Bradykinin Enhances Sympathetic Neurotransmission in Rat Blood Vessels

Yasuo Kansui, Koji Fujii, Kenichi Goto, Isao Abe

Abstract—Bradykinin evokes endothelium-dependent relaxation in some vascular beds; on the other hand, the possibility has been demonstrated that in certain organs, such as the adrenal medulla or atria, bradykinin may enhance transmitter release from the sympathetic nerves. We hypothesized that bradykinin may also enhance postganglionic sympathetic neurotransmission in blood vessels. To test this hypothesis, we recorded excitatory junction potentials (EJPs), a measure of sympathetic purinergic neurotransmission, in rat mesenteric resistance arteries with a conventional microelectrode technique. EJPs were elicited by repetitive perivascular nerve stimulation (1 Hz, 20 to 50 V, 30 to 60 μs, 11 pulses). In this preparation, bradykinin (10^{-7} or 10^{-6} mol/L) significantly enhanced the amplitude of EJPs without altering the resting membrane potential. This effect of bradykinin was blocked by Hoe 140, a bradykinin B2 receptor antagonist, but not by des-Arg^{9}[Leu^{8}]-bradykinin, a bradykinin B1 receptor antagonist. The cyclooxygenase inhibitor indomethacin or NO synthase inhibitor N^{G}-nitro-L-arginine did not alter the effect of bradykinin. Captopril, an ACE inhibitor, but not candesartan, an angiotensin II type 1 receptor antagonist, enhanced the action of a low concentration (10^{-8} mol/L) of bradykinin on EJPs. These findings suggest that in rat mesenteric resistance arteries, bradykinin enhances sympathetic purinergic neurotransmission, presumably through presynaptic bradykinin B2 receptors. The clinical relevance of the present findings remains unclear; however, the fact that the ACE inhibitor, but not the angiotensin II type 1 receptor antagonist, enhanced the action of bradykinin on sympathetic neurotransmission may warrant further investigation. (Hypertension. 2002;39:29-34.)

Key Words: sympathetic nervous system ■ bradykinin ■ rats ■ mesenteric arteries ■ angiotensin-converting enzyme inhibitors

Bradykinin evokes endothelium-dependent relaxation in certain vascular beds through the release of NO, prostaglandins, and putative endothelium-derived hyperpolarizing factor (EDHF). On the other hand, it was demonstrated nearly 40 years ago that bradykinin potentiated the release of epinephrine from the adrenal medulla, implying the excitatory action of bradykinin on sympathetic ganglia. Although several more recent reports concerning the effects of bradykinin on postganglionic sympathetic neurotransmission have appeared, the results have been controversial. Bradykinin was found to potentiate the release of norepinephrine from rat, mouse, and human atria; on the other hand, bradykinin was found to inhibit norepinephrine release in the rabbit heart. Regarding blood vessels, the majority of studies have focused on the vasodilatory action of bradykinin, and only limited information is available concerning the effects of bradykinin on sympathetic nerves innervating blood vessels.

ACE is identical to kininase II and, therefore, promotes the degradation of bradykinin to inactive substrates. Accordingly, ACE inhibitors are expected to increase the local concentration of bradykinin, which has been suggested to partially account for the action of ACE inhibitors. On the other hand, angiotensin II type 1 (AT_{1}) receptor antagonists may not directly affect bradykinin metabolism. Therefore, if bradykinin exhibits a sympathoexcitatory action on blood vessels, ACE inhibitors but not AT_{1} receptor antagonists can be presumed to exert effects on this action of bradykinin. The present study tested the hypothesis that bradykinin enhances postganglionic sympathetic neurotransmission in blood vessels, and if this proved to be the case, the study was to compare the effects of ACE inhibitors and AT_{1} receptor antagonists on the sympathoexcitatory action of bradykinin. For this purpose, we recorded the excitatory junction potentials (EJPs), which are a measure of sympathetic purinergic neurotransmission, in the rat mesenteric resistance arteries by using a conventional microelectrode technique.

Methods

Preparation of Arteries

This study was approved by the Committee on the Ethics of Animal Experimentation of Kyushu University. Six- to 8-week-old male Wistar rats were anesthetized with ether and killed by decapitation. The second or third branches of the mesenteric arteries were excised...
and bathed in Krebs’ solution. Krebs’ solution had the following composition (in mmol/L): Na⁺ 137.4, K⁺ 5.9, Mg²⁺ 1.2, Ca²⁺ 2.5, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2, Cl⁻ 134, and glucose 11.5.

**EJP Recording**

The 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 36°C Krebs’ solution bubbled with 95% O₂/5% CO₂ (pH 7.2 to 7.3) at a rate of 3 mL/min. After at least 60 minutes of equilibration, the membrane potentials of vascular smooth muscle cells were recorded by using conventional glass capillary microelectrodes filled with 3 mol/L KCl, with tip resistances of 50 to 80 MΩ, as previously described. To record the EJPs, the periarterial 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, 30 to 60 μs, 20 to 50 V) every 2 minutes. At this interval, the amplitudes of EJPs remained constant. Signals were amplified through an amplifier (MEZ-7200, Nihon Kohden), monitored on an oscilloscope (VC-11, Nihon Kohden), and recorded on a pen writing recorder (RJG-4002, Nihon Kohden).

**Drugs**

The following drugs were used: bradykinin, indomethacin, N⁶-nitro-L-arginine (L-NNA), captopril, α,β-methylene ATP lithium salt, tetrodotoxin (Sigma Chemical Co), candesartan (CV-11974), an active metabolite of candesartan (cilexetil [TCV-116]) (a gift from Takeda Chemical industry, Ltd, Osaka, Japan), Hoe 140, and des-Arg⁶-[Leu⁷]-bradykinin (Peptide Institute). Stock solutions of bradykinin were prepared at concentrations of 0.1 mmol/L by dissolution in distilled water and were stored at −20°C. Indomethacin was freshly prepared in 10⁻² mol/L Na₂CO₃. Tetrodotoxin was dissolved in 0.1 mmol/L acetic acid. Candesartan was dissolved in 0.9% saline containing 5×10⁻² mol/L Na₂CO₃. L-NNA was dissolved in 0.2 mol/L HCl. At their final chamber concentrations, the solvents used to dissolve drugs did not affect the electrical responses.

**Statistical Analysis**

Data are expressed as mean±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 at the peak effect of bradykinin were used for statistical analysis. Comparison of facilitatory curves of EJPs was performed by 2-way ANOVA. 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 resistance artery was −71.2±0.9 mV (n=23). The membrane potential in the presence of 10⁻⁶ mol/L bradykinin was −70.0±1.5 mV (n=9), which did not significantly differ from the resting membrane potential. Nor did bradykinin at concentrations of 10⁻⁵ and 10⁻⁷ mol/L affect the membrane potential. No other drugs used in the present study significantly altered the resting membrane potential.

Typical recordings of the effect of 10⁻⁶ mol/L bradykinin on EJPs are shown in Figure 1A. The amplitude of EJPs increased with repetitive stimulation, giving rise to the maximum amplitude after ~3 to 5 stimuli (Figure 1A). EJPs were abolished by 3×10⁻⁷ mol/L tetrodotoxin (data not shown). At a concentration of 10⁻⁷ mol/L, bradykinin did not significantly alter the amplitude of EJPs. Higher concentrations of bradykinin (10⁻⁶ and 10⁻⁵ mol/L) did not alter the amplitude of the first EJP (control 10.1±1.0 mV, with 10⁻⁷ mol/L bradykinin 9.9±0.9 mV, n=14; control 8.0±0.7 mV, with 10⁻⁶ mol/L bradykinin 8.4±1.1 mV, n=11; Figure 1A and 1B) but increased the amplitude of the subsequent EJPs (Figure 1A and 1B). Hoe 140 (10⁻⁷ mol/L), a selective
antagonist of the bradykinin B2 receptor, abolished the enhancing effect of bradykinin on EJPs (Figure 2A). On the other hand, des-Arg⁹-[Leu⁸]-bradykinin, an antagonist of the bradykinin B1 receptor, was without effect on the action of bradykinin (Figure 2B).

The effect of bradykinin on the depolarization evoked by /H⁹²⁵¹/H⁹²⁵²-/methylene ATP, a stable analogue of ATP, was tested. Bradykinin (10⁻⁷ mol/L) did not affect the amplitude of depolarization evoked by 3×10⁻⁷ mol/L /H⁹²⁵¹/H⁹²⁵²-/methylene ATP (control 15.0±1.3 mV, with bradykinin 15.0±1.1 mV; n=5; P=NS).

Bradykinin has been shown to stimulate the release of NO and prostaglandins from vascular endothelial cells. To explore the possible involvement of these substances on the effects of bradykinin, we tested the effect of L-NNA, an inhibitor of NO synthase, and indomethacin, an inhibitor of cyclooxygenase (Figure 3A and 3B). In the presence of L-NNA (Figure 3A) or indomethacin (Figure 3B), bradykinin (10⁻⁷ and 10⁻⁶ mol/L) still enhanced the amplitude of EJPs.

Effects of the ACE inhibitor captopril and the angiotensin II type 1 receptor antagonist candesartan on the action of bradykinin on EJPs were examined. Neither agent directly altered the amplitude of EJPs (data not shown). In the presence of captopril (10⁻⁵ mol/L), bradykinin (10⁻⁷ mol/L) significantly enhanced the amplitude of EJPs, an effect not observed with this concentration of bradykinin in the absence of captopril (Figure 4A). On the other hand, even in the presence of candesartan (10⁻⁷ mol/L), bradykinin (10⁻⁸ mol/L) failed to alter the amplitude of EJPs significantly, although higher concentrations of bradykinin (10⁻⁷ and 10⁻⁶ mol/L) enhanced the amplitude of EJPs, as was the case without any pretreatment (Figure 4B).

**Discussion**

The present study demonstrated that bradykinin enhanced the facilitation of EJPs in a train of stimulation in the rat mesenteric artery. Because bradykinin affected neither the membrane potential nor the amplitude of the first EJP in this preparation, bradykinin most likely acted on presynaptic nerve terminals to facilitate the release of neurotransmitters, accounting for EJPs. This effect of bradykinin was blocked by Hoe 140, a bradykinin B2 receptor antagonist, but not by des-Arg⁹,[Leu⁸]-bradykinin, a bradykinin B1 receptor antagonist, suggesting that the effect of bradykinin is mediated by B2 receptors. The ACE inhibitor captopril, but not the AT₁ receptor antagonist candesartan, enhanced the action of bradykinin on EJPs.

Our previous study demonstrated that the EJP in the rat mesenteric artery is mediated by ATP but not by norepinephrine, as is the case in the guinea pig mesenteric artery, the rat tail artery, and the rabbit ear artery. Therefore, it should be mentioned that the present findings primarily represent the effects of bradykinin on sympathetic purinergic neurotransmission, although there is some evidence suggesting that norepinephrine and ATP are released in parallel from sympathetic nerve terminals and that their releases are subject to similar modulations.
In the present study, bradykinin did not affect the amplitude of the first EJP in a train of stimulation but did enhance the amplitudes of subsequent EJPs. Although bradykinin has been shown to elicit smooth muscle hyperpolarization in certain arteries through the release of putative EDHF, bradykinin showed no effect on the membrane potential in the rat mesenteric artery. Furthermore, bradykinin did not affect the magnitude of depolarization evoked by /H9251, /H9252-methylene ATP, a stable analogue of ATP. These findings suggest that bradykinin may not act directly on postsynaptic smooth muscle membrane or alter the responsiveness of muscle cells to the transmitter responsible for EJP in this preparation.

Collectively, it is reasonable to conclude that bradykinin enhanced the amplitude of EJPs through presynaptic facilitation of the transmitter release in the rat mesenteric artery, presumably through the bradykinin B2 receptor.

The present study, for the first time to our knowledge, provided unequivocal evidence that bradykinin enhances postganglionic sympathetic neurotransmission in certain blood vessels. Our findings are consistent with those in some other organs, such as the atria and kidney, in which facilitatory actions of bradykinin on norepinephrine release have been demonstrated, but our findings differ from those in some other studies. Few studies have explored the effect of bradykinin on sympathetic neurotransmission in blood vessels. Thapaliya et al. reported that bradykinin decreased the amplitude of EJPs, with concomitant smooth muscle hyperpolarization in the hamster mesenteric artery. Because these effects were abolished by indomethacin, they concluded that both the hyperpolarization and the inhibition of sympathetic transmission induced by bradykinin were indirect effects mediated by prostaglandins. Similarly, in the rabbit ear artery and the pulmonary artery, bradykinin inhibited neurogenic contractions or norepinephrine release by a prostanoid-dependent mechanism. The reason for the differences between our findings and those of previous studies is unclear, but species differences appear to exist relative to the effects of bradykinin. In the present study, the enhancing effects of bradykinin on EJPs were unaltered in the presence of indomethacin, an inhibitor of cyclooxygenase, suggesting that prostaglandins are unlikely to be involved in the sympathoexcitatory action of bradykinin in the rat mesenteric artery.

Bradykinin has also been shown to stimulate the release of NO from the endothelium, and in the canine temporal artery, NO has been implicated in the modulation of sympathetic neurotransmission. However, in the present study, even in the presence of the NO synthase inhibitor L-NNA, bradykinin still exerted similar enhancing effects on EJPs, thereby excluding the possibility of the involvement of NO in the sympathoexcitatory action of bradykinin. The underlying mechanisms of the action of bradykinin remain to be determined.

ACE inhibitors may increase the local concentration of bradykinin by inhibiting its breakdown. On the other hand, AT₁ receptor antagonists may not interfere directly with bradykinin metabolism, although under the blockade of AT₁ receptors, some increase in bradykinin concentration, possi-
bly through the stimulation of unopposed angiotensin II type 2 receptors, has been reported. Indeed, in the present study, the ACE inhibitor captopril enhanced the action of lower concentrations of bradykinin on EJPs, whereas the AT1 receptor antagonist candesartan was without effect. The lack of significant effects of captopril on higher concentrations of bradykinin might be because the action of bradykinin on EJPs had reached its plateau at these concentrations. Although accumulation of bradykinin by ACE inhibitors may enhance vasodilation through the release of endothelium-derived relaxing factors, it is possible that the sympathoexcitatory action of bradykinin within the vessel wall, as demonstrated in the present study, may partially counteract such an effect.

In human coronary arteries, bradykinin was found to evoke endothelium-dependent hyperpolarization and relaxation, which was augmented by the ACE inhibitor perindoprilat. On the other hand, in the human atria and kidney, bradykinin has been shown to enhance the release of norepinephrine in the presence of ACE inhibitors, although such an effect in human blood vessels remains to be demonstrated. Thus, it appears that multiple aspects of the action of bradykinin should be taken into account when its involvement in the clinical effects of ACE inhibitors is considered.

The present study had several limitations. First, as mentioned previously, recordings of EJPs primarily reflect purinergic transmission and do not involve direct measurement of the amount of transmitters released. Second, the concentrations of bradykinin used in the present study may be relatively high; it is unclear whether local levels of bradykinin could be high enough to alter sympathetic transmission. Third, some of the effects of bradykinin appear to be highly species specific; thus, caution should be exercised in extrapolating the present findings to humans.

In conclusion, bradykinin enhances sympathetic purinergic neurotransmission, presumably through presynaptic bradykinin B2 receptors in the rat mesenteric resistance arteries. The clinical relevance of the present findings remains unclear; however, the fact that the ACE inhibitor, but not the AT1 receptor antagonist, enhanced the action of bradykinin on sympathetic neurotransmission in blood vessels may warrant further investigation.

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References


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