Conversion of B₁ Kinin Receptor–Mediated Vascular Relaxation to Contraction

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SUMMARY We have previously reported that des-Arg⁹-bradykinin can relax the phenylephrine-precontracted rabbit mesenteric artery through B₁ kinin receptor stimulation and the subsequent release of prostaglandins. In the present study, we have found that this relaxant response can be converted to a contractile response by the cyclooxygenase inhibitor indomethacin. Contraction was dose-dependent and was blocked by the B₁ receptor antagonist [Leu⁸]des-Arg⁹-bradykinin, with a pA₂ value obtained by Schild regression similar to that reported for relaxation in the absence of indomethacin. Des-Arg⁹-kallidin (ED₅₀ = 5.0 ± 0.9 x 10⁻⁶ M) was 16 times more potent than des-Arg⁹-bradykinin (ED₅₀ = 8.1 ± 0.8 x 10⁻⁶ M) in contracting the indomethacin-treated artery and was also blocked by [Leu⁸]des-Arg⁹-bradykinin. In contrast, only 13 out of 24 indomethacin-treated vessels contracted in response to bradykinin, which had only one tenth and one 160th the potency (ED₅₀ = 9.9 ± 1.8 x 10⁻⁷ M) of des-Arg⁹-bradykinin and des-Arg¹⁰-kallidin, respectively. B₁ kinin receptor–mediated contraction in the presence of indomethacin was unaffected by the dual cyclooxygenase-Iipoxygenase inhibitor BW 755c. These results indicate that des-Arg-kinins can stimulate both relaxation and contraction of the phenylephrine-precontracted rabbit mesenteric artery through stimulation of B₁ kinin receptors. The relaxation is dependent on the release of prostaglandins, while the contraction may represent a direct effect. (Hypertension 9 [Suppl III]: III-1–III-5, 1987)

KEY WORDS • B₁ kinin receptors • des-Arg⁹-bradykinin • indomethacin

B R A D Y K I N I N and kallidin are potent vasodilator peptides that may be involved in the regulation of both local blood flow and systemic blood pressure.¹⁻³ In addition, Regoli and co-workers⁴⁻⁵ have reported that carboxypeptidase metabolites of bradykinin and kallidin (des-Arg⁹-bradykinin and des-Arg¹⁰-kallidin, respectively) can also have significant cardiovascular effects. Des-Arg-kinins are relatively resistant to degradation by angiotensin converting enzyme,⁶ and human plasma levels of des-Arg⁹-bradykinin are several-fold higher than those of bradykinin.⁷

Des-Arg-kinins are inactive with respect to classic B₂ kinin receptors (i.e., rabbit jugular vein, dog coronary artery), but are significantly more potent than either bradykinin or kallidin in activating B₁ receptors. B₁ kinin receptor stimulation causes contraction of the isolated rabbit aorta, mesenteric vein, and basilar artery.⁸⁻¹⁰ and mediates constrictor effects in isolated perfused rat kidneys.¹⁰ Contractile effects are independent of the release of prostaglandins or leukotrienes and are blocked by the specific B₁ receptor antagonist [Leu⁸]des-Arg⁹-bradykinin.¹⁰,¹¹

In contrast to their constrictor effect in many isolated vascular preparations, des-Arg-kinins produce (and the B₁ receptor antagonist [Leu⁸]des-Arg¹⁰-kallidin blocks) a hypotensive response in vivo in rabbits treated with low doses of lipopolysaccharide.¹² Consistent with such a hypotensive effect is our recent finding that des-Arg⁹-bradykinin will produce relaxation of the rabbit mesenteric artery precontracted with phenylephrine.¹³ This relaxant effect is mediated through stimulation of B₁ kinin receptors, since it is antagonized by [Leu⁸]des-Arg⁹-bradykinin, and involves the subsequent release of prostaglandins, as it is blocked by indomethacin.

During the course of these studies, we observed a slight vasoconstrictor response to des-Arg⁹-bradykinin in indomethacin-treated vessels. Thus, the present study was conducted to determine whether des-Arg-kinin stimulation of B₁ receptors could mediate not only relaxation in control mesenteric arteries but also contraction after treatment with indomethacin.
Methods

All experimental manipulations were carried out as previously described, except that vessels were precontracted with phenylephrine to 30% maximal response. Male New Zealand white rabbits weighing 2 to 3 kg were anesthetized with a mixture of acepromazine maleate (0.5 mg/kg body weight), ketamine HCl (50 mg/kg), and xylazine HCl (10 mg/kg) and were exsanguinated. The superior mesenteric artery was carefully dissected out and immediately placed in cold Krebs solution. The artery was then cleaned of any adherent tissue and cut into a spiral strip (1 x 10 mm). Vessels were handled so as not to stretch the tissue or damage the endothelium. Endothelial integrity was confirmed by demonstrating a relaxant response to acetylcholine (10^-6 M). Tissues were placed in 5-ml tissue baths containing Krebs solution (37°C) and continuously aerated with 95% O₂, 5% CO₂. After a 1-hour equilibration period, vessels were attached to isotonic transducers (Harvard apparatus No. 52-9511; Millis, MA USA) and allowed to equilibrate for an additional 45 minutes. The tissues were washed every 15 minutes during this equilibration period. Changes in length were recorded on a multirecorder (Graphtec, KLS Associates, Cedar Grove, NJ, USA).

The maximal response to phenylephrine was determined for the tissues at the beginning of each experiment. For relaxation experiments, a dose of phenylephrine was chosen to produce 30% maximal contraction (1-3 x 10^-8 M). For cyclooxygenase inhibition experiments, tissues were pretreated (10 minutes) with indomethacin (5 x 10^-6 M) and, as above, precontracted with sufficient phenylephrine to produce 30% maximal contraction. Like Cherry et al., we found that significantly smaller doses of phenylephrine (1-5 x 10^-7 M) were required to contract indomethacin-treated vessels. Test agents were then added to the organ bath to obtain cumulative dose-response curves. Inhibitors were added to the bath 15 minutes before addition of agonists.

Bradykinin was obtained from Peninsula Laboratories (Belmont, CA, USA). Des-Arg²-bradykinin, [Leu⁴,Leu⁶]-Arg²-bradykinin, indomethacin, and phenylephrine were obtained from Sigma Chemical (St. Louis, MO, USA). BW 755c (3-amino-1-[3-trifluoromethylphenyl]-2-pyrazoline HCl) and des-Arg¹⁰-kallidin were gifts from Wellcome Research Laboratories (Beckenham, UK) and Dr. Dominico Regoli (University of Sherbrooke, Sherbrooke, Canada), respectively. The composition of the Krebs solution was (in g/L): NaCl, 6.92; KCl, 0.354; CaCl₂, 0.280; KH₂PO₄, 0.162; MgSO₄-7H₂O, 0.294; NaHCO₃, 2.1; and dextrose, 1.0. Indomethacin was dissolved in 4% NaHCO₃ (5 x 10^-2 M) and was then diluted with Krebs solution to the appropriate final concentration. All data reported are means ± standard error. The ED₅₀ values were compared using a paired Student's t test. Time dependence of the contractile responses was assessed using linear regression and calculated correlation coefficients.

Results

Des-Arg²-bradykinin produced dose-dependent (3 x 10^-9–5 x 10^-8 M) relaxation of mesenteric arteries that were precontracted to 30% of their maximum with phenylephrine (ED₅₀ = 9.2 ± 3.6 x 10^-9 M). These results are similar to those previously reported for vessels precontracted to 70% of their maximum (ED₅₀ = 7.2 ± 1.0 x 10^-9 M). A typical response to des-Arg²-bradykinin before and after indomethacin is shown in Figure 1. Des-Arg²-bradykinin (1.5 x 10^-8 M) produced 80% maximal relaxation of the phenylephrine-precontracted artery. After treatment with indomethacin and precontraction, the same dose of des-Arg²-bradykinin failed to relax the vessel, and a clear contractile response was obtained.

Cumulative dose-response curves were constructed to determine whether the contractile effect of des-Arg²-bradykinin was dose-dependent. Preliminary studies established that the magnitude of the contractile response to des-Arg²-bradykinin increased proportionately with time for the first 5 hours the tissue was suspended in the organ bath (r = 0.91, p<0.001, n = 36). The response to des-Arg²-bradykinin at 5 hours was increased 50 ± 4% over that obtained at 3 hours. This enhanced response was not specific for des-Arg²-bradykinin, since comparable time-related increases to angiotensin II (40 ± 13%, r = 0.80, p<0.001, n = 21) and 5-hydroxytryptamine (5-HT; 5 x 10^-7 M) were also observed.
45 ± 5%,  r = 0.86,  p < 0.001,  n = 20) were also found. However, after 5 hours, the contractile response to all three agonists remained stable. All dose-response studies were performed during the 2-hour period after stability was achieved.

A typical cumulative dose-response curve for des-Arg-bradykinin (5 x 10^-6-1.5 x 10^-8 M)-stimulated vasoconstriction of an indomethacin-treated mesenteric artery is shown in Figure 2. Vasoconstriction was dose-dependent, with an ED50 of 8.1 ± 0.8 x 10^-8 M (n = 13; Figure 3). Des-Arg10-kallidin also produced dose-dependent contractions (5 x 10^-6-5 x 10^-8 M) with the same maximal response as that for des-Arg2-bradykinin. However, after completion of a dose-response curve for des-Arg10-kallidin, the artery became less sensitive to both des-Arg-kinins (but not to 5-HT) in subsequent dose response curves. Thus, single dose-response curves for des-Arg10-kallidin were determined in individual tissues. As shown in Figure 3, des-Arg10-kallidin was found to be 16 times more potent (ED50 = 5.0 ± 0.9 x 10^-9 M, n = 7) than des-Arg2-bradykinin.

In order to determine whether the contractile response to des-Arg8-bradykinin and des-Arg10-kallidin could be attributed to B1, kinin receptor activation, the specific B1 receptor antagonist [Leu8]des-Arg9-bradykinin was used. As is depicted in Figure 4, increasing concentrations of [Leu8]des-Arg8-bradykinin (10-6-10-3 M) produced parallel shifts of the des-Arg8-bradykinin dose-response curve (n = 4-5). Analysis of the data by Schild regression (using each tissue as its own control) gave a slope that was not significantly different from unity and a pA2 value of 6.19 (n = 21; see Figure 4, inset).

Similarly, the contractile response to des-Arg10-kallidin was also inhibited by [Leu8]des-Arg8-bradykinin (10^-6-10^-3 M). Using separate tissues as controls, a comparable pA2 value of 5.8 was obtained for des-Arg10-kallidin (n = 18). In control experiments, [Leu8]des-Arg8-bradykinin (10^-3 M) did not inhibit the contractile effect of angiotensin II.

In contrast to the effect of the des-Arg-kinins, even high doses of bradykinin failed to exert a consistent constrictor effect on the 24 tissues examined. Of the 13 that did contract, the ED50 of bradykinin varied from 0.42 to 1.5 x 10^-8 M with the same maximal response as that of des-Arg2-bradykinin and des-Arg10-kallidin. However, the mean ED50 (9.9 ± 1.8 x 10^-7 M) demonstrated that bradykinin was only one tenth and one 160th as potent as des-Arg2-bradykinin and des-Arg10-kallidin, respectively.

The dual cyclooxygenase-lipoxygenase inhibitor BW 755c (10^-6 M) was used to determine whether lipoxygenase products were mediating the contractile response to des-Arg8-bradykinin in the presence of indomethacin. No significant difference was observed in the des-Arg8-bradykinin dose-response curve after the addition of BW 755c (n = 6).
Discussion

Stimulation of the classic B₃ kinin receptor will produce relaxation of a number of isolated blood vessels either directly or, depending on the tissue and species in question, through the release of mediators such as endothelial-derived relaxing factor or prostaglandins. In contrast, stimulation of the B₁ kinin receptor has been reported to produce vasoconstriction that is neither endothelial-dependent nor mediated by prostaglandins or leukotrienes.

We have recently found that des-Arg²-bradykinin will relax isolated rabbit mesenteric arteries preconstricted to 70% of their maximal tension with phenylephrine. Since no previous studies had reported B₁ receptor-mediated relaxation, we conducted experiments with the B₁ receptor antagonist [Leu⁸]des-Arg⁹-bradykinin and the cyclooxygenase inhibitor indomethacin that demonstrated that this relaxant effect was mediated by B₁ kinin receptor stimulation and the subsequent release of prostaglandins.

During the studies noted above, we observed that des-Arg⁹-bradykinin produced a slight constrictor effect in the presence of indomethacin. Since more significant constriction would have been obscured by the high basal tone of the vessels (70% maximal), studies were conducted on vessels at lower basal tone. Preliminary studies indicated that in the absence of precontraction with phenylephrine, vessels preconstricted to 30% of their maximum, consistent dose-response curves were obtained. Further, a clear reversal of the response from relaxation to constriction was seen in the presence of indomethacin.

The contractile response to des-Arg⁹-bradykinin was inhibited by the B₁ receptor antagonist [Leu⁸]des-Arg⁹-bradykinin. Parallel displacements of the dose-response curve with the same maximum response were observed with increasing concentrations of the antagonist. Analysis of this data by Schild regression gave a slope that did not differ significantly from unity, indicating that [Leu⁸]des-Arg⁹-bradykinin was acting as a competitive antagonist to des-Arg⁹-bradykinin. The observed pA₂ value of 6.16 is similar to that previously found (pA₂ = 6.47) for des-Arg⁹-bradykinin–induced relaxation of the mesenteric artery in the absence of indomethacin. These results support the hypothesis that both relaxation and contraction of the rabbit mesenteric artery by des-Arg⁹-bradykinin occur through stimulation of the same kinin receptor. Therefore, the response of the B₁ kinin receptor stimulation in this tissue appears to depend on the coupling of the B₁ receptor to prostaglandin synthesis, resulting in relaxation (ED₅₀ = 7.2 ± 1.0 × 10⁻⁸ M), and on other events resulting in contraction (ED₅₀ = 8.1 ± 0.8 × 10⁻⁴ M; see Figure 3).

Although the relaxant effect of des-Arg¹⁰-kallidin has not been studied, we have found that des-Arg¹⁰-kallidin is 16 times more potent than des-Arg⁸-bradykinin in contracting indomethacin-treated mesenteric arteries. Further, this contractile effect is inhibited by [Leu⁸]des-Arg⁹-bradykinin with a pA₂ value similar to that obtained for des-Arg⁹-bradykinin. Des-Arg¹⁰-kallidin has also been reported to be more potent than des-Arg⁹-bradykinin in contracting the rabbit aorta, a tissue that contains only B₁ kinin receptors. Collectively, these data indicate that both des-Arg⁸-bradykinin and des-Arg¹⁰-kallidin act through B₁ kinin receptor stimulation and confirm the relative order of potency of des-Arg-kinin–mediated contraction originally reported by Regoli and co-workers.

Vavrek and Stewart have recently identified antagonists to the classic (B₃) kinin receptor. Although the effects of such antagonists have not been examined in the present study, it will be interesting to determine whether they also block the B₁ receptor–mediated effects of des-Arg-kinins in rabbit mesenteric arteries. Some level of inhibition may be likely, considering the recent report of Regoli and co-workers that [Thr³]d-Phe⁴-bradykinin blocks B₁ receptor-stimulated contraction of the rabbit aorta.

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In contrast to the effect of the des-Arg-kinins, even high doses of bradykinin failed to exert a consistent constrictor effect on indomethacin-treated vessels. In
the vessels that did contract (13 out of 24), bradykinin was only one tenth and one 160th as potent as des-Arg<sup>9</sup>-bradykinin and des-Arg<sup>10</sup>-kallidin, respectively. Since the rabbit mesenteric artery may contain both B<sub>1</sub> and B<sub>2</sub> receptors, it remains to be determined whether the inconsistent vasoconstrictor effect of bradykinin is due to B<sub>1</sub> or B<sub>2</sub> receptor stimulation.

Whether B<sub>1</sub> kinin receptor–mediated contraction of the mesenteric artery is occurring through a direct effect or through the release of other mediators is unknown. The reversal of the des-Arg<sup>9</sup>-bradykinin–induced relaxant response in the presence of indomethacin does not appear to be due to shunting of arachidonic acid metabolism to the lipoxygenase pathway, since the response was not blocked by the dual cyclooxygenase–lipoxygenase inhibitor BW 755c.<sup>20</sup> These results are in agreement with those of Regoli and co-workers,<sup>21</sup> who have reported that B<sub>2</sub> kinin receptor–mediated contraction of the rabbit aorta is not blocked by the dual cyclooxygenase–lipoxygenase inhibitor eicosatetraynoic acid.

The mesenteric vessels used in our present study relaxed in response to acetylcholine (10<sup>-8</sup> M), demonstrating the presence of endothelium. However, the participation of endothelium in B<sub>1</sub> receptor–mediated relaxation or contraction (or both) cannot be properly evaluated from the present data, since comparable studies were not conducted with endothelium-denuded vessels. Nevertheless, Regoli and co-workers<sup>21</sup> have reported that B<sub>2</sub> kinin receptor–mediated contraction (rabbit aorta) is not endothelial-dependent.

The sensitivity of both the rabbit aorta and mesenteric vein to B<sub>1</sub> kinin receptor stimulation is reported to increase with time due to de novo synthesis of kinin receptors.<sup>4, 21, 22</sup> In the present study, the magnitude of the des-Arg<sup>9</sup>-bradykinin–induced contractile response in the rabbit mesenteric artery also increased with time (up to 5 hours). However, this increased response was not specific for B<sub>2</sub> kinin receptor stimulation, since comparable increases were seen for both angiotensin II and 5-HT. These data suggest that, unlike in the rabbit aorta and mesenteric vein, no specific induction of B<sub>1</sub> kinin receptors occurred in the mesenteric artery during the course of the experiment. Rather, there was a change in overall smooth muscle sensitivity.

In summary, the present study has demonstrated that B<sub>1</sub> kinin receptor–mediated relaxation of the phenylephrine-precontracted rabbit mesenteric artery depends on the coupling of this receptor to prostaglandin synthesis. If prostaglandin synthesis is inhibited, stimulation of this same kinin receptor will result in contraction either by a direct effect or through the release of other, unidentified mediators.

### References

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