Conversion of \( B_1 \) Kinin Receptor-Mediated Vascular Relaxation to Contraction

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SUMMARY We have previously reported that des-Arg\(^{9}\)-bradykinin can relax the phenylephrine-precontracted rabbit mesenteric artery through \( B_1 \) 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 \( B_1 \) receptor antagonist [Leu\(^{8}\)]des-Arg\(^{9}\)-bradykinin, with a \( pA_2 \) value obtained by Schild regression similar to that reported for relaxation in the absence of indomethacin. Des-Arg\(^{9}\)-kallidin (ED\(_{50} = 5.0 \pm 0.9 \times 10^{-8} \) M) was 16 times more potent than des-Arg\(^{4}\)-bradykinin (ED\(_{50} = 8.1 \pm 0.8 \times 10^{-8} \) M) in contracting the indomethacin-treated artery and was also blocked by [Leu\(^{8}\)]des-Arg\(^{9}\)-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 (ED\(_{50} = 9.9 \pm 1.8 \times 10^{-7} \) M) of des-Arg\(^{9}\)-bradykinin and des-Arg\(^{10}\)-kallidin, respectively. \( B_1 \) kinin receptor-mediated contraction in the presence of indomethacin was unaffected by the dual cyclooxygenase-1ipoxygenase 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 \( B_1 \) 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 • \( B_1 \) kinin receptors • des-Arg\(^{9}\)-bradykinin • 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 \(10^{-6} \text{M}\). Tissues were placed in 5-ml tissue baths containing Krebs solution (37°C) and continuously aerated with 95% O\(_2\), 5% CO\(_2\). 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 \((1-3 \times 10^{-8} \text{M})\). For cyclooxygenase inhibition experiments, tissues were pretreated (10 minutes) with indomethacin \((5 \times 10^{-7} \text{M})\) and, as above, precontracted with sufficient phenylephrine to produce 30% maximal contraction. Like Cherry et al., we found that significantly smaller doses of phenylephrine \((1-5 \times 10^{-7} \text{M})\) 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-Arg\(^9\)-bradykinin, \([\text{Leu}^7\]-des-Arg\(^9\)-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-Arg\(^9\), 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; CaCl\(_2\), 0.280; KH\(_2\)PO\(_4\), 0.162; MgSO\(_4\)-7H\(_2\)O, 0.294; NaHCO\(_3\), 2.1; and dextrose, 1.0. Indomethacin was dissolved in 4% NaHCO\(_3\) \((5 \times 10^{-2} \text{M})\) and was then diluted with Krebs solution to the appropriate final concentration.

All data reported are means ± standard error. The \(ED_{50}\) 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-Arg\(^9\)-bradykinin produced dose-dependent \((3 \times 10^{-9} - 5 \times 10^{-8} \text{M})\) relaxation of mesenteric arteries that were precontracted to 30% of their maximum with phenylephrine \((ED_{50} = 9.2 ± 3.6 \times 10^{-9} \text{M})\). These results are similar to those previously reported for vessels precontracted to 70% of their maximum \((ED_{50} = 7.2 ± 1.0 \times 10^{-9} \text{M})\). A typical response to des-Arg\(^9\)-bradykinin before and after indomethacin is shown in Figure 1. Des-Arg\(^9\)-bradykinin \((1.5 \times 10^{-4} \text{M})\) produced 80% maximal relaxation of the phenylephrine-precontracted artery. After treatment with indomethacin and precontraction, the same dose of des-Arg\(^9\)-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-Arg\(^9\)-bradykinin was dose-dependent. Preliminary studies established that the magnitude of the contractile response to des-Arg\(^9\)-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-Arg\(^9\)-bradykinin at 5 hours was increased 50 ± 4% over that obtained at 3 hours. This enhanced response was not specific for des-Arg\(^9\)-bradykinin, since comparable time-related increases to angiotensin II \((40 ± 13%, r = 0.80, p < 0.001, n = 21)\) and 5-hydroxytryptamine \((5-HT;\)
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 \(E_{D_{50}}\) of bradykinin varied from 0.42 to \(1.5 \times 10^{-6}\) M with the same maximal response as that of des-Arg\(^9\)-bradykinin and des-Arg\(^10\)-kallidin. However, the mean \(E_{D_{50}} (9.9 \pm 1.8 \times 10^{-7} \text{M})\) demonstrated that bradykinin was only one tenth and one 160th as potent as des-Arg\(^9\)-bradykinin and des-Arg\(^10\)-kallidin, respectively.

The dual cyclooxygenase-lipoxygenase inhibitor BW 755c (10\(^{-4}\) M) was used to determine whether lipoxygenase products were mediating the contractile response to des-Arg\(^9\)-bradykinin in the presence of indomethacin. No significant difference was observed in the des-Arg\(^9\)-bradykinin dose-response curve after the addition of BW 755c (n = 6).

Similarly, the contractile response to des-Arg\(^10\)-kallidin was also inhibited by [Leu\(^8\)]des-Arg\(^8\)-bradykinin (10\(^{-6}\)-10\(^{-3}\) M). Using separate tissues as controls, a comparable \(pA_2\) value of 5.8 was obtained for des-Arg\(^10\)-kallidin (n = 18). In control experiments, [Leu\(^8\)]des-Arg\(^8\)-bradykinin (10\(^{-3}\) M) did not inhibit the contractile effect of angiotensin II.
**Discussion**

Stimulation of the classic B₂ 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 precontracted 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 pre-contracture with phenylephrine, vessels did not consistently respond to des-Arg⁹-bradykinin over a dose range of $5 \times 10^{-9}$ to $1.5 \times 10^{-8}$ M. However, in vessels precontracted to 30% of their maximum, consistent dose-response curves were obtained. Further, a clear reversal of the response from relaxation to contraction 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 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 × 10⁻⁴ 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-Arg⁸-kallidin has also been reported to be more potent than des-Arg⁸-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 B₁ contraction originally reported by Regoli and co-workers.

Vavrek and Stewart have recently identified antagonists to the classic (B₂) 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 B₂ 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 [Thr⁵]Phe¹-bradykinin blocks B₂ receptor-stimulated contraction of the rabbit aorta. 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\textsuperscript{9}-bradykinin and des-Arg\textsuperscript{10}-kallidin, respectively. Since the rabbit mesenteric artery may contain both B\textsubscript{1} and B\textsubscript{2} receptors,\textsuperscript{13} it remains to be determined whether the inconsistent vasoconstrictor effect of bradykinin is due to B\textsubscript{2} or B\textsubscript{1} receptor stimulation.

Whether B\textsubscript{1} 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\textsuperscript{9}-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.\textsuperscript{20} These results are in agreement with those of Regoli and co-workers,\textsuperscript{12} who have reported that B\textsubscript{1} 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\textsuperscript{8} M), demonstrating the presence of endothelium. However, the participation of endothelium in B\textsubscript{1} 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\textsuperscript{13} have reported that B\textsubscript{1} kinin receptor-mediated contraction (rabbit aorta) is not endothelium-dependent.

The sensitivity of both the rabbit aorta and mesenteric vein to B\textsubscript{1} kinin receptor stimulation is reported to increase with time due to de novo synthesis of kinin receptors.\textsuperscript{4, 21, 22} In the present study, the magnitude of the des-Arg\textsuperscript{9}-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\textsubscript{1} 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\textsubscript{1} 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\textsubscript{1} 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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