Postischemic anti-inflammatory effects of bradykinin preconditioning

SAKUJI SHIGEMATSU, SHUJI ISHIDA, DEAN C. GUTE, AND RONALD J. KORTHUIS
Department of Molecular and Cellular Physiology, Louisiana State University Health Sciences Center, School of Medicine in Shreveport, Shreveport, Louisiana 71130

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Shigematsu, Sakuji, Shuji Ishida, Dean C. Gute, and Ronald J. Korthuis. Postischemic anti-inflammatory effects of bradykinin preconditioning. Am J Physiol Heart Circ Physiol 280: H441–H454, 2001.—We sought to determine the mechanisms whereby brief administration of bradykinin (bradykinin preconditioning, BK-PC) before prolonged ischemia followed by reperfusion (I/R) prevents postischemic microvascular dysfunction. Intravital videomicroscopic approaches were used to quantify I/R-induced leukocyte/endothelial cell adhesive interactions and microvascular barrier disruption in single postcapillary venules of the rat mesentery. I/R increased the number of rolling, adherent, and emigrated leukocytes and enhanced venular albumin leakage, effects that were prevented by BK-PC. The anti-inflammatory effects of BK-PC were largely prevented by concomitant administration of a B2 receptor antagonist but not by coincident B1 receptor blockade, nitric oxide (NO) synthase inhibition, or cyclooxygenase blockade. However, NO synthase blockade during reperfusion after prolonged ischemia was effective in attenuating the anti-inflammatory effects of BK-PC. Pan protein kinase C (PKC) inhibition antagonized the beneficial effects of BK-PC but only when administered during prolonged ischemia. In contrast, specific inhibition of the conventional PKC isotypes failed to alter the effectiveness of BK-PC. These results indicate that bradykinin can be used to pharmacologically precondition single mesenteric postcapillary venules to resist I/R-induced leukocyte recruitment and microvascular barrier dysfunction by a mechanism that involves B2 receptor-dependent activation of nonconventional PKC isotypes and subsequent formation of NO.

ischemic preconditioning; leukocyte adhesion; leukocyte emigration; venular albumin leakage; protein kinase C; bradykinin B2 receptors

BRIEF PERIODS OF VASCULAR occlusion render tissues resistant to the injury induced by subsequent exposure to prolonged ischemia and reperfusion (I/R), a phenomenon referred to as ischemic preconditioning (IPC). Although the mechanisms whereby IPC exerts its powerful protective effects are not completely understood, a growing body of evidence indicates that release of adenosine A1/A3 or α1-adrenergic receptors, respectively (3,20). In addition, pharmacological preconditioning can be produced by administration of agonists specific for these receptors in lieu of IPC (3,20). Downstream molecular effectors that may be activated by IPC-induced PKC translocation include ATP-sensitive potassium channels, NO synthase, heat shock proteins, and/or 5'-nucleotidase, the relative importance of each depending on the species or organ studied (3,20). Our previous work has uncovered the fact that preconditioning the mesentery or skeletal muscle with ischemia or adenosine attenuates postischemic leukocyte/endothelium adhesive interactions in postcapillary venules (1,18,20). These anti-inflammatory effects of IPC were antagonized by administration of adenosine A1 receptor antagonists or PKC inhibitors (1,18,20). Because leukocyte adhesion and emigration play a major role in the genesis of the reperfusion component of I/R injury in these tissues, the latter observations indicate that inhibition of leukocyte infiltration is involved in the protective mechanism underlying IPC (1,18,22,32).

More recently, the vasoactive proinflammatory peptide bradykinin has been implicated in cardioprotective effects of IPC (4,33,40,48). For example, interstitial bradykinin concentration increases by 200% at the end of 3 or 10 min IPC in swine myocardium and by almost 50-fold in coronary sinus blood after 1 min of balloon inflation in patients undergoing angioplasty (33,40). Moreover, the cardioprotective effects of IPC are substantially reduced by administration of bradykinin receptor antagonists in some models of myocardial I/R (4,33,40). The bulk of available evidence indicates that bradykinin B2 receptors mediate this response (16,33,48), although one report suggests that B1-receptor activation is critical (4). Particularly compelling evidence for a role for bradykinin in IPC is provided by the observations that IPC does not confer cardioprotection in B2 receptor knockout or kininogen-deficient mice (48). Finally, bradykinin preconditioning (BK-PC) appears to be as effective as IPC in preventing myocardial I/R injury (5,16,44).
Although the aforementioned studies clearly indicate that bradykinin may contribute to the cardioprotective effects of IPC in some models and can be used to pharmacologically precondition the heart to resist the deleterious effects of I/R, the contribution of this peptide to IPC in peripheral organs is not clear. Also, it is not known whether BK-PC may exert anti-inflammatory effects in the setting of I/R. Indeed, because bradykinin is well known for its effects to promote adhesion molecule expression, leukocyte activation and infiltration, and the formation of interendothelial gaps and protein extravasation (2, 6, 8, 13, 28, 37–39, 41, 45; unpublished observations), postulating that BK-PC exerts anti-inflammatory effects in I/R might seem counterintuitive. Of course, similar arguments might have been expected to apply in the case of IPC, since ischemia can induce effects similar to bradykinin in the microcirculation. Thus the major aims of this study were to determine whether 1) brief administration of bradykinin before I/R exerts anti-inflammatory effects similar to IPC, and if so, by what mechanism and 2) endogenous bradykinin is involved in the anti-inflammatory effects of IPC. Our results indicate for the first time that BK-PC completely prevents posts ischemic leukocyte rolling, stationary adhesion, and emigration and preserves endothelial barrier function in postcapillary venules of the rat mesentery by a mechanism that is initiated by stimulation of B2 receptors during the period of BK-PC, involves activation of nonconventional PKC isotypes during prolonged ischemia, and is mediated by nitric oxide (NO) production during reperfusion. Although our results indicate that bradykinin can be used to pharmacologically precondition postcapillary venules to resist the deleterious proinflammatory effects of I/R, this peptide does not appear to play a role in beneficial actions of IPC in the mesentery.

METHODS

Surgical Procedure

Male Sprague-Dawley rats (200–250 g) were maintained on a purified laboratory diet and were fasted for 24 h before the experiment. The animals were anesthetized initially by intraperitoneal injection of pentobarbital sodium (65 mg/kg body wt). Upon attaining a surgical level of anesthesia, a tracheotomy was performed to facilitate breathing during the experiment. The right carotid artery was cannulated, and systemic arterial pressure was measured with a Statham P23A pressure transducer connected to the carotid artery catheter. Systemic blood pressure was recorded continuously with a personal computer (Macintosh 8500; Apple) equipped with an analog-to-digital converter (MP 100, Biopac Systems). The left jugular vein was also cannulated for drug administration. After these procedures, a midline abdominal incision was performed to allow for exteriorization and intravital microscopic examination of a section of the mesentery from the small intestine.

Intravital Microscopy

The rats were positioned on a 20 × 30-cm Plexiglas board in a manner that allowed a selected section of mesentery to be placed over a glass slide covering a 4 × 3-cm hole centered in the Plexiglas. The mesentery was superfused at 2.5 ml/min with bicarbonate-buffered saline (BBS, pH 7.4) bubbled with a mixture of 5% CO2–95% N2 to reduce the oxygen tension to the physiological intraperitoneal level (40–50 mmHg). The exposed bowel wall was covered with BBS-soaked gauze to minimize tissue dehydration. The superfusate was maintained at 37 ± 0.5°C by pumping the solution through a heat exchanger warmed with a constant-temperature circulator (model 801; Fisher Scientific). Rectal and mesenteric temperatures were monitored with an electrothermometer (4000A; YSI). Body temperature was kept between 36.5 and 37.5°C with an infrared heat lamp. The board was mounted on the stage of an inverted microscope (TMD-2S, Diaphot; Nikon) and a × 40 objective lens was used to observe the mesenteric microcirculation. The mesentery was transilluminated with a 12-V 100-W direct current-stabilized light source. A video camera (VK-C150; Hitachi) mounted on the microscope projected the image on a color monitor (PVM-2030; Sony), and the images were recorded with a videocassette recorder (SLV-720HF; Sony). The time and date were displayed on both taped and live images with a date-time generator (WJ-810; Panasonic).

Single unbranched venules with diameters of 25–35 μm and lengths >150 μm were selected for study. Venular diameter (Dv) was measured on-line with a video caliper (Microcirculation Research Institute, Texas A&M University). Center-line erythrocyte velocity was measured with an optical Doppler velocimeter (Microcirculation Research Institute, Texas A&M University) that was calibrated against a rotating glass disk coated with erythrocytes. Venular blood flow was calculated from the product of mean erythrocyte velocity (Vmean = center-line velocity/L; see Ref. 10) and microvascular cross-sectional area, with cylindrical geometry assumed. Venular wall shear rate (SR) was calculated from the Newtonian definition as follows: SR = 8(Vmean/Dv).

The number of rolling, stationary (firmly adherent), and emigrated leukocytes was determined off-line during playback of videotaped images. A leukocyte was considered firmly adherent to venular endothelium if it remained stationary for 30 s or longer. Adherent leukocytes were quantified as the number per 100 μm length of venule. Leukocyte emigration was expressed as the number per field of view surrounding the venule. Rolling leukocytes were defined as those leukocytes that moved at a velocity less than that of erythrocytes in the same vessel. Leukocyte rolling velocity was determined from the time required for a leukocyte to traverse a 100-μm distance along the length of the venule and was expressed as micrometers per second. The flux of rolling leukocytes was measured as those leukocytes that could be seen moving within a small (25-μm) viewing area of the vessel with the same area used throughout the experiment.

To quantify albumin leakage across mesenteric venules, 50 mg/kg of FITC-labeled bovine albumin (Sigma) were administered intravenously to the animals 15 min before the baseline recording (23, 42). Fluorescence intensity (excitation wavelength, 420–490 nm; emission wavelength, 520 nm) was detected with a charge-coupled device (CCD) camera (XC-77; Hamamatsu Photonics), a CCD camera control unit (C2400; Hamamatsu Photonics), and an intensifier head (M4314; Hamamatsu Photonics) attached to the camera. The fluorescence intensity of the venule under study (Iv), the fluorescence intensity of contiguous perivenular interstitium within 10–50 μm of the venular wall (Iw), and the background fluorescence (Ib) were measured at various times after the administration of FITC-albumin with a computer-assisted digital imaging processor (NIH Image 1.56b on a Macintosh computer). The windows to measure average fluorescence...
intensities within and along the venule were set at 20 μm length and 10 μm width. An index of vascular albumin leakage was determined from the relation \((I_2 - I_b)/(I_v - I_b)\).

**Measurement of Plasma Nitrite/Nitrate**

Plasma NO levels were estimated by measuring its stable oxidative product, nitrite/nitrate (23). Plasma nitrite/nitrate concentration was measured by using a commercially available assay kit (Nitrate/Nitrite Fluorometric Assay Kit no. 780051; Cayman Chemical). Blood was sampled via a PE-10 tube inserted in the mesenteric vein. Plasma was obtained by centrifuging soon after the blood was sampled and was frozen at −80°C immediately. For the nitrite/nitrate measurement, plasma was thawed and centrifuged at 13,000 rpm for 30 min with an ultrafiltration device (Ultrafree-MC; Millipore). Thereafter, nitrite/nitrate was detected using the assay kit and a luminescence spectrophotometer (SLM AMINCO AB-2; SLM Instruments).

**Experimental Protocols**

Figure 1 illustrates the general design of the experimental protocols. After a stabilization period of 30 min, images from the venule of interest were recorded on videotape for 10 min (baseline recording). In most groups, bradykinin (Sigma) preconditioning was then accomplished by superfusing the mesentery for 5 min with BBS containing the peptide at 1 μM. After 5 min of bradykinin superfusion, the superfusate was then switched to BBS alone, and images from the venule of interest were recorded again for 10 min (preischemia recording). Ischemia was then induced by ligating the superior mesenteric artery (SMA) and vein (SMV) just proximal to the venule of interest by placing a piece of PE-280 tubing next to the SMA and SMV and then tightening a flexible ligature (Tygon, OD 0.30 in.; Norton Performance Plastics) placed around the vessels and the PE-280 tubing. After 20 min of ischemia, the ligature was removed, and venular responses were monitored during minutes 20–30 and 50–60 of reperfusion. Only rats in which ligation of the superior mesenteric vessels induced a decrease in venular center-line erythrocyte velocity of 80% or greater were used in the study.

Fig. 1. Schematic illustration of the experimental protocols employed in each group. Bars located below each time line indicate when in the protocol the 10-min recordings were obtained. Nos. refer to minutes between recording periods. Hatched bar labeled BK, the 5-min period of bradykinin preconditioning (BK-PC); stippled bars labeled ANT, time frames over which the various antagonists (ANT) were superfused over the mesentery, either during the period of bradykinin (BK-PC) or ischemic preconditioning (IPC), prolonged ischemia, or reperfusion, depending on the group; stippled bars labeled HOE-140, 2 subsets of experiments (one subset of mesenteries being treated with HOE-140 during BK-PC and the other subset being treated with the B2 receptor antagonist during reperfusion); solid bars, either the 5-min period of IPC or the 20-min period of prolonged ischemia. See the text for other details. I/R, ischemia-reperfusion.
or during reperfusion after prolonged ischemia (Fig. 1) to evaluate temporal aspects of the signaling cascade induced by BK-PC. Each of the inhibitors was dissolved in BBS and then was added to the BBS superfuse, and doses were selected based on previous studies showing efficacy. Washout of the antagonists was accomplished by switching to a superfuse reservoir that contained only BBS. In the final two groups, mesenteries were preconditioned with ischemia (rather than bradykinin) for 5 min in the absence or presence of a bradykinin B$_2$ receptor antagonist, with all variables recorded at equivalent time points as described for the BK-PC groups. Total leukocyte counts were obtained from blood samples obtained during the basal period and at the end of the experiments and were not different.

**Group 1: Sham (n = 6).** As a time control for the effects of experimental duration, mesenteries in this group were superfused with BBS alone (no drug treatment) throughout the protocol in the absence of preconditioning and I/R.

**Group 2: I/R (n = 6).** Mesenteries in this group were exposed to I/R alone (no bradykinin or IPC) and were superfused with BBS alone (no drug addition) throughout the protocol.

**Group 3: BK-PC (n = 4).** The aim of the studies outlined for this group was to determine the effect of BK-PC alone (no subsequent I/R) on leukocyte adhesion and venular albumin leakage. Mesenteries in this group were briefly exposed to bradykinin (1 μM, 5 min) via the superfuse, with all variables recorded at time points equivalent to that described for the I/R group above.

**Group 4: BK-PC + I/R (n = 7).** To determine the effects of BK-PC on I/R-induced leukocyte adhesion and microvascular barrier function, mesenteries in this group were exposed to bradykinin (1 μM) for 5 min via the superfuse beginning 15 min before I/R.

The experiments outlined for groups 5 and 6 were directed at determining the bradykinin receptor subtype involved in initiating BK-PC.

**Group 5: BK-PC (HOE-140) + I/R (n = 7).** Mesenteries in this group were exposed to bradykinin (1 μM, 5 min) in the presence of HOE-140 (1 μM, a generous gift from Hoechst) before I/R to determine if the effects of BK-PC were initiated by B$_2$ receptor activation during the period of BK-PC. HOE-140 was topically applied to the mesentery beginning 5 min before BK-PC was initiated and was maintained for 15 min.

**Group 6: BK-PC (Des-Arg HOE-140) + I/R (n = 7).** The aim of these studies was to determine the role of bradykinin B$_1$ receptor activation in the beneficial actions of BK-PC. Mesenteries in this group were treated as described for group 5 except that the bradykinin B$_1$ receptor antagonist Des-Arg HOE-140 (1 μM), rather than HOE-140, was topically applied to the mesentery during the period of BK-PC.

The vasodilator responses to bradykinin involve B$_2$ receptor-dependent release of NO, prostacyclin, and an endothelium-derived hyperpolarizing factor (EDHF; see Ref. 17). The studies outlined for groups 7–10 are directed at examining the potential role of NO and prostacyclin as triggers and/or effectors of BK-PC.

**Group 7: BK-PC nitro-l-arginine methyl ester + I/R (n = 7).** Mesenteries in this group were treated as described for group 5 except that the NO synthase inhibitor nitro-l-arginine methyl ester (L-NAME, 10 μM; Calbiochem), rather than HOE-140, was topically applied to the mesentery to determine if the effects of bradykinin were triggered by NO production during the period of BK-PC.

**Group 8: BK-PC + I/R and l-NAME (n = 6).** To determine whether NO served as an effector of the beneficial effects of BK-PC, mesenteries in this group were superfused with L-NAME (10 μM) throughout reperfusion after prolonged ischemia. As a corollary to these studies, a subset of experiments (n = 6) was conducted to determine whether superfusion of the mesentery with the guanylate cyclase inhibitor ODQ (3 μM; Sigma) during reperfusion would also attenuate the beneficial actions of BK-PC.

**Group 9: BK-PC (Indo) + I/R (n = 5).** Because the vasodilatory effects of bradykinin are mediated in part by B$_2$ receptor-dependent prostaglandin production, the aim of these studies was to determine whether cyclooxygenase blockade with indomethacin (Indo, 10 μM) would prevent the beneficial actions of BK-PC. Mesenteries in this group were treated as described for group 5, except that indomethacin (10 μM; Sigma), rather than HOE-140, was topically applied during the period of BK-PC.

**Group 10: BK-PC + I/R and Indo (n = 5).** Mesenteries in this group were treated as described for group 8 except that indomethacin (10 μM), rather than L-NAME, was topically applied to the mesentery throughout reperfusion after prolonged ischemia to determine whether postischemic production of prostaglandins serves as a mediator of BK-PC.

Because bradykinin has been shown to promote the activation of PKC in endothelial cells via stimulation of B$_2$ receptors (7, 15, 47) and PKC-dependent phosphorylation has been implicated in the beneficial actions of IPC (3, 18, 20), the studies outlined for groups 11–14 are directed at determining whether this kinase participates in the anti-inflammatory effects of BK-PC. This question was interrogated by use of antagonists that are structurally unrelated, interfere with PKC activity via distinct mechanisms, and exhibit differential specificities for the different classes of PKC isotypes.

**Group 11: BK-PC (chelerythrine) + I/R (n = 7).** Mesenteries in this group were treated as described for group 5 except that chelerythrine (1 μM; Sigma and Calbiochem) rather than HOE-140 was topically applied during BK-PC to determine whether PKC activation during the period of BK-PC played an important role in its protective effects. Chelerythrine acts to inhibit all PKC isotypes by blocking the substrate recognition domain (19).

**Group 12: BK-PC + I (chelerythrine)/R (n = 7).** To determine whether PKC activation during prolonged ischemia contributed to the beneficial effects of BK-PC, mesenteries in this group were treated with chelerythrine (1 μM) by topical application during the period of prolonged (20 min) ischemia. As a corollary to these studies, a subset of experiments (n = 5) was performed to determine whether chelerythrine (1 μM), administered during the period of prolonged ischemia, would exacerbate the inflammatory responses to I/R in the absence of BK-PC. Mesenteries in this group were treated as described for group 12 except that BK-PC was not produced.

**Group 13: BK-PC + I (bisindolylmaleimide I)/R (n = 6).** Mesenteries in this group were treated as described for group 12 except that bisindolylmaleimide I (10 nM; Calbiochem), rather than chelerythrine, was used to inhibit PKC. Bisindolylmaleimide I is a pan PKC inhibitor that interferes with the ATP-binding site (46).

**Group 14: BK-PC + I (Go-6976)/R (n = 6).** To begin to explore the class of PKC isotypes involved in BK-PC, we used Go-6976, a recently described PKC antagonist that exhibits a high degree of specificity for the classical isotypes PKC-α and PKC-β1 but demonstrates no inhibitory activity toward PKC-δ or PKC-ε, even at millimolar concentrations (27). Mesenteries in this group were treated as described for group 12 except that Go-6976 (10 nM; Calbiochem), rather than chelerythrine, was topically applied during prolonged ischemia.
Because our work suggested that BK-PC prevented post-ischemic inflammatory responses by a mechanism that involved PKC-mediated NO production during I/R, we hypothesized that NO levels in venous blood draining the ischemic region would be reduced during reperfusion on nonpreconditioned mesenteries but maintained in mesenteries exposed to BK-PC before I/R. This latter effect would be abolished by treatment with the PKC antagonist during prolonged ischemia. To address this postulate, we measured plasma nitrite/nitrate in venous blood samples obtained from a catheter that was placed in the mesenteric vein downstream from the mesenteric arcade vessels draining the region of tissue subjected to ischemia. Blood was sampled during baseline conditions and after 60 min of reperfusion in animals subjected to the protocols described for groups 2 (I/R alone, n = 5), 4 (BK-PC + I/R, n = 5), and 12 (BK-PC + 1 (chelerythrine)/I/R, n = 5) above.

Because bradykinin has been implicated in the anti-infarct effects of cardiac IPC in some models (4, 33, 40, 48), we sought to determine whether the peptide plays an important role in the anti-inflammatory effects of IPC in the final two groups of experiments.

**Group 15:** IPC + I/R (n = 7). Mesenteries in this group were preconditioned with 5 min of ischemia followed by 10 min of reperfusion before I/R (i.e., IPC) with all variables recorded at the time points equivalent to those described for BK-PC + I/R (group 4).

**Group 16:** IPC (HOE-140) + I/R (n = 7). To determine if the effects of IPC were initiated via B₂ receptor activation, HOE-140 (1 μM) was topically applied to the mesentery beginning 5 min before IPC was initiated and was maintained for 15 min. In a subset of these experiments (n = 4), HOE-140 (1 μM) was topically applied to the mesentery throughout I/R to determine whether B₂ receptor activation occurred after IPC.

**Statistical Analysis**

The data were analyzed with standard statistical analyses, i.e., ANOVA with Scheffé’s (post hoc) test for multiple comparisons. All values are reported as means ± SE. Statistical significance was set at P < 0.05.

**RESULTS**

Figure 2 shows the numbers of rolling (A), adherent (B), and emigrated (C) leukocytes recorded during the baseline and preischemic periods and after reperfusion in mesenteries subjected to I/R alone, BK-PC alone, and BK-PC plus I/R or at corresponding time points in the nonischemic, time control (sham) group. Very few rolling, adherent, or emigrated leukocytes were observed under baseline conditions or during the preischemic period in any of the groups. The number of rolling, adherent, or emigrated leukocytes did not change over the course of the time control (sham) or BK-PC alone (no I/R) experiments. However, I/R was associated with a marked increase in the number of rolling, adherent, and emigrated leukocytes, effects that were completely prevented by BK-PC.

As depicted in Fig. 3, the inhibitory effects of BK-PC on postischemic leukocyte recruitment were antagonized by coadministration of the bradykinin B₂ receptor antagonist HOE-140 but not by coincident B₁ receptor blockade. Because activation of bradykinin B₂ receptors has been shown to promote the release of endothelium-derived NO and prostacyclin, two potent inhibitors of leukocyte adhesion, mesenteries were treated with either NO synthase (L-NAME) or cyclooxygenase-2 (COX-2) inhibitors.
Exposing mesenteries to the PKC antagonist chelerythrine during prolonged ischemia (Fig. 4) but not during the period of BK-PC (data not shown) prevented the beneficial actions of BK-PC on postischemic leuko...

Fig. 4. Effect of topically applied protein kinase C antagonists [chelerythrine (Chel); bisindolylmaleimide I (Bis); or Go-6976] during the period of prolonged ischemia on the number of rolling (left), adherent (middle), and emigrated (right) leukocytes determined after 30 min (open bars) and 60 min (filled bars) of reperfusion following 20 min of ischemia (I/R) compared with values obtained at corresponding time points in continuously perfused, nonpreconditioned mesenteries (Sham) or mesenteries subjected to I/R alone or BK-PC + I/R in the absence of any other intervention. The BK in parentheses serves to underscore the fact that the various antagonists were topically applied to the mesentery only during the period of prolonged ischemia. *Values that are statistically different from corresponding value in the Sham group at $P < 0.05$. #Values that are statistically different from the corresponding value in the group subjected to BK-PC + I/R at $P < 0.05$. 

Fig. 3. Effect of topically applied bradykinin B₁ receptor (+B1 Block), B₂ receptor (+B2 Block), nitric oxide synthase (+NOS Block), or cyclooxygenase (+COX Block) antagonists during the period of BK-PC on the number of rolling (left), adherent (middle), and emigrated (right) leukocytes determined after 30 min (open bars) and 60 min (filled bars) of reperfusion following 20 min of ischemia (I/R) compared with values obtained at corresponding time points of continuously perfused, nonpreconditioned mesenteries (Sham) or mesenteries subjected to I/R alone or BK-PC + I/R in the absence of any other intervention. The BK in parentheses serves to underscore the fact that the various antagonists were topically applied to the mesentery only during the period of BK-PC. *Values that are statistically different from corresponding value in the Sham group at $P < 0.05$. #Values that are statistically different from the corresponding value in the group subjected to BK-PC + I/R at $P < 0.05$. 

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Fig. 3. Effect of topically applied bradykinin B₁ receptor (+B1 Block), B₂ receptor (+B2 Block), nitric oxide synthase (+NOS Block), or cyclooxygenase (+COX Block) antagonists during the period of BK-PC on the number of rolling (left), adherent (middle), and emigrated (right) leukocytes determined after 30 min (open bars) and 60 min (filled bars) of reperfusion following 20 min of ischemia (I/R) compared with values obtained at corresponding time points of continuously perfused, nonpreconditioned mesenteries (Sham) or mesenteries subjected to I/R alone or BK-PC + I/R in the absence of any other intervention. The BK in parentheses serves to underscore the fact that the various antagonists were topically applied to the mesentery only during the period of BK-PC. *Values that are statistically different from corresponding value in the Sham group at $P < 0.05$. #Values that are statistically different from the corresponding value in the group subjected to BK-PC + I/R at $P < 0.05$. 

Fig. 4. Effect of topically applied protein kinase C antagonists [chelerythrine (Chel); bisindolylmaleimide I (Bis); or Go-6976] during the period of prolonged ischemia on the number of rolling (left), adherent (middle), and emigrated (right) leukocytes determined after 30 min (open bars) and 60 min (filled bars) of reperfusion following 20 min of ischemia (I/R) compared with values obtained at corresponding time points in continuously perfused, nonpreconditioned mesenteries (Sham) or in mesenteries subjected to I/R alone or BK-PC + I/R (BK-PC + I/R) in the absence of any other intervention. The BK in parentheses serves to underscore the fact that the various antagonists were topically applied to the mesentery only during the period of prolonged ischemia. *Values that are statistically different from corresponding value in the Sham group at $P < 0.05$. #Values that are statistically different from the corresponding value in the group subjected to BK-PC + I/R at $P < 0.05$.
cyte recruitment. On the other hand, superfusing the mesentery with chelerythrine in the absence of BK-PC did not alter the response to I/R (data not shown), indicating that PKC antagonism alone does not exacerbate the effects of I/R. These observations suggest that PKC activation is a signaling event that occurs downstream from B2 receptor activation during BK-PC. This notion is supported by the fact that the anti-inflammatory effects of BK-PC were also abolished by topical application of another PKC antagonist, bisindolylmaleimide I, during the prolonged period of ischemia (Fig. 4).

Although the aforementioned observations are consistent with a role for PKC in the signaling cascade induced by BK-PC, they do not provide insight regarding the class of PKC isoforms involved in the anti-inflammatory effects because both chelerythrine and bisindolylmaleimide I block the activity of all PKC isotypes. To determine whether the classic or novel isoform(s) of PKC were activated over the prolonged ischemic period, we treated mesenteries with Go-6976, a highly specific antagonist of the classical isotypes PKC-α and PKC-βI that exhibits no activity toward the novel isotypes PKC-δ or PKC-ε, even at millimolar concentrations (27). In contrast to the marked effect of chelerythrine or bisindolylmaleimide I, treatment with Go-6976 did not alter the effect of BK-PC to prevent postischemic leukocyte rolling, adhesion, or emigration (Fig. 4). Taken together with the data presented in Fig. 3, these results indicate that BK-PC inhibits postischemic leukocyte recruitment by a mechanism that is initiated by stimulation of B2 receptors during the period of bradykinin superfusion and is dependent on activation of one or more of the novel isoforms of PKC during prolonged ischemia.

Figure 5 shows the results of experiments designed to examine the role of NO synthase or cyclooxygenase-derived products as effectors of BK-PC. Superfusion of bradykinin preconditioned mesenteries with indomethacin during reperfusion after prolonged ischemia did not affect the protective actions of BK-PC on postischemic leukocyte rolling, adhesion, or emigration. However, the anti-inflammatory effects of BK-PC were attenuated by topical application of NO synthase or guanylate cyclase inhibitors during reperfusion after prolonged ischemia. These observations indicate that NO produced during reperfusion after prolonged ischemia may serve to mediate the anti-inflammatory effects of BK-PC that were initiated by B2 receptor-dependent PKC activation during the periods of BK-PC and prolonged ischemia.

The data presented above indicate that IPC prevents I/R-induced leukocyte recruitment by a mechanism that involves PKC-mediated NO production during I/R. To confirm this hypothesis, we measured the plasma nitrite/nitrate concentration in venous blood obtained from mesenteric arcade vessels draining the ischemic region of the bowel, which provides a useful index of NO production during the ischemic period. The plasma nitrite/nitrate concentration in venous blood obtained from mesenteric arcade vessels draining the ischemic region of the bowel, which provides a useful index of
local NO production (Fig. 6). Plasma nitrite/nitrate was decreased significantly after I/R. BK-PC maintained plasma NO during reperfusion at preischemic levels, effects that were prevented by administration of chelerythrine during I/R. These results suggest that IPC maintains endothelial NO production via a PKC-dependent mechanism.

Changes in venular albumin leakage in the different groups are shown in Fig. 7. I/R significantly increased venular albumin leakage compared with corresponding time points in the sham or BK-PC alone (no I/R) groups, an effect that was abolished largely by BK-PC. The ability of BK-PC to preserve microvascular barrier function was not affected by topical application of Des-Arg HOE-140, l-NAME, indomethacin, or chelerythrine during the period of preconditioning ischemia. However, coadministration of HOE-140 with bradykinin or topical application of chelerythrine or bisindolylmaleimide (pan PKC inhibitors), but not Go-6976 (an antagonist specific for the classical PKC isoforms), to the mesentery during prolonged ischemia abolished the inhibitory effects of BK-PC on venular protein leakage, as did l-NAME and ODQ (but not indomethacin) when administered during reperfusion.

Like BK-PC, brief exposure (5 min) of the mesentery to ischemia (IPC) before prolonged I/R markedly inhibited postischemic leukocyte recruitment and venular albumin leakage (Fig. 8), as we (1, 18, 20) and others (22) had shown earlier. However, in contrast to BK-PC, the protective effects afforded by IPC were not antagonized by treatment with HOE-140 during IPC or I/R (Fig. 8). These latter results suggest that, while exogenous bradykinin can be used to pharmacologically precondition the mesentery and render it resistant to the deleterious effects of prolonged ischemia, the endogenous peptide does not appear to participate in the protective actions of IPC.

Venular diameters were not different in any of the groups under baseline conditions, averaging 30 μm in all groups, and were not modified by any of the
treatments or by the I/R insult. Changes in wall SR in the different groups are shown in Fig. 9. I/R significantly decreased wall SR, an effect that was prevented by BK-PC. This effect of BK-PC was antagonized by coadministration of HOE-140 with bradykinin, by topical application of chelerythrine or bisindolylmaleimide I during prolonged ischemia, and by superfusion with L-NAME or ODQ during reperfusion, but not by any of the other treatment protocols. The postischemic reduction in wall SR was also prevented by IPC. However, this effect of IPC was not prevented by HOE-140.

**DISCUSSION**

IPC renders tissues remarkably resistant to the deleterious effects of prolonged I/R. The beneficial actions of IPC were originally reported in the canine heart (30) and were subsequently shown to apply in a variety of organs and species (3, 20). Because IPC has been shown to exert such powerful protective actions in so many organs, a major research effort has been directed at elucidating the cellular mechanisms underlying this phenomenon with the goal of developing new and more efficacious treatments for I/R. This work has led to the development of the concept that a variety of endogenous substances such as adenosine, catecholamines, and/or bradykinin are released during the period of preconditioning ischemia and initiate the protective actions of IPC by subsequently inducing isoform-selective PKC (δ and ε) translocation via activation of specific receptor subtypes (A1/A3, α1, and B2 receptors, respectively) for each agent (3, 20, 33).
Although the relative importance of adenosine, catecholamines, or bradykinin as initiating factors in the myocardial protection induced by IPC varies with the species examined, it is now clear that exogenous administration of these agents can be used to pharmacologically precondition hearts to resist the deleterious effects of I/R (3, 20, 33). However, comparatively less attention has been devoted to determining whether pharmacological preconditioning with agents such as bradykinin might prove efficacious in peripheral tissues. In addition, the downstream effector mechanisms that are initiated by BK-PC and mediate its protective actions remain unclear. Moreover, because the beneficial effects of IPC are observed in isolated hearts perfused with crystalloid solutions and in more intact blood-perfused preparations, prevention of leukocyte recruitment by IPC as a potential explanation for the beneficial effects of this phenomenon has been largely ignored. However, we recently demonstrated that IPC completely prevents postischemic leukocyte recruitment by a mechanism that is initiated by stimulation of adenosine receptors and involves PKC activation (1, 18, 20). These are important observations because they indicate that, in addition to protecting against the deleterious effects of ischemia per se, the ability of IPC to induce cellular changes that also prevent leukocyte recruitment to ischemic tissues may limit the reperfusion component of I/R injury, which is primarily leukocyte dependent (1, 18, 20, 32). In view of these considerations, a major goal of the present studies was to determine whether exogenous bradykinin could be used to pharmacologically precondition tissues and prevent leukocyte/endothelial cell adhesive interactions and protein leakage in single postcapillary venules of the mesenteric microcirculation. In addition, we sought to determine whether endogenous bradykinin plays a role as an initiating factor in IPC in the small bowel.

The results of the present study indicate for the first time that BK-PC exerts powerful anti-inflammatory effects, completely preventing I/R-induced leukocyte rolling, adherence, and emigration while maintaining postischemic endothelial barrier function. The protective actions of BK-PC in I/R might be viewed as surprising, given the well-known proinflammatory actions of the peptide. Indeed, bradykinin has been reported to promote the release of chemotactic agents and induces adhesion molecule expression, leukocyte activation and sequestration, and the formation of interendothelial gaps and protein extravasation in postcapillary venules (2, 6, 8, 13, 28, 37–39, 41, 45; unpublished observations). However, we recently reported that the effects of bradykinin on leukocyte sequestration and venular protein leakage are divergent depending on its concentration, that the protective actions of BK-PC noted in our earlier report (42) were for administration of this agent immediately after prolonged ischemia, not as a preconditioning stimulus, as in the present study.

Our findings that coadministration of HOE-140, but not Des-Arg HOE-140, with bradykinin abolished the
beneficial actions of BK-PC indicates that the beneficial actions of preconditioning with the peptide are initiated by activation of B<sub>2</sub> receptors. Similar findings have been reported regarding the anti-infarct effects of BK-PC in the heart (5, 16, 44). Because bradykinin B<sub>2</sub> receptors are coupled to endothelial NO synthase and cyclooxygenase (15, 24, 26), we hypothesized that the protective actions of BK-PC may be initiated by the liberation of NO or prostaglandins such as prostacyclin. However, this seems unlikely because neither NO synthase nor cyclooxygenase inhibition during the period of BK-PC prevented its anti-inflammatory actions.

In addition to stimulating NO synthase and cyclooxygenase, bradykinin B<sub>2</sub> receptor engagement may also stimulate the release of EDHF (11, 12, 14, 36). Indeed, recent evidence suggests that formation of EDHF, which appears to be an endothelium-derived, cytochrome P-450-derived metabolite of arachidonic acid (perhaps an epoxyeicosatrienoic acid), plays a major role in the vasodilator response to bradykinin (14, 36). Although far from understood, the bulk of available evidence supports the concept that bradykinin-induced EDHF release causes hyperpolarization and thus vasodilation of vascular smooth muscle by a mechanism that is initiated by endothelial cell hyperpolarization involving charbydotoxin- and apamin-sensitive potassium channels. This bradykinin-stimulated hyperpolarization in endothelial cells may spread electrotonically to the underlying vascular smooth muscle layer via connexin 40 in myoendothelial gap junctions (11, 12). Although intriguing, we have not evaluated the role of EDHF as an initiating factor in BK-PC. However, future examination of this possibility is clearly warranted given the fact that EDHF serves as an essential component of bradykinin-induced vasomotor responses.

Bradykinin has been shown to promote PKC activation in endothelial cells via stimulation of B<sub>2</sub> receptors (7, 15, 47). Because agonist-induced PKC translocation and activation can be relatively slow (3), we hypothesized that if bradykinin-induced PKC activation contributed to the anti-inflammatory actions of BK-PC, its effects would not become manifest until the prolonged ischemic period. Indeed, Baines and co-workers (3) were among the first to propose that the delay associated with PKC translocation accounted for the memory aspect of IPC. To address this postulate, we superfused the mesentery with two highly specific, structurally unrelated, and mechanistically different PKC antagonists, either chelerythrine (blocks the substrate recognition domain; see Ref. 19) or bisindolylmaleimide (interferes with the ATP-binding site; see Ref. 46), during the period of BK-PC or during prolonged ischemia. The PKC antagonists prevented the beneficial actions of BK-PC when applied topically to the mesentery during prolonged ischemia but not when superfused during the period of BK-PC. These observations are consistent with the concept that BK-PC exerts its anti-inflammatory effects by a mechanism that is initiated by stimulation of B<sub>2</sub> receptors during the period of BK-PC and is dependent on PKC activation during the period of prolonged ischemia.

Although the aforementioned observations are consistent with a role for PKC in the signaling cascade induced by BK-PC, they do not provide insight regarding the class of PKC isoforms involved in its anti-inflammatory effects because both chelerythrine and bisindolylmaleimide I block the activity of all PKC isoforms. Indeed, postulating a role for PKC in the anti-inflammatory effects of BK-PC may be viewed as troublesome given its demonstrated role in leukocyte activation and microvascular barrier disruption (21). This apparent discrepancy may be resolved if one considers the possibility that these divergent effects may be due to isoform-specific PKC activation in the different conditions. At least 11 different subtypes of PKC have been identified, which have been subdivided into three major classes (31). Of these, the isoforms in the classical and novel classes have received the most attention in terms of their regulation and role in different physiological processes. The conventional or classical isoforms (α, βI, βII, and γ) require calcium for their activity (31). On the other hand, the novel isoforms (δ, ε, η, and θ) lack the calcium-binding domain and are thus differentially regulated compared with the classical isoforms (31). A number of recent reports indicate that IPC induces selective translocation and activation of the novel isoforms PKC-δ and PKC-ε in the heart (29, 35), which then act to phosphorylate downstream molecular targets that mediate IPC.

One limitation in establishing a role for a particular PKC isoform(s) relates to the lack of inhibitors that are specific for the different isoforms. However, Go-6976 is a recently described PKC antagonist that exhibits a high degree of specificity for the classical isoforms PKC-α and PKC-βI but demonstrates no inhibitory activity toward the novel isoforms PKC-δ or PKC-ε, even at millimolar concentrations (27). Based on this specificity and the suggested role for the novel isoforms in cardiac IPC, we hypothesized that treatment with Go-6976 would not influence the effectiveness of BK-PC in our model. In contrast to the marked effects of chelerythrine or bisindolylmaleimide I, treatment with Go-6976 did not alter the effect of BK-PC to prevent postischemic venular protein leakage or leukocyte rolling, adhesion, and emigration. Thus our results are consistent with the concept that the beneficial actions of BK-PC involve isoforms other than the classical isoforms, most probably PKC-δ and/or PKC-ε.

We do not believe that the lack of effect of Go-6976 was due to use of an insufficient dose because we have demonstrated that this compound completely prevents hydrogen peroxide-induced endothelial barrier disruption (21) and the increased leukocyte adhesion induced by I/R alone (no preconditioning; see Ref. 18) when administered at the concentration used in the present study (10 nM). It is important to note that, in the latter study, we used the same experimental model (rat mesentery subjected to 20 min of ischemia and 60 min of reperfusion) and dose as employed in the present study but administered Go-6976 during reperfusion. Taken
together, these results suggest that the conventional PKC isotypes mediate the proinflammatory effects of I/R alone (no preconditioning), whereas the novel isoforms play a critical role in the prevention of postischemic leukocyte/endothelial cell interactions in preconditioned mesenteries.

Although our results and the work of others (16, 33) are consistent with the notion that bradykinin B_{2} receptor-dependent activation of PKC plays an important role in initiating the beneficial actions of BK-PC, identification of the end effectors of this phenomenon remain unclear. However, several lines of evidence suggest that NO may fulfill this role in BK-PC. First, basal production of NO by the endothelium serves as an important regulator of leukocyte adhesion and microvascular barrier function in the small bowel (9). Second, NO is a potent antiadhesive agent that limits the expression of P-selectin, an endothelial glycoprotein that participates in leukocyte rolling (9). Third, provision of exogenous NO during reperfusion of ischemic intestine prevents postischemic leukocyte sequestration and microvascular barrier disruption (9). Fourth, PKC-ε activation has been shown to upregulate NO synthase activity in human endothelium (25). Fifth, I/R is associated with a 50% reduction in NO synthase activity and a 98% reduction in NO production in the ischemic region of the intestine (23, 42). Similarly, prostacyclin has also been shown to exert antiadhesive effects. These observations, when coupled with our finding that L-NAME, but not indomethacin, which administered during reperfusion after prolonged ischemia, attenuated the beneficial actions of BK-PC, suggest that preservation of postischemic NO levels may serve as an effector of this phenomenon in the mesentery. However, cyclooxygenase-derived products do not appear to play a role.

The concept that NO serves as a mediator of BK-PC is supported by the changes in plasma nitrite/nitrate noted in the present study, which provide a useful index of NO production (23). As shown in Fig. 6, I/R was associated with a significant reduction in plasma nitrite/nitrate in samples of venous blood draining the ischemic region. BK-PC maintained plasma NO during reperfusion at preischemic levels, an effect that was prevented by administration of chelerythrine during prolonged ischemia. Thus our results suggest that BK-PC maintains postischemic endothelial NO production during reperfusion via a PKC-dependent mechanism.

Our results clearly indicate that NO plays an important role in the anti-inflammatory effects of BK-PC. However, the molecular events that occur downstream from BK-PC-induced NO production are unclear. NO is a physiologically relevant scavenger of superoxide, an oxidant that has been implicated in the formation of chemotactic stimuli in the small intestine and the expression of adhesive ligands by endothelial cells (9). Thus it is possible that preservation of postischemic NO levels by BK-PC may prevent the pathological sequelae related to postischemic superoxide production. However, this explanation seems unlikely in view of our observation that guanylate cyclase inhibition was as effective as NO synthase blockade in preventing the protective actions of BK-PC, a result that suggests that the protective effects of NO are due to guanylate cyclase activation and subsequent formation of cGMP rather than superoxide scavenging by NO. Given our observation that BK-PC completely prevents postischemic leukocyte rolling, it is tempting to speculate that the preservation in NO levels exerts anti-inflammatory effects via modulation of P-selectin expression. This adhesive ligand has been shown to play an important role in postischemic leukocyte rolling in the mesentery, and its expression is modulated by NO (9, 32).

Although bradykinin does not stimulate neutrophilic NADPH oxidase (and thus superoxide production) or phospholipase D (8), the peptide has been shown to increase leukocyte adhesion (our earlier work and Ref. 41; unpublished observations) and cause elastase release (6) and stimulation of several signal transduction pathways (e.g., phosphoinositide phosphorylation) associated with polymorphonuclear leukocyte activation, with the latter being inhibitable by PKC antagonists (8). Thus it is possible that topical application of bradykinin over the mesentery could activate leukocytes during their transit through the superfused region. As a consequence, bradykinin-activated leukocytes could become entrapped in the lung, thereby producing a leukopenia that would limit the number of leukocytes available for recruitment to the ischemic region of the mesentery after reperfusion. Such a scenario would be unrelated to preconditioning. However, this explanation is not likely because the number of circulating leukocytes in blood samples obtained during the control period and at the end of the experimental protocol in animals subjected to BK-PC followed by ischemia-reperfusion were the same.

The likelihood for a leukocyte to adhere to venular endothelium depends on the balance between adhesive forces generated by the leukocyte and the endothelium and the hydrodynamic dispersal forces (e.g., blood flow velocity and SR) that tend to sweep leukocytes away from the vascular wall (34). Because BK-PC and IPC were both effective in preventing the reductions in wall SR induced by I/R, it is possible that maintenance of hydrodynamic dispersal forces by these two forms of preconditioning contributes to the prevention of postischemic leukocyte adhesion. This explanation is unlikely to fully account for anti-inflammatory effects of BK-PC because much larger decreases in wall SR must occur for significant leukocyte adhesion to occur (34). Although the effect of BK-PC on wall SR was prevented by concomitant B_{2}receptor blockade, coincident NO synthase or cyclooxygenase inhibition was without effect. On the other hand, the ability of BK-PC to prevent the postischemic reductions in venular wall SR was abolished by administration of the PKC antagonists chelerythrine or bisindolylmaleimide I during prolonged ischemia and by NO synthase and guanylate cyclase blockade during reperfusion. Thus it appears that BK-PC prevents postischemic reductions in wall
SR by a mechanism that is initiated by stimulation of B2 receptors during the period of BK-PC, involves PKC activation during prolonged ischemia, and is mediated by NO during reperfusion.

We recently obtained evidence indicating that IPC prevents postischemic leukocyte rolling, adhesion, and emigration and maintains endothelial barrier function by a mechanism that is dependent on PKC activation (1, 18, 20). Given the similarities between the anti-inflammatory effects of IPC and BK-PC and the role for PKC in each, we postulated that endogenous bradykinin may be involved in the anti-inflammatory actions of IPC. However, the IPC-induced inhibition of postischemic leukocyte recruitment and venular protein leakage was not blocked by HOE-140, a treatment that effectively antagonized BK-PC. Thus, although exogenous bradykinin can be used to induce preconditioning, endogenous bradykinin does not appear to play a role in IPC. Similar results have been reported for rat and rabbit hearts (5, 9, 44).

In summary, our data indicate that BK-PC prevents postischemic leukocyte rolling, adhesion, and emigration and maintains endothelial barrier function by a mechanism that involves preservation of NO production during I/R secondary to B2 receptor-dependent activation of specific PKC isoforms. Indeed, BK-PC was more efficacious in preventing postischemic leukocyte/endothelial cell adhesive interactions and microvascular barrier disruption as IPC. Although our results indicate that exogenous bradykinin can be used to pharmacologically precondition the mesentery to resist the deleterious proinflammatory effects of prolonged ischemia and reperfusion, it does not appear that the endogenous peptide plays a major role in the beneficial anti-inflammatory effects of IPC in the rat mesenteric microcirculation.

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REFERENCES


24. McPherson M, Vorwerk W, and McLaughlin J. Bradykinin increases intracellular calcium by 10.220.33.3 on September 21, 2017 http://ajpheart.physiology.org/ Downloaded from
