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Triphasic vascular responses to bradykinin in the mesenteric resistance artery of the rat

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Abstract

The vascular effects of bradykinin were studied in rat perfused mesenteric vascular beds with active tone. Bolus injections of bradykinin (1–1000 pmol) but not des-Arg^9-bradykinin (bradykinin B_1 receptor agonist) induced triphasic vascular responses: the initial sharp vasodilation followed by transient vasoconstriction and subsequent gradual vasodilation. The triphasic vascular responses to bradykinin were abolished by FR 172357 (3-bromo-8-[2,6-dichloro-3-[*N*-[(*E*)-4-(*N*,*N*-dimethylcarbamoyl) cinnamidoacetyl]-*N*-methylamino]benzyloxy]-2-metylimidazo[1,2-*a*]pyridine) (bradykinin B_2 receptor antagonist, 0.1 μM). Endothelium removal with sodium deoxycholate and N^{ω}-nitro-L-arginine (300 μM) abolished the bradykinin-induced initial sharp vasodilation. Indomethacin (0.5 μM) and seratrodast (thromboxane A_2 receptor antagonist, 0.5 and 5 μM) abolished the bradykinin-induced second vasoconstriction. The bradykinin-induced third vasodilation was abolished by capsaicin (1 μM) and calcitonin gene-related peptide (CGRP)-(8–37) (CGRP receptor antagonist, 0.5 μM). These findings suggest that the bradykinin-induced initial sharp vasodilation is endothelium dependent, that endogenous thromboxane A_2 is involved in the second vasoconstriction, and that the third slow vasodilation is produced by activation of capsaicin-sensitive CGRP-containing nerves.

Keywords: Bradykinin; Vasodilation; Endothelium dependent; Thromboxane A_2; CGRP (calcitonin gene-related peptide); Mesenteric resistance artery, (Rat)

1. Introduction

Bradykinin, an endogenous nonapeptide, has a wide variety of vascular effects. The action of bradykinin has been shown to be mediated by the activation of two membrane receptors, denoted as bradykinin B_1 and B_2 receptors (Regoli and Barabe, 1980; Burch and Kyle, 1992; Regoli et al., 1998). Bradykinin preferentially acts through constitutive bradykinin B_2 receptors to cause a vascular effect (Berguer et al., 1993; Miyamoto et al., 1999). Bradykinin induces endothelium-dependent vasorelaxation and vasoconstriction, which are mediated via activation of endothelial bradykinin B_1 and B_2 receptors (Drummond and Cocks, 1995; Ohlmann et al., 1997; Ihara et al., 2000). The main endothelium-dependent relaxing and contracting factors in bradykinin-induced responses are nitric oxide (NO) and prostanoids, respectively (Lundgaard et al., 1997; Miyamoto et al., 1999; Ihara et al., 2000). Furthermore, bradykinin has a direct action on vascular smooth muscle, via its action on bradykinin B_1 and B_2 receptors (Persson and Andersson, 1998). Activation of these receptors leads to relaxation, contraction, or a biphasic response, depending on the organs or species (Persson and Andersson, 1998; Bagate et al., 1999).

It is generally accepted that peripheral vascular tone is maintained predominantly by the vasoconstrictor neurotransmitter, norepinephrine, released from perivascular adrenergic nerves. Neuropeptide Y and ATP, which are co-localized in perivascular adrenergic nerves and released along with norepinephrine when these nerves are stimulated, are also involved in the control of vascular tone (Lundberg et al., 1982). Evidence has accumulated that the resistance artery is innervated not only by adrenergic nerves but also by non-adrenergic noncholinergic (NANC) nerves (Kawasaki et al., 1988; Toda and Okamura, 1992). We previously reported that calcitonin gene-related peptide (CGRP) acts as the potential vasodilator transmitter for NANC nerves in the rat mesenteric resistance artery (Kawasaki et al., 1988). Bradykinin has been shown to release endogenous CGRP in the heart, lung and trachea (Geppetti et al., 1990; Hua et al., 1994; Schuligoi et

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al., 1998). However, the role of CGRP in bradykinin-induced vascular responses of the mesenteric resistance artery remains unknown. The present study was, therefore, designed to investigate the vascular effect of bradykinin in mesenteric resistance blood vessels of the rat.

2. Materials and methods

2.1. 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 (light on at 8:00 a.m.).

2.2. 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 (Kawasaki et al., 1988). The superior 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 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-jacketed organ bath maintained at 37 °C and 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). Preparations were 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 was of the following composition (mM): NaCl 119.0, KCl 4.7, $CaCl_2$ 2.4, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, KH_2PO_4 1.2, disodium 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 on a pen recorder (model U-228, Nippon Denshi Kagaku, Tokyo, Japan).

2.3. Periarterial nerve stimulation

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

2.4. 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 in saline for 30 s. This caused a transient increase (20–30 mm Hg) 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, chemical removal of the endothelium was assessed by the lack of a relaxant effect after a bolus injection of 1 nmol acetylcholine, which was injected directly into the perfusate proximal to the arterial cannula with an injection pump (model 975, Harvard Apparatus, Holliston, MA, USA). Volume was 100 μl for 12 s.

2.5. Treatment with capsaicin

In vitro depletion of perivascular CGRP-containing nerves was performed according to the method described by Kawasaki et al. (1988). The isolated mesenteric vascular bed was perfused with Krebs solution containing capsaicin (1 μM) for 20 min and then rinsed with capsaicin-free Krebs solution. After being rinsed for 60 min, the preparation was contracted by perfusion with Krebs solution containing methoxamine (7 μM) and guanethidine (5 μM). After the elevated perfusion pressure stabilized, periarterial nerve stimulation at 1 Hz was performed to check for the presence of peptidergic nerves. Successful depletion of peptidergic nerves was confirmed by the lack of a relaxant effect of periarterial nerve stimulation (1 Hz).

2.6. Experimental protocols

Isolated mesenteric vascular beds were perfused with Krebs solution and perfusion pressure was increased by methoxamine (7 μM) in the presence of guanethidine (5 μM) which was added to block adrenergic neurotransmission. After the elevated perfusion pressure became stabilized, bradykinin and the bradykinin B_1 receptor agonist (des-Arg^9-bradykinin) at concentrations of 1, 3, 10, 30, 100, 300 and 1000 pmol and thromboxane A_2 receptor agonist U46619 (9,11-dideoxy-11$_\alpha$,9$_\alpha$-epoxymethanoprostaglandin $F_{2\alpha}$) at concentrations of 1, 3, 10, 30 and 100 pmol were directly injected into the perfusate with the injection pump. Injection volume was 100 μl for 12 s. The vascular response to bradykinin and des-Arg^9-bradykinin was examined in preparations with intact endothelium. To assess the mechanisms underlying the vascular effect of bradykinin, the bradykinin injection was performed in preparations with intact endothelium during perfusion of bradykinin B_2 receptor antagonist (FR 172357; 3-bromo-8-[2,6-dichloro-3-[*N*-[(*E*)-4-(*N*,*N*-dimethylcarbamoyl) cinnamidoacetyl]-*N*-methylamino]benzyloxy]-2-metylimidazo[1,2-*a*]pyridine, 0.1 μM) (Rizzi et al., 1999), cyclooxygenase inhibitor, indomethacin (0.5 μM), thromboxane A_2 receptor antagonist,

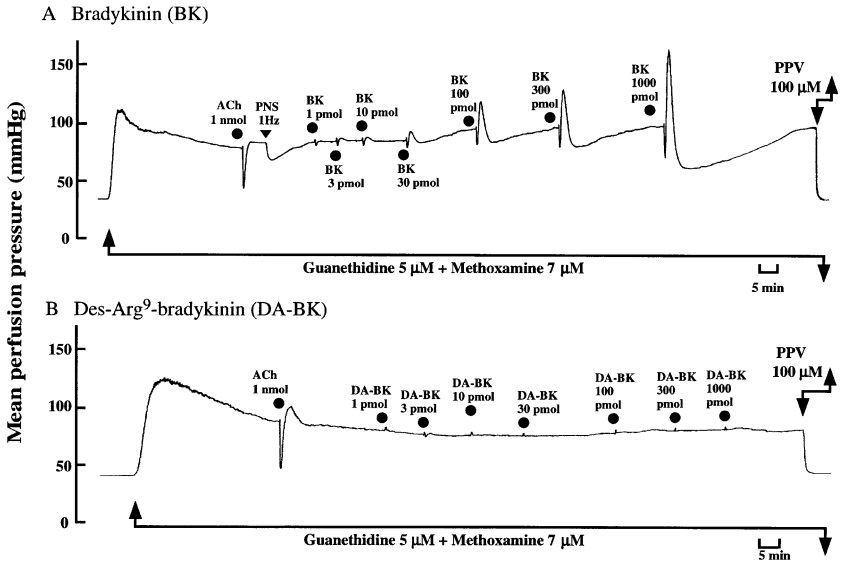

Fig. 1. Typical records showing vascular responses to bolus injections of bradykinin (BK) and des-Arg^9-BK in rat perfused mesenteric vascular bed with active tone. Note that bradykinin but not des-Arg^9-BK induced triphasic vascular responses: the first phase of sharp vasodilation, the second phase of vasoconstriction and third phase of long-lasting vasodilation. ACh, injection of acetylcholine (1 nmol). PNS, periarterial nerve stimulation (1 Hz). BK, injection of bradykinin. PPV, papaverine perfusion.

seratrodast (0.5 and 5 μM) and NO synthase inhibitor, N^{ω}-nitro-L-arginine (L-NA, 300 μM). The effect of the CGRP receptor antagonist, CGRP-(8–37) (0.5 μM), on the bradykinin-induced response was studied in preparations without endothelium.

To assess the influence of endothelium, the bradykinin-induced vascular responses were examined in preparations with the endothelium chemically removed with sodium deoxycholate. After sodium deoxycholate perfusion, active tone of the preparation was produced by perfusion with Krebs solution containing methoxamine (2 μM) and guanethidine (5 μM). After confirming successful removal of the endothelium by the lack of a relaxant effect after 1 nmol acetylcholine injection, bradykinin injections were performed.

The effects of capsaicin (1 μM) on vascular responses to bradykinin were examined in preparations with intact endothelium. The preparation was perfused with Krebs solution containing capsaicin (1 μM) for 20 min, and 60 min after capsaicin treatment, the preparation was contracted by perfusion with Krebs solution containing methoxamine (7 μM) and guanethidine (5 μM). After the elevated perfusion pressure stabilized, periarterial nerve stimulation (1 Hz) was

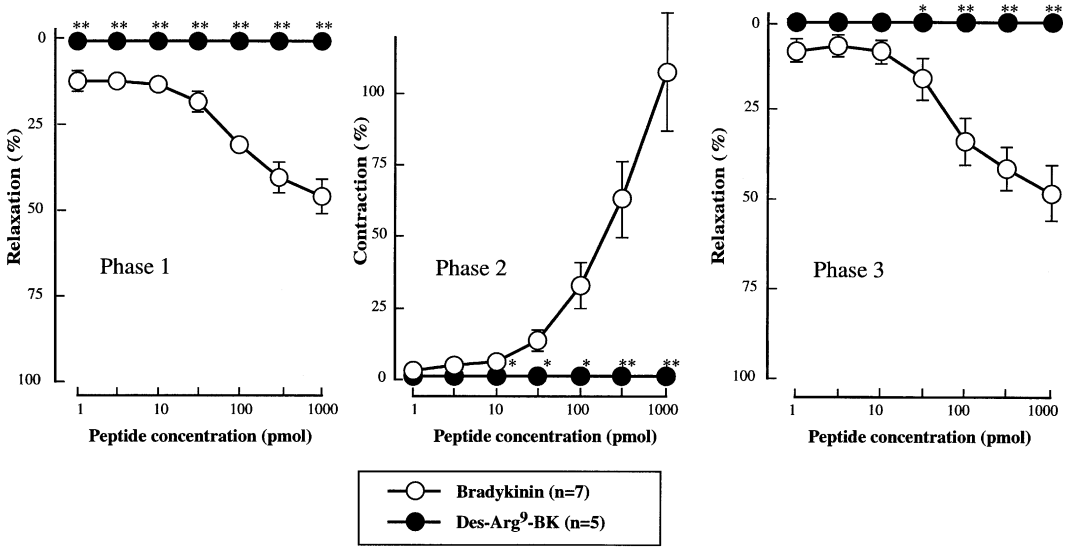

Fig. 2. Vascular responses to bolus injections of bradykinin (BK) and bradykinin B_1 receptor agonist (des-Arg^9-BK) in rat perfused mesenteric vascular beds with active tone. Phase 1, Phase 2 and Phase 3 indicate first phase (vasodilation), second phase (vasoconstriction) and third phase (vasodilation) responses induced by bradykinin injection, respectively. $*P<0.05$, $**P<0.01$, compared with the bradykinin response.

Table 1
Effects of various treatments on vasodilation induced by acetylcholine and periarterial nerve stimulation (PNS) in rat perfused mesenteric vascular beds with active tone

Treatment (μM)	*n*	Vasodilator response (%)	
		Acetylcholine (1 nmol)	PNS (1 Hz)
Control	7	83.7±3.8	36.1±7.1
Endothelium removal	5	11.5±0.1[a]	N.D.
FR 172357 (0.1)	4	89.5±1.5	N.D.
L-NA (300)	5	58.1±9.2[a]	N.D.
Indomethacin (5)	5	85.8±3.3	N.D.
Seratrodast (5)	5	93.2±1.2	N.D.
Capsaicin (1)	5	70.2±7.7	1.0±1.0[a]
CGRP-(8–37) (0.5)[b]	5	N.D.	3.2±3.2[a]

The preparation was routinely perfused with 5 μM guanethidine.
N.D., not determined.
[a] $P<0.01$, compared with control response.
[b] The endothelium of the preparation was removed.

performed to check for the successful removal of CGRP-containing nerves and bradykinin injections were performed.

At the end of each experiment, the preparation was perfused with 100 μM papaverine to cause complete relaxation. Vasodilator responses were expressed as a percentage of the perfusion pressure at maximum relaxation induced by papaverine. Vasoconstrictor responses were expressed as a percentage of the perfusion pressure before bradykinin injection.

2.7. Statistical analysis

Experimental results are presented as the means ± S.E.M. Statistical analysis was done with Student's unpaired or paired *t*-test. A value of $P<0.05$ was considered statistically significant.

2.8. Drugs

The following drugs were used: acetylcholine chloride (Daiichi Pharmaceutical, Tokyo, Japan), bradykinin (Peptide Institute, Osaka, Japan), rat CGRP (Peptide Institute), human CGRP-(8–37) (Peptide Institute), capsaicin (Sigma, St. Louis, MO, USA), des-Arg^9-bradykinin (Peptide Institute), guanethidine sulphate (Sigma), FR 172357 (Fujisawa Pharmaceutical, Tokyo, Japan), indomethacin (Sigma), U46619 (Sigma), seratrodast (Takeda Pharmaceutical, Tokyo, Japan), methoxamine hydrochloride (Nihon Shinyaku, Kyoto, Japan), L-NA (Sigma), papaverine hydrochloride (Dainippon Pharmaceutical, Osaka, Japan), sodium deoxycholate (Sigma). All drugs, except capsaicin and sodium deoxycholate, were dissolved in distilled water and diluted with Krebs solution containing 2–7 μM methoxamine when perfused and injected directly. Capsaicin was dissolved in 50% ethanol and diluted with Krebs solution (final alcohol concentration, 0.4 mg/ml). Sodium deoxycholate was dissolved in 0.9% saline.

3. Results

3.1. Vascular responses to bradykinin and bradykinin B_1 receptor agonist (des-Arg^9-bradykinin) in rat perfused mesenteric vascular beds with active tone

As shown in Fig. 1, a bolus injection of acetylcholine in the mesenteric vascular bed with active tone caused a sharp

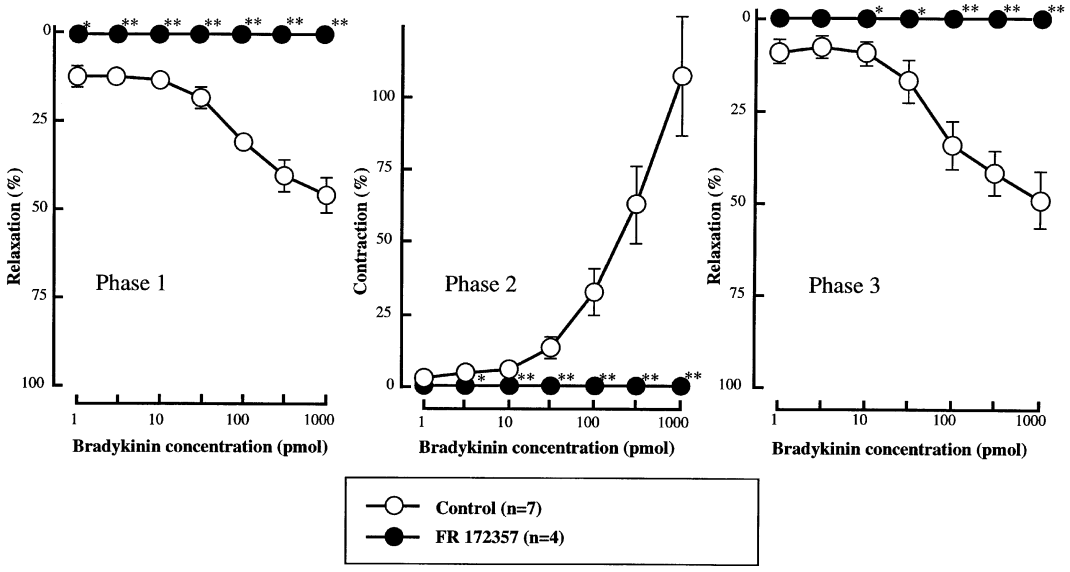

Fig. 3. Effect of bradykinin (BK) B_2 receptor antagonist (FR 172357, 0.1 μM) treatment on triphasic vascular responses to bolus injection of bradykinin in rat perfused mesenteric vascular beds with active tone. Phase 1, Phase 2 and Phase 3 indicate first phase (vasodilation), second phase (vasoconstriction) and third phase (vasodilation) responses induced by bradykinin injection, respectively. * $P<0.05$, * * $P<0.01$, compared with the control bradykinin response.

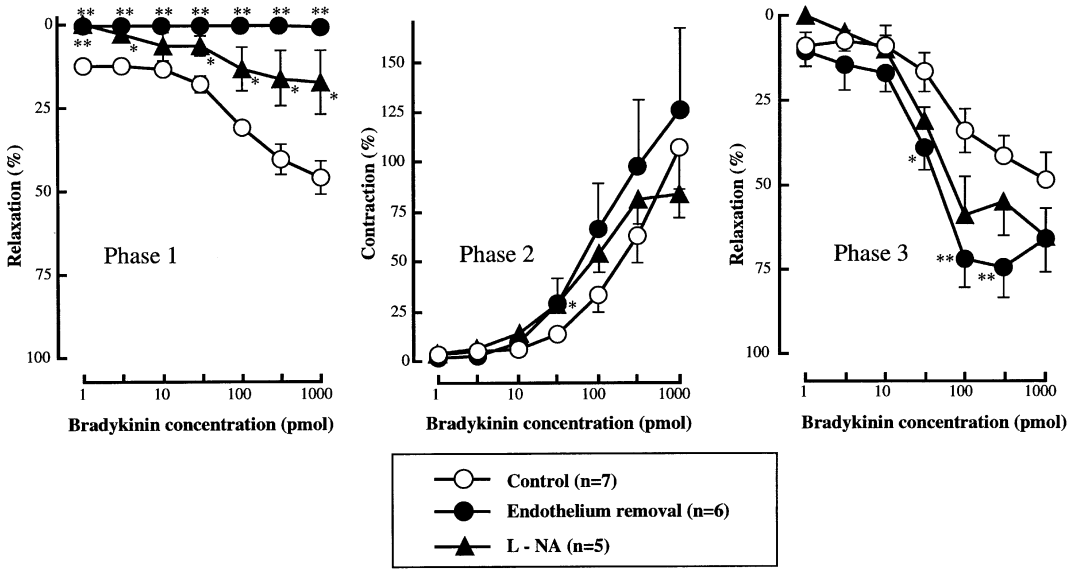

Fig. 4. Effects of endothelium removal and N^{ω}-nitro-L-arginine (L-NA) on vascular responses to bolus injection of bradykinin (BK) in rat perfused mesenteric vascular beds with active tone. Phase 1, Phase 2 and Phase 3 indicate first phase (vasodilation), second phase (vasoconstriction) and third phase (vasodilation) responses induced by bradykinin injection, respectively. $*P<0.05$, $**P<0.01$, compared with the control bradykinin response.

decrease in perfusion pressure due to vasodilation. Periarterial nerve stimulation of the preparation at 1 Hz induced a long-lasting vasodilation (Fig. 1A). The vasodilator responses to acetylcholine and periarterial nerve stimulation have been shown to be mediated by endothelial NO and perivascular CGRP-containing nerves, respectively (Furchgott and Zawadzki, 1980; Kawasaki et al., 1988). In this preparation, as shown in Figs. 1A and 2, bolus injections of bradykinin (1–1000 pmol) caused three phases of vascular responses; the first-phase being an initial short and sharp fall in perfusion pressure due to vasodilation followed by the second-phase, a transient increase in perfusion pressure due to vasoconstriction. Then, this was followed by the third-phase, a gradual decrease in perfusion pressure due to vasodilation.

Bolus injections of des-Arg^9-bradykinin (1–1000 pmol) did not induce vascular responses, as shown in Figs. 1B and 2.

3.2. Effect of bradykinin B_2 receptor antagonist (FR 172357) on vascular responses to bolus injections of bradykinin

The perfusion of FR 172357 (0.1 μM) did not affect the vasodilation in response to acetylcholine injection (Table 1).

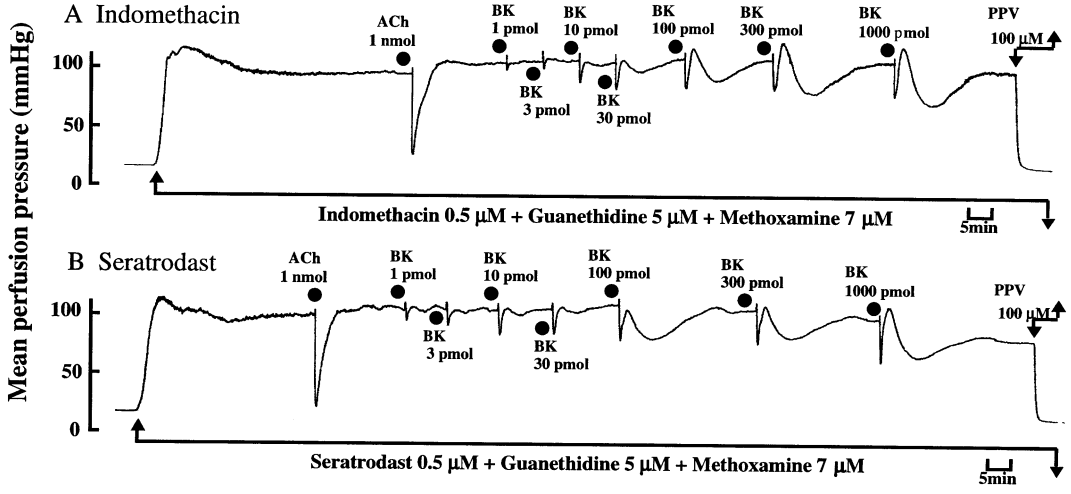

Fig. 5. Typical records showing effects of indomethacin (0.5 μM) and seratrodast (0.5 μM) treatment on vascular responses to bolus injection of bradykinin (BK) in perfused rat mesenteric vascular beds with active tone. (A) Responses in the presence of indomethacin. (B) Responses in the presence of seratrodast. ACh, injection of acetylcholine (1 nmol). BK, injection of bradykinin. PPV, papaverine perfusion.

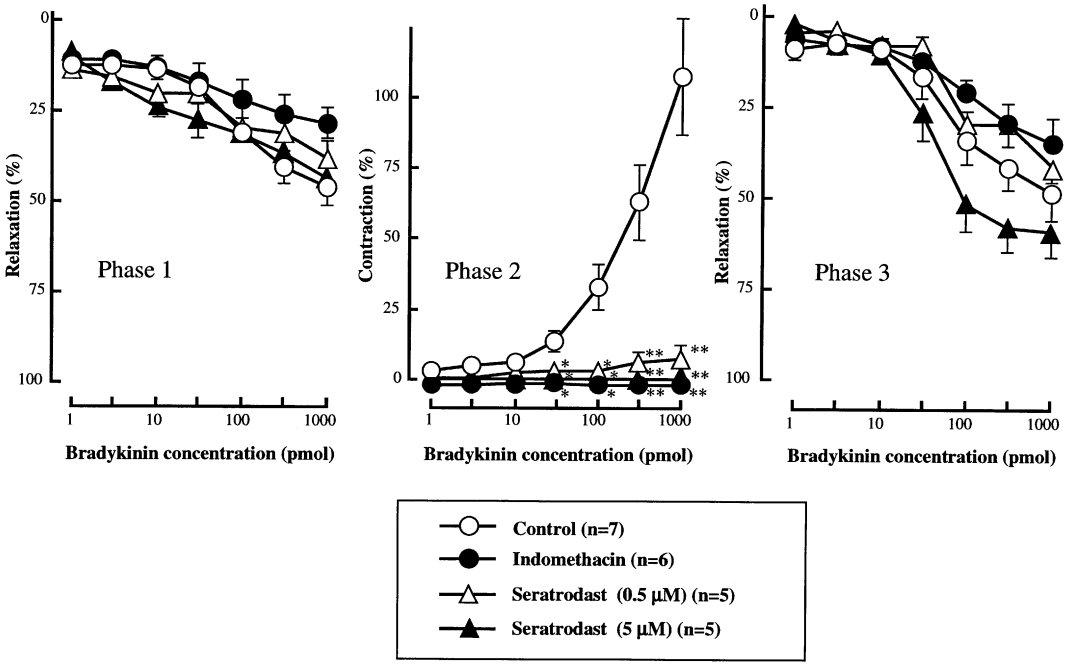

Fig. 6. Effects of indomethacin (0.5 μM) and seratrodast (0.5 and 5 μM) treatment on vascular responses to bolus injection of bradykinin (BK) in rat perfused mesenteric vascular beds with active tone. Phase 1, Phase 2 and Phase 3 indicate first phase (vasodilation), second phase (vasoconstriction) and third phase (vasodilation) responses induced by bradykinin injection, respectively. * $P<0.05$, ** $P<0.01$, compared with the control bradykinin response.

As shown in Fig. 3, the bradykinin B_2 receptor antagonist, FR 172357 (0.1 μM), abolished the vasodilation and vasoconstriction in response to bolus injections of bradykinin.

3.3. Effect of endothelium removal and L-NA on vascular responses to bolus injections of bradykinin

Treatment of the perfused mesenteric vascular bed with sodium deoxycholate abolished the acetylcholine-induced vasodilation, indicating successful removal of the endothelium (Table 1). In this preparation, the first-phase sharp vasodilation in response to bradykinin injection was abolished and the third-phase gradual vasodilation was potentiated (Fig. 4). The second-phase vasoconstriction in response to bradykinin injection was slightly potentiated by endothelium removal and L-NA treatment, but there was no significant difference from control responses (Fig. 4).

Table 2
Effect of seratrodast (5 μM) on vasoconstrictor responses to bolus injection of thromboxane A_2 agonist, U46619, in rat perfused mesenteric vascular beds with active tone

	Vasoconstrictor response (%) to U46619 injection (pmol)[a]			
	1	3	10	30
Control (5)	1.9±0.9	3.0±1.1	10.3±2.7	39.8±8.3
Seratrodast (5)	0	0	0	6.0±6.0[b]

(), number of rats.
[a] The perfusion pressure before U46619 injection was taken as 100%.
[b] $P<0.01$, compared with control response.

As shown in Fig. 4, in preparations with intact endothelium, treatment with L-NA (300 μM) markedly inhibited the first-phase vasodilation in response to bradykinin injection.

3.4. Effect of indomethacin and seratrodast on vascular responses to bolus injection of bradykinin

In preparations with intact endothelium, indomethacin (0.5 μM) and seratrodast (0.5 and 5 μM) did not affect the vasodilation in response to acetylcholine injection (Table 1). Treatment with indomethacin inhibited the second-phase vasoconstriction in response to bradykinin injection without affecting the first- and third-phase vasodilation induced by bradykinin (Figs. 5 and 6).

As shown in Figs. 5 and 6, the thromboxane A_2 receptor antagonist, seratrodast (0.5 and 5 μM), abolished the second-phase vasoconstriction in response to bradykinin injection.

Bolus injection of the thromboxane A_2 receptor agonist, U46619, at concentrations of 1–100 pmol induced a transient increase in perfusion pressure due to vasoconstriction in a concentration-dependent manner. However, U46619 did not cause an initial sharp fall in perfusion pressure as observed by bradykinin injection. The vasoconstrictor responses to U46619 injection were abolished by seratrodast at 1 μM (Table 2).

3.5. Effects of capsaicin and CGRP-(8–37) on vascular responses to bolus injection of bradykinin

In perfused mesenteric vascular beds with intact endothelium, capsaicin treatment abolished the periarterial nerve

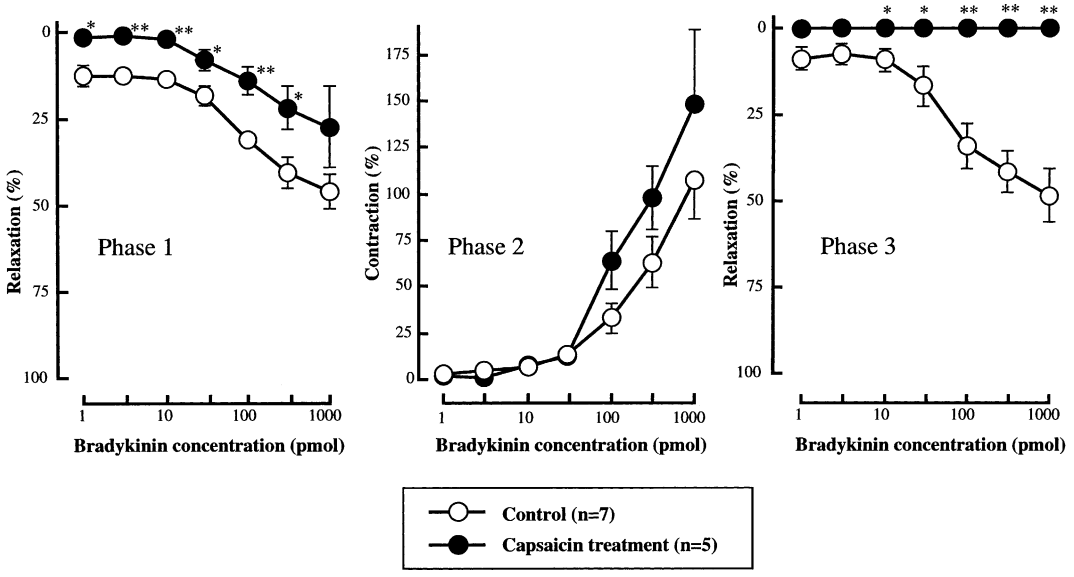

Fig. 7. Effect of capsaicin (1 μM) treatment on vascular responses to bolus injection of bradykinin (BK) in rat perfused mesenteric vascular beds with active tone. Phase 1, Phase 2 and Phase 3 indicate first phase (vasodilation), second phase (vasoconstriction) and third phase (vasodilation) responses induced by bradykinin injection, respectively. $*P<0.05$, $**P<0.01$, compared with the control bradykinin response.

stimulation-induced vasodilation, indicating successful depletion of periarterial CGRP-containing nerves (Table 1). In this preparation, the third slow vasodilation with bradykinin was abolished by treatment with capsaicin. Capsaicin significantly inhibited the first-phase vasodilation, but did not affect the second-phase vasoconstriction induced by bradykinin (Fig. 7).

In preparations without endothelium, perfusion of Krebs solution containing 0.5 μM CGRP-(8–37) caused a transient vasoconstriction. In the presence of CGRP-(8–37), the periarterial nerve stimulation-induced vasodilation was markedly inhibited (Table 1). The third-phase vasodilation in response to the bradykinin injection was significantly inhibited by CGRP-(8–37), but the antagonist potentiated the second-phase vasoconstriction induced by bradykinin (Fig. 8).

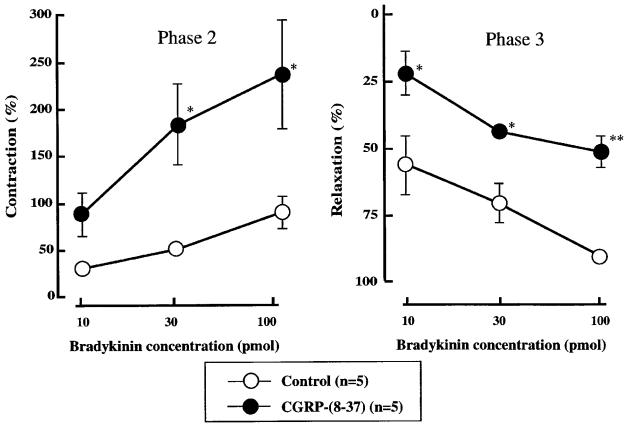

Fig. 8. Effect of CGRP-(8–37) (0.5 μM) treatment on vascular responses to bolus injection of bradykinin (BK) in rat perfused mesenteric vascular beds with active tone and without endothelium. Phase 2 and Phase 3 indicate second phase (vasoconstriction) and third phase (vasodilation) responses induced by bradykinin injection, respectively. $*P<0.05$, $**P<0.01$, compared with the control bradykinin response in denuded preparations.

4. Discussion

The present study demonstrated that exogenous bradykinin in the rat mesenteric artery induces concentration-dependent vascular responses which consisted of three phases; the initial sharp vasodilation followed by the second transient vasoconstriction and subsequently the third gradual vasodilation. However, the selective bradykinin B_1 receptor agonist, des-Arg^9-bradykinin, did not cause vascular responses. Furthermore, pretreatment of preparations with the bradykinin B_2 receptor antagonist, FR 172357 (Rizzi et al., 1999), abolished the bradykinin-induced vasodilation and vasoconstriction. These findings indicate that triphasic vascular responses to exogenous bradykinin in the rat mesenteric artery are mediated by bradykinin B_2 receptors. This agrees with previous findings that bradykinin-induced relaxation of isolated blood vessels in vitro is mediated by bradykinin B_2 receptors (Miyamoto et al., 1999). Recently, the bovine coronary artery and rat kidney vasculature have been reported to have inducible bradykinin B_1 and constitutive bradykinin B_2 receptors (Campos and Calixto, 1994; Drummond and Cocks, 1995; Bagate et al., 1999). These studies demonstrated that long-term perfusion or incubation of the preparation causes vasodilator responses mediated by a selective bradykinin B_1 receptor agonist, whereas the agonist induced less vasodilation in the preparation with a short incubation. Therefore, it is suggested

that bradykinin B_1 receptors were present in these vessels. However, in the present study, a selective bradykinin B_1 receptor agonist did not induce vascular responses even at the end of the experiment (4–6 h after starting the perfusion). Therefore, the role of the bradykinin B_1 receptor in the vascular responses of rat mesenteric artery to exogenous bradykinin is unclear and may be species dependent (Campos and Calixto, 1994; Drummond and Cocks, 1995; Bagate et al., 1999).

In the present study, exogenous bradykinin induced an initial short and sharp vasodilation of the rat mesenteric resistance artery. This first-phase vasodilation in response to bradykinin injection was abolished by endothelium removal, indicating that the initial vasodilation is dependent on intact endothelial function. In addition, the NO synthase inhibitor, L-NA, inhibited the first-phase vasodilation in response to bradykinin injection. Thus, the first-phase vasodilation induced by bradykinin in the rat mesenteric resistance artery is endothelium-dependent and mediated by NO released from the endothelium via activation of the endothelium bradykinin B_2 receptor.

Bradykinin has been shown to cause vasoconstriction (Fasciolo et al., 1990; Aksoy et al., 1990). The bradykinin-induced vasoconstrictor response has been reported to be endothelium independent and indomethacin sensitive or mediated by bradykinin B_2 receptors in the vascular smooth-muscle layer (Persson and Andersson, 1998; Bagate et al., 1999). In addition, other studies have shown that the action of bradykinin was largely dependent on stimulation of the cyclooxygenase pathway to produce prostaglandin H_2 and possibly thromboxane A_2, which can activate a smooth muscle thromboxane A_2/prostaglandin H_2 receptor to elicit vasoconstriction (Aksoy et al., 1990; Weinberg et al., 1997). In the present study, the bradykinin-induced vasoconstriction was not abolished by endothelium removal, indicating that the response is endothelium independent. Furthermore, treatment with indomethacin and seratrodast markedly inhibited the second-phase vasoconstriction induced by bradykinin. Therefore, it is very likely that arachidonic acid metabolites, probably thromboxane A_2, in the vascular smooth muscle are responsible for the vasoconstrictor response to bradykinin. Similar findings were reported for rat mesenteric artery (Fasciolo et al., 1990; Weinberg et al., 1997) and canine jugular and femoral vein (Aksoy et al., 1990).

The findings of the present study demonstrated that bradykinin induced the third-phase vasodilation, which had a slow onset and was long-lasting. This long-lasting vasodilation in response to bradykinin was abolished by capsaicin treatment, indicating that the vasodilation is mediated by capsaicin-sensitive nerves. In addition, the CGRP receptor antagonist, CGRP-(8–37), markedly attenuated the third-phase vasodilation induced by bradykinin. Bradykinin evokes CGRP release from the CGRP-containing nerves (Geppetti et al., 1990; Hua et al., 1994; Schuligoi et al., 1998). Based on these results, it is suggested that the third-phase vasodilation in response to bradykinin is mediated by endogenous CGRP released from CGRP-containing nerves.

CGRP has been shown to be released by prostaglandins such as prostaglandin I_2 and prostaglandin E_2 (Franco-Cereceda, 1989; Hua et al., 1994). Therefore, it is possible that capsaicin-sensitive vasodilation induced by bradykinin results from endogenous CGRP released by prostaglandins, which are produced via the cyclooxygenase pathway stimulated by bradykinin. However, indomethacin treatment did not affect the third-phase vasodilation induced by bradykinin. Thus, it is unlikely that prostaglandins stimulated by bradykinin are involved in the third-phase vasodilation in response to bradykinin.

The present results suggest that the first sharp vasodilation with bradykinin is endothelium-dependent, that thromboxane A_2 is involved in the second-phase vasoconstriction induced by bradykinin and that the third slow vasodilation is mediated by activation of capsaicin-sensitive CGRP-containing nerves.

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