Indomethacin Inhibits the Natriuretic Effects of Neuropeptide Y in Anesthetized Rats

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ABSTRACT

Neuropeptide Y (NPY) is a unique modulator of renal function that enhances urine flow and sodium excretion despite marked reductions in renal blood flow. We investigated whether the cyclooxygenase inhibitor indomethacin alters the renal NPY effects in anesthetized rats. Treatment with 5 mg/kg indomethacin i.p. lowered urinary prostaglandin excretion by 85%. Systemic infusion of NPY elevated mean arterial pressure by 15 mm Hg and renovascular resistance by 8.0 mm Hg/ml/min, whereas the related peptide YY3-36 (PYY3-36) did not. Nevertheless, both peptides enhanced urine flow rate by 250 and 100 μl/min, respectively, and sodium excretion by 15 μmol/15 min. Treatment with indomethacin did not affect NPY- and PYY3-36-induced alterations of systemic and renovascular hemodynamics but completely abolished NPY- and PYY3-36-induced diuresis and natriuresis. Endogenous creatinine clearance was not affected by any treatment. We conclude that cyclooxygenase-derived arachidonic acid metabolites are not involved in the systemic or renal hemodynamic effects of NPY and PYY3-36 but mediate NPY- and PYY3-36-induced diuresis and natriuresis.

TABLE 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Indomethacin</th>
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<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>110 ± 3</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>RBF (ml/min)</td>
<td>7.9 ± 0.3</td>
<td>7.8 ± 0.4</td>
</tr>
<tr>
<td>RVR (mm Hg/ml/min)</td>
<td>14.5 ± 0.6</td>
<td>15.1 ± 0.7</td>
</tr>
<tr>
<td>Urine flow rate (μl/15 min)</td>
<td>108 ± 15</td>
<td>82 ± 9</td>
</tr>
<tr>
<td>Sodium excretion (μmol/15 min)</td>
<td>6.5 ± 1.0</td>
<td>7.4 ± 1.4</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>1.06 ± 0.05</td>
<td>0.94 ± 0.06</td>
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</table>

Data are mean ± S.E.M. MAP, RBF and RVR data are the average of data monitored in the last 3 min, and urinary values are the average of the last three collecting periods before the start of the NPY infusion. None of the observed differences were statistically significant in an unpaired, two-tailed t test.

Moreover, prostaglandins, particularly prostaglandin E2, can induce diuresis and natriuresis by an effect on distal nephron segments (Anderson et al., 1976; Iino and Imai, 1978; Stokes and Kokko, 1977). Although some investigators have doubted that this is due to a direct prostaglandin effect on the tubules (Fine and Trizna, 1977), the overall data suggest that the prostaglandin effect on distal nephron segments is similar to that observed with NPY (Bischoff et al., 1996; Smyth et al., 1988). Therefore, we tested how indomethacin affects the NPY and PYY3-36-induced alterations in RBF and water and electrolyte excretion.

Materials and Methods

Animal surgery and experimental protocol for anesthetized rat experiments. The study followed the principles for labo-
ratory animal care as defined by the National Institutes of Health (Bethesda, MD). The study protocol was approved by the state board for animal welfare at the Regierungspräsidium Düsseldorf. Male Wistar rats (strain, Hsd/Cpb:WU; weight, 270–340 g) were obtained from Harlan CPB (Borchem, Germany) and surgically prepared as previously described (Bischoff et al., 1996). Briefly, rats were unilaterally nephrectomized (left kidney) while under ketamine/xylazine (100 and 6 mg/kg, respectively) anesthesia 10 to 14 days before the experiment. On the day of the experiment, the animals were anesthetized with a single i.p. injection of thiobarbital (100 mg/kg). The animals were placed on a heating pad to maintain the body temperature at 37°C. After tracheotomy to facilitate ventilation, the left femoral artery was cannulated for monitoring MAP via a Statham pressure transducer. After an abdominal midline incision, the ureter was cannulated for urine sampling. Connective tissue was carefully dissected from the right renal artery, and an electromagnetic blood flow sensor (Skalar MDL 1401; Föhr Medical Instruments GmbH, Egelsbach, Germany) was placed on the renal artery for monitoring of RBF. The signals from the flow sensor and the pressure transducer were continuously recorded online using the HDAS hemodynamic data acquisition system (Department of Bioengineering, Rijksuniversiteit Limburg, Maastricht, The Netherlands). RVR was calculated from MAP and RBF. Before the start of the peptide infusion, animals were allowed 3 hr of recovery, during which 60 ml/min of 0.9% saline were infused via the femoral vein. MAP, RVR, RBF, and urine flow rate had stabilized at the end of this period (table 1). Two hours after the completion of surgery, some rats received an i.p. injection of 5 mg/kg indomethacin, whereas control rats received an equal amount of buffer (25 mM NaH₂PO₄, 2.5 mM KH₂PO₄ and 1 mM MgCl₂, pH 7.4). One hour later, vehicle, NPY or PYY₃–₃₆ (2 µg/kg/min each) was infused via the femoral vein at a rate of 60 ml/min for a period of 1 hr. During the whole experimental period, MAP and RBF were measured every 5 min; during the first 5 min of the experimental period, MAP and RBV were quantified every minute. Urine was collected in preweighed tubes in 15-min intervals. At the end of the experiment, a blood sample was taken from the abdominal aorta; subsequently, the rat was killed with an overdose of thiobarbital. Adequate hydration of the animals under these conditions was documented by a constant urine flow rate in the control group during the experimental period. Urine formation was quantified gravimetrically assuming a specific gravity of 1.0 kg/liter, and
samples were stored at 4°C until analysis. Serum was prepared from the aortic blood sample by centrifugation and stored at 220°C until analysis. Urinary sodium concentrations were determined with an Eppendorf flame photometer. Urinary and serum creatinine was determined photometrically with a commercially available test kit. Prostaglandin E2 concentrations were determined with a commercially available radioimmunoassay.

**Chemicals.** Rat and human NPY and PYY3–36 were obtained from Bachem (Heidelberg, Germany). Thiobarbital (Inactin) was from RBI (Natick, MA). Ketamine was from Pittman-Moore GmbH (Burgwedel, Germany). Xylazine (Rompun) was from Bayer (Leverkusen, Germany). Indomethacin was from Sigma Chemical (St. Louis, MO). The test kit for creatinine measurements was obtained from Boehringer-Mannheim (Mannheim, Germany), and the radioimmunoassay for prostaglandin E2 measurements was obtained from New England Nuclear (Brussels, Belgium).

**Data analysis.** The average MAP, RVR and RBF during the last 3 min and urine formation during the last 45 min before the start of the peptide infusion in each animal were taken as base line (table 1). All other data are expressed as alteration relative to the base line. MAP and RBF were determined photometrically with a commercially available test kit. Prostaglandin E2 concentrations were determined with a commercially available radioimmunoassay.

In the first experimental series, the effects of indomethacin on urinary prostaglandin E2 excretion were tested in saline-infused rats. Indomethacin treatment (5 mg/kg i.p.) significantly lowered prostaglandin E2 excretion by ~85% (e.g., from 73 ± 12 to 13 ± 5 pg/min at the 60-min time point; P < .001, fig. 1).

In the second experimental series, we investigated the effects of NPY, PYY3–36 and saline in indomethacin- and vehicle-treated rats. Thus, animals in groups 1 and 4 received 0.9% saline (n = 5 and 6, respectively), groups 2 and 5 received NPY (2 μg/kg/min, n = 8 and 7, respectively) and groups 3 and 6 received PYY3–36 (2 μg/kg/min, n = 7 each). Groups 4 through 6 were treated with indomethacin (5 mg/kg i.p.) at 1 hr before the start of the peptide or saline infusion, whereas groups 1 through 3 received the corresponding vehicle. Indomethacin treatment did not significantly affect basal MAP, RVR, RBF, creatinine clearance or urine and sodium excretion relative to vehicle-treated rats (table 1). In indomethacin-treated, vehicle-infused rats, MAP, RBF, creatinine clearance, urine flow rates and sodium excretion remained stable during the experimental period (see figs. 2–7).

**Results**

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rats (figs. 2 and 3). NPY reduced RBF by ~2 ml/min in control and indomethacin-treated rats (fig. 4). In contrast, PYY$_{3-36}$ significantly increased RBF by ~0.8 ml/min in control and indomethacin-treated rats (fig. 4). Endogenous creatinine clearance was not affected by either peptide in vehicle- or indomethacin-treated animals (fig. 5). NPY enhanced urine flow rates by ~250 µl/15 min (fig. 6) and sodium excretion by ~15 µmol/15 min (fig. 7). PYY$_{3-36}$ enhanced urine excretion by ~100 µl/15 min and sodium excretion by ~15 µmol/15 min (figs. 6 and 7). Indomethacin treatment completely blunted NPY- and PYY$_{3-36}$-induced increases of diuresis (fig. 6) and natriuresis (fig. 7).

**Discussion**

NPY is a unique modulator of renal function (Bischoff and Michel, 1998). Systemically infused NPY reduces RBF and causes diuresis, natriuresis and calciuresis without concomitant alterations in glomerular filtration rate or potassium excretion (Bischoff et al., 1996, 1997a, 1997b; Smyth et al., 1988). At least five subtypes of NPY receptors exist (Michel et al., 1998). In the kidney, NPY effects on the renal vasculature occur via Y$_1$ receptors, whereas those on water and sodium excretion occur largely via Y$_5$ receptors (Bischoff et al., 1997a). Because NPY can enhance urinary prostaglandin excretion (El-Din and Malik, 1988), we investigated whether the cyclooxygenase inhibitor indomethacin alters renal NPY effects.

Cyclooxygenase-derived arachidonic acid metabolites may at least partly mediate NPY-induced vasoconstriction in some vascular beds (Ertl et al., 1993; Martin and Patterson, 1989). Moreover, they can buffer the renal vasoconstriction caused by other agents; for example, indomethacin treatment enhances reductions of RBF by angiotensin II (Chatziantoniou et al., 1990). Although modulation of systemic hemodynamics and renal function by cyclooxygenase inhibitors is well known, the effects of i.p. treatment with 5 mg/kg indomethacin on hemodynamics and renal function did not reach statistical significance in the present study, possibly due to the number of animals or the route of administration. Nevertheless, our indomethacin treatment was clearly effective because it markedly reduced urinary prostaglandin E$_2$ excretion.

The effects of NPY and PYY$_{3-36}$ on MAP, RBF and RVR in the present study are in good agreement with our previous observations under similar experimental conditions (Bischoff et al., 1996, 1997a, 1997b). None of these effects were affected by indomethacin treatment. In agreement with our previous findings (Bischoff et al., 1996), NPY infusion did not significantly affect creatinine clearance. Thus, prostaglandins do not appear to mediate or buffer the systemic or renal vascular effects of NPY and PYY$_{3-36}$. Although indomethacin treatment did not affect basal urine flow rate and sodium excretion, it completely abolished the NPY- or PYY$_{3-36}$-induced diuresis and natriuresis. Thus, prostaglandins may at least partly mediate NPY actions on water and sodium excretion.
NPY can potentially activate the pressure-natriuresis mechanisms due to its MAP-elevating effects, whereas indomethacin can inhibit the pressure-natriuresis mechanism (Firth et al., 1990; Romero and Knox, 1988). However, several pieces of evidence indicate that this is not the basis for the inhibition of NPY-induced diuresis and natriuresis by indomethacin. First, we have previously shown that inhibition of the pressure-natriuresis mechanism by several mechanical and pharmacological maneuvers does not suppress NPY-induced diuresis (Bischoff et al., 1996). Second, two NPY antagonists, PP56 (Bischoff et al., 1997b) and BIBP 3226 (Bischoff et al., 1997a), inhibit NPY-induced MAP elevations but not diuresis and natriuresis. Finally, PYY3–36, which activates Y5 but not Y1 receptors (Michel et al., 1997b) and BIBP 3226 (Bischoff et al., 1997a), inhibit NPY-induced diuresis and natriuresis in the present and a previous study (Bischoff et al., 1997a) but do not elevate MAP. Taken together, these data clearly demonstrate that enhancements of diuresis and natriuresis by NPY occur largely and those by PYY3–36 occur fully independently of the pressure-natriuresis mechanism. Thus, the inhibitory effect of indomethacin cannot be explained by interference with pressure-natriuresis. We conclude that cyclooxygenase-derived arachidonic acid metabolites, possibly prostaglandins, are not involved in the regulation of systemic or renal vascular tone by NPY infusions. However, NPY-induced diuresis and natriuresis are fully blocked by indomethacin treatment. Therefore, we propose that prostaglandins may mediate NPY effects on water and electrolyte excretion.

References


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