Bradykinin may be involved in neuropeptide Y-induced diuresis, natriuresis, and calciuresis

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Bischoff, Angela, Wolfgang Rascher, and Martin C. Michel. Bradykinin may be involved in neuropeptide Y-induced diuresis, natriuresis, and calciuresis. Am. J. Physiol. 275 (Renal Physiol. 44): F502–F509, 1998.—Neuropeptide Y (NPY) can cause diuresis, natriuresis, and calciuresis in rats independently of the pressure-natriuresis mechanism (A. Bischoff and M. C. Michel. Pflügers Arch. 435: 443–453, 1998). Because this is seen in systemic but not intrarenal NPY infusion, we have investigated the possible mediator of tubular NPY effects in anesthetized rats. In the present study, infusion of NPY (2 µg·kg<sup>-1</sup>·min<sup>-1</sup>) enhanced renovascular resistance by -8 mmHg·ml<sup>-1</sup>·min and enhanced urine and sodium excretion by -450 µl/15 min and -60–85 mmol/15 min, respectively. Acute renal denervation did not alter renovascular or tubular NPY effects, indicating that a neurally released mediator is not involved. Treatment with the angiotensin II-receptor antagonist losartan prevented the decline of the renovascular response with time but did not modify tubular NPY effects. The bradykinin B<sub>2</sub>-receptor antagonist icatibant accelerated the decline of the renovascular NPY effects with time; concomitantly, it attenuated NPY-induced diuresis and natriuresis and abolished NPY-induced calciuresis. The converting-enzyme inhibitor ramiprilat prevented the decline of the renovascular response with time; concomitantly, it magnified the NPY-induced diuresis, natriuresis, and calciuresis. We conclude that bradykinin may be involved in NPY-induced diuresis, natriuresis, and, in particular, calciuresis.

renal nerves; renin-angiotensin system; kalikrein-kinin system

Neuropeptide Y (NPY) is a cotransmitter in the sympathetic nervous system and is involved in the regulation of cardiovascular functions at the central, cardiac, vascular, and renal levels (18). Systemic infusion of NPY elevates blood pressure, reduces renal blood flow (RBF), and concomitantly causes diuresis, natriuresis, and calciuresis in anesthetized and conscious rats (5). The tubular NPY actions occur at least partly independently from the pressure-natriuresis mechanism and from alterations of RBF and glomerular filtration rate (3, 4, 6, 27). Studies with several subtype-selective agonists and with the Y<sub>1</sub>-selective agonist BIBP-3226 have demonstrated that RBF reductions occur via a Y<sub>1</sub> receptor. On the other hand, tubular effects occur via a Y<sub>5</sub> receptor (2), although no mRNA for this subtype is detectable in rat kidney (5). Moreover, the RBF reductions were markedly enhanced upon intrarenal administration relative to systemic administration. In contrast, direct intrarenal NPY infusion caused either no enhancement of diuresis and natriuresis or much less enhancement than intravenous administration produced (3). This indicates that an extrarenal NPY receptor and a formed or released mediator of unknown nature may interact to regulate tubular function. This potential mediator may reach the kidney either by release from renal nerves or via the blood stream. A mediator could be released from the sympathetic nerve fibers that densely innervate the kidney and reach intrarenal blood vessels and basement membranes of proximal and distal tubules (10). Possible humoral mediators include components of the renin-angiotensin, kalikrein-kinin, and vasopressin systems. The renin-angiotensin system is a candidate because NPY can inhibit renin release in vitro and reduce the plasma renin activity in vivo (5). Renin-release inhibition and enhancements of diuresis and natriuresis are mediated by an NPY receptor with similar pharmacological characteristics (2), and both are sensitive to pertussis toxin (15, 27). Thus lowering of angiotensin tone to the kidney may contribute to the tubular NPY effects. The kalikrein-kinin system is a candidate because NPY (5) and bradykinin (7) both cause diuresis and natriuresis mainly by altering the function of distal nephron segments. Moreover, the diuretic effects of both agents can be blocked by cyclooxygenase inhibitors (7, 8, 29). Finally, vasopressin can be considered as an additional candidate because it also acts on distal nephron segments; however, vasopressin is not a very likely mediator of NPY effects because it enhances free water clearance (12) whereas NPY does not (3, 27).

To examine the role of neuronally released mediators or one of the above humoral mediators, we have used two experimental designs. First, we have compared the effects of NPY on both kidneys after acute unilateral renal denervation. Second, we have compared the effects of NPY in rats treated with the angiotensin II-receptor antagonist losartan, the bradykinin B<sub>2</sub>-receptor antagonist icatibant, the converting-enzyme inhibitor ramiprilat, and vehicle. In animals from the second study, we have also investigated whether NPY infusion alters plasma vasopressin concentrations.

MATERIALS AND METHODS

Two studies were performed following approval by the state animal welfare board at the Regierungspresident Düsseldorf. In study 1 the animals underwent acute renal denervation, and in study 2 they were treated with pharmacological inhibitors. For both studies male Wistar rats (strain, Hsd/Cpb: WU; weight, 250–350 g in study 1 and 300–450 g in study 2) were obtained from Harlan CPB (Zeist, Netherlands). They...
Table 1. Basal values of RBF, RVR, and urinary parameters after acute unilateral renal denervation in anesthetized rats

<table>
<thead>
<tr>
<th></th>
<th>Denervation Experiments</th>
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<tbody>
<tr>
<td></td>
<td>Denervated</td>
<td></td>
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<tr>
<td>RBF, ml/min</td>
<td>4.0 ± 0.3</td>
<td>5.9 ± 0.3*</td>
</tr>
<tr>
<td>RVR, mmHg·ml⁻¹·min</td>
<td>27.7 ± 3.0</td>
<td>19.3 ± 1.1*</td>
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<tr>
<td>Urine volume, µl/15 min</td>
<td>61 ± 7</td>
<td>76 ± 13</td>
</tr>
<tr>
<td>Sodium excretion, µmol/15 min</td>
<td>6.6 ± 1.8</td>
<td>8.9 ± 2.4</td>
</tr>
<tr>
<td>Calcium excretion, µmol/15 min</td>
<td>0.10 ± 0.01</td>
<td>0.12 ± 0.02</td>
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<tr>
<td>Creatinine clearance, ml/min</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
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Values are means ± SE; n = 18 experiments. Basal values of mean arterial pressure and heart rate were 111 ± 15 mmHg and 376 ± 6 beats/min, respectively. RBF, renal blood flow; RVR, renovascular resistance. *P < 0.05, innervated vs. denervated kidney in paired 2-tailed Student's t-test.

were kept under standardized conditions with free access to standard lab chow and tap water. On the day of the experiment, the animals were anesthetized with an initial intraperitoneal injection of pentobarbital sodium (60 mg/kg). Additional anesthetic was administered in doses of 3 mg iv every 30 min. The animals were placed on a heating pad to maintain the body temperature at 37°C. Catheters were placed into the femoral vein (for saline and drug infusion), the right renal artery (for urine collection), and the right ureter (for urine collection). In addition, an electromagnetic blood flow sensor was placed on the renal artery for measurement of RBF and heart rate. The construction of the catheters and the preparation of the rats have previously been described in detail (3).

Study 1: Acute renal denervation. During these experiments, both kidneys remained in situ and both ureters were canulated. After careful dissection of both renal arteries, one of the vessels was denervated in random order as described (1, 19, 20). Briefly, the adventitia and all visible nerve fibers were removed with a small forceps. Thereafter, the artery was surrounded by a piece of silk soaked with ethanol-phenol solution (10:1) for 4 min. After removal of the silk cloth, electromagnetic blood flow sensors (Skalar MDL 1401; Föhr Medical Instruments, Seeheim/Ober Beerbach, Germany) were placed on the treated and the contralateral renal arteries. Before the start of the experiment, animals were allowed 3 h of recovery, during which 80 µl/min of 0.9% saline was infused.

Sixty minutes before the start of the experiment, the rats were treated with vehicle, the angiotensin II-receptor antagonist losartan (10 mg/kg bolus followed by 1 mg·kg⁻¹·min⁻¹ infusion), the bradykinin B₂-receptor antagonist icatibant (2 µg/kg bolus followed by 0.2 µg·kg⁻¹·min⁻¹ infusion), or the converting-enzyme inhibitor ramiprilat (1 mg/kg bolus followed by 0.1 mg·kg⁻¹·min⁻¹ infusion). All infusions were maintained until the end of the experiment. To verify the effectiveness of the inhibitor treatments, we administered bolus injections of 1 µg/kg angiotensin II (losartan group), 1 µg/kg bradykinin (icatibant group), or 1 µg/kg angiotensin I (ramiprilat group) 20 min before the inhibitor treatment and at the end of the experiment. Angiotensin II-induced mean arterial pressure elevations and RBF reductions were 47 ± 5 and 3 ± 2 mmHg (P < 0.0001) and 89 ± 4 and 35 ± 6% (P < 0.0001), respectively, before losartan treatment and at the end of the experiment. Bradykinin-induced heart rate elevations were 26 ± 4 beats/min before icatibant treatment and 2 ± 4 beats/min (P = 0.0001) at the end of the experiment. Angiotensin I-induced mean arterial pressure increases and RBF reductions were 51 ± 4 and 15 ± 4 mmHg (P < 0.0001) and 96 ± 2 and 63 ± 7% (P < 0.0001), respectively, before ramiprilat treatment and at the end of the experiment. These data indicate effective inhibition of angiotensin II receptors by losartan, bradykinin B₂ receptors by icatibant, and converting-enzyme activity by ramiprilat throughout the experimental period.

Table 2. Basal values of vascular and urinary parameters after treatment with the bradykinin-receptor antagonist icatibant, the angiotensin II-receptor inhibitor losartan, and ACE inhibitor ramiprilat

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 14)</th>
<th>Icatibant (n = 14)</th>
<th>Losartan (n = 16)</th>
<th>Ramiprilat (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>118 ± 5</td>
<td>118 ± 3</td>
<td>107 ± 3</td>
<td>107 ± 4</td>
</tr>
<tr>
<td>RBF, ml/min</td>
<td>8.1 ± 0.4</td>
<td>7.3 ± 0.6</td>
<td>9.6 ± 0.5</td>
<td>9.2 ± 0.5</td>
</tr>
<tr>
<td>RVR, mmHg·ml⁻¹·min</td>
<td>15.5 ± 0.9</td>
<td>15.1 ± 0.6</td>
<td>11.5 ± 0.5*</td>
<td>12.1 ± 0.1*</td>
</tr>
<tr>
<td>Urine volume, µl/15 min</td>
<td>150 ± 25</td>
<td>168 ± 24</td>
<td>126 ± 14</td>
<td>147 ± 33</td>
</tr>
<tr>
<td>Sodium excretion, µmol/15 min</td>
<td>12 ± 2</td>
<td>15 ± 3</td>
<td>8 ± 1</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>Calcium excretion, µmol/15 min</td>
<td>0.39 ± 0.08</td>
<td>0.50 ± 0.08</td>
<td>0.27 ± 0.05</td>
<td>0.40 ± 0.15</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of experiments. ACE, angiotensin-converting enzyme. *P < 0.05 vs. saline-infused (control) rats in 1-way ANOVA followed by Dunnett's test of multiple comparisons.
Chemicals. Rat and human NPY, angiotensin I, angiotensin II, and bradykinin were obtained from Saxon Biochemicals (Hannover, Germany); pentobarbital sodium (Nembutal) from Sanofi (Hannover, Germany); ketamine from Pittman-Moore (Burgwedel, Germany); and xylazine (Rompun) from Bayer (Leverkusen, Germany). Losartan was a kind gift from DuPont Merck (Wilmington, DE), and ramiprilat and icatibant (also known as HOE-140) were provided by Hoechst (Frankfurt-am-Main, Germany).

Data analysis. The averages of mean arterial pressure, heart rate, and RBF during the last 30 min and the average of urine formation during the last 45 min before the start of the experiment in each animal were taken as baseline (Tables 1 and 2). All other experimental data are expressed as alterations relative to the baseline values. Data are means ± SE of alterations relative to baseline values shown in Table 1. NPY was infused from 0 to 90 min. Despite the differences of basal values in innervated and denervated kidney, systemic infusion of NPY rapidly reduced RBF and increased RVR in both kidneys.

Fig. 1. Effects of systemic neuropeptide Y (NPY) infusion (1 µg·kg⁻¹·min⁻¹) on renal blood flow (RBF; left) and renovascular resistance (RVR; right) in innervated (top) and denervated (bottom) kidneys in anesthetized rats (study 1). Data are means ± SE of alterations relative to baseline values shown in Table 1. NPY was infused from 0 to 90 min. Despite the differences of basal values in innervated and denervated kidney, systemic infusion of NPY rapidly reduced RBF and increased RVR in both kidneys.

Fig. 2. Effects of systemic NPY infusion (1 µg·kg⁻¹·min⁻¹) on urine (top), Na⁺ (middle), and Ca²⁺ excretion (bottom) in innervated and denervated kidneys in anesthetized rats (study 1). Data are means ± SE of alterations relative to baseline values shown in Table 1. NPY was infused from 0 to 90 min. Urine formation and electrolyte excretion were significantly increased by NPY infusion compared with vehicle in a 2-way analysis of variance (P < 0.05). NPY-induced elevations of diuresis, natriuresis, and calciuresis were similar in innervated and denervated kidneys.
RESULTS

Study 1: Acute renal denervation. At the end of the equilibration period, basal RBF in denervated kidneys was 50% higher and renovascular resistance (RVR) was 30% lower than in innervated kidneys (Table 1). Water and electrolyte excretion also tended to be higher in denervated kidneys, but these differences failed to reach statistical significance with the given number of animals. Creatinine clearance of both kidneys was very similar (Table 1).

RBF and RVR remained constant in the innervated and the denervated kidney of saline-infused animals throughout the experimental period (Fig. 1). Confirming our previous observations (2, 3, 6), we found that NPY infusion (1 µg·kg\(^{-1}\)·min\(^{-1}\)) elevated mean arterial pressure by \(~15\) mmHg and concomitantly lowered heart rate by \(~25\) beats/min (data not shown). This was accompanied by a rapid reduction of RBF and an increase of RVR in both kidneys (Fig. 1). NPY infusion did not significantly affect creatinine clearance in both kidneys [values during the last urine collection period (in ml/min): innervated saline 0.63 ± 0.10, innervated NPY 0.69 ± 0.09, denervated saline 0.67 ± 0.08, denervated NPY 0.85 ± 0.10]. Water and electrolyte excretion were not significantly altered during saline infusion in innervated or denervated kidneys (Fig. 2). NPY infusion enhanced urine formation and electrolyte excretion to a similar extent in innervated and denervated kidneys (Fig. 2).

Study 2: Pharmacological inhibitors. Icatibant treatment did not significantly affect any of the basal parameters relative to saline-treated rats (Table 2). In contrast, losartan treatment increased basal RBF by 20% and reduced RVR by 25%. Mean arterial pressure and water and electrolyte excretion also tended to be lower, but this failed to reach statistical significance with the given number of animals (Table 2). Ramiprilat treatment enhanced basal RBF by 14% and lowered RVR by 22% but did not significantly alter urine or electrolyte excretion (Table 2).

In all groups mean arterial pressure and RVR remained stable during saline infusion but were transiently increased by infusion of NPY by \(~20\) mmHg and \(~8\) mmHg·ml\(^{-1}\)·min, respectively (Figs. 3 and 4). Although NPY-induced peak elevations of RVR were similar in all groups, the decline of the renovascular response with time was accelerated in the icatibant group and almost abolished in the losartan and ramiprilat groups (Fig. 4).

Water, sodium, and calcium excretion remained stable on saline infusion in all groups throughout the experimental period (Figs. 5–7). In control rats, NPY infusion increased diuresis by \(~450\) µl/15 min (Fig. 5), natriuresis by \(~60–85\) µmol/15 min (Fig. 6), and calciuresis by \(~1\) µmol/15 min (Fig. 7). This was further enhanced by ramiprilat treatment, whereas losartan treatment had no effect (Figs. 5–7). Icatibant treatment attenuated the NPY-induced diuresis (Fig. 5) and natriuresis (Fig. 6) and abolished the alterations of calcium excretion (Fig. 7). Thus total NPY-stimulated diuresis, natriuresis, and calciuresis during the 120-min infusion period in the control group were 2,719 ± 394 µl, 432 ± 85 µmol, and 5.80 ± 1.15 µmol, respectively. In the losartan group the results were 2,369 ± 593 µl, 401 ± 7 µmol, and 7.05 ± 1.35 µmol, respectively. In the icatibant group the results were 1,810 ± 415 µl, 221 ± 75 µmol, and 0.76 ± 0.41 µmol, respectively. In the ramiprilat group the results were 3,721 ± 495 µl, 700 ± 103 µmol, and 7.69 ± 1.88 µmol, respectively. (\(P < 0.05\) in a one-way analysis of variance for all parameters in the icatibant and ramiprilat groups vs. control.)
Plasma concentrations of vasopressin were determined at the end of the experiment. They were not significantly different between the eight experimental groups (in pg/ml: control saline, 26 ± 5; control NPY, 28 ± 7; losartan saline, 30 ± 8; losartan NPY, 41 ± 8; icatibant saline, 25 ± 3; icatibant NPY, 30 ± 4; ramiprilat saline, 28 ± 9; ramiprilat NPY, 16 ± 2).

**DISCUSSION**

Previous work has demonstrated that systemic infusion of NPY concomitantly enhances mean arterial pressure and causes diuresis, natriuresis, and calcirexis (5). The simplest explanation of these data would be an activation of the pressure-natriuresis mechanism (11, 24). However, several lines of evidence demonstrate that this mechanism only plays a small role in NPY-induced diuresis and natriuresis (2–4, 6). First, NPY-induced diuresis and natriuresis are largely maintained when elevations of renal perfusion pressure are prevented mechanically (using an adjustable clamp on the abdominal aorta or renal decapsulation) or pharmacologically (stabilizing blood pressure by concomitant sodium nitroprusside infusion). Second, NPY-receptor antagonists, which block blood pressure elevations, do not affect diuresis and natriuresis. Third, analogs of NPY that do not increase blood pressure [e.g., PYY-(3-
36)] can cause diuresis and natriuresis. These data exclude a major role of the pressure-natriuresis mechanism in NPY-induced diuresis and natriuresis.

The tubular effects of systemic NPY infusion are not mimicked by direct intrarenal NPY administration, and the receptor subtype that mediates them is not detectable in the kidney (5). Therefore, NPY appears to modify tubular function by stimulating a receptor located in an extrarenal site and using a neuronal and/or humoral mediator. Therefore, the present study was designed to identify any such mediators.

Acute denervation experiments were performed to test whether this mediator may be released from the renal nerves. The renal denervation was produced by ethanol-phenol treatment of the dissected renal arteries (1, 19, 20). Its effectiveness in our study was demonstrated by a significantly enhanced RBF and lowered RVR, possibly a consequence of the withdrawal of $\alpha$-adrenoceptor-mediated renal vasoconstriction (10). Acute denervation had no major effects on NPY-induced reductions of RBF or enhancements of RVR, diuresis, natriuresis, or calciuresis. Thus the mediator of tubu-
lar NPY effects does not appear to be released from the renal nerves. Therefore, our further experiments were designed to identify a possible humoral mediator of tubular NPY effects.

Three possible humoral mediator systems were investigated: the renin-angiotensin, kallikrein-kinin, and vasopressin systems. Because we have not detected significant effects of NPY on plasma vasopressin levels, our data do not support a role for vasopressin in tubular NPY effects. This is consistent with the observation that a lowering of vasopressin tone to the kidney primarily causes diuresis without major alterations to sodium excretion, whereas NPY concomitantly causes diuresis, natriuresis, and calciuresis (i.e., it does not alter free water clearance (5)).

A role for the renin-angiotensin system in tubular NPY effects was investigated by use of the angiotensin II-receptor antagonist losartan and the converting-enzyme inhibitor ramiprilat. Whereas losartan is selective for the AT1 subtype of angiotensin II receptors (28), ramiprilat suppresses the formation of angiotensin II and should therefore prevent the effects of angiotensin II regardless of the receptor subtype by which they occur. The effectiveness of our losartan and ramiprilat treatment regimens throughout the experimental period was demonstrated by marked attenuation of the effects of bolus injections of angiotensin II and angiotensin I, respectively. Because both losartan and ramiprilat abolished the decline of renovascular NPY effects over time. Because losartan or ramiprilat did not inhibit the NPY-induced enhancements of water and electrolyte excretion, a withdrawal of angiotensin II tone to the kidney does not appear to mediate the tubular NPY effects.

Ramiprilat actually magnified the diuresis, natriuresis, and calciuresis caused by NPY. Because this cannot be attributed to the withdrawal of angiotensin, it is likely to occur by the other known effect of converting-enzyme inhibition, reduced bradykinin degradation. This is supported by our data with the bradykinin B2-receptor antagonist icatibant, which inhibited NPY-induced diuresis and natriuresis and abolished enhancements of calcium excretion. Therefore, the combined ramiprilat and icatibant data suggest that bradykinin may be involved in the tubular NPY effects. Its role in the control of calciuresis may be even more important than its role in the control of diuresis and natriuresis.

Several other findings also support the idea that bradykinin may mediate tubular NPY effects. Bradykinin causes diuresis and natriuresis on intrarenal infusion in conscious and anesthetized dogs (7, 13, 16, 26). This is also seen on systemic infusion in conscious rats, which had been rendered hypertensive by deoxycorticosterone treatment (21). The diuretic and natriuretic effects of bradykinin mainly occur in distal nephron segments (9, 25). NPY also appears to cause diuresis mainly by altering the function of distal nephron segments (5). Finally, bradykinin stimulates renal prostaglandin formation (14, 17), and its diuretic and natriuretic effects can be inhibited by cyclooxygenase inhibitors (7, 8). Similarly, the cyclooxygenase inhibitor indomethacin also inhibits the diuretic and natriuretic NPY effects (4). Taken together these data demonstrate that bradykinin may be an important mediator of tubular NPY effects. In this model it is theoretically possible that stimulation of the extrarenal Y5 NPY receptors causes bradykinin release, which then reaches the kidney via the bloodstream to act intrarenally on prostaglandin formation. The very short plasma half-life of bradykinin (23), however, casts doubt on this mode of action. Alternatively, it is possible that the extrarenal Y5 receptor activates yet another mediator that causes intrarenal bradykinin formation and/or release. Discrimination of these possibilities will require additional studies.

In summary, our data demonstrate that renal nerves, vasopressin, and the renin-angiotensin system do not play an important role in tubular NPY effects. In contrast, enhancements and inhibition of NPY-induced diuresis, natriuresis, and calciuresis by ramiprilat and the bradykinin-receptor antagonist icatibant, respectively, demonstrate that bradykinin may be involved in the tubular NPY effects.

We thank the respective drug companies for providing losartan, icatibant, and ramiprilat.

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