Disappearance of Bradykinin in the Renal Circulation of Dogs

EFFECTS OF KININASE INHIBITION

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ABSTRACT

In chloralose-anesthetized dogs, we investigated the disappearance of bradykinin on passage across the renal circulation. The peptide was infused into a renal artery at various doses (5-200 ng/kg min⁻¹); renal blood flow and the concentration of kinins in renal venous blood were then determined and the percent survival of bradykinin on passage through the kidney calculated. Bradykinin caused a dose-related increase in renal blood flow, urine flow, sodium excretion, and kinin content of renal venous blood. Intravenous administration of BPP₉₋₉ (300 μg/kg), a peptide kininase II inhibitor, potentiated the renal vasodilator, diuretic, and natriuretic actions of bradykinin and augmented the survival of the kinin on passage through the kidney from 12.72 ± 1.64% in control dogs to 53.92 ± 7.48% (P < 0.001). Furthermore, the values of peptide survival were positively correlated with the increases in renal blood flow (r = 0.92, P < 0.01), urine flow (r = 0.75, P < 0.01), and sodium excretion (r = 0.68, P < 0.01) produced by bradykinin. In addition, BPP₉₋₉ by itself increased renal blood flow (16%, P < 0.01), urine flow (115%, P < 0.005), and sodium excretion (167%, P < 0.02). Similarly, the concentration of kinin in renal venous blood and the excretion of urinary kinins rose from 0.11 ± 0.03 ng/ml and 4.4 ± 1.1 ng/min to 0.24 ± 0.05 ng/ml (P < 0.005) and 38.5 ± 12.2 ng/min (P < 0.02). These studies suggest that kinins generated intrarenally play a role in the regulation of renal blood flow and salt-water excretion and that variations in the capacity of the kidney to inactivate kinins may be a determinant of the intrarenal activity of the kallikrein-kinin system.

The demonstration that urine contains large amounts of kinins (1) and an active kallikrein indistinguishable from the active form of renal kallikrein (2) suggests that kinins may be generated within the kidney. Since this organ is also a rich source of kininases (3), the intrarenal activity of the kallikrein-kinin system is probably a function of both kinin-generating and kinin-inactivating mechanisms. The importance of the latter cannot be overlooked when one is considering the local activity of hormones that are as readily inactivated by renal enzymes as are kinins. In comparison to other tissues, the kidney is one of the richest sources of kininases; it has at least three types of kinin-inactivating enzymes (3). Because these enzymes are predominantly located in subcellular structures (3), the in vitro inactivation of kinin by a renal extract may bear no relationship to the in vivo inactivation of the peptide. For example, homogenized lungs destroy bradykinin more slowly than do renal extracts (4), whereas intact lungs appear to remove circulating kinins more efficiently than do kidneys (5). Ferreira and Vane (5) have estimated the ability of the kidney to remove kinins in vivo. In two experiments they found that 72% of the bradykinin infused intrarenally disappeared during passage through the renal circulation of the cat. Their experiments, however, did not indicate whether the peptide was enzymatically degraded or excreted into the urine as suggested by the finding of large quantities of labeled material in the kidney and the urine of rats infused with tritiated bradykinin (6).

Recent evidence suggesting the involvement of the renal kallikrein-kinin system in the regulation of blood pressure and sodium-water homeostasis emphasizes the need for defining the mechanisms and the functional implications of kinin-inactivating processes within the kidney. To evaluate intrarenal destruction of kinins as a mechanism which modulates the activity of the kallikrein-kinin system of the kidney, we investigated the disappearance of bradykinin on passage across the renal circulation before and after the administration of a kininase II inhibitor. In addition, we correlated the renal responses to intrarenal infusions of bradykinin with
the survival of the peptide on passage across the kidney. Finally, the effects of kininase II inhibition on renal blood flow and sodium excretion were examined and related to variations in the kinin content of urine and renal venous blood.

Methods

Male mongrel dogs (23–27 kg) which were fasted overnight but allowed free access to water were anesthetized with morphine sulfate (2 mg/kg, sc) and chloralose (100 mg/kg, iv). The trachea was cannulated, and the lungs were ventilated with a positive-pressure pump. The artery, vein, and ureter of the right kidney were exposed through a midline incision. A multichannel direct writer (Hewlett-Packard, model 7720) recorded (1) mean aortic blood pressure measured with a Statham transducer (model P23Db) via a catheter inserted in a retrograde direction into the left brachial artery and (2) renal blood flow measured with an electromagnetic flowmeter (Statham, model FC400). A polyethylene catheter (2.0 mm, o.d.) was passed into the right renal vein via the femoral vein to obtain blood samples. Samples of urine were obtained through polyethylene tubing (PE 160) placed in the ureter. A 25-gauge needle attached to a polyethylene catheter was introduced into the right renal artery distal to the probe and used for intrarenal infusions (Brown Infusion Apparatus, model Unita 1). Patency was ensured by a continuous infusion of saline (0.9% NaCl) at a rate of 0.2 ml/min. The experiments were begun 30 minutes after surgery. Bradykinin (Sandoz) was dissolved in saline and infused at a rate of 0.2 ml/min. Since the vasodilator, diuretic, and natriuretic actions of bradykinin were observed only during infusion of the peptide, several doses of the agent could be infused in the same dog by alternating periods of bradykinin infusion with periods of saline infusion (30 minutes). In one group of nine dogs five different doses of bradykinin (10, 20, 50, 100 and 200 ng/kg min\(^{-1}\)) were infused into the renal artery for periods of 10 minutes each. The kinin content of renal venous blood was determined on samples obtained during the fifth minute of infusion and used to calculate the survival of bradykinin on passage across the kidney. In addition, the concentrations of sodium, potassium, and kinins were determined in urine collected throughout the experimental period.

In a second group of six dogs, the kininase II inhibitor (7, 8) and bradykinin-potentiating peptide (BPP\(_{90}\)) Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro (SQ-20881, Squibb) was dissolved in saline and given intravenously (forelimb vein) as a bolus (300 \(\mu\)g/kg) followed by a continuous infusion at a rate of 10 \(\mu\)g/kg min\(^{-1}\) in 0.5 ml of 0.9% NaCl until the experiments were terminated. The kinin content of urine and of renal venous blood was measured before and 30 minutes after administration of the kininase inhibitor. This procedure was followed by intrarenal infusions of bradykinin at three different rates (5, 10, and 20 \(\mu\)g/kg min\(^{-1}\)) for periods of 10 minutes each. Kinin levels in the renal venous effluent and the survival of bradykinin on passage across the renal vascular bed were determined at the midpoint of the bradykinin infusion. The concentrations of sodium, potassium, and kinins were determined in urine collected throughout the experimental period.

Intervals of 30 minutes were allowed between infusions of bradykinin. In both groups of dogs, the measurements of blood and urinary kinins during infusions of bradykinin were compared with values obtained during a control period at the beginning of the experiments (group 1) or 30 minutes after BPP\(_{90}\) had been given (group 2). All other parameters were compared with control values obtained immediately before each bradykinin infusion.

Analytical Techniques

The kinin content of renal venous blood was determined by the method of Binia et al. (9). All of the glassware used in the purification procedure was siliconized. Blood (50 ml) was rapidly collected in flasks containing four volumes of 95% ethanol to avoid in vitro formation or destruction of kinins. After filtration and evaporation, the samples were acidified (pH 1) and extracted with ether (20 ml). The aqueous phase was then evaporated and the pH adjusted to 7-7.4. Following centrifugation, the kinins present in the supernatant fluid were purified by extraction with butanol. After lyophilization, the sample was dissolved in 1 ml of 0.15M NaCl and bioassayed. Recoveries of added bradykinin (0.2, 0.5, and 1 ng/ml) were 67 ± 4% (N = 10); they were not increased by the addition of BPP\(_{90}\) (4 \(\mu\)g/ml) to the blood samples. The results were corrected for losses and expressed as nanograms of bradykinin equivalents per milliliter of blood (ng/ml).

The procedure for renal venous blood (9) was adapted to determine the kinin content of urine. Urine samples were collected in plastic tubes immersed in an ice bath containing 0.2 ml of 5N HCl to bring the pH to 2-3 and thereby prevent destruction of kinins by urinary kininases. After adjusting the volume to 10 ml with 0.15M NaCl, the pH was readjusted to 1, and the sample was extracted with 20 ml of ether. Kinins present in the aqueous phase were purified (twice) by extraction with 10 ml of butanol. The aqueous layer was discarded, and the butanol was extracted three times with 10 ml of 0.001N HCl. After lyophilization of the aqueous phase, the samples were dissolved in 0.15M NaCl (2 ml), adjusted to pH 7.4, and bioassayed. Recoveries of added bradykinin (50 ng) were 75 ± 7% (N = 10); they were not increased by addition of BPP\(_{90}\) (4 \(\mu\)g) to the samples. The results corrected for losses were expressed as nanograms of bradykinin equivalents per milliliter of urine (ng/ml). Kinin excretory rates, calculated by multiplying the urinary kinin concentration by the urine flow, were expressed as nanograms of bradykinin equivalents excreted per minute (ng/min).

The biological assay used to determine the kinin content of blood and urine was based on the comparison of the vasodilator responses to femoral arterial injections of the unknown samples with those produced by a reference standard of synthetic bradykinin (Sandoz) (9). Male mongrel dogs (20–27 kg) were anesthetized with sodium pentobarbital (30 mg/kg, iv), and their lungs were ventilated mechanically. Mean blood pressure was measured by a Statham transducer via a catheter placed in the left brachial artery. The femoral arteries were exposed below the inguinal ligaments, and their blood
flows were measured with noncannulating electromagnetic flowmeters. Blood pressure and blood flows were recorded with a multichannel direct writer. The femoral arteries were punctured with a 26-gauge needle 2 cm distal to the probes, and a polyethylene catheter (PE 50) was placed 4 cm inside the lumen of the vessel without obstructing the flow. The unknown samples and the standards were injected through the femoral catheter in a volume of 0.5 ml. The dead space was rinsed with 0.5 ml of 0.9% NaCl. Doses of 0.5, 1, 2.5, 5, 10, and 15 ng of bradykinin standard were used. The increases in femoral blood flow produced by bradykinin were linearly related to the logarithm of the dose (between 1 and 25 ng). Bradykinin in doses up to 100 ng did not recirculate as indicated by the absence of effects on systemic blood pressure and contralateral femoral blood flow. The kininlike activity of the unknown sample was determined in duplicate by bracketing its vasodilator effect between the reference standard. The sensitivity of this bioassay procedure permitted the detection of 0.05 ng/ml of blood kinins when the previously described standards were injected through the femoral catheter in a volume of 0.5 ml. The mean aortic blood pressure at the start of the experiments was 169 ± 18 mm Hg, respectively. When the experiments were terminated, renal blood flow and mean aortic blood pressure did not differ from control (159 ± 19 ml/min and 108 ± 6 mm Hg, respectively). The infusion of bradykinin into one renal artery (Fig. 1) caused a prompt and sustained increase in renal blood flow, urine flow, and sodium excretion. Figure 1: Changes in renal blood flow (RBF), sodium excretion (U_{Na}, V), potassium excretion (U_{K}, V), and urine flow (UV) during ipsilateral infusions of bradykinin into the renal artery of normal dogs and of dogs treated with the kininase II inhibitor BPP. Each point represents the mean difference in at least four experiments between control values and values obtained during bradykinin infusion. Vertical bars indicate the SE.

**Results**

**Survival of Bradykinin on Passage through the Kidney**

In nine dogs, the average renal blood flow and mean aortic blood pressure at the start of the experiments were 169 ± 18 ml/min (3.13 ml/g wet tissue) and 111 ± 6 mm Hg, respectively. When the experiments were terminated, renal blood flow and mean aortic blood pressure did not differ from control (159 ± 19 ml/min and 108 ± 6 mm Hg, respectively). The infusion of bradykinin into one renal artery (Fig. 1) caused a prompt and sustained increase in renal blood flow, urine flow, and sodium content of the specimens.

The concentrations of sodium and potassium in urine were measured by flame photometry.

Survival of bradykinin on passage across the kidney was calculated using the following formula:

Bradykinin survival (%) = \[ \frac{(K_e \cdot \text{RBF}_{e}) - (K_c \cdot \text{RBF}_{c})}{\text{BK}} \times 100 \]  

where \( K_e = \) kinin concentration in renal venous blood during the control period (ng/ml), \( K_c = \) kinin concentration in renal venous blood during the infusion of bradykinin (ng/ml), \( \text{RBF}_{e} = \) renal blood flow at the time the control sample \( (K_c) \) was obtained (ml/min), \( \text{RBF}_{c} = \) renal blood flow at the time the experimental sample \( (K_e) \) was obtained (ml/min), and \( \text{BK} \) = amount of bradykinin infused (ng/min). This formula is adequate to calculate the survival of bradykinin on passage through the kidney, since simultaneous measurements of kinins in brachial arterial (0.10 ± 0.02 ng/ml) and renal venous blood (0.13 ± 0.04 ng/ml) of control dogs \( (N = 4) \) failed to show a significant arterial-venous difference. Moreover, the concentrations of kinins in brachial arterial blood of dogs \( (N = 4) \) before (0.10 ± 0.02 ng/ml) and during (0.15 ± 0.04 ng/ml) intrarenal arterial infusions of bradykinin (200 ng/kg min⁻¹, \( N = 4 \)) were not significantly different, thereby suggesting failure of the peptide to recirculate.

All of the results are expressed as means ± SE. Statistical significance was determined by the t-test based on unpaired or paired observations when appropriate. A P value of 0.05 or less was considered statistically significant. Statistical analyses were performed according to methods described by Steel and Torrie (13).

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[For a detailed understanding, please refer to the original scientific article.]
excretion, whereas systemic blood pressure did not change even when doses of 200 ng/kg min⁻¹ were infused. In three experiments in which these parameters were measured in the contralateral kidney, they were not altered during renal arterial infusion of bradykinin. The changes in renal blood flow, urine flow, and sodium excretion elicited by the peptide were dose related (Fig. 1). In contrast, the effects of bradykinin on potassium excretion were unrelated to the dose, although kaliuresis was usually observed (Fig. 1). Within 5–10 minutes after cessation of infusion, all measured variables returned to preinfusion levels. The concentration of kinins in renal venous blood during the control period was 0.16 ± 0.03 ng/ml. Intrarenal arterial infusions of bradykinin resulted in dose-related increases in the kinin concentration of renal venous blood (Fig. 2). However, the average survival of bradykinin during these infusions (10–200 ng/kg min⁻¹) was 13.56 ± 1.27%, indicating that almost 86% of the infused peptide was removed or inactivated in the kidney (Fig. 2). This inactivation or removal was unrelated to the concentration of kinin in renal arterial blood, since during the infusions of bradykinin at rates of 20 and 200 ng/kg min⁻¹ the average survival of the peptide on passage across the renal circulation was 12.72 ± 1.64% and 12.59 ± 2.24%, respectively (Fig. 2). The rate of excretion of urinary kinins during the control period was 2.70 ± 1.09 ng/min; it was not altered by infusions of bradykinin at 20 and 50 ng/kg min⁻¹. Higher rates of administration (200 ng/kg min⁻¹) caused a twofold rise in the excretion of the peptide (P < 0.01) (Fig. 2).

**EFFECT OF KININASE II INHIBITION ON THE SURVIVAL OF BRADYKININ ON PASSAGE ACROSS THE KIDNEY**

Following intravenous administration of the kininase II inhibitor BPP₉α, renal blood flow increased by 16% (P < 0.01) and mean aortic blood pressure fell from 104 to 96 mm Hg (P < 0.02) (Table 1). Simultaneously, urine flow, sodium excretion, and potassium excretion were augmented by 115%, 167% and 40%, respectively (Table 1). Thirty minutes after administration of BPP₉α, the mean concentration of kinins in renal venous blood increased from 0.11 ± 0.03 to 0.24 ± 0.05 ng/ml (P < 0.005) (Table 1). Similarly, the concentration and excretion of urinary kinins rose from 14.6 ± 3.2 ng/ml and 4.4 ± 1.1 ng/min to 53.7 ± 13.6 ng/ml (P < 0.02) and 38.5 ± 12.2 ng/min (P < 0.02), respectively (Table 1). Levels of kinins in renal venous blood before and after administration of BPP₉α were positively correlated with renal blood flow (r = 0.77, P < 0.01), sodium excretion (r = 0.82 P < 0.01), and urine flow (r = 0.85, P < 0.01). Similarly, the excretion of urinary kinins was positively correlated with renal blood flow (r = 0.65, P < 0.05), sodium excretion (r = 0.92, P < 0.01), and urine flow (r = 0.82, P < 0.01). In contrast, neither the excretion of urinary kinins nor the level of kinins in renal venous blood was correlated with potassium excretion (r = 0.39 and r = 0.09, respectively.

After administration of BPP₉α, the renal actions of bradykinin infused intrarenally were potentiated (Fig. 1). At 20 ng/kg min⁻¹ the increases in renal blood flow, urine flow, and sodium excretion...
caused by bradykinin were augmented by 90% \((P < 0.01)\), 173% \((P < 0.01)\), and 146% \((P < 0.05)\), respectively. The dose-response curves of bradykinin were shifted to the left in a parallel fashion, but in no instance was mean aortic blood pressure altered by the infusions of the kinin.

The concentration of kinins in renal venous blood increased severalfold during bradykinin infusion, the levels being twofold greater \((P < 0.001)\) than those found during identical infusion rates \((20 \text{ ng/kg min}^{-1})\) in the absence of \(\text{BPP}_9\) (Fig. 2). Similarly, the survival of bradykinin on passage across the kidney was increased by \(\text{BPP}_9\) from \(12.72 \pm 1.64\%\) to \(53.92 \pm 7.48\% \((P < 0.001)\) (Fig. 2). Increases in renal blood flow, urine flow, and sodium excretion produced by bradykinin before and after administration of \(\text{BPP}_9\) were positively correlated with the survival of bradykinin (Fig. 3).

**Discussion**

When bradykinin was infused into the renal artery of dogs, a little more than 10% of the peptide was recovered in the renal venous effluent. This fact suggests that almost 90% of the kinin was inactivated or excreted on passage through the kidney. Our estimation of bradykinin survival, although less than what Ferreira and Vane (5) found in cats using the blood-bathed organ technique, is nonetheless comparable. Disappearance of kinin on passage across the renal circulation may be due to urinary excretion of the peptide (6) as well as to inactivation by blood and renal kininases. The former possibility is remote, since during infusions of 200 ng/kg min\(^{-1}\) of bradykinin the urinary excretion of the peptide increased by only twofold, the difference representing a mere 0.1% of the amount infused (5 \(\mu\text{g}\)). Although destruction of bradykinin by blood kininases is a

### TABLE 1

<table>
<thead>
<tr>
<th></th>
<th>Control ((N = 6))</th>
<th>(\text{BPP}_9) ((N = 6))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{MABP} \text{ (mm Hg)})</td>
<td>104 ± 3</td>
<td>96 ± 2</td>
<td>(&lt; 0.02)</td>
</tr>
<tr>
<td>(\text{RBF} \text{ (ml/min)})</td>
<td>158 ± 24</td>
<td>184 ± 29</td>
<td>(&lt; 0.01)</td>
</tr>
<tr>
<td>(U_{\text{Na}}V \text{ ((\mu\text{Eq/min)})})</td>
<td>36.8 ± 10.1</td>
<td>98.4 ± 26.6</td>
<td>(&lt; 0.02)</td>
</tr>
<tr>
<td>(U_kV \text{ ((\mu\text{Eq/min)})})</td>
<td>24.0 ± 3.2</td>
<td>33.8 ± 5.5</td>
<td>(&lt; 0.02)</td>
</tr>
<tr>
<td>(\text{UV} \text{ (ml/min)})</td>
<td>0.29 ± 0.06</td>
<td>0.64 ± 0.12</td>
<td>(&lt; 0.05)</td>
</tr>
<tr>
<td>(\text{Blood kinins (ng/ml)})</td>
<td>0.11 ± 0.03</td>
<td>0.24 ± 0.05</td>
<td>(&lt; 0.005)</td>
</tr>
<tr>
<td>(\text{Urinary kinin excretion (ng/min)})</td>
<td>4.4 ± 1.1</td>
<td>38.5 ± 12.2</td>
<td>(&lt; 0.02)</td>
</tr>
</tbody>
</table>

Results are means ± se. \(P\) indicates significance of the difference between control and experimental periods. \(N\) = number of dogs tested, \(\text{MABP}\) = mean aortic blood pressure, \(\text{RBF}\) = renal blood flow, \(U_{\text{Na}}V\) = sodium excretion, \(U_kV\) = potassium excretion, and \(\text{UV}\) = urine flow.

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certain possibility, it is unlikely to account for a disappearance rate as high as we found, since the half-life of bradykinin in blood is 17 seconds (5, 12) and the mean renal transit time is about 6 seconds (14). Thus, a disappearance of more than 17% may indicate participation by renal tissue in the removal of bradykinin. In support of this view, we have shown that the isolated rabbit kidney perfused with Krebs-bicarbonate solution inactivates more than 99% of the infused bradykinin (unpublished observations). The abundance of renal kininases is well established (3) and may account, to a large extent, for the disappearance of bradykinin on passage across the renal circulation. Our observation that BPP9a increased by fourfold the survival of bradykinin infused intrarenally strongly supports this interpretation. BPP9a is a potent inhibitor of kininase II (7, 8), a peptidyl-dipeptide hydrolase occurring in the kidney and other tissues (3, 8) which cleaves the C-terminal dipeptide from bradykinin (3.4.15.1 is the code number assigned to kininase II by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of Biochemistry).

After administration of BPP9a, the renal vasodilator, diuretic, and natriuretic actions of bradykinin were augmented. This potentiation may be attributed to diminished inactivation of the kinin within the kidney, resulting in enhanced concentration of the peptide at its site of action. Although the intrarenal site of bradykinin inactivation is unknown, the positive correlation between peptide effects and survival suggests that the site(s) of action is distal to the site(s) of degradation.

Intrarenal infusions of 20 and 50 ng/kg min⁻¹ of bradykinin failed to increase the excretion of urinary kinins. Increments in the dose to establish kinin levels in arterial blood at least a hundredfold higher than normal caused a mere twofold rise in kinin excretion, which was equivalent to 0.1% of the amount of kinin infused. These observations, which were not modified after inhibition of kininase II activity, are consistent with the view that urinary kinins originate intrarenally (24) and increases urinary kallikrein (25) and the blood concentration of a kininlike material of renal origin (25). Our studies provide evidence which supports this concept. Inhibition of kininase II activity with BPP9a resulted in vasodilation, diuresis, and natriuresis. Furthermore, the levels of kinin in renal venous blood and the excretion of urinary kinins were positively correlated with renal blood flow, urine flow, and sodium excretion. These observations, which are indicative of an involvement of kinins as mediators of renal vasodilation and natriuresis, are consistent with the recent demonstration that bradykinin antibodies attenuate the natriuretic response to an acute saline load in rats (26). They are difficult to reconcile, however, with the occurrence of augmented excretion of urinary kallikrein in sodium-depleted states (21, 22), a condition in which sodium excretion is suppressed.

Although our results do not permit definite assignment of a physiological role to the kallikrein-
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kinin system of the kidney, they do suggest that kinins generated intrarenally, either acting alone or in concert with other intrarenal systems (27), play a role in the regulation of renal blood flow and sodium excretion.

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References


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