Importance of Sympathetic Innervation in the Positive Inotropic Effects of Bradykinin and Ramiprilat

Richard D. Minshall, Vishnu P. Yelamanchi, Andreja Djokovic, David J. Miletich, Ervin G. Erdös, Sara F. Rabito, Stephen M. Vogel

Abstract Isolated rat left atria or right ventricular strips were electrically stimulated at a constant frequency. The amplitude of twitch contractions, thus elicited, rose as a function of stimulation intensity because of increases in the evoked release of sympathetic catecholamines. Bradykinin had no effect on contractile force in preparations paced at a minimal intensity (threshold). By contrast, bradykinin (1 nmol/L to 1 μmol/L) markedly increased twitch contractile force when the preparations were paced at a high intensity (two to three times threshold). The EC50 for the positive inotropic action of bradykinin averaged 42 nmol/L. Ramiprilat (1 μmol/L), an angiotensin I–converting enzyme/kininase II inhibitor, shifted the EC50 for bradykinin to ≈2 nmol/L. Ramiprilat (1 μmol/L) per se also produced a modest positive inotropic effect. The effects of bradykinin and/or ramiprilat were inhibited by HOE 140 (300 nmol/L), a bradykinin B2-receptor antagonist. Propranolol (1 μmol/L), a β-adreno-

ceceptor blocker, abolished the effects of bradykinin. After the destruction of sympathetic nerve endings by use of 6-hydroxydopamine, bradykinin no longer exerted a positive inotropic action. Cocaine (10 μg/mL), an inhibitor of catecholamine reuptake, potentiated the effect of bradykinin. Bradykinin did not affect the positive inotropic response to tyramine (10 μmol/L), whereas cocaine blocked it. Furthermore, bradykinin did not modify the dose-response curves for added norepi-
nephrine. α-Conotoxin (100 nmol/L) inhibited the positive inotropic effect of intensified stimulation and bradykinin poten-
tiation. Bradykinin is suggested to facilitate the evoked release of sympathetic catecholamines and thereby cause a positive inotropic effect. (Circ Res. 1994;74:441-447.)

Key Words • angiotensin-converting enzyme inhibitors • sympathetic nerve endings • kininase II • kinin antagonist • β-blocker

The clinical benefits of angiotensin I–converting enzyme (ACE) or kininase II inhibition, as an effective therapy for heart failure, are now increasingly appreciated.1 ACE inhibitors, such as ramipril, exert their vasodilatory effects by decreasing the formation of angiotensin II and protecting bradykinin from inactivation.2 ACE inhibitors also increase cardiac output in failing hearts more than other vasodilator substances.3 Thus, ACE inhibitors not only reduce the afterload but act by additional mechanisms as well. Little consideration has been given thus far to the possibility that ACE inhibitors may act as cardiotoxic agents. Angiotensin II increases cardiac contractility,4,5 but the formation of angiotensin II from angiotensin I is blocked in the presence of an ACE inhibitor. By contrast, ACE inhibitors may increase the concentration of bradykinin, at least locally. Therefore, the present inves-
tigation was undertaken to see if bradykinin affects the force of contraction in heart muscle.

We wanted to establish whether bradykinin has a card-
tonic effect in isolated myocardial preparations and, if so, by what mechanism. Our results suggest that bradyki-
nin, by acting presynaptically at sympathetic nerve end-
ings, evokes a positive inotropic response through the release of sympathetic agonists, catecholamines.

Materials and Methods

Measurement of Contractile Force in Isolated Heart Muscle

Adult Sprague-Dawley rats were anesthetized with halothane, and the hearts were rapidly excised. The blood was removed by a brief aortic perfusion. Isolated left atria or right ventricular strips (=5 mm in length and 2 mm in width) were then removed from the perfused hearts. Subsequent steps of the experimental proto-

Received July 19, 1993; accepted November 10, 1993.


Correspondence to S. Vogel, PhD, Department of Anesthesiology, University of Illinois College of Medicine, 1740 W Taylor St (M/C 515), Chicago, IL 60612.
determined during both normal and intensified electrical stimulation. Care was taken in the experiments conducted to ensure that the stimulation level used to excite sympathetic nerve endings produced inotropic responses of no more than 50% of maximum. The twitch contractions were recorded on a Grass model 7 polygraph and simultaneously displayed on the video monitor of a computer (model G/AT 286-10, Gems Computers) after being digitized (Labmaster board, Tecmar, Inc). On line, automated measurements of the peak amplitude of twitch contractions were made, and when desired, the measured values were stored in a file for later analysis. All experimental values reported are mean±SEM. Statistical comparisons were made using Student’s t test; differences in mean values were considered significant at P<.05.

**Solutions and Drugs**

**Solutions**

Krebs-Henseleit solution contains (mmol/L) NaCl 118, KCl 4.7, CaCl₂ 2, HEPES (acid) 5.55, Na⁺-HEPES 4.45, MgCl₂ 1, glucose 10, and Na₂-EDTA 0.025.

**Drugs**

Ramiprilat was a gift from The Upjohn Co, Kalamazoo, Mich, and D-arginyl-L-arginyl-L-prolyl-L-[4R]-4-hydroxyproplyl]-glycyl-L-[3-(2-thienyl)alanyl]-L-ery-yl-[(1,2,3,4-tetrahydroisooquinol-3-ylcarbonyl)-L-[(3α, 7α)-octahydroindol-2-ylcarbonyl]-L-arginine acetate (HOE 140) was a gift from Hoechst-Roussel Pharmaceuticals, Inc, Somerville, NJ. Bradykinin acetate, DL-propranolol hydrochloride, atropine sulfate, 6-hydroxydopamine hydrobromide, α-conotoxin GVIA, and tyramine were purchased from Sigma Chemical Co, St Louis, Mo. Cocaine was purchased from the University of Illinois Hospital Pharmacy.

**Results**

In our experiments, Bradykinin increased the force of contraction in the myocardial preparations when nerve endings within the tissue were excited by intensified field stimuli. We wanted to study only the effects of Bradykinin on sympathetic nerve endings; thus, atropine was present in all the experiments to block possible cholinergic effects of Bradykinin.

**Positive Inotropic Effect of Stimulation on the Isolated Rat Left Atria**

Increasing the intensity of field stimuli in itself produces a positive inotropic effect. First, we tested whether this effect is due to the action of endogenous sympathetic agonists, ie, norepinephrine. Fig 1a shows original recordings of twitch contractions from a paced (3 Hz) rat left atrium. In the time interval specified (indicated by asterisk pairs), the electrical stimulation was intensified from 1.1 to 2 times the contractile threshold. After the sudden increment in stimulation, a positive inotropic response developed gradually and tended to achieve a stable plateau. After 0.7 minute, the intensity of stimulation was reset to the control level (ie, 10% above threshold), and the contractile force recovered to the prestimulation baseline level.

Fig 1b shows the effects of propranolol (1 μmol/L), a blocker of β-adrenoceptors, on the contractile response to intense stimulation. Propranolol (added at arrow), within 5 minutes, markedly reduced (70%, n=3) and later abolished the effects of intensified (three times threshold) stimulation. These observations suggest that the inotropic effect produced by intense field stimulation is due to the electrically evoked release of sympathetic agonists.

**Influence of Bradykinin on the Force of Contraction During Stimulation**

Fig 1a also illustrates the effect of Bradykinin (1 μmol/L) on the contractile force in an isolated rat left atrium. Bradykinin (added at arrow) did not affect the baseline force. By contrast, Bradykinin did increase the contractile force during the period of intensified (two times threshold) stimulation by 49±6% (P<.0001, n=21). This effect of Bradykinin appeared rapidly (with-
in 5 minutes of its application) and was completely reversed (within 10 to 15 minutes) after washout of bradykinin (not illustrated).

The effects of bradykinin on responses to intensified field stimulation were also examined in right ventricular strips (n=3), and the results were qualitatively the same as in the left atrial preparation. Bradykinin, in ventricular muscle, increased the positive inotropic effects of intensified electrical stimulation (by 25% to 75%) without affecting the baseline contractile force elicited with near-threshold stimulation.

**Influence of Propranolol**

In the presence of propranolol (1 μmol/L), bradykinin (1 μmol/L) no longer affected contractile force during intensified (three times threshold) electrical stimulation of the atrial preparation (Fig 1c). As shown, intensified stimulation per se was also ineffective in the presence of the β-receptor antagonist.

**Influence of HOE 140**

Fig 1d shows the blocking of the effects of bradykinin in an isolated rat left atria by HOE140 (300 nmol/L), a bradykinin B2-receptor antagonist. In the presence of HOE 140, intensified field stimulation (two times threshold) still produced a normal positive inotropic effect, but addition of bradykinin (1 μmol/L) no longer increased the positive inotropic effect of intensified stimulation (n=4), indicating the involvement of B2-receptors.

**Effect of Bradykinin and Ramiprilat on the Rate of Increase of Contractile Force During Intensified Electrical Stimulation**

In addition to increasing steady-state contractile force during intensified field stimulation, bradykinin also increased the initial slope (milligrams per stimulus) of the envelope of twitch contractions. A straight line was fitted to the first 5 to 7 seconds of the rising phase to obtain the slope. Further quantitative analysis of bradykinin effects was made on the basis of the slope values. With stimulation of two to three times threshold intensity, slopes of 1 to 7 mg per stimulus were obtained, depending on the preparation tested (mean, 4.7±1.1; n=7 atria).

The relative slope is described as the ratio of twitch envelopes obtained immediately after and before drug treatment. In a series of untreated atria, two successive stimulations of equal intensity (two to three times the normal threshold) were compared. The ratio of second to first responses averaged 0.92±0.04 (n=19) (Fig 2, control); i.e., successive control responses tended to decline slightly. Bradykinin increased the relative slope of twitch contractions to 1.7±0.1 (P<.001, n=7) (Fig 2, BK). Similar results were obtained in three right ventricular strips (mean ratio, 1.8±0.2; not illustrated). HOE 140 blocked the effects of bradykinin on the slope in atrial (Fig 2, HOE+BK) and ventricular preparations.

Ramiprilat (1 μmol/L), an ACE inhibitor, caused a rather small, but statistically significant, increase in the slope to 1.2±0.1 (P<.01, n=8) (Fig 2, RAM) in experiments conducted without the addition of bradykinin. To see if potentiation of endogenous bradykinin could be responsible for the effect of ramiprilat, HOE 140 was tested in a separate group of atria. HOE 140 (300 nmol/L) prevented the effects of ramiprilat (Fig 2, HOE+RAM), suggesting the involvement of the B2-receptor in mediating its inotropic effect via endogenous kinins.

**Concentration-Dependent Effect of Bradykinin**

Fig 3 summarizes the effects of bradykinin as a function of concentration. The minimal concentration of bradykinin producing a detectable increase in the slope was ≈1 nmol/L. The effects of bradykinin increased further at cumulative concentrations of 10 nmol/L to 1 μmol/L. The EC50 for bradykinin was estimated to be 42±11 nmol/L (n=5).

Treatment with ramiprilat (1 μmol/L) potentiated the effects of added bradykinin (Fig 3). In the presence of the ACE inhibitor, the EC50 for bradykinin was shifted to a 20-fold lower concentration (1.9±0.5 nmol/L).
nmol/L, n=5, P<.01). Thus, the real potency of bradykinin in eliciting inotropic responses may be underestimated in the absence of an ACE inhibitor.

** Destruction of Sympathetic Nerve Endings by 6-Hydroxydopamine**

The blocking effect of propranolol (eg, Fig 1c) suggested that the positive inotropic responses to bradykinin should be mediated by catecholamines. Therefore, we determined whether intact nerve endings are required for the positive inotropic response to bradykinin. The atrial preparation was pretreated for 60 minutes with 6-hydroxydopamine (100 μmol/L) to destroy the sympathetic nerve endings. After this period, 6-hydroxydopamine was washed out, and the effects of bradykinin were tested. In untreated atria, the addition of bradykinin (1 μmol/L) during continuous intensified stimulation (five times threshold) caused a sustained positive inotropic response (Fig 4a). In preparations exposed to 6-hydroxydopamine, intensification of field stimulation no longer produced the usual cardiotonic effect (not illustrated), and the addition of bradykinin (arrow) failed to elicit a positive inotropic effect (Fig 4b). In four isolated atria, after 6-hydroxydopamine treatment, the positive inotropic effect of bradykinin was only 8±3% of baseline, as compared with 41±8% obtained in four untreated atria. The difference in mean values was statistically significant (P<.01).

**Potentiation by Cocaine**

We investigated whether cocaine, by inhibiting neuronal catecholamine reuptake, could potentiate the positive inotropic effect of bradykinin. In these experiments, the pacing frequency was lowered to 2 Hz to minimize the cardiac depressant action of cocaine, which was more pronounced at 3 Hz. Fig 5 illustrates the effect of bradykinin on contractile force in the presence or absence of cocaine. Here, bradykinin (1 μmol/L) increased the positive inotropic effect of intensified (five times threshold) stimulation by 195 mg (Fig 5a). In the presence of cocaine (10 μg/mL), bradykinin increased the positive inotropic effect of intensified stimulation (three times threshold) by 465 mg. The lower stimulation intensity used in the presence of cocaine produced a submaximal inotropic response, approximately equivalent to the untreated preparation (see legend to Fig 5). In three separate atrial preparations, the positive inotropic effect of bradykinin approximately doubled (116±12% of control level, P<.05) in the presence of cocaine. This potentiation by cocaine supports the thesis that bradykinin increases the evoked release of catecholamines.

**Bradykinin and Tyramine**

Tyramine enters sympathetic nerve endings via the monoamine transporter and produces a positive inotropic action by releasing catecholamines stored within the nerve ending (for review, see Reference 6). To see if bradykinin inhibits the monoamine transporter, we studied the incorporation of tyramine (10 μmol/L) into sympathetic nerve endings. The positive inotropic response to tyramine was taken as an indirect measure of its incorporation. Fig 6 illustrates the positive inotropic response of an atrium to tyramine in the absence (Fig 6a) or presence of bradykinin (Fig 6b). Between applications of tyramine, there was an intervening washout period of 15 minutes; bradykinin (1 μmol/L) was added 5 minutes before the second exposure to tyramine. As shown in Fig 6, in the presence of bradykinin a large positive inotropic response to tyramine was obtained. Control experiments in four separate preparations dem-

---

**Fig 4.** Loss of bradykinin effects after chemical sympathectomy. Original recordings for one experiment are depicted. a, Bradykinin (1 μmol/L), added at arrow, elicited a positive inotropic effect in an isolated rat left atria continuously stimulated at five times the contractile threshold. b, After treatment of the same atrium for 60 minutes with 100 μmol/L 6-hydroxydopamine (to destroy nerve endings), the positive inotropic effect of bradykinin was abolished.

**Fig 5.** Recordings showing potentiation of bradykinin (BK) effects by cocaine. Rat left atria (paced at 2 Hz to minimize the local anesthetic-like effects of cocaine) were electrically stimulated in the absence (a) and presence (b) of 10 μg/mL cocaine. Lower dashed lines in a and b indicate force levels achieved by intensified stimulation before the addition of BK (1833 and 1794 mg in a and b, respectively). These levels were made approximately the same by using a stimulation intensity of five times threshold (a) and three times threshold (b). Upper dashed lines in a and b mark the additional effect caused by BK. In this example, BK (1 μmol/L) increased the twitch amplitude by 195 mg in atria in the absence of cocaine (a) and 465 mg in atria treated with cocaine (b).
Bradykinin and Response to Norepinephrine

To determine if bradykinin alters the postsynaptic sensitivity of the myocardium to norepinephrine, we tested whether bradykinin could affect the positive inotropic response to added norepinephrine. The experiments were carried out in isolated rat left atria paced at near-threshold intensity. In each preparation, two cumulative dose-response curves of norepinephrine (10 nmol/L to 30 μmol/L) were generated in the presence and absence of 1 μmol/L bradykinin (data not shown). The EC50 values for the positive inotropic effect of norepinephrine alone (0.95 ± 0.2 μmol/L) and in the presence of bradykinin (1.0 ± 0.2 μmol/L) were not significantly different (n = 8). The EC50 of repeated norepinephrine dose-response curves in an additional control group did not differ either (1.1 ± 0.6 versus 1.4 ± 0.9 μmol/L, n = 4), indicating that bradykinin could not have acted by increasing the postsynaptic sensitivity of the myocardial cells to norepinephrine.

Effect of \( \omega \)-Conotoxin

Given that bradykinin does not affect the catecholamine uptake mechanism but, rather, seems to potentiate evoked transmitter release, we investigated whether the n-type \( \mathrm{Ca}^{2+} \) channel is involved in the evoked release of sympathetic agonists. Therefore, we tested the specific n-type \( \mathrm{Ca}^{2+} \) channel blocker, \( \omega \)-conotoxin, for an ability to block the positive inotropic effect of intensified stimulation and bradykinin potentiation (Fig 7). Fig 7a illustrates, in the absence of the toxin, the usual positive inotropic effect of intensified (four times threshold) field stimulation and the added effect of bradykinin, which was 170 mg. Treatment of the preparation for 15 minutes with 100 nmol/L \( \omega \)-conotoxin nearly abolished the inotropic response to intensified stimulation (Fig 7b) and reduced bradykinin potentiation to only 50 mg (compare Fig 7a with Fig 7b). The slope of the envelope of twitch contractions averaged 9.5 ± 1.6 mg per stimulus (n = 13) in untreated atria and 3.5 ± 0.7 mg per stimulus (n = 11) after exposure to \( \omega \)-conotoxin (P < .01). Further, the increase in the twitch amplitude that bradykinin normally caused, averaging 161 ± 6 mg (n = 3), was significantly reduced in the three

![Graph](http://circres.ahajournals.org/lookup/suppl/doi:10.1161/01.RES.0000041858.84922.4f/-/media/fig7.jpg)
atrial preparations treated with \(\omega\)-conotoxin (48.5\(\pm\)1.5, \(P<0.01\)). These results suggest that the n-type \(Ca^{2+}\) channel may be involved in the evoked release of sympathetic agonists in rat atria.

**Discussion**

We found that bradykinin increased the contractility of the myocardial preparations only during intensified stimulation, which excited nerve endings, and this effect was abolished by a \(\beta\)-adrenergic receptor antagonist. In addition, after the destruction of sympathetic nerve endings by \(6\)-hydroxypoline, bradykinin no longer exerted a positive inotropic action. Further, cocaine (10 \(\mu\)g/mL), a known inhibitor of catecholamine uptake at sympathetic nerve endings, potentiated the inotropic responses to bradykinin, and the effects of bradykinin were abolished by the bradykinin B2-receptor blocker HOE 140. These findings suggest that bradykinin, acting on presynaptic B2-receptors, augments the evoked release of neurotransmitter from sympathetic nerve endings, resulting in a positive inotropic effect.

Some additional experiments were performed to gain information on the mode of action of bradykinin at sympathetic nerve endings. In preparations paced at threshold intensity, bradykinin did not affect the positive inotropic response to tyramine (10 \(\mu\)mol/L), whereas cocaine blocked it. Since tyramine gains access to the interior of the nerve ending via the monoamine transporter and bradykinin did not alter the effects of tyramine, bradykinin very likely does not act by inhibiting the transporter. Bradykinin did not modify the dose-response curves of norepinephrine, indicating that bradykinin did not alter the postsynaptic sensitivity of the myocardium to \(\beta\)-adrenoceptor agonists. \(\omega\)-Conotoxin (100 \(\mu\)mol/mL) inhibited the positive inotropic effect of intensified stimulation and the potentiating effect of bradykinin, suggesting the n-type calcium channels to be important in the evoked release of sympathetic catecholamines.

In contrast to the present results, bradykinin directly affected the contraction of isolated guinea pig atria, even in the presence of propranolol, when relatively weak electrical stimuli were used. Since bradykinin, in our experiments, did not affect the myocardial contractility during threshold stimulation, the functional role of the postsynaptic receptor may vary with the species under investigation.

A positive inotropic effect of bradykinin via the B2-receptor was observed in dogs, anesthetized with a barbiturate, after the denervation of the heart and in isolated perfused rat hearts.\(^3\) In these myocardial preparations, characterized presumably by low sympathetic activity, the inotropic effect of bradykinin is related to its action as a potent coronary vasodilator. Because isolated atria of the present study do not contain vascular tissue and were not perfused, changes in coronary flow cannot explain the functional effects observed.

The ACE inhibitor, ramiprilat, markedly potentiated the actions of exogenous bradykinin.\(^10\)\(^12\) The assumption that ACE is present in heart muscle is supported by reports of the presence of components of the renin-angiotensin and kallikrein-kinin systems\(^13\)\(^17\) in the heart. Our results indicate that the activity of endogenous ACE could modulate the concentration of bradykinin in the vicinity of its receptor population in the rat myocardium.

Even though the effects of ramiprilat alone were quite small, they might have been mediated by endogenous bradykinin because they were abolished by HOE 140.\(^11\) The lack of a pronounced inotropic effect of ramiprilat suggests either that the endogenous production of bradykinin under relatively normal conditions is low and/or that a ramiprilat-resistant peptidase is responsible for the bulk of its degradation.\(^12\) The kininogenase generating the bradykinin that was potentiated by ramiprilat could have been plasma or tissue (glandular) kallikrein present in contaminating residual plasma or actual local tissue kallikrein, as recently reported.\(^13\) In response to myocardial injury (eg, ischemia), not only are the bradykinin levels elevated in the heart,\(^14\)\(^15\) but they are further enhanced in the presence of ramiprilat.\(^16\) Such observations support the view that the myocardium is capable of releasing bradykinin locally.

Angiotensin II is well known to activate receptors on sympathetic nerve endings, thereby augmenting the evoked release of neurotransmitter.\(^17\) In our preparations, we found the \(EC_{50}\) with respect to the positive inotropic effect of angiotensin II to be 8.4 \(\pm\) 3.8 \(\mu\)mol/L (n = 3; R.D. Minshall and S.M. Vogel, unpublished observations). Hence, angiotensin II proved to be more potent than bradykinin (EC\(_{50}\), 42 \(\pm\) 11 \(\mu\)mol/mL). In contrast, endothelin-1 (10 \(\mu\)mol/L to 100 \(\mu\)mol/mL) did not modulate stimulation-evoked transmitter release in three rat atria preparations, even in the presence of 10 \(\mu\)g/mL cocaine (R.D. Minshall and S.M. Vogel, unpublished observations). Consistent with the report of Takanashi and Endow,\(^18\) a significant postsynaptic positive inotropic effect of endothelin-1 was observed (with threshold stimulation), with an \(EC_{50}\) between 1 and 10 \(\mu\)mol/L.

In conclusion, evidence has been presented to support the concept that functional bradykinin B2-receptors exist on presynaptic sympathetic effenter fibers. Thus, bradykinin is suggested to facilitate the evoked release of sympathetic catecholamines and thereby to cause a positive inotropic effect. A possible implication of this finding is that the positive inotropic action of bradykinin (and perhaps of ACE inhibitors) could become more pronounced in conditions (eg, congestive heart failure) characterized by elevated sympathetic activity.

**Acknowledgments**

This study was supported in part by a grant from The Upjohn Co (Dr Rabio) and by National Institutes of Health grants HL-36473 and HL-36082 (Dr Erdős).

**References**


Importance of sympathetic innervation in the positive inotropic effects of bradykinin and ramiprilat.
R D Minshall, V P Yelamanchi, A Djokovic, D J Miletich, E G Erdös, S F Rabito and S M Vogel

_Circ Res._ 1994;74:441-447
doi: 10.1161/01.RES.74.3.441
_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/74/3/441