Bradykinin prevents postischemic leukocyte adhesion and emigration and attenuates microvascular barrier disruption

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

Shigematsu, Sakui, Shuji Ishida, Dean C. Gute, and Ronald J. Korthuis. Bradykinin prevents postischemic leukocyte adhesion and emigration and attenuates microvascular barrier disruption. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H161–H171, 1999.—Although a number of recent reports indicate that bradykinin attenuates ischemia-reperfusion (I/R)-induced tissue injury, the mechanisms underlying its protective actions are not fully understood. However, because bradykinin induces endothelial nitric oxide (NO) production and NO donors have been shown to attenuate postischemic leukocyte adhesion, endothelial barrier disruption, and tissue injury, we hypothesized that bradykinin may act to reduce I/R-induced tissue injury by preventing leukocyte recruitment and preserving microvascular barrier function. To address this postulate, we used intravital videomicroscopic approaches to quantify leukocyte-endothelial cell interactions and microvascular barrier function in single postcapillary venules in the rat mesentery. Reperfusion after 20 min of ischemia significantly decreased wall shear rate and leukocyte rolling velocity, increased the number of rolling, adherent, and emigrated leukocytes, and disrupted the microvascular barrier as evidenced by enhanced venular albumin leakage. Superfusion of the mesentery with bradykinin (10 nM) during I/R significantly reduced these deleterious effects of I/R. Although these inhibitory effects of bradykinin were not affected by cyclooxygenase blockade with indomethacin (10 µM), coadministration with NO synthase inhibitor N-nitro-L-arginine methyl ester (10 µM) or bradykinin B2-receptor blocker (HOE-140, 1 µM) antagonized the protective actions of bradykinin. Plasma NO concentration was measured in the mesenteric vein and was significantly decreased after I/R, an effect that was prevented by bradykinin treatment. These results indicate that bradykinin attenuates I/R-induced leukocyte recruitment and microvascular dysfunction by a mechanism that involves bradykinin B2-receptor-dependent NO production.

Bradykinin is a vasoactive, proinflammatory neuropeptide that induces relaxation of vascular smooth muscle in arteries and arterioles (26) and promotes adhesion molecule expression (33), leukocyte infiltration (16, 35, 38), and the formation of interendothelial gaps and protein extravasation in postcapillary venules (8, 29). These proinflammatory effects appear to involve the generation of nitric oxide (NO) (6), prostaglandin synthesis (2, 6), release of cytokines (32), and production of platelet-activating factor (PAF) (30, 34, 35). On the other hand, the results of a number of recent studies indicate that administration of bradykinin at comparatively low dosages attenuates ischemia-reperfusion (I/R) injury. For example, intracoronary administration of bradykinin reduces myocardial infarct size after reperfusion in canine (23, 28), rabbit (13), and pig (43) hearts. This infarct-limiting effect of bradykinin was antagonized by the pretreatment with HOE-140, a bradykinin B2-receptor blocker (13, 25, 28). Administration of bradykinin has also been reported to improve postischemic ventricular function (23, 50), an effect that was antagonized by pretreatment with the NO synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME) or cyclooxygenase blockade with indomethacin (50). Bradykinin also reduces the incidence of ventricular fibrillation after I/R (25, 44, 45). This antiarrhythmic effect of bradykinin was blocked by HOE-140 (25) or L-NAME (44). Finally, I/R injury is also attenuated in preparations treated with angiotensin-converting enzyme inhibitors that act to prevent the degradation of endogenous bradykinin by kininase II (24, 36). That bradykinin exerts such powerful protective effects in I/R is surprising because of its well-known proinflammatory actions, especially because I/R is now recognized as one form of acute inflammation in which activated leukocytes play a key role (14).

Although the aforementioned studies suggest that bradykinin may exert protective effects in postischemic tissues by a B2-receptor-activated NO-dependent pathway, the downstream effector of bradykinin-induced protection is unclear. Moreover, it is not clear why this vasoactive neuropeptide exerts proinflammatory effects in some studies but is protective in the setting of I/R. We hypothesized that bradykinin may act to reduce postischemic tissue injury by preventing I/R-induced leukocyte adhesion and that this effect was mediated by bradykinin-induced formation of NO or prostacyclin. This postulate was based on the following observations. First, leukocyte adhesion is a prerequisite to the production of postischemic tissue dysfunction in a variety of models (14, 27, 40). Second, NO and prostacyclin are potent antiadhesive agents (1, 3, 21). Third, administration of NO donors or prostacyclin attenuates I/R-induced leukocyte infiltration and tissue injury (17), and bradykinin induces the formation of NO and prostacyclin (2, 6). Furthermore, we hypothesized that the effects of bradykinin on leukocyte adhesion and microvascular barrier function might be dose dependent, with low doses being anti-inflammatory via the release of NO and/or prostacyclin, whereas higher doses induce leukocyte adhesion and venular protein leakage by a PAF-dependent mechanism. This latter notion was addressed in a companion study (38) and is...
Intravital Microscopy

performed to allow exteriorization and intravital microscopic
left jugular vein was also cannulated for drug administration.
eter. Systemic blood pressure was continuously recorded with
otomy was performed to facilitate breathing during the
When a surgical level of anesthesia was attained, a trache-
experiment. The animals were initially anesthetized by intra-
concentration of 10 nM.
maximal bradykinin-induced increase in blood flow
adhesion (30, 34, 35). The concentration of bradykinin
based on the observations that bradykinin can induce
H162 BRADYKININ AND POSTISCHEMIC MICROVASCULAR DYSFUNCTION

METHODS

Surgical Procedure

Male Sprague-Dawley rats (200–250 g) were maintained
on a purified laboratory diet and fasted for 24 h before
the experiment. The animals were initially anesthetized by intra-
one study was selected on the basis of results
in the companion study (38), in which a
maximal bradykinin-induced increase in blood flow
with no change in leukocyte adhesion was noted at a
concentration of 10 nM.

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 main-
tained 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 tempera-
tures were monitored with an electrotetherometer (4000A,
Yellow Springs Instruments). Body temperature was kept
between 36.5 and 37.5°C with an infrared heat lamp. The
board was mounted onto the stage of an inverted microscope
(TMD-25, 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 onto 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-910, 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). Centerline
red blood cell velocity was measured with an optical Doppler
velocimeter (Microcirculation Research Institute) that was
calibrated against a rotating glass disk coated with red blood
cells. Venular blood flow was calculated from the product of
mean red blood cell velocity \(V_{\text{mean}}\) and microvascular cross-sectional area, with cylindrical
graphics assumed. Venular wall shear rate (SR) was calcu-
larized from the Newtonian definition: \(SR = 8V_{\text{mean}}/D_v\).

The number of rolling, stationary (firmly adherent), and
emigrated leukocytes was determined off-line during playback
of videotaped images. A leukocyte was considered to
firmly adhere to venular endothelial if it remained station-
ary for 30 s or longer (11). Adherent leukocytes were quanti-
ified 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
white blood cells that moved at a velocity significantly 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 is expressed
as micrometers per second. The flux of rolling leukocytes was
measured as those white blood cells 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 adminis-
tered intravenously to the animals 15 min before the baseline
recording (19, 20). Fluorescence intensity (excitation wave-
length, 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 fluores-
ce intensity of the venule under study \(I_v\), the fluores-
cence intensity of contiguous perivascular interstitium within
10 to 50 µm of the venular wall \(I_v\), and the background
fluorescence \(I_b\) 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 \((I_v - I_b)/(I_v - I_b))\).

Experimental Protocols

A schematic illustration of the experimental protocols is
shown in Fig. 1. After a stabilization period of 30 min, images
from the venule of interest were recorded on videotape for 10
min (baseline recording). Thereafter, the specific drug(s) to be
tested was administered, and 5 min later a preischemic
recording \(I/R\) but in preparations not subjected to I/R.

Group 1. Control.

Group 2. Experimental Protocols

A schematic illustration of the experimental protocols is
shown in Fig. 1. After a stabilization period of 30 min, images
from the venule of interest were recorded on videotape for 10
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recording \(I/R\) but in preparations not subjected to I/R.
Group 2. Bradykinin alone. To determine the effect of bradykinin superfusion alone, mesenteries in this group (n = 6) were superfused with BBS containing bradykinin [10 nM; Research Biochemicals International (RBI)] in the absence of I/R.

Group 3. L-NAME alone. After we obtained baseline measurements, mesenteries in this group (n = 8) were superfused with BBS containing L-NAME (10 µM; Sigma) throughout the remainder of the protocol and were not subjected to I/R.

Group 4. I/R alone. Mesenteries in this group (n = 6) were exposed to I/R superfused with BBS alone (no drug addition).

Group 5. I/R (bradykinin). To determine the effect of bradykinin on I/R-induced leukocyte adhesion and microvascular barrier function, mesenteries in this group (n = 7) were exposed to I/R in the presence of bradykinin, which was added to the superfusate to achieve a concentration of 10 nM.

Group 6. I/R (bradykinin-L-NAME). Mesenteries in this group (n = 7) were exposed to I/R in the presence of bradykinin (10 nM) and L-NAME (10 µM) via the superfusate to determine the role of NO in the protective actions of bradykinin.

Group 7. I/R (bradykinin-indomethacin). To examine the role of cyclooxygenase-derived products in the protective actions of bradykinin, mesenteries in this group (n = 6) were exposed to I/R in the presence of bradykinin (10 nM) and indomethacin (10 µM; Sigma) via the superfusate, as described for group 5.

Group 8. I/R (bradykinin-HOE-140). Mesenteries in this group (n = 6) were exposed to bradykinin (10 nM) and HOE-140 (1 µM; RBI) via the superfusate during I/R to determine whether the protective actions of bradykinin were dependent on B2-receptor activation.

Group 9. I/R (L-NAME). Because high doses of L-NAME induce leukocyte adhesion, which might exacerbate the adhesion response noted in I/R, mesenteries in this group (n = 7) were exposed to I/R in the presence of L-NAME at the relatively low dose (10 µM) we employed in these studies to ensure that such exacerbation was not present.

The concentration of bradykinin used in this study was selected on the basis of results obtained in the companion study (38), in which a maximal bradykinin-induced increase in blood flow with no change in leukocyte adhesion was noted at a concentration of 10 nM.

In a final set of experiments, plasma nitrite-to-nitrate concentration ratios were determined from venous blood samples obtained from a catheter that was placed in the mesenteric vein that emptied into the collecting mesenteric arcade vessel in the region of tissue subjected to ischemia before and after I/R in the absence or presence of bradykinin.
(10 nM). Blood was sampled during the preischemic period, 15 min after the administration of bradykinin (or after an equivalent time period in mesenteries not superfused with bradykinin), and 30 and 60 min after reperfusion.

Measurement of Plasma Nitrite and Nitrate

NO was estimated as its stable oxidative product, nitrite and nitrate (49). Plasma levels of nitrite/nitrate were measured by using a commercially available assay kit (Nitrate/Nitrite Fluorometric Assay Kit 780051; Cayman Chemical). Blood was sampled via polyethylene P-10 tubing inserted into the mesenteric vein. Plasma was obtained by centrifugation after the blood was sampled and 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).

Statistical Analysis

The data were analyzed with standard statistical analyses, i.e., ANOVA with Scheffé's (post hoc) test for multiple comparisons or paired or unpaired t-test where appropriate. All values are reported as means ± SE. Statistical significance was set at P < 0.05.

RESULTS

Changes in the number of adherent and emigrated leukocytes during the preischemic period and after reperfusion (group 4) or at corresponding time points in control (group 1, no ischemia) mesenteries or in preparations exposed to bradykinin alone (group 2, i.e., bradykinin in the absence of I/R) are shown in Fig. 2. Very few adherent or emigrated leukocytes were observed under baseline (i.e., preischemia) conditions in any of the groups. The number of adherent or emigrated leukocytes did not change over the course of the time-control experiments (group 1) or in mesenteries exposed to bradykinin alone (group 2), at the relatively low dose of the neuropeptide used in these studies, as we showed earlier (38). However, I/R was associated with a marked increase in the number of adherent and emigrated leukocytes (group 4; I/R), effects that were completely prevented by superfusion of the mesenteries with bradykinin (group 5; I/R (BK)). The inhibitory effects of bradykinin on postischemic leukocyte adhesion and emigration were antagonized by coadministration of l-NAME (group 6; I/R (BK-l-NAME)) or HOE-140 (group 8; I/R (BK-HOE-140)) with the neuropeptide. In contrast, cyclooxygenase blockade with indomethacin failed to modify the effect of bradykinin to reduce postischemic leukocyte adhesion and emigration (group 7; I/R (BK-Indo)).

Figure 3 compares the changes in the number of rolling leukocytes (Fig. 3A) and leukocyte rolling velocity (Fig. 3B) before and after I/R. These variables did not change in the time-control group (group 1) or during bradykinin superfusion in the absence of I/R (group 2). I/R significantly increased the leukocyte flux and decreased the leukocyte rolling velocity, effects that were prevented by the administration of bradykinin. The effect of bradykinin to prevent leukocyte rolling was also antagonized by coadministration with l-NAME or HOE-140 but not by indomethacin.

![Fig. 2. Average number of adherent (A) and emigrated (B) leukocytes determined during baseline conditions (preischemia) and after 30 min (rep 30 min) and 60 min (rep 60 min) of reperfusion (rep) in mesenteries subjected to I/R alone, BK superfusion during I/R (I/R (BK)), and concomitant treatment with l-NAME (I/R (BK-l-NAME)), indomethacin (I/R (BK-Indo)), or HOE-140 (I/R (BK-HOE-140)) during BK-superfusion during I/R. Control and BK refer to values obtained at equivalent time points in time-control (no ischemia) and BK superfusion-alone (no ischemia) experiments, respectively. *Values that are statistically different from preischemic value at P < 0.05.](http://ajpheart.physiology.org/)
Changes in venular albumin leakage in the different groups are presented in Fig. 4. I/R significantly increased venular albumin leakage compared with corresponding time points in the time control group. Although superfusion of the mesentery with bradykinin (10 nM) did not affect venular albumin leakage in the absence of I/R, topical application of the neuropeptide during I/R attenuated postischemic leakage. The ability of topically applied bradykinin to maintain microvascular barrier function in mesenteries exposed to I/R was prevented by coadministration with L-NAME or HOE-140 but not by indomethacin.

Average values for venular diameter and venular red blood cell velocity obtained before and after I/R are compared in Table 1. Average values for venular diameter were the same in all groups under baseline conditions and were not modified by any of the treatments administered. However, I/R was associated with a significant reduction in venular red blood cell velocity, an effect that was prevented by administration of bradykinin. Coadministration of L-NAME or HOE-140, but not indomethacin, with bradykinin antagonized the effect of the neuropeptide to restore venular erythrocyte velocity. The changes in wall shear rate in the different groups are presented in Fig. 5 and mirror the pattern discussed for venular erythrocyte velocity.

The data presented above indicate that bradykinin prevents I/R-induced leukocyte recruitment and venular albumin leakage by a mechanism that involves B2-receptor-mediated NO production. To confirm this...
Table 1. Change in venular diameter and RBC velocity in mesentery subjected to 20 min of ischemia and 60 min of reperfusion

<table>
<thead>
<tr>
<th>Venular diameter, µm</th>
<th>RBC velocity, mm/s</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Preischemia</strong></td>
</tr>
<tr>
<td>I/R</td>
<td>31.4±0.32</td>
</tr>
<tr>
<td>I/R (BK)</td>
<td>31.5±3.36</td>
</tr>
<tr>
<td>I/R (BK + L-NAME)</td>
<td>30.4±0.16</td>
</tr>
<tr>
<td>I/R (BK + Indo)</td>
<td>29.3±0.10</td>
</tr>
<tr>
<td>I/R (BK + HOE-140)</td>
<td>30.7±0.19</td>
</tr>
</tbody>
</table>

Values are means ± SE. RBC, red blood cells; I/R, ischemia and reperfusion; BK, bradykinin; L-NAME, N-nitro-L-arginine methyl ester; Indo, indomethacin. Mesentery was subjected to 20 min of ischemia and 60 min of reperfusion in absence of drug or in presence of bradykinin, bradykinin plus L-NAME, bradykinin plus indomethacin, or bradykinin plus HOE-140. Baseline, values obtained during baseline conditions before drug or vehicle administration. Preischemia, values obtained 15 min after drug or vehicle administration. Reperfusion 30 min and 60 min, values obtained after 30 and 60 min reperfusion, respectively. * Values that were statistically different from preischemia and I/R, respectively, at P < 0.05.

Discussion

The results of a large number of studies indicate that I/R is one form of acute inflammation in which leukocytes play a key role in inducing tissue dysfunction (10, 14, 27, 40). For example, leukocytes specifically infiltrate postischemic tissues in the regions that were exposed to ischemia (10). Moreover, neutrophil depletion or prevention of leukocyte adhesion with monoclonal antibodies directed against specific membrane-associated adhesive glycoproteins dramatically attenuates I/R injury (14, 27, 40). Recognition of the fact that activated neutrophils are largely responsible for the production of microvascular dysfunction induced by I/R has led to a considerable research effort directed at evaluating the potential for inhibition of leukocyte adherence to postcapillary venular endothelium as a novel approach to the treatment of reperfusion injury. A growing body of evidence indicates that administration of the vasoactive neuropeptide bradykinin may also be effective in reducing organ dysfunction induced by I/R (13, 23, 28, 43). Because the beneficial actions of bradykinin are antagonized by bradykinin B2-receptor antagonists (13, 25, 28) or inhibitors of NO synthase and cyclooxygenase in postischemic myocardium (50), it appears that this neuropeptide confers protection by a mechanism that is dependent on activation of B2 receptors and the stimulation of NO and prostaglandin synthesis. Because the downstream effectors of bradykinin B2-receptor-dependent NO and/or prostaglandin (e.g., prostacyclin) synthesis are uncertain, we postulated that bradykinin may exert its beneficial actions via inhibition of leukocyte adhesion. This hypothesis was especially appealing because of...
In addition, the effects of bradykinin to increase vascular permeability appear to be mediated by NO in the hamster cheek pouch (29). From these proinflammatory actions and the demonstrated role for leukocytes in the genesis of I/R (14), it is surprising that bradykinin would reduce postischemic leukocyte infiltration, maintain the integrity of the microvascular barrier, and attenuate tissue injury. One explanation for these apparently contradictory effects may be that the microvascular responses to bradykinin are divergent and strongly concentration dependent, with lower concentrations of bradykinin serving beneficial actions by inducing the formation of NO, whereas higher concentrations of bradykinin are proinflammatory via the formation of chemotactic substances that induce leukocyte recruitment. The results of the present study and its companion (38) are consistent with this concept. That is, high concentrations of bradykinin (>10^-7 M) induced leukocyte adhesion by a PAF-dependent mechanism (34, 38), whereas low doses (<10^-8 M) of the neuropeptide prevented postischemic leukocyte adhesion and venular protein leakage via the production of NO (present study).

Our observations are also consistent with the results of several recent studies that have demonstrated that NO plays important roles in the regulation of leukocyte-endothelial adhesive interactions and maintaining microvascular barrier function in postcapillary venules under both baseline conditions and in the setting of I/R. For example, inhibition of endogenous NO production with the NO synthase inhibitor L-NAME induces leukocyte adhesion to postcapillary venular endothelium and increases vascular albumin leakage in control (normoxic) conditions (1, 19, 21). In addition, I/R-induced leukocyte adhesion is associated with a 98% reduction in NO production in the rat mesentery (20). These observations suggest that decreased NO production after I/R may contribute to I/R-induced leukocyte recruitment, microvascular barrier dysfunction, and organ dysfunction (22). If this were the case, then replenishment of NO levels by exogenous administration should attenuate I/R injury. Indeed, exogenously applied NO prevents postischemic leukocyte adhesion, venular protein leakage, and tissue dysfunction (17, 39). In the present study, we measured venous plasma nitrite/nitrate in samples obtained from mesenteric arcade vessels draining the ischemic region before and after I/R in the presence and absence of bradykinin to determine whether the exogenously applied low dose of the neuropeptide would maintain postischemic NO levels in plasma draining the ischemic region at pres ischemic levels. I/R was associated with a significant reduction in plasma NO levels as indicated by the decline in the plasma nitrite/nitrate concentration. However, administration of bradykinin maintained this concentration at pres ischemic levels. This result supports the notion that bradykinin acts in a fashion similar to NO donors to maintain postischemic NO levels and thus inhibit I/R-induced leukocyte recruitment and microvascular barrier disruption.

The following observations. First, leukocyte adhesion plays a critical role in the pathogenesis of I/R (14, 27, 40). Second, the cardioprotective effects of bradykinin appear to involve NO and prostacyclin (23, 50). Third, NO or prostacyclin administration inhibits postischemic leukocyte adhesion and tissue dysfunction (3, 17).

In the present study, we demonstrated that administration of low-dose (10 nM) bradykinin attenuated I/R-induced leukocyte rolling, stationary adhesion, and emigration and maintained the integrity of the microvascular barrier. Moreover, we showed that the inhibitory effects of bradykinin on leukocyte recruitment and albumin leakage were completely antagonized by coadministration of L-NAME or HOE-140 but not indomethacin. Thus the results of our study are consistent with the notion that bradykinin attenuates I/R injury by a mechanism that involves inhibition of postischemic leukocyte infiltration via B2-receptor-dependent NO production. However, cyclooxygenase-derived products such as prostacyclin do not appear to contribute to the protective actions of this neuropeptide in our model.

Although the aforementioned studies clearly demonstrate that bradykinin attenuates postischemic leukocyte adhesion and microvascular barrier disruption by an NO-dependent mechanism, this neuropeptide is also well known for its powerful proinflammatory effects. For example, bradykinin can cause the formation of interendothelial cell gaps and increased protein extravasation (8, 29), induces P-selectin expression (33) and the production of PAF by endothelial cells (30, 34, 35), and releases chemotactic activity for neutrophils (16).
The mechanism whereby bradykinin-induced NO production inhibits leukocyte recruitment and microvascular barrier disruption is uncertain. However, one possibility is suggested by the well-known action of bradykinin as an NO-dependent vasodilator. That is, the tendency for a leukocyte to become adherent to the venular wall is dependent on the balance between the proadhesive forces generated by the interaction of adhesion molecules present on the leukocyte and endothelial cell surface and the hydrodynamic forces (e.g., venular wall shear rate, which is proportional to blood flow) that tend to sweep the leukocyte away from the venular wall. Thus it is possible that the bradykinin-induced, NO-mediated increase in wall shear rate would tend to strip leukocytes away from the venular wall, thereby decreasing postischemic leukosequestration. Although this hydrodynamic mechanism may account, perhaps in part, for the increased velocity of leukocyte rolling and the reduction in the number of rolling and adherent leukocytes noted in bradykinin-treated postischemic mesenteries, Kubes et al. (17) have presented evidence that suggests that NO-mediated increases in venular wall shear rate and leukocyte rolling velocity at baseline levels. However, another NO donor, 3-morpholinosydnonimine-N-ethyl-carbamide (SIN-1), also inhibited the postischemic leukocyte adhesion but failed to prevent the postischemic decreases in wall shear rate and rolling velocity (17).

A more likely explanation for the antiadhesive effects of bradykinin is suggested by the fact that I/R-induced expression of endothelial P-selectin, an adhesive glycoprotein that participates in leukocyte rolling, is inhibited by the administration of NO donors (7). Thus bradykinin may act to limit leukocyte rolling, which is a prerequisite to firm adhesion and emigration, via NO-mediated inhibition of P-selectin expression. The notion is consistent with our observation that the effect of bradykinin to reduce leukocyte rolling was prevented by prior administration of L-NAME, an NO synthase inhibitor. Interestingly, a preliminary report indicates that high doses of bradykinin promote P-selectin expression (33), a result that is consistent with our observations that large doses of this neuropeptide promote leukocyte rolling and stationary adhesion (38).

Other mechanisms whereby bradykinin-induced NO production may act to limit leukocyte sequestration relate to its ability to act as an oxidant scavenger or chelate iron (4, 15). This notion is related to the facts that reactive oxygen species appear to play an important role in the generation of chemotactic substances.
during reperfusion (42) and that NO can act as a physiologically relevant oxidant scavenger (47). NO may also act to reduce neutrophilic oxidant production by inhibition of NADPH oxidase (4) and may decrease the production of hydroxyl radicals via the Fenton reaction by chelating iron (4, 15).

Although our results indicate that bradykinin may protect tissues from the deleterious effects of I/R via NO-mediated reductions in leukocyte infiltration, this is probably not the sole mechanism whereby this neuropeptide confers protection. Indeed, bradykinin improves cell viability in isolated myocytes subjected to anoxia and reoxygenation (A/R, an in vitro model of I/R) in the absence of neutrophils (12). Although it is not clear how bradykinin confers protection independent of an effect on leukocytes, it is known that B2-receptor-mediated NO production increases intracellular cGMP level in ventricular myocytes (37), which leads to the inhibition of sarcosomal voltage-gated calcium channels (31). This results in decreased cytosolic calcium concentrations, which, in turn, decreases contractility and preservation of intracellular high-energy phosphates. Development of lethal cellular calcium overload might also be preventable by this mechanism, which may explain why NO donors prevent A/R-induced dysfunction in endothelial monolayers in the absence of neutrophils (39, 46).

Administration of agents that prevent postischemic leukocyte adhesion and emigration such as adenosine, NO donors, and monoconal antibodies directed against CD11/CD18, P-selectin, or intercellular adhesion molecule 1 also prevents venular protein leakage induced by reperfusion. Intravital microscopic observation of postcapillary venules during reperfusion indicates that the sites along the length of the vessel where leukocytes adhere and emigrate are where the leakage of fluorescently labeled albumin is the greatest (18). These observations suggest that the microvascular barrier is disrupted by leukocytes as they emigrate across the barrier. However, it is clear that this is not the sole mechanism underlying I/R-induced disruption of the microvascular barrier because administration of such treatments at the onset of reperfusion or leukocyte depletion only partially protects microvascular barrier function. In the present study, we observed that bradykinin completely prevented postischemic leukocyte adhesion and emigration but only partially ablated the microvascular barrier disruption induced by I/R. This result indicates that leukocyte emigration only partially accounts for the increased venular protein leakage induced by reperfusion in our model. This notion is consistent with the concept that leukocytes appear to be largely responsible for the reperfusion component of I/R-induced microvascular barrier disruption but do not contribute to the dysfunction induced by ischemia per se (18).

A large body of evidence is accumulating that indicates that treatment with NO donors such as spermine-NO or SIN-1 prevents postischemic microvascular dysfunction and tissue injury (17). These observations suggest that use of NO donors may prove efficacious in the treatment of I/R injury. Our results with bradykinin lend additional support to this concept. In addition, this neuropeptide has been implicated in the cardioprotective effects of ischemic preconditioning (a phenomenon whereby the deleterious effects of prolonged ischemia are prevented by prior exposure to brief periods of vascular occlusion) in some models (9, 48). It has also been shown that this neuropeptide can be used to pharmacologically precondition ischemic myocardium (41) and protect against the damage induced by subsequent exposure to prolonged I/R. Although the mechanisms whereby bradykinin may induce these protective effects are unclear, we have obtained preliminary evidence that indicates bradykinin preconditioning prevents postischemic microvascular dysfunction (unpublished observations). That is, superfusion of the mesentery with bradykinin for a 5-min period followed by a 10-min superfusion period without bradykinin (i.e., bradykinin preconditioning) completely prevents postischemic leukocyte adhesion and emigration and maintains microvascular barrier function. Thus it appears that bradykinin can prevent postischemic microvascular dysfunction whether administered during I/R (present study) or as a preconditioning stimulus. By understanding the mechanisms whereby bradykinin confers protection in these settings, new treatment regimens for I/R may be uncovered.

In summary, the administration of bradykinin at a relatively low dose attenuated I/R-induced leukocyte adhesion and emigration and venular albumin leakage. Our results are consistent with the concept that the anti-inflammatory effects of low-dose bradykinin are mediated by a B2-receptor-dependent NO-mediated mechanism that may involve inhibition of P-selectin expression by venular endothelial cells. These observations are in stark contrast to those presented in the companion paper (38), in which high doses of bradykinin were shown to induce leukocyte adhesion by a PAF-dependent mechanism. Taken together, the results of these studies indicate that the effects of bradykinin are highly dependent on the concentration used, with low doses acting to inhibit leukocyte adhesion via an NO-dependent mechanism whereas high doses exert proinflammatory effects that are mediated by PAF production.

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