Effect of Bradykinin on Isolated Mesenteric Arteries of the Rat

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Bradykinin is a potent vasodilator peptide; however, its half-life in vivo is very short because of various plasma and tissue peptidases that hydrolyze bradykinin to inactive fragments. We studied the role of kininase II (angiotensin converting enzyme) and neutral endopeptidase 24.11 (enkephalinase) in the catabolism of bradykinin in vascular tissue by determining the effect of inhibitors of kininase II (captopril) and of endopeptidase 24.11 (phosphoramidon) on the action of bradykinin on rat isolated mesenteric arteries. Because bradykinin may induce prostaglandin formation and release, we also studied the effect of a cyclooxygenase inhibitor, indomethacin, on the action of bradykinin. The mesenteric bed was isolated from rats (250–300 g) with rats under ether anesthesia and was perfused with Krebs' solution (4 ml/min) containing phenylephrine (0.5–1.0 µg/ml) to produce a mean perfusion pressure of 120–130 mm Hg. Bradykinin (2.5–40.0 ng), injected as a bolus, produced a dose-dependent decrease in perfusion pressure. In the presence of indomethacin (1.0 µg/ml), the amplitude of the vasodilator responses to bradykinin was not significantly affected, although the duration of the responses was increased approximately two to four times. In the presence of captopril (1.0 µg/ml), bradykinin elicited either a vasodilator or a biphasic effect. The vasodilator effect was greatly potentiated by captopril, whereas the duration of the response was unchanged when compared with control experiments. When present, the pressor responses were also dose related. In the presence of indomethacin plus captopril, bradykinin produced only a fall in perfusion pressure that lasted five to six times longer than without any treatment. Phosphoramidon (1–200 ng/ml) did not affect the responses to bradykinin. We conclude that bradykinin induces vasodilation of rat isolated mesenteric arteries followed by a vasoconstrictor effect due to prostaglandin release. The contribution of neutral endopeptidase 24.11 to bradykinin inactivation seems to be negligible, whereas kininase II plays an important role in the metabolism of bradykinin in vascular tissue. (Hypertension 1992;19[suppl II]:II-251–II-254)

Bradykinin is a vasoactive peptide that produces systemic vasodilation and a fall in blood pressure.1 However, its half-life in vivo is very short because of various plasma and tissue-bound kininases that hydrolyze kinins to inactive fragments. The contribution of several known kininases to bradykinin inactivation may depend on the source and species studied. In human plasma, kininase I, also known as carboxypeptidase N, may account for as much as 90% of kininase activity,2 whereas in rat plasma, another carboxypeptidase, kininase II or angiotensin converting enzyme, plays a major role in the catabolism of bradykinin.3 Tissue kininase activity, particularly in the lung and kidney, is considered to be mainly due to kininase II,4 although Ura et al5 indicated that neutral endopeptidase 24.11 plays a major role in kinin metabolism in the rat nephron.

There is evidence1 suggesting that kinins may act as a paracrine hormone. Thus, the intravascular concentration of kinins may depend on both production and destruction of this peptide. To investigate the importance of two membrane-bound peptidases, kininase II and endopeptidase 24.11, in the metabolism of bradykinin in vascular tissue, we studied the effect of their inhibitors on the action of bradykinin in the rat isolated mesenteric artery. Because bradykinin may elicit vasodilation by inducing formation and release of prostaglandins,6 we also investigated the effect of indomethacin, an inhibitor of prostaglandin formation, on bradykinin action.

Methods

Bradykinin was synthesized by A.C.M. Paiva, Escola Paulista de Medicina, São Paulo, Brazil.
methacin, phenylephrine, and acetylcholine were purchased from Sigma Chemical Co., St. Louis, Mo.; phosphoramidon from the Peptide Institute, Protein Research Foundation, Osaka, Japan; and captopril from the Squibb Institute, Princeton, N.J.

The mesenteric vascular bed was removed from male Wistar rats weighing 250–300 g as described by McGregor. The rats were anesthetized with ether, and after the abdominal cavity was opened, the animals were heparinized (1,000 units) and the superior mesenteric artery was perfused through a cannula (PE-50; Clay Adams, Parsippany, N.J.) inserted into the artery at its origin from the aorta. The cecal, ileocolic, colic, and pancreaticoduodenal branches from the superior mesenteric artery were tied off. The intestine was severed from the mesentery by cutting close to the intestinal border, and the mesentery was isolated from the rat and placed ready for perfusion in a water-jacketed organ bath maintained at 37°C. The mesenteric arteries were perfused with Krebs’ solution (millimolar concentration: NaCl 120.0, KCl 4.7, CaCl₂ 3.0, MgCl₂ 1.43, NaHCO₃ 25.0, KH₂PO₄ 1.17, glucose 11.0, and EDTA 0.03) equilibrated with a 95% O₂-5% CO₂ mixture at 37°C at a constant rate of 4 ml/min, and the perfusion pressure was recorded with a Hewlett-Packard (Palo Alto, Calif.) transducer (HP 1280) and recorder (HP 7754B). After a 30-minute stabilization period, phenylephrine was added to the perfusion solution at increasing concentrations in the range of 0.5–1.0 μg/ml until a stable perfusion pressure (120–130 mm Hg) was achieved. The vasodilator effect of acetylcholine (100 ng) was first established to assure that the endothelium was functionally intact.

Dose–response curves for bradykinin (2.5–40.0 ng) were obtained by bolus injection of 10–40 μl solution into the perfusion stream close to the superior mesenteric artery cannula. For the assessment of the contribution of prostaglandin formation and kininase activities to the bradykinin actions, the following specific inhibitors were added to the perfusion solution: indomethacin (1.0 μg/ml) to inhibit prostaglandin release, captopril (1.0 μg/ml) to inhibit kininase II, and phosphoramidon (1–200 ng/ml) to inhibit endopeptidase 24.11. This concentration of captopril inhibited the pressor response elicited by angiotensin I in this preparation (data not shown); phosphoramidon concentrations were in the range of those used by Ura et al. that demonstrated a major role for endopeptidase 24.11 in the metabolism of kinins in the rat nephron in vivo.

Data are expressed as mean±SEM. Changes in perfusion pressure and duration of vasodilator responses to different doses of bradykinin were determined by analysis of variance. Differences between groups were evaluated using multivariate analysis of variance for repeated measures. Differences were considered significant at a value of p<0.05.

### Results

Bradykinin (2.5–40.0 ng) elicited a dose-related fall in perfusion pressure of phenylephrine-preconstricted mesenteric arteries (n=7; Figures 1A and 2).

![Figure 1](image1.png)

**Figure 1.** Typical recordings show example of the effects of bradykinin on preconstricted rat mesenteric arteries in the control period (panel A), in the presence of captopril (1 μg/ml) (panel B), in the presence of indomethacin (1 μg/ml) (panel C), and in the presence of indomethacin plus captopril (panel D).

![Figure 2](image2.png)

**Figure 2.** Line plots show effect of bradykinin on perfusion pressure (top panel) and duration of vasodilator responses to bradykinin (bottom panel) in rat mesenteric arteries perfused with Krebs’ solution (control) or Krebs’ solution containing indomethacin (1 μg/ml) or captopril (1 μg/ml). *Significant difference between experimental and control groups (p<0.001).
The addition of captopril to the perfusion solution produced a decrease in basal perfusion pressure such that more phenylephrine (1.2–1.5 μg/ml) was required to maintain perfusion pressure at the same levels as before treatment (120–130 mm Hg). In the presence of indomethacin, a small but not significant increase in the amplitude of the vasorelaxant effect of bradykinin was observed. Moreover, the vasodilator responses to bradykinin lasted twofold to fourfold longer (p<0.001) during indomethacin treatment compared with controls (Figures 1C and 2).

The addition of captopril to the perfusion solution produced no change in the perfusion pressure but induced a twofold to threefold increase (p<0.001) in the vasorelaxant action of bradykinin on the perfused mesenteric arteries, although the duration of the vasodilator responses did not differ from that observed in controls (Figures 1B and 2). In the presence of captopril, the potentiated vasodepressor response was followed by a vasopressor effect in six of seven experiments when the higher dose of bradykinin (40 ng) was used. This biphasic response was observed in only one preparation when the lower dose of bradykinin (2.5 ng) was administered. When present, the pressor response was also dose related.

In another series of experiments (n=6), bradykinin was injected into mesenteric arteries perfused with Krebs’ solution containing indomethacin before and after the addition of captopril (Figures 1D and 3). In the presence of captopril plus indomethacin, bradykinin produced only a vasorelaxant effect that lasted twofold to threefold longer (p<0.001) compared with the indomethacin-only treatment period, or approximately six times longer than in the control group.

The addition of phosphoramidon (1–200 ng/ml), a specific inhibitor of endopeptidase 24.11, to the perfusion solution produced no significant change in the response of mesenteric arteries to bradykinin.

Discussion

When bradykinin is injected into the systemic circulation, its most conspicuous effects are lowering of arterial pressure and dilation of peripheral arterioles. However, its vasodilator activity is not consistently reflected in studies performed on isolated blood vessels; depending on the species and anatomic origin of the blood vessel, bradykinin may elicit relaxation, contraction, or no response. Bradykinin induces relaxation of isolated arteries by releasing an endothelium-derived relaxing factor or by releasing vasodilator prostaglandins, or both, depending on the particular blood vessel being studied. The relaxation of isolated cat and rabbit arteries elicited by bradykinin is mediated by prostaglandin release, but bradykinin relaxes the arteries of dogs and humans by an endothelium-dependent mechanism. Both mechanisms are involved in the relaxation induced by bradykinin in bovine intrapulmonary arteries.

In the present study, with preconstricted perfused mesenteric arteries isolated from rats, bradykinin elicited a dose-related vasodilation that was potentiated by indomethacin, an inhibitor of prostaglandin synthesis. These findings indicate that the relaxing effect of bradykinin on rat mesenteric arteries does not appear to derive from prostaglandin release but rather to be blunted by the release of vasoconstrictor prostaglandins. This finding confirms a previously reported study with the same preparation, in which the vasoconstrictor response elicited by bradykinin was inhibited by cyclooxygenase inhibitors and an endoperoxide H2/thromboxane A2 receptor antagonist, but contrasts with the available in vitro data on the interaction between the two systems, kinins and prostaglandins. The reason for this discrepancy is not clear, but most previous in vitro studies have been performed on large vessels and in species other than rats. However, such a sequence of events in response to bradykinin found in the rat isolated mesenteric bed—that is, vasodilation, probably by the release of endothelium-relaxant factor, and contraction, due to prostaglandin release—might explain why the pressor response to a bradykinin antagonist becomes apparent only after prostaglandin inhibition in intact rats.

Although in our study bradykinin elicited vasodilation only in preconstricted mesenteric arteries, Faciolo et al demonstrated with the same preparation that bradykinin elicited a pronounced vasoconstriction usually preceded by a small vasodilation. This apparent discrepancy between their and our results may be due to differences in
assay conditions. For example, in our study, basal perfusion pressure was maintained at 120–130 mm Hg, approximately the same perfusion pressure observed in vivo, whereas in their study, perfusion pressure was kept at 40–80 mm Hg. It has been shown\(^5\) that blood pressure responsiveness to bradykinin is markedly affected by the level of pressure, probably because of the degree of vasoconstriction or alterations of the endothelial function due to perfusion pressure.

Captopril, a kininase II inhibitor, greatly potentiated the vasodilator effect of bradykinin on the mesenteric arteries, indicating the important role of kininase II (angiotsensin converting enzyme) in metabolizing kinins in vascular tissue. However, in the presence of captopril, bradykinin induced, in most of the experiments, a biphasic response in the perfused mesenteric bed, whose pressor component was completely blocked by indomethacin. Moreover, when prostaglandin synthesis was inhibited, captopril induced a sixfold longer lasting vasodilator response to bradykinin than in controls. These data indicate that captopril, by protecting bradykinin from being inactivated, enhanced the bradykinin-induced release of vasoconstrictor prostaglandins.

Neutral endopeptidase 24.11, an enzyme found at many of the same sites as kininase II,\(^6\) is of little or no importance as a kinin-degrading enzyme in rat mesenteric arteries, because phosphoramidon, which, so far, appears to be a specific inhibitor for this particular enzyme in mammals, did not affect the responses to bradykinin. This finding does not exclude the possibility that endopeptidase 24.11 may exist in this particular vascular bed, because the action of this endopeptidase may be of minor significance when the action of other peptidases predominates.

In conclusion, bradykinin induces a vasodilator response in rat mesenteric arteries, probably by releasing an endothelium-derived relaxing factor, that is partially blunted by the release of vasoconstrictor prostaglandins. Whereas the contribution of neutral endopeptidase 24.11 seems to be negligible, kininase II (or angiotensin converting enzyme) plays an important role in the metabolism of bradykinin in vascular tissue.

References

KEY WORDS • kinins • prostaglandins • angiotensin converting enzyme inhibitors • angiotensin converting enzymes • peptide peptidohydrolases
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Hypertension. 1992;19:II251
doi: 10.1161/01.HYP.19.2_Suppl.II251
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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