Adrenergic Stimulation of Renal Prostanoids
in the Lyon Hypertensive Rat

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Madeleine Vincent, and Jean Sassard

Young, genetically hypertensive Lyon (LH) rats exhibited an increased renal in vivo turnover of norepinephrine and an elevated urinary excretion of thromboxane B₂ when compared with normotensive (LN) and low blood pressure (LL) controls. Therefore, the effects of norepinephrine (1.2×10⁻⁸ to 9.6×10⁻⁷ M) and of phenylephrine (5×10⁻⁸ to 1.9×10⁻⁶ M) on renal function and the urinary excretion of prostanoids were assessed in isolated perfused kidneys of 8-week-old LH, LN, and LL rats. In addition, the effects of norepinephrine were assessed before and during thromboxane A₂/prostaglandin H₂ receptor blockade by AH23848 (4×10⁻⁶ M). Before drug infusion, LH kidneys differed from those of LN and LL controls by having an elevated renal vascular resistance and a decreased natriuresis and glomerular filtration rate; the urinary output of prostaglandin E₂ and F₂α, of 6-ketoprostaglandin F₁α, and of thromboxane B₂ was similar in the three strains. The constrictor effects of norepinephrine and phenylephrine were significantly increased in LH rat kidneys compared with LL but not with LN controls, and their pressure–natriuresis was markedly reduced. Norepinephrine and phenylephrine induced a 10- to 20-fold dose-dependent increase in the synthesis of the four prostanoids, which was more pronounced in LH than in LN and LL rats for thromboxane B₂ only. AH23848 infusion significantly reduced the vascular effects of norepinephrine and increased the natriuretic response of LH but not of LN and LL rat kidneys. In conclusion, isolated perfused kidneys from LH rats exhibit an increased production of thromboxane A₂, which enhances the renal effects of norepinephrine and therefore could participate in the development of hypertension of the Lyon model. (Hypertension 1991;17:296–302)

We have previously observed that the urinary excretion of renal thromboxane (Tx) B₂, used as an index of the renal synthesis of TxA₂, was increased in 5- and 9-week-old genetically hypertensive (LH) rats of the Lyon strain. The same abnormality has also been described in vivo and in vitro in spontaneously hypertensive rats (SHR) of the Japanese strain. Because TxA₂ is a potent vasoconstrictor and promotes platelet aggregation, such an increase is likely to play a pathogenic role. This hypothesis is supported by the observation that TxA₂ synthesis inhibition or blockade of TxA₂ receptors delays the onset or reduces the severity of hypertension in SHR and in LH rats. However, the mechanisms underlying the TxA₂ increase remain to be elucidated. Among them, the sympathetic nervous system could be of major importance since 1) intrarenal administration of norepinephrine (NE) and of phenylephrine (PHE) or electrical renal nerve stimulation is followed by an increase in prostaglandin synthesis and 2) the in vivo turnover of norepinephrine is increased in the kidney cortex of 5-week-old LH rats. Therefore, the present study was undertaken to determine the influence of adrenergic stimulation on the renal production of TxA₂ in LH rats. It was conducted by using isolated perfused kidneys, which we have shown to be a valuable model for the study of renal prostanoids.

Methods

Animals

Eight-week-old male LH, normotensive (LN), and low blood pressure (LL) rats were used. Animals were housed under constant conditions of temperature (21±1°C), lighting (8:00 AM to 8:00 PM), and humidity (60±10%). They were fed a standard diet (Elevage UAR, Villemoisson s/Orge, France) and had free access to tap water. Systolic blood pressure...
Renal Function Parameters

Renal Function Parameters

Kidney Preparation

After rats were anesthetized with sodium pentobarbital (45 mg/kg i.p.), the right kidney was isolated according to Schmidt and Imbs.18 Briefly, after a midline abdominal incision, the right adrenal artery and the small lumbar arteries were tied off. The right kidney was removed from peripheral fat pads and transferred without interruption of the renal blood flow to a small metallic double-walled cup, which was maintained at 37°C with a constant thermostated water circulation. After an injection of heparin (1,000 units i.v.) was administered, four polyethylene catheters were inserted into 1) the mesenteric artery, facing the origin of the right renal artery, to ensure perfusion of the kidney; 2) the intrarenal aorta to allow measurement of the renal perfusion pressure (RPP); 3) the suprapleural vena cava to collect the renal venous effluent; and 4) the ureter. The left renal artery was then ligated, and immediately after the beginning of the perfusion, the infrarenal vena cava and the suprapleural aorta were tied off. The whole right kidney was then excised, trimmed of adhering tissue, and completely isolated. The left kidney was removed and weighed.

Perfusion Medium

The perfusate used was a blood-free modified Krebs-Henseleit solution containing 35 g/l of a gelatin derivative (Polygeline, Behring, Marburg, FRG) as an oncotic agent. The final electrolyte composition (mM) NaCl 100, KCl 3.8, CaCl2 1.1, MgCl2 0.6, KH2PO4 1.2, and NaHCO3 25.0. In addition, the medium contained (mM) D-glucose 10.0, sodium pyruvate 2.0, oxaloacetic acid 1.0, sodium DL-lactate 5.0, L-glutamic acid 5.0, and urea 6.0. Just before use, the perfusate was filtered through two millipore filters (1.2 um and 0.8 um, successively) and added to 0.5 g/l polyfructosan (Inutest, Laevosan, Linz, Austria). The solution was continuously bubbled with a 95% O2–5% CO2 mixture and perfused in an open thermostatically controlled circuit with a peristaltic pump (Minipuls 2, Gilson, Paris) at a constant rate, providing a perfusion pressure of about 90 mm Hg for all preparations.

Renal Function Parameters

The RPP (mm Hg) was continuously recorded (model BS 273, Gould Inc., Cleveland, Ohio) through the aortic catheter placed at the bifurcation of the renal and mesenteric arteries and connected to a pressure transducer (model P23ID, Statham Instrument Division, Gould Inc., Oxnard, Calif.). Renal plasma flow (RPF) (ml/min/g) was measured by precollection of the peristaltic pump and urinary flow rate by weight. Renal vascular resistance (RVR) (mm Hg/ml/min/g) was calculated as the ratio RPF/RF. Glomerular filtration rate (GFR) (ml/min/g) was measured by polyfructosan clearance. Sodium concentration was determined by flame photometry (IL 243).

Measurement of Prostanoids

TXB2 and 6-ketoprostaglandin (PG) F1α (the stable breakdown products of TXA2 and PGI2, respectively), PGE2, and PGF2α, urinary concentrations were measured using previously described radioimmunoassays.19–21 In this technique, prostanoids were first extracted with ethyl acetate and then submitted to a high-performance liquid chromatographic step that allows separation of prostanoids and their metabolites. The sensitivity of our assays was 0.7 pg for TXB2, 1.4 pg for 6-keto-PGF1α, 1.1 pg for PGE2, and 0.9 pg for PGF2α. The urinary excretion of prostanoids was corrected for the weight of the unperfused left kidney (pg/min/g).

Experimental Protocols

Protocol 1: Effect of adrenergic stimulation on renal function and prostanoids. According to previous experiments,16 baseline parameters were obtained during a 10-minute period after a stabilization period of 30 minutes. Then graded concentrations of NE (Levophed, Winthrop, Clichy, France) were infused (Precidor 5003, Inforos A.G., Bäle, Switzerland) for 3 minutes at 20-minute intervals via a catheter connected to the main perfusion line in six animals of each strain; PHE (Sigma Chemical Co., St. Louis) was given according to the same protocol to the six remaining rats. Final concentrations reached in the perfusate ranged from 1.2 x 10−8 to 9.6 x 10−7 M for NE and from 5 x 10−8 to 1.9 x 10−6 M for PHE. For each dose, urine samples were collected during the 7 minutes before (control value) and during the first 6 minutes after the start of drug infusion. The concentration of agonist that evoked 50% of the maximal response (EC50) was used as an index of the sensitivity of the isolated perfused kidneys.

Protocol 2: Effect of thromboxane A2/prostaglandin H2 receptor blockade on vascular response to norepinephrine. This experiment was performed in six other kidneys of each strain to determine whether the NE-induced release of TXA2 could influence the renal effects of NE. After 30 minutes of stabilization, increasing concentrations of NE (3.5 x 10−8 to 3.2 x 10−7 M) were infused for 3 minutes at 20-minute intervals before and during a continuous perfusion of AH23848 (Glaxo, Greenford, Kent, England), a specific TXA2/PGH2 receptor antagonist,22 at a final concentration of 4 x 10−6 M. At the end of each experiment, the TXA2/PGH2 receptor blockade was verified by administration of U46619 (Sigma Chemical Co., St. Louis), a specific TXA2 agonist.23 The final concentration of U46619 reached in the perfusate was 1.4 x 10−7 M which, in absence of AH23848, produced a submaximal increase in RVR. For each dose, urine samples were collected during the 3
TABLE 1. Baseline Characteristics of Isolated Perfused Kidneys

<table>
<thead>
<tr>
<th>Variables</th>
<th>LL</th>
<th>LN</th>
<th>LH</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mm Hg)</td>
<td>114±2*</td>
<td>123±3</td>
<td>154±3†</td>
</tr>
<tr>
<td>BW (g)</td>
<td>234±5</td>
<td>205±5</td>
<td>274±4†</td>
</tr>
<tr>
<td>KW (g)</td>
<td>1.03±0.03</td>
<td>1.03±0.03</td>
<td>1.19±0.02†</td>
</tr>
<tr>
<td>RPP (mm Hg)</td>
<td>81.8±1.7</td>
<td>83.0±2.1</td>
<td>89.6±2.3*</td>
</tr>
<tr>
<td>RVR (mm Hg/ml/min/g)</td>
<td>5.16±0.18*</td>
<td>6.03±0.29</td>
<td>8.35±0.51†</td>
</tr>
<tr>
<td>GFR (ml/min/g)</td>
<td>0.63±0.06</td>
<td>0.67±0.05</td>
<td>0.42±0.02†</td>
</tr>
<tr>
<td>UNaV (µmol/min/g)</td>
<td>22.3±2.9</td>
<td>22.8±2.6</td>
<td>12.6±1.1†</td>
</tr>
<tr>
<td>TxB2 (pg/min/g)</td>
<td>10.4±0.6</td>
<td>10.1±0.7</td>
<td>10.1±0.9</td>
</tr>
<tr>
<td>6-keto-PGF1α (pg/min/g)</td>
<td>69.6±6.1</td>
<td>75.6±4.8</td>
<td>74.6±5.8</td>
</tr>
<tr>
<td>PGF2α (pg/min/g)</td>
<td>50.0±8.5</td>
<td>55.4±7.8</td>
<td>43.1±4.5</td>
</tr>
<tr>
<td>PGE2 (pg/min/g)</td>
<td>78.3±8.0</td>
<td>80.1±6.3</td>
<td>69.5±5.5</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, number of rats of each strain; LL, Lyon low blood pressure rats; LN, Lyon normotensive rats; LH, Lyon hypertensive rats; SBP, systolic blood pressure; BW, body weight; KW, weight of the unperfused left kidney; RPP, renal perfusion pressure; RVR, renal vascular resistance; GFR, glomerular filtration rate; UNaV, urinary sodium excretion; TxB2, urinary output of thromboxane B2; 6-keto-PGF1α, urinary output of 6-ketoprostaglandin F1α; PGF2α, urinary output of prostaglandin F2α; PGE2, urinary output of prostaglandin E2.

* p<0.05; † p<0.001 vs. LN rats.

results

minutes before (control value) and during the 3 minutes after the start of NE infusion.

Statistical Analysis

Values are mean±SEM. The data were analyzed by the nonparametric Mann-Whitney test to assess the interstrain differences and the Wilcoxon test to compare the responses obtained before and after AH23848 infusion.

Results

As indicated in Table 1, LH rats exhibited higher and LL rats lower indirect SBP and RVR than LN controls. In the three strains of rats, RVR was positively related to indirect SBP (r=0.53, n=54, p<0.001). GFR and urinary sodium excretion were decreased in LH rat kidneys compared with both LN and LL controls. No interstrain difference could be distinguished in the baseline values of the urinary excretion of prostanoids.

Graded concentrations of NE and PHE (Figure 1) elicited a dose-related increase in the RVR of the three strains. The maximal RVR values observed during the infusion of the highest concentration of NE or PHE were significantly greater in LH rat kidneys (28.6±2.1 and 28.1±3.7 mm Hg/ml/min/g for NE and PHE, respectively) than in LN (15.5±1.5 and 18.7±1.5 mm Hg/ml/min/g for NE and PHE, respectively) or LL (17.0±0.9 and 16.8±0.7 mm Hg/ml/min/g for NE and PHE, respectively) rat kidneys. The EC50 of NE and PHE was significantly lower in LH compared with LL rats but not with LN rats. As shown by Figure 2, GFR remained stable during infusion of NE and PHE. The pressure-natriuresis, expressed by the slope of the relation between urinary sodium excretion and RPP, was significantly
Concentration (10^-8 M)

Phenylephrine

Concentration (10^-8 M)

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FIGURE 2. Line graphs showing effects of increasing concentrations of norepinephrine (left panel) and of phenylephrine (right panel) on the glomerular filtration rate (GFR) and pressure-natriuresis of six isolated perfused kidneys from 8-week-old Lyon male rats (protocol 1). *p<0.05 vs. Lyon low blood pressure (LL) rat; †p<0.05 vs. Lyon normotensive (LN) rat. LH, Lyon hypertensive rat.

(\(p<0.05\)) reduced in LH rat kidneys (slope 0.11±0.02 and 0.06±0.02 for NE and PHE, respectively) compared with LN (slope 0.41±0.03 and 0.10±0.01 for NE and PHE, respectively) and LL (slope 0.22±0.06 and 0.11±0.03 for NE and PHE, respectively) rat kidneys. Figure 3 shows that the two doses of NE and PHE, which induced approximately 50% and 100% of the maximum increase in RVR, strikingly elevated the urinary excretion of the prostanoids measured. This effect was more marked for PGE_2, PGF_2\alpha, and TxB_2 than for 6-keto-PGF_1\alpha. In that respect, NE was more potent than PHE. Above all, LH rat kidneys differed from both LL and LN controls by a significantly more pronounced increase in the urinary excretion of TxB_2 only.

As shown in Figure 4, AH23848 did not modify the NE-induced increases of RVR in LL and LN rats but significantly reduced those of LH rat kidneys. Similarly, the GFR and the pressure-natriuresis of LL and LN rat kidneys remained unchanged after TxA_2/PGH_2 receptor blockade but were enhanced in LH rats.

Discussion

To determine the relation between the increased renal NE in vivo turnover and TxA_2 synthesis previously observed in young LH rats, the influence of graded concentrations of NE and of PHE on renal function and prostanoid urinary excretion was studied in isolated perfused kidneys. It was demonstrated that in baseline conditions, isolated perfused kidneys from LH rats differed from those of LN and LL controls by an enhanced vascular resistance and a decreased natriuresis. The urinary TxB_2 excretion of unstimulated kidneys from the three strains did not differ, but it was more markedly stimulated by NE and PHE in LH than in LN and LL rat kidneys. Finally, in LH kidneys only, TxA_2/PGH_2 receptor...
blockade significantly reduced the vascular responses to NE and enhanced the GFR and the pressure–natriuresis.

We used the isolated perfused kidney because we have demonstrated it to be a valuable preparation for the study of prostanoid synthesis\textsuperscript{16} that allows control of factors that may influence renal function. In addition, the use of an open perfusion circuit allowed us to avoid the recirculation of renally produced prostanoids and to separate the administration of NE and PHE by washout periods so as to prevent tachyphylaxis. The kidneys were removed from 8-week-old rats. At that age, the SBP of the three strains significantly differed. The kidney weight of LH rats was higher than that of the two control strains. However, when corrected for body weight, the kidney weight of LH rats (0.43±0.01%) was similar to that of LL (0.44±0.01%) and lower than that of LN (0.50±0.01%) rats.

Under baseline conditions, RVR was significantly increased (38%) in LH and decreased (14%) in LL rats compared with LN controls. In addition, RVR values were positively related to the SBP levels. Elevated RVR was also reported in isolated perfused kidneys of SHR.\textsuperscript{24} Such an increase cannot be explained by differences in renal vascular reactivity since the cell-free medium used for perfusion was devoid of vasoactive agents. Similarly, the renal autoregulatory process is unlikely to be involved since in our experimental conditions the perfusion rate was selected to obtain similar RPP. Therefore, the most likely explanation for increased RVR relies on structural alterations of the renal vasculature that are already present in the young LH rat.\textsuperscript{25} GFR was decreased in LH kidneys compared with LN and LL controls. This abnormality, which is not present in young SHR,\textsuperscript{26} could also reflect the structural alterations of the glomeruli previously reported in that model.\textsuperscript{25}

To study the effects of NE and PHE, we used 3-minute infusions of each concentration followed by a 17-minute washout period to avoid the development of tachyphylaxis. NE and PHE concentrations increased the RVR in the kidneys of the three strains. The maximal response was significantly higher in LH than in LN and LL rat kidneys. The EC\textsubscript{50} for both NE and PHE was decreased in LH compared with LN and LL rat kidneys. Because
these differences, as discussed below, were reduced after TxA2/PGF2α receptor blockade, they could not solely be explained by the structural alterations already described. Increased perfusion pressure increased the sodium excretion due to a decrease in fractional sodium reabsorption since GFR remained stable. This pressure–natriuresis, which plays an important role in the control of blood pressure, was significantly lower in LH compared with LN and LL rat kidneys. Similar observations were made using isolated perfused kidneys of SHR and of salt-sensitive Dahl rats once hypertension had developed. In addition, these interstrain differences were more pronounced after NE than after PHE administration. NE stimulates the α1-adrenergic receptors, which induce a pressure-dependent natriuresis, the α2 subtype, which also increases the natriuresis but mainly via a direct tubular effect, and finally, to a lesser extent, the β-receptors, which decrease the sodium excretion. Because PHE acts on the α1 subtype only, its natriuretic effect should be lower than that of NE. This is effectively observed in LL and LN but not in LH kidneys. Such a difference may be explained by the higher density of renal α1-adrenergic receptors exhibited by LH rats compared with LN and LL rats.

The urinary excretion of prostanoids, which has previously been demonstrated to be a reliable index of their renal synthesis, did not differ among kidneys of the three strains during the baseline period. Such a finding demonstrates that the increased TxA2 renal synthesis observed in vivo in LH rats of the same age is not a primary event but results from an extrarenal stimulation of Tx synthetase or of arachidonic acid availability. Adrenergic stimulation strikingly increased the prostanooid excretion in the three strains. This increase reached 20 times control levels for PGF2α, PGF2α, and TxB2 but only 10 times control levels for 6-keto-PGF1α. In agreement with Cooper and Malik, but at variance with Vandongen et al, this stimulatory effect did not seem related to changes in RPP since, in spite of similar increases in RPP, NE was twofold more potent that PHE on prostanooid synthesis. For the same reason, the α2-adrenergic receptor is probably not the only receptor type involved in that response, as suggested by Cooper and Malik. Above all, the TxA2 synthesis was more sensitive to NE and PHE in LH than in LN or LL rat kidneys. Because α-adrenergic stimulation promotes the first step of eicosanoid synthesis by acting on the phospholipase A2, the enhanced TxA2 response of LH rat kidneys may be due to an elevated activity of Tx synthetase. Using in vitro experiments, Shibouta et al reported an increased activity of this enzyme in kidney homogenates from SHR. Such an increased activity of TxA2 synthesis appears of functional importance since in LH but not in LN and LL rat kidneys the TxA2/PGF2α receptor blockade significantly reduced the effects of NE on RVR and natriuresis. Because this latter effect was associated with an increase in GFR but not in the fractional sodium reabsorption, it is likely to result from an increased filtered sodium load. Taken together, these results indicate that in LH rats, TxA2 is involved in the enhanced RVR, the lower GFR, and in the decreased pressure–natriuresis observed after adrenergic stimulation.

In conclusion, the present study shows that adrenergic stimulation is a potent stimulus for prostanooid synthesis by the rat kidney. It demonstrates that the increased renal synthesis of TxA2 observed in vivo in young LH rats reflects an elevated activity of the Tx synthetase, which is revealed by extrarenal stimuli such as adrenergic receptor activation. Because TxA2/PGF2α receptor blockade, which has been shown to decrease the blood pressure of LH rats, also depresses the effects of α-adrenergic receptor stimulation in the isolated kidneys, the above described abnormality is likely to be involved in the pathogenesis of hypertension in that model.

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References


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