Bradykinin Promotes Ischemic Norepinephrine Release in Guinea Pig and Human Hearts

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ABSTRACT

We previously reported that bradykinin (BK; 1–1000 nM) facilitates norepinephrine (NE) release from cardiac sympathetic nerves. Because BK production increases in myocardial ischemia, endogenous BK could foster NE release and associated arrhythmias. We tested this hypothesis in guinea pig and human myocardial ischemia models. BK administration (100 nM) markedly enhanced exocytotic and carrier-mediated NE overflow from guinea pig hearts subjected to 10- and 20-min ischemia/reperfusion, respectively. Ventricular fibrillation invariably occurred after 20-min global ischemia; BK prolonged its duration 3-fold. The BK B2 receptor antagonist HOE140 (30 nM) blocked the effects of BK, whereas the B1 receptor antagonist des-Arg9-Leu8-BK (1 μM; i.e., 2.5 × pA2) did not. When serine proteinase inhibitors (500 KIU/ml aprotinin and 100 μg/ml soybean trypsin inhibitor) were used to prevent the formation of endogenous BK, NE overflow and reperfusion arrhythmias were diminished. In contrast, when kininase I and II inhibitors (ω-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid and enalaprilat, each 1 μM) were used to prevent the degradation of endogenous BK, NE overflow and reperfusion arrhythmias were enhanced. B2 receptor blockade abolished these effects but was ineffective if kininases were not inhibited. B2 receptor stimulation, by either exogenous or endogenous BK, also markedly enhanced carrier-mediated NE release in the human myocardial ischemia model; conversely, inhibition of BK biosynthesis diminished ischemic NE release. Because atherosclerotic heart disease impairs endothelial BK production, in myocardial ischemia BK could accumulate at sympathetic nerve endings, thus augmenting exocytotic and carrier-mediated NE release and favoring coronary vasoconstriction and arrhythmias.

Bradykinin (BK) is a potent releaser of catecholamines from cultured bovine adrenal chromaffin cells (Owen et al., 1989) and of [3H]norepinephrine (NE) from rat vas deferens (Llona et al., 1991) and human neuroblastomas (McDonald et al., 1994). Furthermore, BK potentiates the inotropic response of rat left atrium to sympathetic stimulation (Minshall et al., 1994) and facilitates NE exocytosis from cardiac sympathetic nerve endings (Seyedi et al., 1997), and when its degradation is prevented by kininase II/angiotensin-convert- ing enzyme (ACE) inhibitors, BK enhances the release of [3H]NE elicited by electrical field stimulation from slices of human atrium (Rump et al., 1997).

Short-lived myocardial ischemia causes NE exocytosis (Schömig, 1990; Imamura et al., 1994). In contrast, in protracted ischemia, the much greater NE release is “carrier mediated” due to reversal of the NE transporter in an outward direction, triggered by Na+/H+ exchanger activation and Na+ and NE accumulation in sympathetic nerve endings (Schömig, 1990; Imamura et al., 1996).

Because BK production is known to increase in the ischemic heart (Kimura et al., 1973; Matsuki et al., 1987; Lamontagne et al., 1995) and BK stimulates the Na+/H+ exchanger in endothelial cells (Fleming et al., 1994), BK could potentiate NE release in myocardial ischemia. Thus, the protective endothelium-dependent coronary-dilating effects of BK (Scici, 1994; Rubin and Levi, 1995; Giannella et al., 1997) could be offset by a BK-evoked facilitation of NE release and its attending vasoconstricting and arrhythmogenic effects.

Accordingly, the purpose of this study was to investigate whether BK promotes NE release and associated arrhythmias in myocardial ischemia/reperfusion. Furthermore, inasmuch as the cardiac metabolism of BK is species related and kininase II/ACE is a major BK-degrading enzyme in human cardiac membranes (Blais et al., 1997), we sought to determine whether BK plays a role in ischemic NE release in the human heart and, if so, whether such a role might be accentuated by ACE inhibitors.

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ABBREVIATIONS: ACE, angiotensin-converting enzyme; BK, bradykinin; DMI, desipramine; EIPA, 5-(N-ethyl-N-isopropyl)-amiloride; ANOVA, analysis of variance; KHS, Krebs-Henseleit solution; MERGETPA, ω-2-mercaptomethyl-3-guanidinoethylthiopropanoic acid; NE, norepinephrine; SBTI, soybean trypsin inhibitor.
Materials and Methods

Ischemia/Reperfusion Experiments in Guinea Pig Heart. Male Hartley guinea pigs (300–350 g) were sacrificed by cervical dislocation while under light anesthesia with CO2 vapor. The hearts were rapidly excised and perfused through the aorta at constant pressure (40 cm H2O) with Ringer’s solution at 37°C saturated with 100% O2 (pH 7.5) (Imamura et al., 1996). The composition of the Ringer’s solution was 154.0 mM NaCl, 5.61 mM KCl, 5.55 mM NaHCO3, 2.16 mM CaCl2, and 5.95 mM dextrose (pH 7.5). The electrocardiogram was continuously recorded with surface electrodes from the right atrium and the left ventricle. Hearts were perfused for 30 min before the experiments were begun to allow the heart rate to stabilize. After a 30-min preischemic stabilization period, normothermic global ischemia (10 and 20 min) was induced by complete interruption of coronary perfusion. This was followed by a 45-min reperfusion period. The coronary effluent was collected into tubes. In the preischemic period, tubes were replaced every 5 min. In the first 10 min of reperfusion, tubes were replaced every 2 min and every 5 min during the last 35 min. The volume of effluent collected for each period was measured and subsequently analyzed for NE content. All drugs were added to the perfusion solution. Hearts were perfused with a given drug, or drug combination, for the entire duration of the experiment, beginning 30 min before global ischemia. Hearts were weighed at the end of the experiment.

Arrhythmias were analyzed from the continuously recorded electrocardiogram tracings, according to the guidelines defined by the Lambeth Conventions (Walker et al., 1989). Ventricular fibrillation was the most common and persistent type of reperfusion arrhythmia, whereas ventricular premature beats and ventricular tachycardia were rare and inconsistent. Thus, only ventricular fibrillation was taken as an index of reperfusion arrhythmia.

Human Model of Protracted Myocardial Ischemia. Specimens of right atrium (i.e., surgical waste tissue) were obtained from 38 patients undergoing cardiopulmonary bypass (35 men and 3 women; age, 66.8 ± 1.3 years; coronary artery bypass graft surgery, 35; valve replacement, 3) according to a protocol approved by our Institutional Review Board. Twenty-three of the 35 patients undergoing coronary artery bypass graft surgery were chronically treated with atorvastatin; age, 66.8 ± 6.7 years; male, 33; female, 2; coronary artery bypass graft surgery, 35; valve replacement, 3). In the absence or presence of BK. When BK receptor antagonists and/or kininase I and II inhibitors or serine proteinase inhibitors were used, they were added 15 min after the beginning of the stabilization period.

Anoxia was induced by incubating the atrial fragments for 70 min in glucose-free KHS gassed with 95% O2 and 5% CO2 (pO2 0 mm Hg, pH 7.4) containing the monoamine oxidase inhibitor pargyline (1 mM). After the 45-min stabilization period, fragments were incubated for an additional 20 min in oxygenated KHS in the absence or presence of BK. When BK receptor antagonists and/or kininase I and II inhibitors or serine proteinase inhibitors were used, they were added 15 min after the beginning of the stabilization period.

Anoxia was induced by incubating the atrial fragments for 70 min in glucose-free KHS gassed with 95% O2 and 5% CO2 and containing the reducing agent sodium dithionite (3 mM; PO2 0 mm Hg, pH 7.3; anoxic period; in contrast, in the absence of sodium dithionite, PO2 70). Matched control fragments were incubated for an equivalent length of time with oxygenated KHS (normoxic NE release). When drugs were used, they were continued throughout the entire anoxic period.

NE Assay. NE was assayed in the coronary perfusate by high-performance liquid chromatography coupled to electrochemical detection and expressed in either pmol/g or nmol/g of wet heart weight (Imamura et al., 1996).

Drugs. L-NE bitartrate and 5-(N-ethyl-N-isopropyl)-amiloride (ethyliosopropylamiloride; EIPA) were purchased from Research Biochemicals International (Natick, MA). Desipramine hydrochloride (DMI), BK, des-Arg9-Leu9-BK, pargyline hydrochloride, aprotinin, and soybean trypsin inhibitor (SBTI) were purchased from Sigma Chemical Co. (St. Louis, MO). nL-2-Mercaptomethyl-3-guaninoeth-ythiopropanoic acid (MERGETPA) was purchased from Calbiochem-Novobiochem (San Diego, CA). Enalaprilat was a gift from Merck Sharp & Dohme Research Laboratories (West Point, PA). Hoe140 was a gift from Hoechst AG (Frankfurt, Germany). EIPA and DMI were dissolved in 99.5% dimethyl sulfoxide, and further dilutions were made with perfusion buffer. At the concentration used (0.1%), dimethyl sulfoxide did not affect NE release.

Statistical Analyses. Values are expressed as mean ± S.E.M. Comparison between two groups was done by paired or unpaired Student’s t test (depending on the specific circumstance), whereas comparisons among more than two groups were performed by analysis of variance (ANOVA), with the Bonferroni t test used for post-hoc analysis. A value of p < .05 was considered statistically significant.

Results

Effects of Exogenous BK on Exocytotic NE Release Associated with Reperfusion after Short-Lasting Ischemia in Guinea Pig Hearts. In isolated guinea pig hearts subjected to 10-min ischemia followed by 45-min reperfusion,
NE overflow into the coronary effluent increased from an undetectable preischemic level to a total of ~5 pmol/g in the first 2 min of reperfusion (Fig. 1A). No arrhythmias occurred. The NE transporter inhibitor desipramine (10 nM) potentiated NE overflow during reperfusion by 55%, whereas the N-type Ca\(^{2+}\) channel inhibitor ω-conotoxin GVIA (100 nM) inhibited NE overflow by 70%, demonstrating the exocytotic nature of NE release in this model (Fig. 1A).

BK administration (100 nM) caused a ~12-fold increase in NE overflow during reperfusion following 10-min ischemia (Fig. 2A). Ventricular fibrillation occurred in one of five experiments. At lower BK concentrations (10 nM), NE release increased ~3-fold but without attaining a level of statistical significance. No arrhythmias occurred (n = 4; data not shown). The BK B\(_1\) receptor antagonist des-Arg\(^9\)-Leu\(^8\)-BK (1 
\mu M) failed to modify the effects of BK on NE overflow (Fig. 2A). In contrast, the B\(_2\) receptor antagonist Hoe140 (30 nM) prevented the BK-induced potentiation of NE release (Fig. 2A).

Effects of Exogenous BK on Carrier-Mediated NE Release Associated with Reperfusion after Protracted Ischemia in Guinea Pig Hearts. When guinea pig hearts were subjected to 20-min ischemia followed by 45-min reperfusion, NE overflow into the coronary effluent increased from an undetectable preischemic level to ~800 pmol/g during reperfusion (Fig. 1B). Ventricular fibrillation invariably occurred and lasted ~2 min (see below). Desipramine (10 nM) inhibited NE overflow during reperfusion by ~85% (Fig. 1B), indicating that a reversal of the NE transporter was responsible for the increase in NE overflow in the 20-min ischemia/45-min reperfusion model. The Na\(^{+}\)/H\(^{+}\) exchanger inhibitor EIPA (10 \mu M) attenuated the increase in NE overflow during reperfusion by ~65% (Fig. 1B). Furthermore, desipramine and EIPA each prevented the occurrence of ventricular fibrillation (see below).

BK administration (100 nM) caused a 50% increase in NE overflow (Fig. 2B) and a 3-fold increase in the duration of ventricular fibrillation in the 20-min ischemia/45-min reperfusion model (see Fig. 5). At 10 nM, BK caused a moderate, but not significant, increase in NE overflow (651 ± 58 and 972 ± 71 pmol NE/g/min in the absence and presence of BK 10 nM, respectively; n = 3 + 3; N.S.). The duration of ventricular fibrillation also was not significantly changed. Hoe140 (30 nM) blocked the BK-induced augmentation of NE release and abbreviated ventricular fibrillation (see Figs. 2B and 5). Both of these effects of BK were also prevented by EIPA (see Fig. 5). In fact, EIPA inhibited not only the NE release associated with reperfusion after protracted ischemia (Fig. 1) but also the BK-induced potentiation of NE release (see Fig. 5; EIPA decreased total NE overflow by 533 ± 73 and 779 ± 59 pmol/g/min in the absence and presence of BK, respectively; P < .05, by unpaired t test). In contrast the BK B\(_1\) receptor antagonist des-Arg\(^9\)-Leu\(^8\)-BK (1 \mu M) failed to modify the effects of BK (Fig. 2B).

Endogenous BK and Exocytotic NE Release Associated with Reperfusion after Short-Lasting Ischemia in Guinea Pig Hearts. Perfusion with the serine proteinase inhibitors aprotinin (500 KIU/ml) and SBTI (100 \mu g/ml) in combination decreased exocytotic NE release by ~43% (Fig. 3A); no arrhythmias occurred.

Perfusion with either the kininase II/ACE inhibitor enalaprilat (1 \mu M) or the kininase I/carboxypeptidase M inhibitor MERGETPA (1 \mu M) to prevent the degradation of endogenous BK did not significantly affect exocytotic NE release during reperfusion after 10-min global ischemia (Fig. 4A); also, no arrhythmias occurred. However, when enalaprilat and MERGETPA were used in combination, NE overflow increased ~15-fold (Fig. 4A) and ventricular fibrillation occurred in one of six experiments. These effects were prevented by the BK B\(_2\) receptor antagonist Hoe140 (30 nM; Fig. 4A). When hearts were perfused with Hoe 40 (30 nM) in the absence of kininase I and II inhibitors, NE overflow at reperfusion was 8.1 ± 0.5 and 7.0 ± 1.6 pmol/g/min in the absence and presence of Hoe140, respectively (n = 3 + 3; N.S.).

Endogenous BK and Carrier-Mediated NE Release Associated with Reperfusion after Protracted Ischemia in Guinea Pig Hearts. Perfusion with the serine proteinase inhibitors aprotinin (500 KIU/ml) and SBTI (100 \mu g/ml) in combination in the 20-min ischemia/45-min reperfusion model decreased NE overflow by ~55% (Fig. 3B); the duration of ventricular fibrillation decreased from ~2 min to ~30 s (Fig. 5).

Perfusion with either enalaprilat (1 \mu M) or MERGETPA (1 \mu M) in the 20-min ischemia/45-min reperfusion model affected neither NE overflow (Fig. 4B) nor associated arrhythmias (data not shown). However, when enalaprilat and MERGETPA were used in combination, carrier-mediated NE overflow increased by ~70% (Fig. 4B) and the duration of ventricular fibrillation increased from 2 to ~11 min (Fig. 5). These effects were prevented by the BK B\(_2\) receptor antagonist Hoe140 (30 nM; Figs. 4B and 5). When hearts were

![Fig. 2. NE overflow into the coronary effluent in isolated guinea pig hearts subjected to 10-min (A) and 20-min (B) global ischemia followed by 45-min reperfusion. Hearts were perfused without any drug (control) or with BK (100 nM), alone or in combination with the BK B\(_1\) receptor antagonist des-Arg\(^9\)-Leu\(^8\)-BK (1 \mu M) or the BK B\(_2\) receptor antagonist Hoe140 (Hoe; 30 nM). Each bar represents NE overflow into the coronary effluent during 45 min of reperfusion (mean ± S.E.M., n = 5 in A, and n = 5 or 6 in B). NE overflow was undetectable in preischemic conditions. *p < .05 and **p < .01, significantly different from control, and †p < .05 and ††p < .01, significantly different from BK alone by ANOVA with Bonferroni’s t test used for post-hoc analysis. Drugs were added to the perfusion medium 30 min before ischemia.](https://jpet.aspetjournals.org/content/10/6/921.full.png)
perfused with Hoe140 (30 nM) without kininase I and II inhibitors, NE overflow at reperfusion was 755 ± 656 and 706 ± 43 pmol/g/min in the absence and presence of Hoe140, respectively (n = 6 or 7; N.S.); the duration of ventricular fibrillation was 2.1 ± 0.7 and 2.2 ± 1.1 min in the absence and presence of Hoe140, respectively (n = 4 + 4; N.S.).

BK, Carrier-Mediated NE Release, and Reperfusion Arrhythmias after Protracted Ischemia in Guinea Pig Hearts. Figure 5 demonstrates the relationship between NE overflow and duration of ventricular fibrillation in 61 hearts subjected to 20-min global ischemia followed by 45-min reperfusion in either the absence or presence of various agents. It is evident that arrhythmias lasted progressively longer as NE overflow increased. Moreover, when NE release was enhanced by 50% or 70% through the addition of exogenous BK or through prolongation of the half-life of endogenous BK by preventing its destruction with a combination of kininase I and II inhibitors, NE overflow was decreased and ventricular fibrillation was shortened or abolished (Fig. 5).

Effects of Exogenous BK on Carrier-Mediated NE Release from Human Myocardium. As reported previously (Hatta et al., 1997a), the incubation of human right atrial tissue in glucose-free KHs in anoxic conditions (Po2 0 mm Hg; pH 7.3) caused a pronounced carrier-mediated NE release. As shown in Fig. 6A, after 70 min of anoxia, NE release was ~6-fold greater than that in normoxic controls. At 10 nM, BK caused a modest increase in anoxic NE release (509 ± 272 and 725 ± 161 pmol NE/g in the absence and presence of BK, respectively; n = 3 + 3; N.S.). In contrast, 100 nM BK caused a ~2-fold increase in anoxic NE release (Fig. 6B). This effect of BK was insensitive to BK B1 receptor blockade with des-Arg^9-Leu^8-BK (1 μM; Fig. 6C) but was prevented by the BK B2 receptor antagonist Hoe140 (30 nM; Fig. 6D).

Effects of Endogenous BK on Carrier-Mediated NE Release from Human Myocardium. The incubation of anoxic human heart specimens with the serine proteinase inhibitors aprotinin (500 KIU/ml) and SBTI (100 μg/ml). Each bar represents NE overflow into the coronary effluent during 45 min of reperfusion (mean ± S.E.M., n = 6 in A, and n = 6 or 7 in B. *p < .05 and **p < .01, significantly different from control by unpaired Student’s t test.

Fig. 4. NE overflow into the coronary effluent in isolated guinea pig hearts subjected to 10-min (A) or 20-min (B) global ischemia followed by 45-min reperfusion in the absence (control) and the presence of the kininase I and II inhibitors MERGETPA (MER; 1 μM) and enalaprilat (Enal; 1 μM), each alone or in combination with the other kininase inhibitor, in the absence or presence of Hoe140 (30 nM). Each bar represents NE overflow into the coronary effluent during 45 min of reperfusion (mean ± S.E.M., n = 5 or 6). **p < .01, significantly different from control, and ††p < .01, significantly different from enalaprilat in combination with MERGETPA by ANOVA with Bonferroni’s t test used for post-hoc analysis.

Fig. 5. Correlation between the magnitude of NE overflow into the coronary effluent and the duration of ventricular fibrillation in isolated guinea pig hearts subjected to 20-min global ischemia followed by 45-min reperfusion. Each point is the mean of 5 to 8 experiments (±S.E.M.) except for the control point, which is the mean of 14 experiments. The line was calculated by regression analysis: r = correlation coefficient. Concentrations were DMI, 10 nM; EIPA, 10 μM; aprotinin (apro), 500 KIU/ml; SBTI, 100 μg/ml; BK, 100 nM; Hoe140 (HOE), 30 nM; MERGETPA (MER) 1 μM; and enalaprilat (Enal), 1 μM.
inhibitors aprotinin (500 KIU/ml) and SBTI (100 μg/ml) in combination decreased carrier-mediated NE release by 40% (Fig. 7).

Incubation of anoxic human heart specimens with either enalaprilat (1 μM) or MERGETPA (1 μM) did not affect NE release (Fig. 8A). However, when enalaprilat and MERGETPA were used in combination, carrier-mediated NE release increased almost 2-fold, and this effect was prevented by the BK B2 receptor antagonist Hoe140 (30 nM; Fig. 8B). In contrast, in the absence of enalaprilat and MERGETPA, anoxic NE release was not significantly modified by Hoe140 (i.e., anoxic NE release was 682 ± 197 and 599 ± 244 pmol/g in control conditions and in the presence of Hoe140 30 nM, respectively; n = 3 + 3; N.S.).

Discussion

Our findings demonstrate that the administration of BK to isolated guinea pig hearts subjected to ischemia/reperfusion enhances both exocytotic and carrier-mediated NE release and aggravates reperfusion arrhythmias by activating B2 receptors. Prevention of BK production with serine proteinase inhibitors markedly reduced NE release and reperfusion arrhythmias. Conversely, when degradation of endogenously formed BK was averted by a combination of kininase I and II inhibitors, NE release was enhanced and arrhythmias were aggravated. B2 receptor blockade prevented the effects of the kininase inhibitors. Furthermore, in a human model of protracted myocardial ischemia, carrier-mediated NE release was attenuated by inhibition of BK synthesis and potentiated by inhibition of BK degradation or the administration of exogenous BK. Thus, local BK production is likely to modulate NE release and associated arrhythmias in myocardial ischemia.

When isolated hearts are subjected to a short period of global ischemia followed by reperfusion, NE spillover into the coronary effluent increases due to enhanced exocytosis from adrenergic nerve terminals (Schömig, 1990; Imamura et al., 1994). The N-type Ca2+ channel inhibitor ω-conotoxin GVIA (Sher et al., 1991) blocked NE overflow after 10 min of global ischemia, whereas the NE transporter inhibitor desipramine (Imamura et al., 1994) potentiated the exocytotic nature of NE release in our model. In these conditions, perfusion with BK at 100 nM caused a striking increase in NE overflow during reperfusion. In the nanomolar to micromolar range, BK is known to facilitate peripheral noradrenergic transmission (Schwieler and Hjemdahl, 1992; Minshall et al., 1994; Rump et al., 1995) and to evoke [3H]NE release from neuroblastoma cells (McDonald et al., 1994) and isolated...
atrial sources, as well as protein kinase C activation and inhibition of NE release from the ischemic rat heart, which heart synaptosomes (Seyedi et al., 1997) but not with theduced NE release from isolated rat atria (Chulak et al., 1995).

Neuronal acidosis activates the Na

\(^{+}\)ion entry from extra-
cellular sources, as well as protein kinase C activation and Ca

\(^{+}\) release from intracellular stores (McDonald et al., 1994; Purkiss et al., 1995).

In protracted myocardial ischemia, NE is released by a nonexocytotic, "carrier-mediated" mechanism (McDonald et al., 1994; Schöning, 1990). Reversal of the NE reuptake process results from an impeded NE storage into synaptic vesicles leading to NE accumulation into the axoplasm, coupled with a decreased pH due to an ATP deficit and failure of the H

\(^{-}\)/ATPase pump (Schöning, 1990). Intraneuronal acidosis activates the Na

\(^{+}\)/H

\(^{-}\) exchanger, leading to an increase in [Na

\(^{+}\)]. This, combined with the increased axoplasmic NE, causes a reversal of the NE transporter in an outward direction, thus eliciting a carrier-mediated NE release (Schöning, 1990). Typically, the NE transporter inhibitor desipramine inhibited NE overflow after 20 min of global ischemia, verifying that NE release in these conditions was carrier mediated. Moreover, in keeping with a carrier-mediated mechanism, the Na

\(^{+}\)/H

\(^{-}\) exchanger inhibitor EIPA (Vigne et al., 1983) markedly attenuated the increase in NE overflow in the 20-min global ischemia/45-min reperfusion model. We found that perfusion with 100 nM BK significantly enhanced this carrier-mediated NE release, a process most certainly mediated by B

\(_{2}\) receptors, because Hoe140 blocked it, whereas des-Arg

\(_{9}\)-Leu

\(_{8}\)-BK did not. Notably, EIPA prevented the BK-induced potentiation of NE release, suggesting that BK may foster the activation of the Na

\(^{+}\)/H

\(^{-}\) exchanger in sympathetic nerve endings in the setting of protracted myocardial ischemia. In support of this hypothesis, BK has been found to activate the Na

\(^{+}\)/H

\(^{-}\) exchanger in other cells and tissues (Frelin et al., 1988; Fleming et al., 1994). Because agents that increase intracellular Ca

\(^{+}\) activity are known to activate the Na

\(^{+}\)/H

\(^{-}\} exchanger (ODonnell and Owen, 1994), this could be a mechanism by which BK ultimately facilitates NE release in the protracted ischemia/reperfusion model.

Consistent with the arrhythmogenic effects of NE (Schöning, 1990), the BK-induced increase in NE overflow, whether exocytotic or carrier mediated, resulted in an increased severity of reperfusion arrhythmias. Moreover, it is conceivable that BK, independent of its effects on NE release, also may directly contribute to the increased incidence and duration of arrhythmias. Indeed, BK stimulates inositol-1,4,5-triphosphate production by cardiac myocytes via high-affinity B

\(_{2}\) receptors (Minshall et al., 1995), and inositol-1,4,5-triphosphate production plays a pivotal role in reperfusion arrhythmias (Du et al., 1995). Thus, BK administration may exacerbate reperfusion arrhythmias by acting on both myocytes and adrenergic nerve terminals. Another potential mechanism by which BK may increase the severity of reperfusion arrhythmias is via activation of axoaxonal reflexes and sympathetic afferents as part of a cardiovascular reflex to enhance sympathetic efferent activity to the heart (Geppetti, 1993; Veelken et al., 1996; Wang and Zucker, 1996).

Our findings that exogenous BK enhances NE release in myocardial ischemia/reperfusion and aggravates associated arrhythmias do not concur with data from another laboratory (Chahine et al., 1993; Ribou et al., 1994). In models unlike ours (i.e., isolated rat heart subjected to 30-min global ischemia/5-min reperfusion and anesthetized dog with a 60-min LAD occlusion/30-min reperfusion), the authors found that BK infusion attenuates NE release and reperfusion arrhythmias. These results were attributed to B

\(_{1}\) receptor activation in the rat heart, whereas no indication of BK receptor activation was given for the findings in the dog. It is difficult to reconcile these findings with those in the literature consistently indicating that the proarrhythmic effects of BK are mediated by B

\(_{2}\) receptors (McDonald et al., 1994; Minshall et al., 1994, 1996; Chulak et al., 1985, 1995, 1996; Dendorfer et al., 1996; Seyedi et al., 1997).

BK production is known to increase in the ischemic heart (Kimura et al., 1973; Matsuki et al., 1987; Lamontagne et al., 1995), and tissue levels of this order of magnitude may well be attained in pathophysiological conditions (Regoli and Barabé, 1980; Bhoola et al., 1992), as also was suggested by our findings with serine proteinase inhibitors (see Figs. 3 and 7).

The BK-induced increase in NE overflow was prevented by the B

\(_{2}\) receptor antagonist Hoe140 (Hock et al., 1991) but not by the B

\(_{1}\) receptor antagonist des-Arg

\(_{9}\)-Leu

\(_{8}\)-BK (Regoli and Barabé, 1980), indicating that only B

\(_{2}\) receptor activation is involved in the facilitation of NE exocytosis. This agrees with the B

\(_{2}\) receptor-mediated enhancement of electrically induced NE release from isolated rat atria (Chulak et al., 1995), human kidney (Rump et al., 1995), and guinea pig heart synaptosomes (Seyedi et al., 1997) but not with the inhibition of NE release from the ischemic rat heart, which appears to be mediated by B

\(_{1}\) receptors (Chahine et al., 1993).

Analogous to BK-evoked release of [\(^{3}\)H]NE from neuroblastosoma cells (McDonald et al., 1994; Purkiss et al., 1995), the BK-induced potentiation of NE exocytosis in myocardial ischemia/reperfusion also may involve an increase in intraneuronal Ca

\(^{+}\). This could result from Ca

\(^{+}\) entry from extracellular sources, as well as protein kinase C activation and Ca

\(^{+}\) release from intracellular stores (McDonald et al., 1994; Purkiss et al., 1995).

Although exogenous BK facilitates ischemic NE release by activating B

\(_{2}\) receptors on sympathetic nerve endings (see
Figs. 2 and 6) and the production of endogenous BK is increased in myocardial ischemia (Kimura et al., 1973; Matsuki et al., 1987; Lamontagne et al., 1995), Hoe140 did not inhibit ischemic NE release in the absence of enalaprilat and MERGETPA. This suggests that unless BK metabolism is prevented by the prior inhibition of kininase I and II, the concentration of endogenous BK attained at the effector sites may not be sufficiently high to facilitate ischemic NE release.

Nevertheless, we found that the prevention of BK formation with serine proteinase inhibitors reduced ischemic NE release. A likely explanation for this apparent discrepancy is that cardiac serine proteinases can generate both BK and angiotensin II (Arakawa, 1996). Thus, aprotinin and SBTI inhibit not only the formation of BK, via kallikrein, but also the formation of angiotensin II, via the ACE-independent pathway (Arakawa, 1996). Accordingly, the effectiveness of the aprotinin-SBTI combination in reducing ischemic NE release and associated arrhythmias may be due not only to the inhibition of BK production but also to the prevention of the NE-releasing effects of ACE-independent angiotensin II. Indeed, in preliminary experiments, we have found that the angiotensin IIAT1 receptor antagonist EXP 3174 inhibits the ischemic facilitation of NE release and that EXP 3174 and Hoe140 in combination are more effective than EXP 3174 alone (Hatta et al., 1997b). Thus, although local BK production increases in myocardial ischemia, the effects of BK on adrenergic nerve terminals are more likely to be uncovered when BK half-life is prolonged, when the effects of angiotensin II are suppressed, and/or in pathophysiological conditions, when endothelial BK production is impaired, as occurs in atherosclerotic coronary disease (Busse and Fleming, 1996) and congestive heart failure (Kubo et al., 1991).

Because the responses of cardiac sympathetic nerves to BK vary among different animal species (Chahine et al., 1993; Ribout et al., 1994; Seyedi et al., 1997; Rump et al., 1997), we determined whether BK potentiates ischemic NE release in the human heart. For this, we used a human model of protracted myocardial ischemia previously established in our laboratory (Hatta et al., 1997a). In this system, NE release is typically carried out. Mediated by B2 receptors as in the guinea pig heart, 100 nM BK markedly potentiated NE release in this human model. Moreover, as indicative of a role played by local BK production in cardiomediated NE release, the blockade of BK synthesis with serine proteinase inhibitors attenuated NE release, whereas preservation of BK by kininase inhibition (Blais et al., 1997) potentiated it. Thus, BK is likely to play an important part in the regulation of NE release in human myocardial ischemia.

The BK-induced promotion of ischemic NE release may seem to be in conflict with previous reports from our and other laboratories focusing on the cardioprotective effects of BK. In this context, we had shown that BK acts in the isolated guinea pig heart as a physiological antagonist of anaphylactic vasoconstriction (Rubin and Levi, 1995) and participates in the protection afforded by ischemic preconditioning against the loss of hypoxic coronary vasodilation caused by ischemia/reperfusion (Giannella et al., 1997). Other investigators reported that the administration of BK to the isolated rat heart alleviates reperfusion arrhythmias (Linz et al., 1989; Minshall et al., 1997). When given to vivo models of coronary occlusion/reperfusion, BK was found to either worsen the signs of myocardial ischemia in the pig (Tio et al., 1991) or attenuate reperfusion arrhythmias in the dog (Vegh et al., 1994).

Modest reductions in reperfusion arrhythmias were also observed on the administration of ACE/kininase II inhibitors, some with (van Gilst et al., 1986) and some without (Linz et al., 1989; Liu et al., 1996) free radical-scavenging activity. These protective effects were attributed to an increased availability of endogenous BK because in most instances, they were attenuated by Hoe140. In contrast, other investigators have reported that ACE inhibitors can actually increase infarct size in a canine ischemia/reperfusion model (de Lorgeril et al., 1992).

Accordingly, it is possible that depending on the site or sites of predominant formation in the heart, whether the coronary endothelium (Busse et al., 1994) or adrenergic nerve endings (Seyedi et al., 1997), BK will promote protective or deleterious effects, mediated by nitric oxide/prostacyclin/endothelium-derived hyperpolarizing factor (Mombouli et al., 1992; Vegh et al., 1994; Liu et al., 1996) and NE (Seyedi et al., 1997), respectively. The balance between these two opposite effects may vary with experimental conditions and animal species, generating the apparent discrepancies between our findings and those of other investigators. Notably, our ischemic guinea pig and human heart models were very similar in their adrenergic responses to BK.

Because endothelial function is often compromised in atherosclerotic heart disease (Busse and Fleming, 1996) and congestive heart failure (Kubo et al., 1991), the cardioprotective effects of BK are likely to be curtailed in these conditions, due to the decreased accumulation of BK at the luminal endothelial surface of the coronary vasculature. The persisting production of BK at adrenergic nerve endings (Seyedi et al., 1997) may then favor the NE-releasing effects of BK, promoting arrhythmias and coronary vasoconstriction (Hatta et al., 1997c). In fact, coronary arteries with dysfunctional endothelium are known to be hypersensitive to the vasoconstricting effects of catecholamines (Vita et al., 1992).

After several large clinical trials, the potential of ACE inhibitors to reduce mortality rates and ischemic events in patients with coronary artery disease remains controversial, with studies reporting cardioprotection (e.g., SAVE, AIRE, ISIS-4, GISSI-3) and others demonstrating no beneficial effects or actually a harmful trend (e.g., CONSENSUS II, QUIET, ELITE 1) (Megarry et al., 1997; Brown and Vaughan, 1998). Furthermore, when ACE inhibitors are compared with angiotensin receptor blockers in large clinical trials such as ELITE 1 (Pitt et al., 1997), captopril is found to be less effective than losartan in reducing mortality rates in elderly patients with post-myocardial infarction congestive heart failure. One likely explanation for the higher mortality rate with captopril is that, whereas losartan blocks the cardiotoxic effects of Ang II, independently of its formation (i.e., by both ACE-dependent and independent pathways), captopril prevents only the ACE-dependent Ang II formation. On the other hand, our findings offer another explanation: captopril not only allows the ACE-independent formation of AII to continue, but also may allow the emergence of the deleterious effects of BK by inhibiting its metabolism.

In conclusion, we report here that BK promotes both exocytotic and carrier-mediated NE release associated with brief and protracted periods of ischemia/reperfusion, respectively. In either case, arrhythmias are aggravated.
The effects of BK are mediated by activation of prejunctional B2 receptors on sympathetic nerve endings, which probably results in an increase in intraneuronal Ca++. Enhanced (Ca++), would then be responsible for augmenting exocytosis and activating the Na+/H+ exchanger, a pivotal signal for initiating carrier-mediated NE release. In view of our recent discovery that cardiac sympathetic nerve endings harbor a kallikrein-kinin system capable of generating locally effective kinin concentrations (Seyedi et al., 1997), and that serine proteinase inhibitors attenuate NE release and alleviate arrhythmias in the isolated heart subjected to ischemia/reperfusion, it is conceivable that in pathophysiological conditions such as atherosclerotic heart disease and congestive heart failure, BK will accumulate at sympathetic nerve endings. Via an autocrine mechanism, BK will then augment exocytotic and carrier-mediated NE release, favoring coronary vasoconstriction and arrhythmias.

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