Concentration-dependent effects of bradykinin on leukocyte recruitment and venular hemodynamics in rat mesentery

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Shigematsu, Sakui, Shuji Ishida, Dean C. Gute, and Ronald J. Korthuis. Concentration-dependent effects of bradykinin on leukocyte recruitment and venular hemodynamics in rat mesentery. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H152–H160, 1999.—The results of several recent studies indicate that bradykinin protects tissues against the deleterious effects of ischemia-reperfusion (I/R). However, other studies indicate that bradykinin can act as a proinflammatory agent, inducing P-selectin expression, the formation of chemotactic stimuli, and endothelial barrier disruption. In the present study, we used intravital microscopic techniques to examine the dose-dependent effects of bradykinin on leukocyte-endothelial cell interactions, the formation of platelet-leukocyte aggregates, and venular hemodynamics in rat mesentery in an attempt to explain these divergent findings. Superfusion of the mesentery with low concentrations of bradykinin (â‰ˆ10^{-7} M) increased venular erythrocyte velocity (V_{RBC}) without increasing the number of adherent leukocytes, whereas higher concentrations (â‰ˆ10^{-6} M) decreased V_{RBC}, increased the number of platelet-leukocyte aggregates, and induced leukocyte adhesion in single postcapillary venules. The formation of platelet-leukocyte aggregates and increased leukocyte adhesion induced by high-dose bradykinin were attenuated by administration of a B_2-receptor (HOE-140) or a platelet-activating factor (PAF, WEB-2086) antagonist. Thus these adhesive interactions induced by high-dose bradykinin appear to be mediated by a mechanism that is dependent on B_2-receptor activation and the formation of PAF or PAF-like lipids. The effects of bradykinin on venular V_{RBC} and blood flow were also concentration dependent, with low doses producing nitric oxide-mediated vasodilation, whereas high doses decreased V_{RBC} by a mechanism that is PAF independent.

leukocyte adhesion; postcapillary venules; erythrocyte velocity; B_2 receptors; platelet-activating factor; nitric oxide synthase; cyclooxygenase

A GROWING BODY OF EVIDENCE indicates that the administration of bradykinin protects the heart from ischemia-reperfusion (I/R)-induced injury. For example, bradykinin treatment improves postischemic ventricular function in isolated rat heart, an effect that was blocked by coadministration with a nitric oxide (NO) synthase inhibitor (37). Bradykinin has also been reported to decrease myocardial infarct size in anesthetized dogs and reduced the likelihood of ventricular fibrillation after reperfusion of isolated working rat hearts (23). These protective effects of bradykinin were abolished by HOE-140, a bradykinin B_2-receptor antagonist (23). Bradykinin also maintains the viability of isolated rat cardiomyocytes exposed to anoxia and reoxygenation (15).

Although the mechanisms underlying the protective actions of bradykinin are not fully understood, the aforementioned studies are consistent with the notion that bradykinin B_2-receptor-mediated NO production plays an important role. Because bradykinin is a well-known NO-dependent vasodilator (13, 24), the protective actions of this agent may be related to improved blood flow during reperfusion. In addition, bradykinin-induced NO release may be cardioprotective by virtue of its ability to inhibit leukocyte adhesion and emigration and maintain endothelial barrier function (20–22). However, this intriguing possibility is yet to be evaluated.

Although the aforementioned studies clearly demonstrate that the protective actions of bradykinin may involve the production of NO, this neuropeptide is also well known for its potent proinflammatory effects. For example, this agent causes endothelial barrier dysfunction, induces P-selectin expression, and releases chemotactic activity for neutrophils (3, 19, 29). Because of these effects and the demonstrated role for leukocytes in the genesis of I/R (16, 25, 35), it is surprising that bradykinin would reduce postischemic tissue injury. We hypothesized that the microvascular effects of 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 may be related to inflammatory via the formation of chemotactic substances that induce leukocyte recruitment. Thus the aim of the present study was to investigate the concentration-dependent effects of bradykinin on the microvascular hemodynamics and leukocyte-endothelial interactions. Our results indicate that low doses of bradykinin (â‰ˆ10^{-7} M) increase venular erythrocyte velocity (V_{RBC}) by a B_2-receptor NO-dependent mechanism but do not influence leukocyte-endothelial cell adhesive interactions. On the other hand, higher doses (â‰ˆ10^{-6} M) increased leukocyte adhesion via B_2-receptor activation and the formation of platelet-activating factor (PAF). In a companion study (33), we demonstrated that low doses of bradykinin prevent postischemic leukocyte adhesion by an NO-dependent mechanism. Taken together, the results of these studies indicate that bradykinin may exert anti- or proinflammatory effects, depending on the dose used.

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 intraperitoneal pentobarbital injection (65 mg/kg body wt). After a surgical plane of anesthesia was attained, a tracheotomy was performed to facilitate breathing during the experiment. The right carotid artery was cannulated, and systemic arterial pressure was continuously measured with a pressure transducer (P23A, Statham) connected to a personal computer (Macintosh 8500, Apple) equipped with an analog-to-digital converter (MP 100, Biopac Systems). A midline abdominal incision was then performed to allow exteriorization 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 temperature was monitored with an electrothermometer (4000A, Yellow Springs Instruments), and body temperature was kept 36 ± 0.5°C with an infrared heat lamp. The Plexiglas board was mounted onto the stage of an inverted microscope (TMD-25, 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) connected to 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-810, Panasonic).

Single unbranched venules with diameters of 25–35 µm and lengths >150 µm were selected for the study. Venular diameter (Dv) was measured on-line with a video caliper (Microcirculation Research Institute, Texas A&M University). Cell velocities were calculated from the cross-sectional area, with cylindrical geometry assumed. Centerline velocity (Vmean = centerline velocity/1.6) (8). Venular wall shear rate (SR) was calculated from the Newtonian definition: SR = 8Vmean/Dv. In some experiments, paired arterioles with diameters of 15–25 µm and lengths >150 µm were used to observe the mesenteric microcirculation. Arteriolar diameter and velocity were also measured in these experiments as described for the venules. The number of adherent leukocytes was determined off-line during playback of videotaped images. A leukocyte was considered adherent to venular endothelium if it remained stationary for 30 s or longer (14). Adherent leukocytes were quantified as the number per 100-µm length of venule. A rolling leukocyte was defined as a white cell that moved slower than the stream of flowing erythrocytes. The number of rolling leukocytes was quantified as the number of white cells that passed a fixed point (25-µm window) on the television monitor.

Experimental protocols. After a stabilization period of 30 min, images from the mesenteric preparation were recorded on videotape for 10 min (baseline recording). Thereafter, continuous dose-response curves were conducted by superfusing the mesentery with bradykinin at increasing concentrations. Each concentration of bradykinin was superfused for 15 min. Video recordings were made during bradykinin superfusion at the different dosages, with each recording beginning 5 min after the onset of a given dose of bradykinin and continuing for 10 min (i.e., from minutes 5 to 15 after each increase in bradykinin dose). Vmean and venular diameter measurements were recorded at the end of each dose superfusion. To minimize the cumulative effects of bradykinin, the dose-response study of each preparation was terminated when Vmean decreased by >20% or when the number of adherent leukocytes increased to ≥10. The effects of higher doses of bradykinin were evaluated in separate experiments. The concentration-dependent effects of bradykinin were examined in the absence of blockers (control) or in the presence of an NO synthase inhibitor [Nω-nitro-L-arginine methyl ester (L-NAME), 10 µM, Sigma], a cyclooxygenase inhibitor (indo-methacin, 10 µM, Sigma), a β2-receptor antagonist (HOE-140, 1 µM, Research Biochemicals International), or a PAF inhibitor (WEB-2086, 10 µM, a gift from Boehringer Ingelheim). Each blocker was topically applied to the mesentery superfusing the superfusate beginning 15 min before the baseline recording.

Measurement of plasma nitrite/nitrate concentration. To monitor changes in plasma NO, plasma levels of nitrite/nitrate were measured (30, 36) using a commercially available assay kit (Nitrate/Nitrite Fluorometric Assay Kit no. 780051, Cayman Chemical). Blood was sampled via PE-10 tubing inserted into the mesenteric vein during, immediately before (baseline), and 15 min after superfusion of the mesentery with bradykinin at either 10−6 or 10−5 M. Plasma was obtained from the blood by centrifugation immediately after the blood samples were collected and was frozen at −80°C. For the nitrite/nitrate measurement, plasma was thawed and centrifuged 13,000 rpm for 30 min with ultrafiltration device (Ultrafree-MC, Millipore). Thereafter, nitrite/nitrate was measured using the assay kit and a luminescence spectrophotometer (SLM AMINCO AB-2, SLM Instruments).

Statistical analysis. The data were analyzed by unpaired t-test or ANOVA followed by Scheffé’s post hoc test where appropriate. Fisher’s test was used for evaluation of correlation. All values are expressed as means ± SE. Statistical significance was set at P < 0.05. The number of observations for each variable was obtained from 5–13 rats.

RESULTS

Figure 1 illustrates the effect of superfusing the mesentery with increasing concentrations of bradykinin on leukocyte adherence to single postcapillary venules in the rat mesentery. The number of adherent leukocytes was measured during brief (15 min) application of each dose of bradykinin. No significant change in the number of adherent leukocytes was seen when lower concentrations (≤10−7 M) of bradykinin were administered. In contrast, higher concentrations (≥10−6 M) of bradykinin significantly increased the number of adherent leukocytes (Fig. 1A). The increased leukocyte adherence induced by high-dose bradykinin was not affected by blockade of NO synthase with L-NAME (Fig. 1B) or by cyclooxygenase inhibition with indomethacin (Fig. 1C). However, pretreatment with a bradykinin B2-receptor (HOE-140) or a PAF (WEB-2086) antagonist abolished the effect of high doses of bradykinin to induce leukocyte adhesion (Fig. 1, D and E, respectively). None of the antagonists affected baseline leuko-
cyte adhesion (i.e., in the absence of bradykinin). A similar pattern was noted with regard to the effect of bradykinin on the weaker adhesive interactions associated with leukocyte rolling, as depicted in Fig. 2.

Although bradykinin is known to be an endogenous vasodilating neuropeptide, the effect of bradykinin on venular V_{RBC} was divergent depending on the concentration applied. Typical examples are shown in Fig. 3, in which the mesentery was superfused with two different concentrations of bradykinin. Superfusion with a low concentration of bradykinin (10^{-8} M) increased V_{RBC}, which returned to baseline levels after the perfusate was switched to one lacking bradykinin to wash out the neuropeptide from the mesentery. On the contrary, topically applied bradykinin at 10^{-6} M resulted in a sustained decrease in V_{RBC} below control levels after an initial brief increase. As noted for the lower dose of bradykinin, V_{RBC} returned to baseline levels after cessation of superfusion with the higher dose.

Figure 4 shows the average changes in V_{RBC}, venular diameter, and mean systemic blood pressure measured at the end of each 15-min superfusion period with increasing concentrations of bradykinin. Topically applied bradykinin over a dose range of 10^{-10} to 10^{-6} M progressively increased V_{RBC} (Fig. 4A). However, higher concentrations of bradykinin produced a progressive decrease in V_{RBC}. No changes in venular diameter or mean systemic blood pressure were noted at any dose of bradykinin (Fig. 4, B and C, respectively). To examine the contribution of NO and prostacyclin to the bradykinin-induced changes in venular V_{RBC}, the effects of the neuropeptide were examined in the presence of L-NAME (10 µM) or indomethacin (10 µM; Fig. 4A).

Although coadministration with indomethacin did not affect bradykinin-induced changes in V_{RBC} at any bradykinin dose, the increased velocity noted at lower bradykinin doses was completely blocked by L-NAME. However, the decrease in V_{RBC} noted at higher concentrations of bradykinin was not affected by L-NAME.

The L-NAME data suggest that the increases in V_{RBC} induced by lower doses of bradykinin were most likely due to NO-induced relaxation of vascular smooth muscle. However, because the wall of postcapillary venules that are 25–35 µm in diameter lacks smooth muscle, the increase of V_{RBC} most likely reflects the effects of bradykinin-induced precapillary arteriolar vasodilation. This notion is supported by the observation that the bradykinin-induced change in venular
V_{RBC} was well correlated with the change in arteriolar V_{RBC} (Fig. 5). However, when we measured the diameter of arterioles that were present in the same microscopic field as the venule of interest, arteriolar caliber during bradykinin superfusion at any dose relative to control (i.e., in the absence of bradykinin) was not different. This implies that changes in vascular diameter occurred in larger upstream arterioles, the result of which was altered V_{RBC} in the smaller arterioles that we viewed. Because venular diameter was not affected by any concentration of bradykinin or inhibitor tested and venular blood flow is the product of venular cross-sectional area (which is proportional to venular diameter) and V_{RBC}, alterations in V_{RBC} most likely reflect changes in venular blood flow.

As noted previously, the increase in blood flow induced by lower concentrations of bradykinin was abolished with L-NAME, a result consistent with the fact that bradykinin is an NO-dependent vasodilator. Because augmented endothelial NO production by bradykinin is reported to be B2-receptor dependent (10), we examined the effect of HOE-140 (1 µM), a B2-receptor antagonist, on the bradykinin-induced changes in V_{RBC}. Pretreatment with HOE-140 completely blocked not only the bradykinin-induced increase in V_{RBC} noted at

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![Fig. 2. Effect of increasing concentrations of bradykinin on number of leukocytes rolling along walls of single postcapillary venules of rat mesentery in absence (control, A) and presence of L-NAME (10 µM, B), indomethacin (10 µM, C), HOE-140 (1 µM, D), or WEB-2086 (10 µM, E).](image)

![Fig. 3. Typical examples of effect of superfusion of mesentery with low (10^{-8} M, A) and high (10^{-6} M, B) concentration of bradykinin on venular erythrocyte velocity (V_{RBC}).](image)
low concentrations but also the decrease in $V_{RBC}$ noted at high concentrations (Fig. 6A). On the other hand, there is evidence that bradykinin can induce the formation of PAF and/or PAF-like lipids that occupy the same receptor as authentic PAF (26, 32), which can act as a vasoconstrictor in the mesentery. We thus examined the role of PAF formation in the effect of higher doses of bradykinin to decrease $V_{RBC}$. However, coadministration of WEB-2086 (10 µM), an inhibitor of PAF, was without effect on the bradykinin-induced hemodynamic changes (Fig. 6A). Neither HOE-140 nor WEB-2086 influenced venular diameter or systemic arterial blood pressure at any concentration of bradykinin tested (Fig. 6, B and C, respectively). Table 1 summarizes changes in venular wall shear rate in the various groups. Venular wall shear was altered in a manner that mirrored the changes in $V_{RBC}$.

During our experiments, we observed that bradykinin superfusion induced the formation of platelet-leukocyte aggregates. Their formation was dose dependent, with significant numbers of platelet-leukocyte aggregates appearing at bradykinin concentrations $\geq 10^{-6}$ M in the superfusate (Fig. 7A). We hypothesized that these large aggregates, which interact with the vascular wall in a fashion similar to that of rolling leukocytes and often became stationary for brief periods (1–2 s), could impede flow and thus reduce $V_{RBC}$. If this were the case, we would expect that the formation of these aggregates would be modified in a pattern similar to that noted with regard to the effect of high-dose bradykinin on $V_{RBC}$ presented in Figs. 4 and 6. Indeed, pretreatment with a B2-receptor (HOE-140) antagonist, but not an NO synthase (L-NAME) or cyclooxygenase (indomethacin) inhibitor, prevented the appearance of these aggregates (Fig. 7B) in response to high doses of bradykinin, a pattern similar to that noted for the $V_{RBC}$ responses. However, topical application of a PAF antagonist (WEB-2086) prevented the formation of platelet-leukocyte aggregates (Fig. 7B) but failed to modify the decrease in $V_{RBC}$ induced by high-dose bradykinin (Fig. 6A). Thus it does not appear likely that the formation of these aggregates accounts for the reduction in $V_{RBC}$ noted at high doses of bradykinin.

Our L-NAME data indicate that the increase in the $V_{RBC}$ by lower doses of bradykinin was due to increased NO production. On the other hand, one may speculate that the decrease in $V_{RBC}$ noted at higher concentrations of bradykinin could be due to decreased NO production. To examine this postulate, we measured the plasma nitrite/nitrate, which provides a useful index of NO production, in the mesenteric vein during superfusion with bradykinin at a low ($10^{-8}$ M) and a high ($10^{-5}$ M) concentration (Fig. 8). However, both concentrations of bradykinin produced a significant increase in plasma nitrite/nitrate levels. This result indicates that the decrease in $V_{RBC}$ during superfusion with a high concentration of bradykinin was not secondary to decreased production of NO.
DISCUSSION

The results of a large number of studies indicate that bradykinin is an endogenously produced, NO-dependent vasodilator neuropeptide that exerts powerful proinflammatory effects. For example, bradykinin releases chemotactic activity, induces the expression of P-selectin, and causes the formation of interendothelial gaps and extravasation of plasma proteins (3, 19, 29). On the other hand, a growing body of evidence indicates that bradykinin treatment prevents the tissue injury that is induced by prolonged I/R (15, 23, 37). Because I/R is one form of acute inflammation in which leukocytes play a key role (16, 25, 35), it is surprising that an agent so well known for its proinflammatory actions would limit postischemic tissue injury. We hypothesized that these dichotomous effects of bradykinin might be strongly concentration dependent, with low doses of bradykinin being anti-inflammatory via the production of NO, whereas higher doses induced leukocyte adhesion by a mechanism that involves the liberation of PAF.

To address this postulate, we evaluated the concentration-dependent effects of bradykinin on leukocyte-endothelial cell interactions in single postcapillary venules of the rat mesentery. Our results indicate that bradykinin at concentrations of \( \leq 10^{-7} \) M did not alter leukocyte adhesion relative to baseline conditions, whereas higher concentrations (\( \geq 10^{-6} \) M) induced a progressive increase in the numbers of rolling and firmly adherent leukocytes in single postcapillary venules.

The latter results are consistent with the observations that bradykinin at concentrations in excess of \( 10^{-4} \) M can induce macrophages to release mediators that are chemotactic for neutrophils, eosinophils, and monocytes (19), as well as promote leukocyte adhesion to cultured endothelial monolayers (32). In addition, a recent preliminary report indicates that a high-dose bradykinin can induce the expression of P-selectin (29), an adhesive glycoprotein that plays an important role in leukocyte rolling. These observations indicate that higher doses of bradykinin are proinflammatory. In a companion study (33), we have shown that low doses of bradykinin are anti-inflammatory in that they prevent I/R-induced leukocyte adhesion and microvascular barrier disruption by a mechanism that is NO dependent.

Although the present study clearly demonstrates that bradykinin can induce leukocyte adhesion at concentrations of \( \geq 10^{-6} \) M, the mechanisms responsible for this proinflammatory effect are not clear. Indeed, the finding that bradykinin induces leukocyte adhesion is quite surprising because of its well-known action as an NO-dependent vasodilator (10) and the fact that NO is a powerful antiadhesive agent (20, 21, 22). Moreover, bradykinin can also induce the production of prostacyclin, which has also been reported to inhibit leukocyte adhesion (5). Some insight regarding this dilemma is provided by the following observations. First, bradykinin induces the production of PAF or PAF-like lipids by cultured endothelial cells when administered at concentrations similar to those that evoked leukocyte adhesion.

### Table 1. Effects of superfusion of mesentery with increasing concentrations of bradykinin on venular wall shear rate in absence (control) and presence of L-NAME, indomethacin, HOE-140, or WEB-2086

<table>
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<th>0</th>
<th>10^{-10}</th>
<th>10^{-9}</th>
<th>10^{-8}</th>
<th>10^{-7}</th>
<th>10^{-6}</th>
<th>10^{-5}</th>
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<tr>
<td>Control</td>
<td>100</td>
<td>106 ± 2</td>
<td>109 ± 3*</td>
<td>108 ± 2</td>
<td>103 ± 6</td>
<td>81 ± 6*</td>
<td>74 ± 5*</td>
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<tr>
<td>L-NAME (10 µM)</td>
<td>100</td>
<td>98 ± 2</td>
<td>97 ± 2</td>
<td>92 ± 5*</td>
<td>80 ± 3*</td>
<td>77 ± 7*</td>
<td>58 ± 5*</td>
</tr>
<tr>
<td>Indomethacin (10 µM)</td>
<td>100</td>
<td>106 ± 2</td>
<td>108 ± 2*</td>
<td>105 ± 2</td>
<td>88 ± 3*</td>
<td>77 ± 7*</td>
<td>64 ± 3*</td>
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<tr>
<td>HOE-140 (1 µM)</td>
<td>100</td>
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<td>99 ± 2</td>
<td>101 ± 1</td>
<td>100 ± 3</td>
<td>99 ± 3</td>
</tr>
<tr>
<td>WEB-2086 (10 µM)</td>
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<td>117 ± 4*</td>
<td>115 ± 6</td>
<td>95 ± 4</td>
<td>80 ± 2*</td>
<td>68 ± 3*</td>
</tr>
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*Values are means ± SE of percent value before administration of bradykinin (0 M); n = 5–13 rats/group. L-NAME, Nω-nitro-L-arginine methyl ester. *P < 0.05 vs. 0 M bradykinin.
sion in our study (26, 32). Second, these lipids promote leukocyte adhesion to endothelial cells both in vitro and in vivo (4, 26, 32). Third, bradykinin-induced generation of chemotactic activity by macrophages is partially blocked by coadministration of a PAF antagonist (31). From these observations, we postulated that high-dose bradykinin provoked leukocyte adhesion by a PAF-dependent mechanism. As shown in Figs. 1 and 2, bradykinin-induced leukocyte rolling and stationary adhesion were blocked by coadministration of the PAF antagonist WEB-2086 as well as the bradykinin B2-receptor antagonist HOE-140. Thus our results support the concept that high-dose bradykinin causes leukocyte adhesion to postcapillary venular endothelium by a mechanism that involves activation of B2 receptors and is PAF dependent.

Bradykinin is well known for its ability to relax vascular smooth muscle and increase blood flow (10), an action that is dependent on activation of B2 receptors on the endothelium (7, 10, 11). This action results in an increase in intracellular calcium which in turn activates endothelial constitutive NO synthase to produce NO. The NO produced after B2-receptor activation subsequently activates guanylate cyclase in vascular smooth muscle, resulting in increased cGMP levels and relaxation (7, 10, 11, 27). From this mechanism, one would suspect that bradykinin would have produced an increase in V_{RBC} and blood flow at all doses tested in our study. However, increased mesenteric blood flow (as reflected by increased V_{RBC} in venules of unchanging diameter) was observed only at lower concentrations (10^{-7} M) of bradykinin in the present study. Conversely, higher concentrations of bradykinin (10^{-6} M) decreased the mesenteric blood flow. Because the increases in venular V_{RBC} induced by low-dose bradykinin were completely blocked by L-NAME or HOE-140, B2-receptor-mediated endothelial NO production is responsible for the increase in V_{RBC} and blood flow, as has been reported previously (7, 10, 11, 27). Because postcapillary venules in the size range examined in our study lack smooth muscle, the NO-dependent increase in venular V_{RBC} probably resulted from B2 receptor-dependent arteriolar vasodilation, a contention supported by the strong correlation between the change of blood velocity in venules versus arterioles depicted in Fig. 5.

In addition to producing NO-dependent relaxation of vascular smooth muscle, bradykinin-induced, B2-receptor-dependent generation of prostacyclin also contributes to the vasodilating action of this neuropeptide, at least in some vessel types and species (1). However, the vasodilatory effects of low-dose bradykinin were not affected by cyclooxygenase blockade in our study, suggesting that bradykinin-induced relaxation of mesenteric vascular smooth muscle is independent of prostacyclin (or other vasodilatory prostaglandins produced by cyclooxygenase). Indeed, the complete abrogation of
the vasodilatory effects of low-dose bradykinin by NAME noted in the present study is consistent with reports indicating that the vasorelaxation induced by bradykinin in the rat mesentery is exclusively mediated by NO (2).

The change in venular $V_{RBC}$ induced by higher doses of bradykinin was biphasic. That is, after an initial and very transient vasodilation, continued superfusion of the mesentery with high-dose bradykinin resulted in a decline in velocity to levels below baseline (Fig. 3B). Vasoconstriction by bradykinin has been reported in both the isolated perfused rat mesentery (6, 12) and chronically catheterized rat mesenteric artery in vivo (18). It is unlikely that the sustained decrease in venular $V_{RBC}$ induced by high-dose bradykinin was due to a decrease in perfusion pressure secondary to systemic vasodilation because systemic arterial blood pressure was not affected except at the highest ($10^{-5}$ M) concentration of bradykinin tested. However, it is possible that the bradykinin-induced decrease of $V_{RBC}$ was due to $\alpha$-adrenergic vasoconstriction that might arise secondary to activation of the baroreceptors and that this vasoconstriction maintained blood pressure at normal levels. This mechanism is also unlikely because $\alpha$-adrenergic receptor blockade with phentolamine (1 $\mu$M) did not modify the response to high-dose bradykinin, i.e., $V_{RBC}$ decreased by 72 $\pm$ 6% after superfusion with $10^{-5}$ M bradykinin in the presence of phentolamine ($P < 0.01$, n = 5), a decrease similar to that noted in the presence of $10^{-5}$ M bradykinin alone. Another possibility is that plasma NO levels induced by high-dose bradykinin may not have increased to the same extent as that produced by low-dose bradykinin. Such a scenario might also contribute to the increased leukocyte adhesion noted with high-dose bradykinin, given the antiadhesive properties of NO. This potential explanation is consistent with the observation that bradykinin can induce the formation of oxidants such as superoxide by endothelial cells (17, 34). Bradykinin-induced formation of superoxide could reduce the bioavailability of NO via their interaction to form peroxynitrite, thereby inducing vasoconstriction and decreased $V_{RBC}$. However, these explanations seem unlikely because bradykinin-induced oxidant formation occurs via a cyclooxygenase-dependent mechanism (17, 34) and indomethacin failed to modify $V_{RBC}$ at any dose of bradykinin in our experiments. In addition, the plasma nitrite/nitrate concentration, which provides an index of NO production, in blood samples obtained from the mesenteric vein, increased to a similar extent in response to both low ($10^{-4}$ M) and high ($10^{-5}$ M) concentrations of bradykinin. However, this latter observation must be interpreted with caution, because scavenging of NO by superoxide may not affect nitrate levels.

Another potential explanation for the decreased $V_{RBC}$ with high-dose bradykinin was suggested by reports that indicate that bradykinin results in PAF generation in cultured endothelium (26, 32). This proinflammatory lipid produces vasoconstriction when applied to the hamster cheek pouch microcirculation (9). However, the decrease in $V_{RBC}$ associated with topical application of high doses of bradykinin in the present study was not prevented by coadministration of a PAF antagonist (WEB-2086). A final possibility may relate to the ability of high-dose bradykinin to induce the formation of platelet-leukocyte aggregates. Because these aggregates are relatively large and interact with the vascular wall to slow their transit through the venular lumen, they may impede flow leading to reduced $V_{RBC}$. In addition, it is possible that these aggregates physically impact in the capillary, plugging the lumen and thereby reducing downstream (venular) $V_{RBC}$. However, both of the aggregation scenarios seem unlikely because WEB-2086 treatment markedly reduced the number of circulating leukocyte-platelet aggregates seen in the presence of high concentrations of bradykinin but failed to modify venular hemodynamics.

Whatever the mechanism responsible for the effect of high-dose bradykinin on $V_{RBC}$, it is important to consider the potential effects of reduced wall shear rate as a potential contributor to the increased leukocyte adhesion induced by high-dose bradykinin. This is an important consideration because 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 shear rate) that tend to sweep white cells away from the vascular wall (28). However, this explanation seems unlikely because coadministration of a PAF antagonist with high-dose bradykinin prevented the increase in leukocyte adhesion but did not prevent the reduction in venular $V_{RBC}$ and wall shear rate.

In summary, the results of our study indicate that the effects of bradykinin on leukocyte rolling and adhesion are highly concentration dependent. High doses of this neuropeptide increased leukocyte adhesion and the formation of platelet-leukocyte aggregates by a mechanism that is dependent on the activation of bradykinin B$_2$ receptors and is mediated by the formation of PAF or PAF-like lipids. In direct contrast, the results of the companion study (33) indicate that low doses of bradykinin prevent the increased leukocyte adhesion induced by I/R by a mechanism that involves B$_2$-receptor activation and the formation of NO. Interestingly, the effects of bradykinin on venular $V_{RBC}$ and blood flow were also concentration dependent; with low doses producing NO-dependent vasodilation, whereas high doses decreased venular $V_{RBC}$ and blood flow by a PAF-independent mechanism.

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H160  BRADYKININ AND LEUKOCYTE RECRUITMENT


