Biphasic Effect of Bradykinin on Rabbit Afferent Arterioles

Hong Yu, Oscar A. Carretero, Luis A. Juncos, Jeffrey L. Garvin

Abstract—Bradykinin plays an important role in the regulation of renal hemodynamics. However, there have been few studies of the effect of bradykinin on isolated afferent arterioles, vascular segments that are important for the regulation of renal blood flow and glomerular filtration rate. Our purpose was to study (1) the effects of bradykinin on isolated perfused rabbit afferent arterioles and (2) the mechanisms of actions. Afferent arterioles dissected from rabbits were perfused in vitro at 60 mm Hg. In afferent arterioles preconstricted with phenylephrine, 10^{-12} to 10^{-10} mol/L bradykinin increased luminal diameter from 9.0±1.0 to 14.3±1.2 μm (P<0.003). In contrast, 10^{-9} and 10^{-8} mol/L bradykinin decreased luminal diameter to 10.8±1.4 and 9.7±1.2 μm, respectively (P<0.001). Bradykinin added to the bath had no effect on preconstricted afferent arterioles. The addition of [des-Arg^{9}] bradykinin (10^{-9} and 10^{-8} mol/L), a B₁ receptor agonist, to the lumen decreased diameter from 9.7±1.2 to 6.7±1.2 μm at 10^{-8} mol/L (P<0.002). Icatibant (Hoe 140), a B₂ receptor antagonist, blocked both the vasodilation and vasoconstriction induced by bradykinin as well as the vasooconstriction induced by [des-Arg^{9}] bradykinin. L-NAME had no effect on bradykinin-induced dilation or constriction. Indomethacin blocked both the dilation induced by 10^{-12} to 10^{-10} mol/L bradykinin and the constriction induced by 10^{-9} to 10^{-8} mol/L bradykinin. In fact, in the presence of indomethacin, 10^{-4} and 10^{-8} mol/L bradykinin increased luminal diameter from 6.2±0.7 to 10.7±0.6 μm at 10^{-8} mol/L (P<0.001), which was attenuated by L-NAME. Finally, in the presence of SQ29548, a prostaglandin H₂/thromboxane A₂ receptor antagonist, bradykinin caused dilation at all concentrations tested. In conclusion, bradykinin has a biphasic effect on afferent arterioles. Both dilation and constriction may be mediated by bradykinin B₂ receptors. The mechanisms of vasodilation and vasoconstriction are due to cyclooxygenase products, not nitric oxide. (Hypertension. 1998;32:287-292.)

Key Words: prostaglandins ■ nitric oxide ■ renal blood flow ■ receptors ■ thromboxane

The kallikrein-kinin system has been reported to play a role in the regulation of renal hemodynamics and thereby in sodium and water excretion, blood pressure, and renin release.1-8 Previous studies have shown that kallikrein from the connecting tubule enters the vascular space9-11 as well as the interstitium,8 where it produces bradykinin. Thus, both plasma bradykinin and interstitial bradykinin may be significant regulators of afferent arteriole resistance.

Several investigators have shown that bradykinin increases renal blood flow,12-19; however, few have directly investigated the effects of bradykinin on the afferent arteriole without the confounding effects of the proximal and distal vasculature. Edwards et al20 studied the effect of bradykinin on afferent arterioles in vivo but found none. These studies focused primarily on the effects of bradykinin added to the bath. Because the results of Edwards et al20 clearly conflict with the in vivo data, we investigated the effect of bradykinin on isolated perfused afferent arterioles in the presence of flow.

In addition to the uncertainty regarding the action of bradykinin on the afferent arteriole, the mechanisms by which it acts are also unclear. There are at least two significant bradykinin receptors: B₁ and B₂. A majority of studies have shown that the effects of bradykinin are due to activation of the B₂ receptor.21-28 However, Lortie et al12 have shown that the increase in renal blood flow induced by bradykinin is due to both B₁ and B₂ receptors. In addition, the second messenger cascades activated by these receptors are also in question. There are data that support roles for NO,13 prostaglandins and NO,26 and P450–arachidonic acid products.14 The explanation for these disparate results is unclear; however, they may be due to differences in experimental design.

Methods

Kidneys of young male New Zealand White rabbits (1.4 to 2.2 kg) were removed and sliced longitudinally along the corticomedullary axis. The slices were placed in ice-cold minimum essential medium (MEM; Gibco Laboratories) containing 5% BSA (Intergen Co), and a single superficial afferent arteriole with its glomerulus intact was dissected. This arteriole was transferred to a perfusion chamber mounted on an inverted microscope and cannulated with an array of glass pipettes as described previously.27,28 The afferent arteriole was perfused with oxygenated MEM containing 5% BSA, with intraluminal pressure maintained at 60 mm Hg throughout the experiment. Luminal flow was approximately 300 μL/min. The bath was similar to the perfusate except that it contained 0.1% BSA and was exchanged continuously. All studies were in accordance with the guidelines of the Henry Ford Hospital Animal Care and Use Committee.

Microdissection and cannulation of the afferent arteriole were completed within 90 minutes at 8°C, after which the bath was gradually warmed to 37°C for the rest of the experiment. Once the temperature was stable, a 30-minute equilibration period was al-
lowed before any measurements were taken. Images of the afferent arterioles were displayed at magnifications up to ×1980 and recorded with a video system. The diameter was measured with an image analysis system.

Phenylephrine, L-NAME (an NO synthase inhibitor), and indomethacin (a cyclooxygenase inhibitor) were purchased from Sigma Co. SQ29548, a TXA2/PGH2 receptor antagonist, was obtained from Biomol. Bradykinin and (des-Arg9)-bradykinin were purchased from Biomol. We found that standard BSA had the least kininogenase activity in several different preparations of BSA. We found that standard BSA had the least kininogenase activity; thus, we used standard BSA in all solutions throughout the experiments. In addition, bradykinin was added to the perfusate immediately before its use to avoid time-dependent breakdown. Measurements were made 5 to 10 minutes later. Icatibant and (des-Arg9)-bradykinin were prepared in the same manner as bradykinin. Phenylephrine was used to preconstrict the arterioles to approximately 50% of their initial diameter. The range of concentrations from 10^{-6} to 10^{-8} mol/L was 0.1 to 3 μmol/L.

Statistics
Data are expressed as mean±SEM. Paired t tests were used to examine whether the diameter at a given concentration was different from the control value within each group. Univariate repeated-measures ANOVA with the Greenhouse-Geisser sphericity correction was used to test whether the groups (treated versus nontreated) differed with respect to the rate of change across the various periods. For this analysis, P<0.05 was considered significant. If a significant or borderline interaction effect was detected, Student’s two-sample t test (or Welch’s test in the event of unequal variances) was used to examine whether the change in diameter at a given concentration differed between 2 groups. When more than 1 measurement was made, Bonferroni’s multiple comparison adjustment was used to reduce the significance level.

Results
Because in vivo studies have demonstrated that bradykinin increases renal blood flow, we first examined whether it dilates preconstricted arterioles. Afferent arterioles were preconstricted with phenylephrine to approximately 50% of basal diameter, after which increasing doses of bradykinin (10^{-12} to 10^{-8} mol/L) were added to the lumen. Bradykinin concentrations from 10^{-12} to 10^{-10} mol/L increased luminal diameter in a dose-dependent manner as shown in Figure 1. Maximum dilation occurred at 10^{-10} mol/L, a concentration that increased diameter from 9.9±1.0 to 14.3±1.2 μm. In contrast, higher concentrations of bradykinin decreased luminal diameter, with 10^{-8} mol/L bradykinin reducing it from 14.3±1.2 to 9.7±1.2 μm (P<0.001; Figure 1). Time controls showed no significant change in diameter. These data suggest that bradykinin has a biphasic effect on preconstricted afferent arterioles. Because 10^{-8} to 10^{-8} mol/L bradykinin reduced luminal diameter in preconstricted afferent arterioles, we next determined whether the latter concentration reduced the diameter of nonpreconstricted afferent arterioles. For this, 10^{-8} mol/L bradykinin was added to the perfusate of nonpreconstricted afferent arterioles. Basal luminal diameter was 19.3±0.7 μm and did not change when bradykinin was added (19.6±0.8 μm; n=3). Taken together, these data indicate that bradykinin only constricts afferent arterioles when vascular tone is present.

Because bradykinin only exerted its effects in preconstricted afferent arterioles, in the remaining protocols all arterioles were preconstricted to ~50% of their basal diameter before the addition of bradykinin. Next, we tested whether 10^{-8} mol/L bradykinin induces vasoconstriction without the confounding dilator effects of the low doses of bradykinin. When 10^{-8} mol/L bradykinin was added to the lumen of preconstricted arterioles, it decreased diameter from 10.7±0.9 to 8.3±0.8 μm (P<0.02).

Bradykinin may be released into the interstitium as well as the vascular space; therefore, we next studied the effect of 10^{-12} to 10^{-8} mol/L bradykinin in the bath. Bradykinin had no significant effect on preconstricted afferent arterioles when added to the bath (Figure 2). To demonstrate that these vessels would respond to bradykinin, we then washed the bradykinin from the bath and added it to the lumen. When added to the lumen, bradykinin induced dilation.
Because bradykinin exerts most of its vascular actions through B₁ and B₂ receptors, the biphasic effect of bradykinin could be explained by activation of 2 receptor subtypes. We tested the ability of the B₂ receptor antagonist icatibant to block the effects of bradykinin. First, 10⁻²⁷ mol/L icatibant was added to the perfusate, and afferent arterioles were preconstricted with phenylephrine. Bradykinin was then added to the perfusate in increasing concentrations from 10⁻¹² to 10⁻⁸ mol/L. Icatibant blocked both the vasodilation induced by low concentrations of bradykinin and the vasoconstriction induced by high concentrations (Figure 3).

To examine whether the B₁ receptors are also involved in the action of bradykinin, we first tested whether [des-Arg⁹]-bradykinin, a B₁ receptor agonist, mimics the effect of bradykinin. The addition of [des-Arg⁹]-bradykinin (10⁻⁹ mol/L) to the lumen reduced diameter from 9.7±1.1 to 7.0±1.1 μm, and 10⁻⁸ mol/L reduced it further to 6.7±1.2 μm. Next, we tested whether the B₁ receptor agonist exerts its effect via the B₂ receptor. In the presence of 10⁻⁷ mol/L icatibant, [des-Arg⁹]-bradykinin had no effect (Figure 4). These data suggest that the vasodilator and vasoconstrictor actions of bradykinin may be mediated by the bradykinin B₂ receptor.

Next, we examined the mechanism of bradykinin’s actions. Because bradykinin is known to stimulate NO release in other vascular beds, resulting in vasodilation, we first studied the role of NO in mediating bradykinin-induced dilation of afferent arterioles. Initially, luminal diameter was 16.6±0.6 μm. After 10⁻⁴ mol/L of L-NAME, an inhibitor of NO synthesis, was added to the perfusate, luminal diameter decreased to 12.8±1.3 μm. Afferent arterioles were then preconstricted, and the effect of bradykinin was examined as before. Bradykinin diluted L-NAME–treated afferent arterioles to the same extent as arterioles not treated with L-NAME (Figure 5). Maximal dilation occurred at 10⁻¹⁰ mol/L in both groups. Higher concentrations of bradykinin caused similar vasoconstriction in both groups. These data suggest that NO does not play a major role in the response of the afferent arteriole to bradykinin.

Bradykinin is also known to stimulate the release of both vasodilator and vasoconstrictor cyclooxygenase products. Because NO did not appear to be important in the dilation induced by bradykinin, we tested whether prostaglandins mediate bradykinin-induced dilation and/or constriction. Initially, luminal diameter was 18.7±1.1 μm. When indomethacin (5 μmol/L) was added to the lumen and bath, diameter did not change. As shown in Figure 6, indomethacin blocked both the dilation of preconstricted arterioles induced by low concentrations of bradykinin and the constrictor action of high concentrations. In fact, a vasodilator effect of

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**Figure 3.** Effect of bradykinin on preconstricted afferent arteriolar diameter in the presence and absence of icatibant, a B₂ receptor antagonist. Icatibant, n=8; vehicle, n=6. CON indicates diameter after constriction with phenylephrine. *P<0.003 vs CON. **P<0.001 vs BK 10⁻¹⁰ mol/L.

**Figure 4.** Effect of [des-Arg⁹]-bradykinin, a B₁ receptor agonist, on the diameter of preconstricted afferent arterioles in the presence of B₂ receptor antagonist icatibant. Icatibant, n=6; vehicle, n=7. CON indicates diameter after constriction with phenylephrine. *P<0.02 vs CON.

**Figure 5.** Effect of bradykinin on preconstricted afferent arteriolar diameter in the presence and absence of L-NAME. Vehicle, n=10; L-NAME, n=8. CON indicates diameter after constriction with phenylephrine. With L-NAME: *P<0.001 vs CON, **P<0.05 vs BK 10⁻¹⁰ mol/L. Without L-NAME: *P<0.003 vs CON, **P<0.001 vs BK 10⁻¹⁰ mol/L.

**Figure 6.** Effect of bradykinin on preconstricted afferent arteriolar diameter in the presence of indomethacin. CON indicates diameter after constriction with phenylephrine (n=8). *P<0.001 vs BK 10⁻¹⁰ mol/L.
10⁻⁹ and 10⁻⁸ mol/L bradykinin was now observed, with luminal diameter increasing from 6.2 ± 0.7 to 10.7 ± 0.6 μm at 10⁻⁸ mol/L. (P<0.001). These data suggest that cyclooxygenase products mediate both bradykinin-induced vasodilation and constriction.

Because we found that indomethacin unmasked a vasodilator effect of high doses of bradykinin, we next investigated the mechanism involved. We postulated that the vasodilation produced by high doses of bradykinin was mediated by NO. To test this, indomethacin was added as before (to unmask the dilator action of high doses of bradykinin), and then 10⁻⁴ mol/L L-NAME was added to the perfusate. With 10⁻⁹ to 10⁻⁸ mol/L bradykinin, luminal diameter rose from 6.2 ± 0.7 to 10.7 ± 0.6 μm (P<0.001) in the untreated group but did not increase significantly in the L-NAME–treated group (P>0.1). Thus, L-NAME prevented the vasodilation induced by 10⁻⁹ and 10⁻⁸ mol/L bradykinin in indomethacin-treated afferent arterioles (Figure 7). These data suggest that bradykinin releases NO from rabbit afferent arterioles but only at concentrations >10⁻⁹ mol/L.

Finally, we investigated whether the cyclooxygenase product(s) involved in bradykinin–induced vasoconstriction were acting via the TXA₂/PGH₂ receptor by examining the ability of SQ29548, a TXA₂/PGH₂ receptor antagonist, to block vasoconstriction. Initially, luminal diameter was 17.0 ± 2.0 μm. Pretreatment of afferent arterioles with SQ29548 did not alter basal diameter. Concentrations of bradykinin from 10⁻¹² to 10⁻¹⁰ mol/L induced vasodilation, as seen in untreated preconstricted arterioles; however, 10⁻⁹ and 10⁻⁸ mol/L bradykinin caused further dilation in the presence of SQ29548 rather than vasoconstriction (Figure 8). These data suggest that the vasoconstrictor effect of bradykinin takes place via activation of the TXA₂ receptor.

Discussion

In our studies, when bradykinin was added to the lumen, it had a biphasic effect on diameter in preconstricted vessels. by B₂ receptors. Furthermore, our results suggest that bradykinin-induced vasodilation and vasoconstriction are due to metabolites of the cyclooxygenase pathway rather than NO.

In the present study, we examined the direct action of concentrations of bradykinin ranging from 10⁻¹² to 10⁻⁸ mol/L on isolated microperfused afferent arterioles of rabbits. We chose these concentrations because concentrations of bradykinin reported in the literature range from 2×10⁻¹² mol/L to 5×10⁻⁵ mol/L. When bradykinin was added to the lumen, it dilated preconstricted arterioles at concentrations ranging from 10⁻¹² to 10⁻¹⁰ mol/L, whereas concentrations of 10⁻⁹ and 10⁻⁸ mol/L induced vasoconstriction. Because 10⁻⁸ mol/L induced vasoconstriction, we next examined whether it would do so without the arterioles first being treated with low concentrations of bradykinin. The decrease in diameter induced by 10⁻⁸ mol/L was similar in preconstricted arterioles whether or not they were first treated with low concentrations of bradykinin. Finally, we tested whether 10⁻⁸ mol/L bradykinin could constrict afferent arterioles that had not been preconstricted with phenylephrine. This concentration of bradykinin had no effect on diameter in the absence of phenylephrine. These data suggest that either bradykinin induces vasoconstriction only when there is basal tone or else there is some synergism between bradykinin and phenylephrine.

Our finding that luminal bradykinin has a biphasic effect on afferent arteriole diameter indicates that it should also have a biphasic effect on renal vascular resistance, since the afferent arteriole accounts for a large portion of the latter parameter. Previous reports of the effects of bradykinin on renal blood flow have indicated only that bradykinin induces vasodilation. However, Guimaraes et al did report that lysylbradykinin has a temporal biphasic effect on renal vascular resistance in vivo. The reason for the disparate results is unclear, but it should be noted that in many of the aforementioned studies only 1 concentration of bradykinin was used and/or animals were pretreated with a cyclooxygenase inhibitor. Our data indicate that if afferent arterioles are first treated with a cyclooxygenase inhibitor, they only dilate when challenged with bradykinin. In our studies, when bradykinin was added to the lumen, it had a biphasic effect on diameter in preconstricted vessels.
These results differ from those of Edwards et al. This is undoubtedly due to differences in experimental design. In our experiments, arterioles were exposed to flow and luminal bradykinin (from $10^{-12}$ to $10^{-7}$ mol/L) after the vessels were preconstricted. In the study by Edwards et al., there was no flow through the lumen of the arterioles and only 0.1 mmol/L bradykinin was used. Edwards et al reported that bradykinin did not alter arteriolar diameter when added to vessels that were not preconstricted, or when added to the lumen simultaneously with norepinephrine. The former results are similar to ours; however, according to our data, the latter protocol should have induced vasoconstriction. Two possible explanations for the discrepancy are that (1) the reduction in diameter induced by bradykinin, when added simultaneously with norepinephrine, may not have been large enough to distinguish from that of norepinephrine alone; or (2) the differences may be due to the presence or absence of flow. There was no luminal flow in the experiments of Edwards et al, but in our studies there was. The response of the afferent arteriole to vasoactive agents has been shown to be modified by flow.

Because bradykinin may also be formed in the interstitial space as well as the luminal space, we added bradykinin to the bath. When this was done, bradykinin had no effect on preconstricted afferent arterioles. This result was similar to that reported by Edwards et al. While it is unclear why bradykinin alters afferent arteriolar diameter when added to the lumen but not the bath, possible explanations may be that (1) bradykinin acting via receptors on smooth muscle cells induces constriction while receptors on endothelial cells induce dilation, and the two effects cancel each other; (2) bradykinin induces only very weak contractions in the smooth muscle of arterioles and does not reach the endothelium when added to the bath because of degradation; or (3) bradykinin has no direct effect on vascular smooth muscle of the afferent arteriole, and the receptors on the endothelium are localized to the luminal membrane.

Most of the effects of bradykinin are mediated via B₁ and B₂ receptors. In the present study, we found that both the vasodilation and vasoconstriction induced by bradykinin were mediated by the B₂ receptor. The bradykinin B₂ receptor is thought to mediate not only endothelium-dependent vasodilation of canine carotid, bovine or porcine coronary arteries and canine renal arteries but also venoconstriction and smooth muscle contraction. These results are consistent with immunohistochemical studies, which have shown that afferent arterioles have numerous B₂ receptors. Our results indicate that the effects of B₁ receptor agonists may actually be mediated via the B₂ receptor. However, concern about the selectivity of icatibant and [des-Arg⁹]-bradykinin for the appropriate receptors remains an issue. Consequently, we cannot completely eliminate a role for the B₁ receptor in bradykinin’s action.

The mechanisms by which bradykinin exerts its effects are unclear. In this study, we found that blocking cyclooxygenase with indomethacin abolished both bradykinin-induced dilation and constriction of preconstricted afferent arterioles. These data indicate that cyclooxygenase products are responsible for most of the effects of bradykinin on the afferent arteriole. We also found that the vasoconstriction induced by bradykinin was blocked by a PGH₂/TXA₂ receptor antagonist, indicating that I or both of these constrictor compounds is responsible for the reduction in arteriolar diameter induced by 1 and 10 mmol/L bradykinin. Bradykinin also has been found to stimulate the release of vasodilator prostaglandins from cultured endothelial cells and is known to stimulate phospholipase A₂, causing the release of arachidonic acid and the subsequent synthesis of cyclooxygenase or lipoxygenase products.

If the ability of bradykinin to release prostaglandins is blocked, we found that bradykinin-induced release of NO will dilate the afferent arteriole. After indomethacin blocked the vasoconstriction induced by high doses of bradykinin, vasodilation was unmasked that could be blocked by an NO synthesis inhibitor, L-NAME. In the absence of indomethacin, pretreatment of arterioles with L-NAME failed to affect the response of afferent arterioles to bradykinin. Taken together, these data suggest that low concentrations of bradykinin induce the release of vasodilator cyclooxygenase products, whereas higher doses release vasoconstrictor cyclooxygenase products, probably PGH₂ or TXA₂. Finally, cyclooxygenase products mask the effect of NO release by bradykinin at concentrations $>10^{-7}$ mol/L on afferent arterioles. Whereas bradykinin stimulates the production of several classes of prostaglandins, tissue responsiveness to a particular class of prostaglandin (ie, vasoconstrictor or vasodilator) may determine whether the net response is constriction or relaxation.

Our results are similar to those of Guimaraes et al. Siragy et al. and Quilley et al. who showed that the dilation induced by bradykinin is mediated by prostaglandins. These findings can also be reconciled with those of Lahera et al. who have shown that the renal vasodilator response to bradykinin is mediated by NO, since the dogs were first treated with meclofenamate, a cyclooxygenase inhibitor.

In conclusion, bradykinin has a biphasic effect on afferent arterioles. Both dilation and constriction may be mediated by the bradykinin B₂ receptor. The mechanisms of vasodilation and vasoconstriction are primarily due to cyclooxygenase products, not NO. Bradykinin-induced NO release dilates vessels if cyclooxygenase is inhibited.

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References
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