ORIGINAL ARTICLE

Facilitation of sympathetic neurotransmission by phosphatidylinositol-4,5-bisphosphate-dependent regulation of KCNQ channels in rat mesenteric arteries

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Sympathetic nerves regulate vascular tone by releasing neurotransmitters into the vasculature. We previously demonstrated that bradykinin facilitates sympathetic neurotransmission in rat mesenteric arteries. Although little is known about the intracellular mechanism modulating this neurotransmission, recent cell line experiments have shown that the KCNQ channel, which is inhibited by the depletion of membrane phosphatidylinositol-4,5-bisphosphate (PIP₂), participates in the control of neurotransmission by bradykinin. In the present study, we examined the mechanism regulating neurotransmitter release from rat perivascular sympathetic nerves. Excitatory junction potentials (EJPs) elicited by repetitive nerve stimulation (1 Hz, 11 pulses, 20 μ s, 20–50 V), a measure of sympathetic purinergic neurotransmission, were recorded with a conventional microelectrode technique in rat mesenteric arteries. Bradykinin (10^{-7} mol l⁻¹) significantly enhanced the amplitude of EJPs ($n = 22$, $P < 0.05$). This enhancing effect was abolished by N-type calcium-channel inhibition with ω -conotoxin GVIA (2×10^{-9} mol l⁻¹, $n = 8$). The blockade of phospholipase C with U-73122 (10^{-6} mol l⁻¹, $n = 17$) also eliminated the facilitatory effect of bradykinin. In addition, the effects of bradykinin were diminished by the prevention of PIP₂ resynthesis with wortmannin (10^{-5} mol l⁻¹, $n = 7$) or KCNQ channel inhibition with XE-991 (10^{-5} mol l⁻¹, $n = 7$). On the other hand, depletion of intracellular calcium stores with cyclopiazonic acid (3×10^{-6} mol l⁻¹, $n = 6$) or the inhibition of protein kinase C with bisindolylmaleimide-I (10^{-6} mol l⁻¹, $n = 9$) did not alter the action of bradykinin. These data demonstrate that the hydrolysis of PIP₂ by phospholipase C, which is activated by G_{q/11}-coupled receptors, and subsequent KCNQ channel inhibition enhance sympathetic purinergic neurotransmission presumably via the activation of N-type calcium channels in rat mesenteric arteries.

Hypertension Research (2012) 35, 909–916; doi:10.1038/hr.2012.61; published online 17 May 2012

Keywords: excitatory junction potential; KCNQ channel; phosphatidylinositol-4, 5-bisphosphate; purinergic neurotransmission; rat mesenteric artery

INTRODUCTION

Arterial tone is regulated not only by endothelial cells but also by perivascular nerves. Stimulation of these perivascular nerves, including sympathetic, parasympathetic and sensory nerves, causes the release of various transmitters, such as norepinephrine, adenosine 5'-triphosphate (ATP), nitric oxide and calcitonin gene-related peptide (CGRP), to regulate vascular tone.^{1,2} Several substances, including kinins, have been reported to modulate the release of transmitters from perivascular nerves.^{3–5}

Kinins are proinflammatory, vasoactive peptides that influence vascular relaxation via the release of endothelial mediators. Bradykinin also causes a pain response to tissue injury by acting on sensory neurons.⁶ We previously showed that bradykinin enhances purinergic neurotransmission by sympathetic nerves in rat mesenteric arteries.³ Additionally, the action of bradykinin was increased by captopril, an

angiotensin-converting enzyme (ACE) inhibitor.³ Although the accumulation of bradykinin secondary to ACE inhibitor therapy may enhance endothelium-dependent relaxation in human arteries,⁷ our previous findings raised the possibility that bradykinin may partially counteract such a vasodilatory effect. Therefore, different aspects of bradykinin should be considered when ACE inhibitor therapy is considered, and it is important to elucidate the mechanisms that regulate the action of bradykinin. The facilitatory effect of bradykinin was mediated by bradykinin B₂ but not B₁ receptors; however, the subsequent intracellular mechanism was not clear, and neither nitric oxide nor prostaglandins were likely to be involved in the effect of bradykinin in that study. The kinin B₂ receptors belong to the G-protein-coupled receptor family, and it was recently shown that bradykinin acts on more than one G protein in a neuronal cell line, although B₂ receptors are reported to be mainly coupled to G_{q/11}.⁸

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Received 22 December 2011; revised 17 February 2012; accepted 1 March 2012; published online 17 May 2012

Voltage-gated Kv7 (KCNQ) channels, underlying the M-type current, have been found throughout the peripheral and central nervous systems.^{9–12} The name of this M-current is derived from its suppression by muscarinic acetylcholine receptors in sympathetic neurons. The M-current of neurons is a voltage-gated K⁺ current that has a crucial role in regulating neuronal excitability,^{13,14} and the inhibition of KCNQ channels leads to membrane potential depolarization in neurons, which causes neural excitation. The open state of KCNQ channels is maintained by membrane phosphatidylinositol-4,5-bisphosphate (PIP₂); these channels are closed upon stimulation of receptors that induce PIP₂ hydrolysis through G_{q/11}-coupled phospholipase C activation.¹⁵ Although KCNQ channels are inhibited by muscarinic acetylcholine receptor stimulation, it has been suggested that angiotensin II and bradykinin also act on this process of G_{q/11}-coupled KCNQ channel inhibition in neurons.^{16–18} Thus, it is plausible to speculate that bradykinin facilitates sympathetic neurotransmission through its inhibitory effect on neural KCNQ channels.

In the present study, we evaluated bradykinin-induced sympathetic neurotransmission in blood vessels to elucidate the intracellular mechanism by which bradykinin modulates perivascular sympathetic nerves. We also showed that this neurotransmission was influenced by KCNQ channels. For this purpose, we recorded the excitatory junction potentials (EJPs), a measure of sympathetic purinergic neurotransmission, in rat mesenteric arteries using a conventional microelectrode technique.

METHODS

Preparation of arteries

This study was approved by the Animal Care Committee of Kyushu University. Six-eight-week-old male Wistar rats were killed by cervical dislocation. The second or third branches of the mesenteric arteries were excised and bathed in Krebs solution having the following composition (in mmol l⁻¹): NaCl 121.8, KCl 4.7, MgCl₂ 1.2, CaCl₂ 2.6, NaHCO₃ 15.5, KH₂PO₄ 1.2 and glucose 11.5.

EJP recording

The second or third branches of the mesenteric arteries were pinned out on a rubber base fixed at the bottom of the experimental chamber (capacity 2 ml). The chamber was superfused with Krebs solution at 36 °C aerated with 95% O₂/5% CO₂ (pH 7.2–7.3) at a rate of 3 ml min⁻¹. After at least 60 min of equilibration, the membrane potentials of vascular smooth muscle cells were recorded using a conventional glass capillary microelectrode filled with 3 mol l⁻¹ KCl with a tip resistance of 50–80 MΩ, as previously described.^{3,19} Criteria for successful impalement included the following: an abrupt drop in voltage upon impalement of the microelectrode into the vascular smooth muscle cell, a stable membrane potential for at least 2 min and a sharp return to zero potential upon withdrawal of the electrode. To record the EJPs, the periaxillary nerves were stimulated by drawing the proximal part of the artery into a suction electrode (Ag-AgCl). An electric stimulator (SEN-3201, Nihon Kohden) was used to supply a train of pulses (1 Hz, 11 pulses, 20 μs, 20–50 V) every 2 min. At this interval, the amplitudes of EJPs remained constant. Signals were amplified (VC-11, Nihon Kohden) and recorded (RJG-4002, Nihon Kohden).

Drugs

The following drugs were used: bradykinin (10⁻⁷ mol l⁻¹),³ U-73122 (10⁻⁶ mol l⁻¹),²⁰ U-73433 (10⁻⁶ mol l⁻¹),²⁰ cyclopiazonic acid (3 × 10⁻⁶ mol l⁻¹),²¹ HC-030031 (5 × 10⁻⁶ mol l⁻¹),²² H-89 (10⁻⁶ mol l⁻¹),^{8,23} XE-991 (10⁻⁵ mol l⁻¹),²⁴ bisindolylmaleimide-I (10⁻⁶ mol l⁻¹),²⁵ capsaicin (10⁻⁵ mol l⁻¹),²⁶ capsazepine (5 × 10⁻⁶ mol l⁻¹),²⁷ and phentolamine (10⁻⁴ mol l⁻¹)²⁸ from Sigma-Aldrich (St Louis, MO, USA) arachidonyl trifluoromethyl ketone (3 × 10⁻⁵ mol l⁻¹, AACOCF₃)²⁹ from Calbiochem (San Diego, CA, USA) ω-conotoxin GVIA (2 × 10⁻⁹ or 10⁻⁶ mol l⁻¹)^{30,31}

and Calcitonin Gene-Related Peptide (Human, 8–37) (CGRP8-37, 10⁻⁷ mol l⁻¹)³² from Peptide Institute (Osaka, Japan) and wortmannin (10⁻⁵ mol l⁻¹)³³ and AP-18 (10⁻⁶ mol l⁻¹)³⁴ from Biomol (Plymouth Meeting, PA, USA). Stock solutions of bradykinin, phentolamine, ω-conotoxin GVIA, H-89 and CGRP8-37 were dissolved in distilled water. Capsaicin was dissolved in ethanol. The other drugs were dissolved in 9.95% dimethylsulfoxide. If not otherwise specified, the inhibitors were added to the incubation solution, and the arteries were equilibrated for at least 20 min before obtaining responses. At their final chamber concentrations, the solvents used to dissolve the drugs did not affect electrical responses.

Statistical analysis

Data are expressed as means ± SEM; *n* refers to the number of animals examined. The values were accepted only when continuous recordings of EJPs were obtained throughout the application of bradykinin. The values of the peak effect of bradykinin were used for statistical analysis. Comparison of facilitatory curves of EJPs was performed by two-way analysis of variance (ANOVA). For additional reference, the area between the two facilitatory curves of EJPs was expressed in arbitrary units and was compared by one-way ANOVA followed by Dunnett's multiple comparison test. Other variables were analyzed by paired or unpaired Student's *t*-test. Values of *P* < 0.05 were considered statistically significant.

RESULTS

The resting membrane potential of the rat mesenteric artery was -70.4 ± 0.8 mV (*n* = 36). The membrane potential in the presence of 10⁻⁷ mol l⁻¹ bradykinin was -69.3 ± 1.2 mV (*n* = 18), which was not significantly different from the specimen without bradykinin treatment. Representative recordings of the effect of 10⁻⁷ mol l⁻¹ bradykinin are shown in Figure 1a. Repetitive perivascular field stimulation facilitated the amplitude of EJPs with a maximal effect around 3–5 stimuli. Bradykinin significantly enhanced the amplitude of EJPs without affecting the resting membrane potential or the amplitude of the first EJP (Figure 1b, *n* = 22). In subsequent experiments, the resting membrane potential and the amplitude of the first EJP were not altered by bradykinin except when otherwise stated. In addition, because bradykinin showed tachyphylaxis regarding its effect on EJPs, becoming weaker or absent upon the second application (data not shown), repeated use of bradykinin for the same arterial segment was avoided in the present study. Pretreatment with 10⁻⁶ mol l⁻¹ ω-conotoxin GVIA, an N-type calcium-channel inhibitor,^{30,31} substantially decreased the amplitude of EJPs, and bradykinin no longer increased the amplitude of EJPs (Figure 1c, *n* = 4). When a low dose of ω-conotoxin GVIA (2 × 10⁻⁹ mol l⁻¹), which mildly inhibits EJP amplitude, was added, a substantial EJP amplitude was still observed. Under this condition, the enhancing effect of bradykinin remained absent (Figures 1d and 2e, *n* = 8).

To examine the involvement of the G_{q/11}-dependent mechanism, arteries were treated with U-73122 (10⁻⁶ mol l⁻¹), a phospholipase C inhibitor.²⁰ The enhancing effect of bradykinin was substantially inhibited in the presence of U-73122 (Figures 2a and e, *n* = 17). U-73433 (10⁻⁶ mol l⁻¹), a negative analog of U-73122,²⁰ showed no effect (Figures 2b and e, *n* = 8). On the other hand, inhibition of protein kinase A or phospholipase A₂ with H-89 (10⁻⁶ mol l⁻¹, *n* = 5)^{8,23} or AACOCF₃ (3 × 10⁻⁵ mol l⁻¹, *n* = 8),²⁹ respectively, did not alter the effects of bradykinin (Figures 2c–e).

The activation of phospholipase C, which is activated by bradykinin, degrades PIP₂ to produce intracellular molecules. To explore the possible involvement of these substances in the effect of bradykinin, we tested the effect of wortmannin, which inhibits resynthesis of PIP₂,³³ and XE-991, a KCNQ channel inhibitor.²⁴ Wortmannin (10⁻⁵ mol l⁻¹) and XE-991 (10⁻⁵ mol l⁻¹) mildly depolarized the

Figure 1 Effects of bradykinin on EJPs elicited by repetitive nerve stimulation in the rat mesenteric artery. (a) Representative tracings before and after application of 10^{-7} mol l⁻¹ bradykinin. (b–d) Line graphs showing the effect of 10^{-7} mol l⁻¹ bradykinin on EJPs in the absence of inhibitors (b), or in the presence of 10^{-6} mol l⁻¹ (c) or 2×10^{-9} mol l⁻¹ (d) ω -conotoxin GVIA, an N-type calcium channel inhibitor. * $P < 0.05$ vs. control by two-way ANOVA.

resting membrane potential of vascular smooth muscle cells (control -67.4 ± 1.0 mV, with wortmannin -59.9 ± 1.0 mV, $n = 9$, $P < 0.05$; control -66.2 ± 1.9 mV, with XE-991 -61.1 ± 1.4 mV, $n = 15$, $P < 0.05$). In the presence of wortmannin, the effect of bradykinin on EJPs was abolished without affecting the resting membrane potential (Figures 3a and e, $n = 7$). In addition, XE-991 also significantly reduced the facilitation by bradykinin (Figures 3b and e, $n = 11$). In contrast, neither cyclopiazonic acid (3×10^{-6} mol l⁻¹, $n = 6$), a sarcoplasmic reticulum calcium ATPase inhibitor,²¹ nor bisindolylmaleimide-I (10^{-6} mol l⁻¹, $n = 9$), a protein kinase C inhibitor,²⁵ altered the action of bradykinin (Figure 3c–e).

Neither capsaicin (10^{-5} mol l⁻¹, $n = 8$), which acts on sensory nerve terminals to release and ultimately deplete neuropeptides,²⁶ nor CGRP8-37 (10^{-7} mol l⁻¹, $n = 11$), a CGRP antagonist,³² altered the effect of bradykinin (Figures 4a, b and g). Similarly, in the presence of AP-18 (10^{-6} mol l⁻¹, $n = 4$)³⁴ or HC-030031 (5×10^{-6} mol l⁻¹, $n = 12$),²² both of which inhibit transient receptor potential ankyrin 1 (TRPA1), the amplitude of EJPs facilitated by bradykinin was not altered (Figures 4c, d and g). Capsazepine (5×10^{-6} mol l⁻¹, $n = 8$), a transient receptor potential vanilloid 1 (TRPV1) antagonist,²⁷ also did not affect the action of bradykinin (Figures 4e and g). In addition, in the presence of phentolamine (10^{-4} mol l⁻¹, $n = 10$), exogenously applied bradykinin continued to enhance the amplitude of EJPs (Figures 4f and g).

DISCUSSION

The present study has demonstrated that the inhibition of KCNQ channels via depletion of PIP₂ by phospholipase C activation contributes to enhanced sympathetic neurotransmission of bradykinin in rat mesenteric arteries. In addition, this enhancing effect of bradykinin is likely to depend on the activation of N-type calcium channels. On the other hand, the downstream metabolites of phospholipase C might not be involved in the effect of bradykinin because neither the sarcoplasmic reticulum calcium ATPase inhibitor nor the protein kinase C inhibitor had any effect on the amplitude of EJPs facilitated by bradykinin. This is the first study to show that KCNQ channels have a key role in purinergic sympathetic neurotransmission in rat mesenteric arteries.

In our previous studies, we demonstrated that EJPs observed in rat mesenteric arteries are mediated by ATP but not by norepinephrine,³¹ although several studies have shown that ATP and norepinephrine are released from sympathetic nerves simultaneously.^{35,36} Therefore, it

should be mentioned that EJPs recorded in the present study were mediated by purinergic sympathetic neurotransmission.

Bradykinin did not alter the first EJP in a train of repetitive nerve stimulations, nor did it alter the resting membrane potential after incubation with inhibitors shown to regulate intracellular mechanisms, in the present study; however, bradykinin continued to facilitate subsequent EJPs. Furthermore, in our previous study, bradykinin did not affect the magnitude of depolarization by α, β -methylene ATP, a stable analog of ATP.³ These findings indicate that bradykinin may not directly affect the postsynaptic smooth muscle membrane or the responsiveness of myocytes to the transmitter responsible for EJPs in the study. Collectively, bradykinin probably facilitated the amplitude of EJPs by affecting presynaptic nerve endings around the rat mesenteric artery and not the postsynaptic smooth muscle membrane.

Norepinephrine released from nerve endings in response to nerve stimulation is known to inhibit the release of neurotransmitters via α_2 adrenoceptors in presynaptic nerve terminals.^{37,38} Indeed, when phentolamine was used to inhibit adrenergic α -receptors, the facilitation of EJPs was enhanced in the absence of bradykinin (data not shown). However, in arteries treated with phentolamine, bradykinin still showed a facilitatory effect on EJPs, suggesting that the action of bradykinin was unrelated to presynaptic α_2 -auto-inhibition of neurotransmitter release.

Bradykinin binds to bradykinin B₁ or B₂ receptors.⁶ The B₁ receptors are expressed as a result of tissue injury and presumably have a role in inflammation.⁶ By contrast, it has been demonstrated that B₂ receptors are constitutively expressed and participate in the physiologic effects of kinins.^{6,39} We previously showed that bradykinin facilitates purinergic sympathetic neurotransmission in rat mesenteric arteries. In addition, this effect of bradykinin was mediated by presynaptic B₂ but not B₁ receptors.³ The B₂ receptors are G-protein-coupled receptors and are mainly described as coupled with G_{q/11},^{40,41} although this receptor interacts with other G proteins, namely G_s and G_i.^{42–44} In the present study, the blockade of phospholipase C, which is activated by G_{q/11}, substantially diminished the action of bradykinin. Meanwhile, pretreatment with H-89 or AACOCF₃ to inhibit the G_s-cAMP-protein kinase A pathway or G_i-dependent phospholipase A2 activation, respectively, did not alter the effect of bradykinin. These findings suggest that in rat perivascular sympathetic nerves, the enhancing effect of bradykinin is probably mediated by G_{q/11}, but not G_i or G_s.

Figure 2 Line graphs showing the effects of 10^{-7} mol/L bradykinin on EJPs in the rat mesenteric artery in the presence of the following: U-73122, a phospholipase C inhibitor (a); U-73433, a negative analog of U-73122 (b); H-89, a protein kinase A inhibitor (c); and AACOCF₃, a phospholipase A2 inhibitor (d). (e) Bar graph showing bradykinin-dependent response (the area between two facilitatory curves of EJPs in each graph from Figures 1 and 2, expressed as arbitrary units) in the presence of each inhibitor. * $P < 0.05$ vs. control by two-way ANOVA. † $P < 0.05$ vs. no inhibitors by one-way ANOVA followed by Dunnett's multiple comparison test.

Activation of bradykinin receptors coupled to $G_{q/11}$ leads to activation of phospholipase C to metabolize PIP₂. It has been shown that PIP₂ activates KCNQ channels in cell line studies and that depletion of PIP₂ with a high concentration of wortmannin or the activation of phospholipase C inhibits KCNQ channel activity, which may cause neuronal depolarization.⁴⁵ To elucidate the involvement of KCNQ channels in the present study, a high dose of wortmannin (10^{-5} mol/L) was employed to deplete PIP₂ in the perivascular sympathetic nerves, resulting in the abolition of bradykinin-induced EJP facilitation. Furthermore, the prevention of KCNQ channel activity with XE-991 also substantially inhibited the enhancing effect of bradykinin. These findings support the involvement of

KCNQ channels in the facilitatory effect of bradykinin in rat perivascular sympathetic nerve activity.

KCNQ channels have increasingly been demonstrated to have an important role in arterial function. In vascular smooth muscle cells, KCNQ1, KCNQ4 and KCNQ5 are predominantly expressed with a minimal contribution of KCNQ2 and KCNQ3.^{46–48} In addition, Jepps *et al.*⁴⁹ recently demonstrated that KCNQ channel activators induce arterial relaxation, and the expression of KCNQ4 is decreased in the vascular smooth muscle cells of hypertensive rodent models. The present study also suggested that perivascular sympathetic neurotransmission might also be influenced by KCNQ channels in rat mesenteric arteries. Thus, it appears that multiple action sites of

Figure 3 Line graphs showing the effects of 10^{-7} mol l^{-1} bradykinin on EJP in the rat mesenteric artery in the presence of the following: wortmannin, a resynthesis inhibitor of phosphatidylinositol-4,5-phosphate (**a**); XE-991, a KCNQ channel inhibitor (**b**); cyclopiazonic acid, a sarcoendoplasmic reticulum calcium ATPase inhibitor (**c**); and bisindolylmaleimide-I, a protein kinase C inhibitor (**d**). (**e**) Bar graph showing bradykinin-dependent response (the area between two facilitatory curves of EJPs in each graph from Figure 3, expressed as arbitrary units) in the presence of each inhibitor. * $P < 0.05$ vs. control by two-way ANOVA. † $P < 0.05$ vs. no inhibitors by one-way ANOVA followed by Dunnett's multiple comparison test.

KCNQ channel modulators should be taken into account when their clinical use is considered.

Activation of phospholipase C yields inositol triphosphate and diacylglycerol. Inositol triphosphate induces Ca^{2+} release from the endoplasmic reticulum. It has been suggested that Ca^{2+} release from intracellular Ca^{2+} stores acts on KCNQ channels to activate or inhibit via phosphatidylinositol-4-kinase-dependent PIP_2 synthesis or direct activation of calmodulin, respectively.⁴⁵ Furthermore, it has been suggested that TRPA1 is also sensitized by bradykinin-induced release of Ca^{2+} from intracellular calcium stores.⁸ To evaluate the role of

intracellular Ca^{2+} stores, cyclopiazonic acid was used in the present study. Pretreatment with cyclopiazonic acid did not alter the action of bradykinin in rat mesenteric arteries. In addition, the inhibition of protein kinase C, which is activated by diacylglycerol, did not exhibit an effect. These findings suggest that the downstream metabolites of phospholipase C are not likely to be involved in bradykinin-induced facilitation of EJPs in our study.

In the presence of ω -conotoxin GVIA (10^{-6} mol l^{-1}), the amplitudes of EJPs were nearly abolished, and the enhancing effect of bradykinin could not be detected. A lower concentration of

Figure 4 Line graphs showing the effects of 10^{-7} mol l^{-1} bradykinin on EJPs in the rat mesenteric artery in the presence of the following: capsaicin, which desensitizes sensory nerves (a); CGRP8-37, a CGRP inhibitor (b); AP-18, a TRPA1 inhibitor (c); HC-030031, a TRPA1 inhibitor (d); capsazepine, a TRPV1 inhibitor (e); and phentolamine, an α -adrenoceptor inhibitor (f). (g) Bar graph showing bradykinin-dependent response (the area between two facilitatory curves of EJPs in each graph from Figure 4, expressed as arbitrary units) in the presence of each inhibitor. * $P < 0.05$ vs. control by two-way ANOVA.

ω -conotoxin GVIA (2×10^{-9} mol l^{-1}) significantly, but mildly, decreased the amplitude of EJPs. Again, no enhancing effect of bradykinin was detected even under these conditions. Thus, calcium influx through N-type voltage-dependent calcium channels may have a crucial role in perivascular sympathetic nerve

function, and KCNQ channels inhibited by bradykinin might contribute to the depolarization of presynaptic membrane potentials, which both activate Ca^{2+} influx through N-type calcium channels and trigger exocytosis of neurotransmitters from sympathetic neurons.

Transient receptor potential channels are non-selective cation channels and cause an influx of Na^+ and Ca^{2+} into the cell, resulting in neural excitation. It has been shown that protein kinase C and TRPV1 may contribute to sensory neuron excitation and sensitization by bradykinin.^{8,50} Furthermore, intracellular Ca^{2+} release has been demonstrated to be associated with sensitization of TRPA1.⁸ These transient receptor potential channels are mainly reported to work in sensory nerves; however, perivascular sympathetic nerves co-exist with sensory nerves. Thus, sensory nerves might modulate the function of sympathetic nerves.⁵¹ In the present study, inhibition of TRPA1 with AP-18 or HC-030031 did not alter the action of bradykinin. Furthermore, incubation with capsazepine, a TRPV1 antagonist, did not show any effect on the facilitatory action of bradykinin. Desensitization of sensory nerves with capsaicin was also without effect. These findings suggest that the facilitatory effect of bradykinin is independent of sensory nerves as well as TRPA1 and TRPV1.

One may argue that the concentration of bradykinin used in this study was relatively high. It is unclear whether the local concentration of bradykinin was high enough to cause an enhancement of sympathetic nerve activity. In our previous study, the enhancing effect of bradykinin was not statistically significant at $10^{-8} \text{ mol l}^{-1}$, although $>10^{-7} \text{ mol l}^{-1}$ bradykinin substantially increased the sympathetic neurotransmission in rat mesenteric arteries.³ In addition, several studies have demonstrated that the interstitial concentration of bradykinin ranges from approximately $10^{-11} \text{ mol l}^{-1}$ to $10^{-7} \text{ mol l}^{-1}$, and the concentration of bradykinin actually reaches approximately $10^{-7} \text{ mol l}^{-1}$, the dose used in the present study, during exercise or ischemic preconditioning.^{52–54}

The present study had several limitations. First, we did not evaluate the subtypes of KCNQ channels involved in the actions of bradykinin in perivascular sympathetic nerves because all types of KCNQ channels were shown to be inhibited by XE-991, an effective and relatively selective inhibitor of KCNQ channels at a concentration of $10^{-5} \text{ mol l}^{-1}$.⁵⁵ Several studies have demonstrated that the M-current is associated with KCNQ3 and KCNQ4 channels in sympathetic neurons,^{9,17,56} but this has not been evaluated in perivascular sympathetic nerves. Further investigation would be needed to determine the involved subtypes of KCNQ channels in this study. Second, as mentioned above, the EJPs evaluated in this study reflect purinergic sympathetic neurotransmission, but they are not a direct measurement of neurotransmitter release; however, in a recently conducted study, it was demonstrated that the release of norepinephrine is facilitated by bradykinin in rat mesenteric arteries and veins.⁵⁷ Third, little evidence is available concerning the effect of bradykinin on perivascular sympathetic neurotransmission, especially in humans. The effects of bradykinin on sympathetic nerves are likely to be highly species- and tissue-specific;^{57–60} thus, the relevance of the present findings to human arteries remains to be determined.

In conclusion, degradation of PIP_2 by phospholipase C via presynaptic bradykinin B_2 receptors enhances purinergic neurotransmission in rat mesenteric arteries. The inhibition of KCNQ channels following reduced PIP_2 may be essential for the PIP_2 -dependent regulation of sympathetic neurotransmission. In addition, N-type calcium channels activated via membrane depolarization triggered by inhibited KCNQ channels may contribute to enhanced sympathetic neurotransmission.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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