaptic vanilloid receptor-1 located on CGRPergic nerves to augment neurogenic vasodilation.

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