Attenuated Renal Response to Dopaminergic Drugs in Spontaneously Hypertensive Rats

Robin A. Felder, Mouin G. Seikaly, Peter Cody, Gilbert M. Eisner, and Pedro A. Jose

Activation of renal dopamine-1 receptors decreases sodium transport. However, the spontaneously hypertensive rat retains sodium despite increased renal dopamine concentration. We tested the hypothesis that the abnormal sodium handling in spontaneously hypertensive rats (Okamoto-Aoki strain) is related to a decreased dopaminergic response by studying the effects of the intrarenal infusion of the dopamine-1 agonist SKF-38393 and the dopamine-1 antagonist SCH-23390 in hypertensive and in normotensive Wistar-Kyoto rats. Rats (9–16 weeks old) were studied with renal nerves intact under pentobarbital anesthesia (n=5–6 in each group). Specificity of dopamine-1 effects of SKF-38393 was verified because its natriuretic effect was blocked in a dose-related manner by the dopamine-1 antagonist SCH-23390 (n=5). Intrarenal arterial infusion of the dopamine-1 agonist SKF-38393 did not affect glomerular filtration rate but resulted in a dose-related natriuresis and diuresis in normotensive but not in hypertensive rats. Intrarenal arterial infusion of the dopamine-1 antagonist SCH-23390 alone induced an antinatriuresis, without affecting glomerular filtration rate, in normotensive but not in hypertensive rats. Addition of the dopamine-2 antagonist YM-09151 to the dopamine-1 antagonist infusion did not enhance the effect of the dopamine-1 antagonist. The lack of response to the dopamine-1 agonist or antagonist in hypertensive rats was not due to differences in renal dopamine-1 receptor density (U±0.2 pmol/mg protein for spontaneously hypertensive rats, n=4; l±0.2 for Wistar-Kyoto rats, n=4) or affinity; distribution determined by autoradiography was also similar. The abnormal renal sodium handling in 9–16-week-old spontaneously hypertensive rats is in part due to decreased response distal to the dopamine-1 receptor. (Hypertension 1990;15:560–569)
sodium excretion of dopamine endogenously produced in the kidney was studied by examining the effects on the intrarenal administration of the benzazepine DA₁ antagonist SCH-23390. Based on the observation that a combined DA₁ and DA₂ agonist was necessary to inhibit sodium-potassium adenosine triphosphatase (Na⁺K⁺-ATPase) activity in the proximal convoluted tubule of the rat, Bertorello and Aperia suggested a synergism between the dopamine receptor subtypes in the natriuretic response to dopamine. Therefore, the effect of the DA₁ antagonist YM-09151 was also studied during DA₂ blockade. In addition, renal DA₁ receptors were studied by using radioligand binding and autoradiographic techniques with the DA₁ radioligand [³²P]SCH-23982 (New England Nuclear, Boston, Massachusetts) as previously reported from our laboratory.

Methods

In Vivo Studies

Male SHR of the Okamoto-Aoki strain and control WKY rats (Harlan Sprague Dawley, Inc., Indianapolis, Indiana), aged 9–16 weeks and weighing 200–350 g, were maintained on standard rat chow with ad libitum access to water. The rats were anesthetized and prepared for bilateral renal clearances as described previously. A polyethylene tubing (tip outside diameter, 125 μm; inside diameter, 60 μm) was introduced into the left carotid artery and into the left renal artery through the descending aorta.

Then an intrarenal arterial infusion of heparinized saline was started at 0.5 ml/hr. Glomerular filtration rate was determined by the clearance of [³H]iminulin (New England Nuclear) infused at 0.1 μCi/100 ml and delivered in normal saline at a rate of 3 ml/100 g body wt over 30 minutes, followed by 2 ml/100 g body wt/hr until the termination of the experiment.

After an equilibrium period of 2–4 hours, a 40-minute control urine collection was obtained. The rats were then divided into separate groups for study: groups 1, 2A, 2B, and 3 consisted of WKY rats (Tables 1–4) and groups 4 and 5 (Tables 5 and 6) consisted of SHR. To determine the effect of exogenous DA₁ on sodium excretion, the effects of intrarenal administration of the DA₁ agonist SKF-38393 were studied. After the control period, the rats (group 1 [WKY rats, n=5] and group 4 [SHR, n=6]) received an intrarenal infusion of DA₁ agonist SKF-38393; the dose was increased from 1.2 to 12 to 120 ng/g kidney wt/min; each was delivered at the same rate of 0.5 ml/hr. A 40-minute urine collection was obtained beginning 10 minutes (time needed to clear dead space) after each change of drug concentration and 10 minutes after stopping the drug infusion, designated as the recovery period. In another group of WKY rats (group 2A [n=5]), the specificity of SKF-38393 to the DA₁ receptor was tested by the infusion of the DA₁ antagonist SCH-23390. After the initial control period, SCH-23390 was infused at 1.2 ng/g kidney wt/min. Ten minutes after the infusion was started, a 40-minute urine collection was begun. Then, the DA₁ agonist SKF-38393 (120 ng/g kidney wt/min) was infused into the renal artery; 10 minutes into the infusion, a 40-minute urine collection period was begun. Thereafter, while the infusion of SKF-38393 was continued, collections were obtained while SCH-23390 was infused at rates of 12 and 120 ng/g kidney wt/min. The infusion rate was kept at 0.5 ml/hr, the same rate of intrarenal fluid administration in all periods. In another group of WKY rats (group 2B [n=4]), the DA₁ agonist SKF-38393 was infused alone at 120 ng/g kidney wt/min after the control period and served as the time control for group 2A rats.

The role of endogenous renal dopamine on sodium excretion was studied in another group of rats (group 3 [WKY rats, n=7] and group 5 [SHR, n=5]) by examining the effects of the intrarenal administration of the DA₁ antagonist SCH-23390. After the control period, SCH-23390 (120 ng/min/g kidney wt) in normal saline was infused at the same rate of 0.5 ml/hr as in the control period. According to the receptor occupation theory, this dose of SCH-23390 is expected to occupy 96% of the renal DA₁ receptors. Ten minutes into the infusion, a 40-minute urine collection period was begun. The possible synergism between DA₁ and DA₂ receptors on renal function was studied by the addition of the DA₂ receptor antagonist YM-09151 (120 ng/min/g kidney wt) to the DA₁ antagonist infusion. The rate of infusion was kept at the same rate of 0.5 ml/hr. Ten minutes into the infusion, a 40-minute urine collection period was obtained. Thereafter, the infusate was changed to the vehicle (normal saline) at the same rate of 0.5 ml/hr; 10 minutes into the infusion, a 40-minute urine collection period (recovery period) was begun. Blood (0.5 ml) was collected during the control period, between the control period and SCH-23390 infusion, between the infusion of SCH-23390 plus YM-09151 and the recovery period (no drugs), and just before they were killed. The blood removed was replaced (vol/vol) with normal saline.

At the end of the experiment, a 1 ml bolus of 10% lissamine green was injected into the renal artery to verify patency of the renal arterial catheter and uniform delivery of drugs to all segments of the kidney. The kidney was weighed at the end of the experiment, and the drug infusion was expressed in nanograms per gram kidney weight. Time control studies revealed stable renal functional parameters. Stability of the rats was also assured as recovery to control values occurred after the agonist drug infusions were stopped.

Radioligand Binding Studies

The rats were decapitated; the kidneys were rapidly removed after a midline abdominal incision and placed immediately on ice. All tissues were used fresh within 2 hours of decapitation and kept at 4°C until incubation for the radioligand binding study. The kidneys were bisected longitudinally, and the

Felder et al Renal DA₁ Defect in Hypertension 561

Downloaded from http://hyper.ahajournals.org/ by guest on May 1, 2017
medulla was removed from the cortex at the corticomедullary junction. The renal cortex was then minced to a fine paste, and the glomeruli were separated from cortical tubular tissue by a sieving procedure as previously described, except that the incubation buffer used was Tris-chloride buffer containing (mM) Tris-chloride 80, MgSO₄ 1, and EGTA 0.8. The tubular tissue was then homogenized in a glass Teflon Dounce homogenizer (clearance 0.095–0.115 mm, Braun GmbBH, Melsungen, Germany) with a motor-driven pestle at 1,200 rpm for 20 strokes. The homogenate was centrifuged at 500 g for 5 minutes in a refrigerated centrifuge.

The membranes obtained from the 30,000 g centrifugation procedure as previously described, except that the incubation buffer used was Tris-chloride buffer (to determine total binding) or 50 µM SCH-23390, a DA, antagonist, (Schering Corporation, Kenilworth, New Jersey) (to determine nonspecific binding). After 40 minutes of incubation, the incubation buffer was replaced by a buffer containing either radioligand (20 nM) or radioligand and competing drug (5 µM SCH-23390, to determine nonspecific binding). After 40 minutes of incubation, the slides were subjected to a final, 1-second deionized water rinse at 4° C. The slices were then rapidly dried under a stream of warm air and exposed to LKB ultrafilm (LKB, Uppsala, Sweden) for approximately 24 hours. Exposure times were optimized for the linear response of the film to 125I by including 125I microscales (Amersham, Arlington Heights, Illinois) with each exposure. Autoradiographic density was quantitated on a digital videodensitometry acquisition system developed at the Biomedical Processing Center at the University of Virginia, Charlottesville, Virginia. Density units were converted to radioactive decay units and subsequently to drug concentrations with each exposure. Autoradiographic density was quantitated from known densities determined from the microscales included in each autoradiographic exposure. The microscales were calibrated from 125I SCH-23982 was added in known quantities as previously described from our laboratory.

Analytical Procedures

[3H]Inulin was measured in a liquid scintillation spectrometer with an efficiency of 60%. Sodium concentration was measured by ion-specific electrode. Protein concentration was measured by using human serum protein standard according to the method of Lowry et al.

Calculations

Dissociation constant and maximum receptor density were calculated according to Rosenthal by nonlinear regression (Lundon Software Inc., Cleveland, Ohio) as previously described.

Statistical Analyses

Results are expressed as mean±SEM. Differences between control values and drug treatments within groups were compared by using repeated-measures analysis of variance; significance was tested by Scheffe's test or by paired t test with Bonferroni correction as noted in the results. Differences between groups were compared by one-
way analysis of variance; significance was tested by Duncan multiple range test. A value of $p<0.05$ was considered significant.

## Results

**In Vivo Studies**

**Effect of exogenous DA$_1$ agonist.** In WKY rats (group 1), infusion of SKF-38393 had no effect on mean arterial pressure or glomerular filtration rate but increased urine flow in a dose-related fashion and showed a similar effect on absolute and fractional sodium excretion. Recovery to pre-drug conditions was indicated (Table 1). The contralateral kidney was unaffected; thus, absence of significant spillover into the systemic circulation was indicated (Table 1). Intrarenal arterial infusion of vehicle alone did not influence ipsilateral or contralateral renal function (data not shown). To verify that SKF-38393 exerted its action by means of the DA$_1$ receptor, studies were repeated in the presence of the DA$_1$ antagonist SCH-23390 (group 2A). The antagonist alone, infused at the low dose of 1.2 ng/g kidney wt/min, had no effect on any of the parameters studied (Table 2, period A). However, the natriuretic and diuretic effects of SKF-38393 were blocked in a dose-related fashion by the DA$_1$ antagonist (Table 2, periods B–D). The contralateral kidney was again unaffected; thus, absence of significant spillover into the circulation was indicated (Table 2). However, the possibility that any effect of DA$_1$ antagonist on sodium excretion in the contralateral kidney was masked by an equivalent spillover of the DA$_1$ agonist cannot be ruled out. The decreases in urine flow and absolute and fractional sodium excretion associated with the infusion of SCH-23390 were not due to tachyphylaxis as the infusion of 120 ng/g kidney wt SKF-38393 alone was associated with persistent diuresis and natriuresis (Table 3). Thus, the natriuretic and diuretic effects of SKF-38393 in WKY rats were due to occupation of DA$_1$ receptors.

In contrast to the results in WKY rats (group 1), intrarenal administration of SKF-38393 in SHR (group 4) with intact renal nerves had no effect on mean arterial pressure, glomerular filtration rate, urine flow, absolute sodium excretion, or fractional sodium excretion (Table 4).

**Effect of endogenous dopamine on renal function.** The effect of endogenous dopamine on renal function was evaluated by the intrarenal arterial infusion of the DA$_1$ antagonist SCH-23390.$^{5,10}$ The dose of 120 ng/min/g kidney wt was chosen because, according to the receptor occupation theory,$^{28}$ 96% of DA$_1$ receptors are expected to be occupied in this situation. As shown in Table 2, SCH-23390 at 1.2 ng/min/g kidney wt had no effect on renal function (in this instance occupation of about 18% of the DA$_1$ receptors is expected). The infusion of SCH-23390 at 120 ng/min/g kidney wt decreased sodium excretion in WKY rats (group 3, Table 5) but not in SHR (group 5, Table 6). At the dose of 120 ng/min/g kidney wt SCH-23390, there was spillover into the circulation as sodium excretion was decreased in the right kidney (Table 4). Urine flow was slightly but not significantly decreased in the right kidney in SHR (Table 6) and WKY rats (Table 5). Based on the ability of DA$_2$ agonist to inhibit Na$^+$,K$^+$-ATPase activity in the presence of DA$_1$ agonist, Bertorello and Aperia$^{16}$ have suggested a synergism between the dopamine

### Table 1. Effects of Presence and Absence of Intrarenal Infusion of Dopamine-1 Agonist SKF-38393 in Wistar-Kyoto Rats (Group 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP (mm Hg)</th>
<th>V (µl/min)</th>
<th>GFR (µl/min/g)</th>
<th>U$_{NaV}$ (µeq/min)</th>
<th>Fe$_{Na}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infused left kidney (n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>120±4</td>
<td>9.1±0.4</td>
<td>771±30</td>
<td>1.4±0.2</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>SKF-38393</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2 ng/g kidney wt/min</td>
<td>119±7</td>
<td>10.5±0.3</td>
<td>727±20</td>
<td>1.7±0.2</td>
<td>1.7±0.1</td>
</tr>
<tr>
<td>12.0 ng/g kidney wt/min</td>
<td>118±4</td>
<td>11.6±0.3</td>
<td>751±21</td>
<td>2.0±0.2</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>120.0 ng/g kidney wt/min</td>
<td>118±6</td>
<td>12.4±0.2</td>
<td>729±20</td>
<td>2.1±0.3</td>
<td>2.3±0.1</td>
</tr>
<tr>
<td>Recovery</td>
<td>120±5</td>
<td>9.7±0.5</td>
<td>747±22</td>
<td>1.3±0.2</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>Noninfused right kidney (n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>...</td>
<td>8.8±0.4</td>
<td>738±13</td>
<td>1.3±0.2</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Period 2</td>
<td>...</td>
<td>8.7±0.5</td>
<td>703±20</td>
<td>1.4±0.2</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>Period 3</td>
<td>...</td>
<td>8.6±0.5</td>
<td>720±23</td>
<td>1.4±0.3</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>Period 4</td>
<td>...</td>
<td>8.5±0.4</td>
<td>693±18</td>
<td>1.3±0.3</td>
<td>1.1±0.2</td>
</tr>
<tr>
<td>Period 5</td>
<td>...</td>
<td>8.4±0.5</td>
<td>725±32</td>
<td>1.5±0.1</td>
<td>1.1±0.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MAP, mean arterial pressure; V, urine flow; GFR, glomerular filtration rate; U$_{NaV}$, sodium excretion; Fe$_{Na}$, fractional sodium excretion; control, 2–4-hour equilibrium period with no drugs; recovery, period after cessation of drug infusion. Periods 1–5 correspond to time periods for the infused left kidney.

*p<0.05 vs. control.

*p<0.05 vs. 1.2 ng/g kidney wt/min SKF-38393.

*p<0.05 vs. recovery.

*p<0.05 vs. 12.0 ng/g kidney wt/min SKF-38393.
TABLE 2. Effects of Presence of Intrarenal Arterial Infusion of Graded Doses of Dopamine-1 Antagonist SCH-23390 During Intrarenal Arterial Infusion of Dopamine-1 Agonist SKF-38393 and Absence of Infusion in Wistar-Kyoto Rats (Group 2A)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP  (mm Hg)</th>
<th>V  (μl/min)</th>
<th>GFR (μl/min/g)</th>
<th>U_{Na}V (μeq/min)</th>
<th>F_{en}V (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infused left kidney (n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>125±4</td>
<td>10.9±0.4</td>
<td>705±18</td>
<td>2.3±0.3</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>A</td>
<td>124±3</td>
<td>10.5±0.5</td>
<td>665±13</td>
<td>2.1±0.2</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>B</td>
<td>124±5</td>
<td>13.3±0.2±‡</td>
<td>712±15</td>
<td>3.2±0.3±‡</td>
<td>2.8±0.3‡</td>
</tr>
<tr>
<td>C</td>
<td>125±4</td>
<td>11.7±0.3‡</td>
<td>642±20</td>
<td>2.4±0.3‡</td>
<td>2.3±0.3‡</td>
</tr>
<tr>
<td>D</td>
<td>124±5</td>
<td>10.9±0.3</td>
<td>651±17</td>
<td>2.0±0.4</td>
<td>2.0±0.3</td>
</tr>
<tr>
<td>Noninfused right kidney (n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>...</td>
<td>11.1±0.9</td>
<td>677±17</td>
<td>2.3±0.2</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td>Period 2</td>
<td>...</td>
<td>11.0±1.0</td>
<td>650±18</td>
<td>2.2±0.2</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>Period 3</td>
<td>...</td>
<td>10.9±1.0</td>
<td>657±28</td>
<td>2.3±0.2</td>
<td>2.1±0.3</td>
</tr>
<tr>
<td>Period 4</td>
<td>...</td>
<td>11.2±1.0</td>
<td>647±14</td>
<td>2.3±0.2</td>
<td>2.2±0.2</td>
</tr>
<tr>
<td>Period 5</td>
<td>...</td>
<td>11.0±1.0</td>
<td>651±18</td>
<td>2.4±0.3</td>
<td>2.2±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MAP, mean arterial pressure; V, urine flow; GFR, glomerular filtration rate; U_{Na}V, sodium excretion; Fe_{en}V, fractional sodium excretion; control, 2-4-hour equilibrium period with no drugs; A, 1.2 ng/g kidney wt/min SCH-23390; B, 1.2 ng/g kidney wt/min SCH-23390+120 ng/min/g kidney wt SKF-38393; C, 12 ng/g kidney wt/min SCH-23390+120 ng/min/g kidney wt SKF-38393; D, 120 ng/g kidney wt/min SCH-23390+120 ng/min/g kidney wt SKF-38393. Periods 1-5 correspond to time periods for infused left kidney (with period 1 corresponding with control, period 2 with A, and so forth). Values of p were determined by repeated-measures analysis of variance and Scheffe's test.

*p<0.05 vs. control.
†p<0.05 vs. A.
‡p<0.05 vs. D.
§p<0.05 vs. B.

receptor subtypes. Therefore, we studied the effect of the DA₂ antagonist YM-09151 in combination with the DA₁ antagonist (Tables 5 and 6). In WKY rats, the addition of YM-09151 to SCH-23390 resulted in an increase in urine flow; sodium excretion tended to increase. After both drugs were stopped, left kidney urine flow and sodium excretion remained elevated but tended to return to control values. In SHR, DA₁ blockade did not alter urine flow or sodium excretion. Although there was a tendency for sodium excretion to decrease with DA₁ blockade, the effect was neither consistent nor significant. These drugs did not affect blood pressure in SHR and WKY rats although mean arterial pressure did decrease slightly in SHR after both DA₁ and DA₂ blockers were discontinued.

Radioligand Binding Studies

Nonlinear regression analysis of Rosenthal plots revealed no differences in dissociation constant or maximum receptor density between renal cortical homogenates from SHR and WKY rats (Figure 1). The plots of both SHR and WKY rats best fit binding characteristics for one receptor type. There were also no apparent differences in the location of [^{125}I]SCH-23982-specific binding sites (Figure 2). The specific binding in a randomly selected 1-mm² area in the cortex was 1.05±0.06 pmol/mg protein in WKY rats (n=3) and 1.19±0.19 in SHR (n=3). The striatum of both SHR and WKY rats had high specific binding for [^{125}I]SCH-23982.

Discussion

The intrarenal arterial infusion of the DA₁ agonist SKF-38393 induced a dose-related increase in urine flow and sodium excretion without affecting glomerular filtration rate in WKY rats with intact renal nerves. This effect may be attributed to stimulation of receptor subtypes.
TABLE 4. Effects of Presence and Absence of Intrarenal Infusion of Dopamine-1 Agonist SKF-38393 in Spontaneously Hypertensive Rats (Group 4)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP (mm Hg)</th>
<th>V (µl/min)</th>
<th>GFR (µl/min/g)</th>
<th>UnV (µeq/min)</th>
<th>FeNa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infused left kidney (n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>156±4</td>
<td>10.1±1.4</td>
<td>573±32</td>
<td>1.2±0.2</td>
<td>1.3±0.2</td>
</tr>
<tr>
<td>SKF-38393</td>
<td>154±6</td>
<td>10.0±1.4</td>
<td>577±30</td>
<td>1.3±0.2</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td></td>
<td>150±4</td>
<td>10.0±1.3</td>
<td>591±27</td>
<td>1.4±0.2</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td></td>
<td>120.0 ng/kidney wt/min</td>
<td>10.1±1.3</td>
<td>544±30</td>
<td>1.5±0.1</td>
<td>1.4±0.2</td>
</tr>
<tr>
<td>Recovery</td>
<td>154±3</td>
<td>10.0±1.3</td>
<td>541±27</td>
<td>1.3±0.2</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>Noninfused right kidney (n=6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>...</td>
<td>9.8±1.5</td>
<td>518±20</td>
<td>1.2±0.1</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>Period 2</td>
<td>...</td>
<td>10.1±1.5</td>
<td>550±26</td>
<td>1.3±0.1</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>Period 3</td>
<td>...</td>
<td>10.2±1.2</td>
<td>558±23</td>
<td>1.3±0.1</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>Period 4</td>
<td>...</td>
<td>10.4±1.4</td>
<td>581±18</td>
<td>1.4±0.1</td>
<td>0.9±0.1</td>
</tr>
<tr>
<td>Period 5</td>
<td>...</td>
<td>10.2±1.7</td>
<td>543±12</td>
<td>1.3±0.1</td>
<td>0.8±0.1</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MAP, mean arterial pressure; V, urine flow; GFR, glomerular filtration rate; UnV, sodium excretion; FeNa, fractional sodium excretion; control, 2-4-hour equilibrium period with no drugs; recovery, period after cessation of drug infusion. Periods 1-5 correspond to time periods for infused left kidney.

renal DA1 receptors since the DA1 antagonist SCH-23390 blocked the natriuretic and diuretic effects of SKF-38393 in a dose-related manner. This is in agreement with previous reports that the natriuretic effects of dopamine and DA1 agonists are due to renal DA1 receptor occupancy. In contrast, in SHR SKF-38393 had minimal effect on the renal functional parameters studied. Although the glomerular filtration rate was less in this group of SHR than in WKY rats (group 4 vs. group 1), this alone does not explain the differences in the responses to DA1 effects, as in the subsequent studies the glomerular filtration rate in SHR (group 5) was similar to that noted in WKY rats (group 3), yet the responses to endogenous DA1 blockade were still different (see below).

In the rat, increased sodium intake is associated with increased renal dopamine production; dopamine has been suggested as a paracrine substance that can regulate sodium excretion in normotensive animals. The natriuretic effect of endogenous dopamine becomes apparent only during a sodium load. Inhibition of dopamine production attenuates the natriuresis of chronic sodium loading. We have previously reported that dopamine blockade in hydropenia does not affect sodium excretion. However, dopamine blockade did attenuate the natriuresis associated with saline loading. This effect of dopamine blockade on sodium excretion is due primarily to occupation of DA1 receptors, since DA1 antagonists more consistently de-

TABLE 5. Effects of Presence of Intrarenal Infusion of Dopamine-1 Antagonist SCH-23390 Alone or in Combination With Dopamine-2 Antagonist YM-09151 and Absence of Infusion in Wistar-Kyoto Rats (Group 3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MAP (mm Hg)</th>
<th>V (µl/min)</th>
<th>GFR (µl/min/g)</th>
<th>UnV (µeq/min)</th>
<th>FeNa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infused left kidney (n=7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>103±4</td>
<td>16.3±2.0</td>
<td>1,307±162</td>
<td>3.16±0.41</td>
<td>1.62±0.21</td>
</tr>
<tr>
<td>SCH-23390</td>
<td>104±3</td>
<td>14.3±2.3*</td>
<td>1,174±160</td>
<td>2.22±0.39*</td>
<td>1.17±0.18*</td>
</tr>
<tr>
<td>SCH-23390+YM-09151</td>
<td>104±3</td>
<td>24.1±5.0*</td>
<td>1,159±145</td>
<td>3.55±0.80</td>
<td>1.75±0.37</td>
</tr>
<tr>
<td>Recovery</td>
<td>103±4</td>
<td>22.5±4.4</td>
<td>997±127</td>
<td>3.31±0.72</td>
<td>1.91±0.26*</td>
</tr>
<tr>
<td>Noninfused right kidney (n=7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Period 1</td>
<td>...</td>
<td>15.5±2.2</td>
<td>1,304±172</td>
<td>3.22±0.38</td>
<td>1.65±0.21</td>
</tr>
<tr>
<td>Period 2</td>
<td>...</td>
<td>14.8±2.3</td>
<td>1,210±131</td>
<td>2.38±0.26*</td>
<td>1.24±0.14</td>
</tr>
<tr>
<td>Period 3</td>
<td>...</td>
<td>25.7±5.1*</td>
<td>1,206±113</td>
<td>3.34±0.71</td>
<td>1.62±0.32</td>
</tr>
<tr>
<td>Period 4</td>
<td>...</td>
<td>24.7±5.0</td>
<td>1,149±130</td>
<td>3.49±0.72</td>
<td>1.77±0.21</td>
</tr>
</tbody>
</table>

Values are mean±SEM. MAP, mean arterial pressure; V, urine flow; GFR, glomerular filtration rate; UnV, sodium excretion; FeNa, fractional sodium excretion; control, 2-4-hour equilibrium period with no drugs; recovery, period after cessation of drug infusion. Periods 1-4 correspond to time periods for infused left kidney.

*p<0.05 vs. control by paired t test with Bonferroni correction for multiple comparison.

**tp<0.05 vs. SCH-23390 by paired t test with Bonferroni correction for multiple comparison.

***tp<0.05 vs. period 1 by paired t test with Bonferroni correction for multiple comparison.

****p<0.05 vs. period 2 by paired t test with Bonferroni correction for multiple comparison.
crease sodium excretion than nonselective or DA₂ antagonists.²⁹ In the current studies, the intrarenal administration of the DA₁ antagonist SCH-23390 decreased sodium excretion in WKY rats. These results are in agreement with Siragy et al.,¹³ who reported that SCH-23390 given into the renal artery of conscious dogs on 40 meq sodium/day decreased sodium excretion by occupation of renal DA₁ receptors.¹³ In WKY rats, the addition of the DA₂ antagonist LY-171555 induced a natriuresis when renal nerves were intact; however, he noted an antinatriuresis in the acutely denervated kidney. Haloperidol, a nonselective dopamine antagonist (DA₂>DA₁) also increased sodium excretion in the isolated perfused kidney.³¹ Under our experimental conditions, DA₂ exerted an opposite rather than a synergistic effect on sodium excretion, which was in agreement with previous reports.²²,³¹ These data are consistent with the lack of a synergistic effect of a DA₂ agonist on the ability of a DA₁ agonist to stimulate adenylate cyclase or phospholipase C activities, the enzymes involved in the generation of signal transducers of dopamine.³² These studies agree with other reports¹³,¹⁵,¹⁸ and indicate a role of endogenous dopamine in increasing renal sodium excretion by means of the DA₁ receptor.¹³ The ability of selective dopaminergic drugs to influence sodium and water excretion in WKY rats is in pronounced contrast to the absence of such effects in SHR. Weinstock et al.³³ have also reported a decreased natriuretic effect of the DA₁ agonist fenoldopam in SHR.³³ The lack of effect of a DA₂ antagonist in SHR is also in agreement with the report of Tsuda et al.³⁴ of a decreased ability of dopamine to inhibit the release of norepinephrine in mesenteric vessels (a DA₂-mediated event). The decreased effect of both the DA₁ agonist and the DA₂ antagonist on sodium excretion is probably not a nonspecific effect. The ability of the DA₁ agonist fenoldopam to increase renal blood flow is similar in SHR and WKY rats.³³ The magnitude of the renal vascular and tubular responses to α-adrenergic stimulation or blockade is also similar in SHR and WKY rats.³⁶ In contrast, calcium channel blockers increase renal blood flow to a greater extent in SHR than WKY rats.³⁷

Several investigators¹⁹,³⁸,³⁹ have reported that dopamine excretion is increased in the early stages of hypertension at a time when urinary excretion of
sodium in SHR is lower than in WKY rats. The decreased dopaminergic response in SHR might have been explained by down-regulation of D_A receptors induced by the high renal concentrations of dopamine in SHR, especially since renal tubular D_A receptors (but not glomerular D_A receptors) were decreased in 32-week-old SHR. However, in the younger rats used in this study, D_A receptor affinity and density in renal cortical homogenates were similar; thus, no down-regulation of renal D_A receptors by intrarenal dopamine in SHR was indicated. Autoradiography in kidney slices confirmed our previous observation that specific binding of [125I]SCH-23982 was found in renal cortex but not in medulla in both SHR and WKY rats. In the microdissected renal proximal convoluted tubule, Kinoshita et al. also found no difference in D_A receptor affinity or density between the SHR and WKY rats. However, the ability of D_A agonists to stimulate adenylyl cyclase activity in the proximal convoluted tubule of SHR was significantly attenuated compared with WKY rats. Apparently, the decreased ability of D_A agonists to stimulate adenylyl cyclase activity in SHR is due to a defective D_A receptor G protein coupling mechanism. Thus, defective transduction of the D_A receptor signal may well be the mechanism for the decreased renal dopaminergic effect in SHR.

Lee has previously suggested that abnormalities in the renal dopaminergic system may be an important pathogenic mechanism in essential hypertension. SHR have been reported to be in a hyperdopaminergic state. Van den Buuse et al. have suggested that the increased central dopaminergic activity in SHR is a cause of the hypertension. In our studies, however, we showed that the increased dopaminergic activity in the intermediate lobe of the pituitary gland is a consequence rather than a cause of the hypertension. In the periphery, the increased dopamine synthesis may be a compensatory mechanism for a defective dopamine effect. When peripheral synthesis of dopamine was inhibited with carbidopa in SHR, salt loading accelerated the development of hypertension. The higher blood pressures were correlated with lower urinary excretions of dopamine and sodium but with higher urinary excretions of norepinephrine. Bromocriptine, a dopamine agonist, attenuated the development of deoxycorticosterone acetate–salt hypertension. Thus, decreasing peripheral dopaminergic activity (including the kidney) accelerates hypertension, and the reverse attenuates the development of hypertension.

Because in normotensive rats dopamine, by means of the D_A receptor, plays a role in the natriuresis of moderate sodium loading, a defective D_A receptor in SHR should be associated with an attenuated natriuretic response to volume expansion. In some strains of SHR (12-week-old rats), sodium chloride can aggravate the hypertension. There is also preliminary evidence to suggest that even the “salt-resistant” SHR becomes sensitive to sodium chloride when potassium intake is deficient. In SHR with established hypertension (12-week-old rats), the ability to excrete an acute sodium load is not impaired and is actually increased probably because of mechanisms that can overcome the D_A defect (e.g., pressure natriuresis). However, even the renal perfusion–natriuresis phenomenon is blunted in the SHR; this occurrence suggests the presence of intrinsic changes in the kidney that enhance sodium reabsorption. Indeed, when sodium-sensitive SHR are placed on a high sodium diet before the acute sodium load, their ability to excrete sodium is impaired compared with WKY rats. Because the D_A receptor defect has been noted as early as 3 weeks of age, it is possible that the sodium retention that occurs early in SHR is related to this defect.

Acknowledgments

We thank Smith Kline & French Laboratories, Philadelphia, Pennsylvania, and Schering Corporation,
Hypertension *Vol 15, No 6, Part 1, June 1990*

Kenilworth, New Jersey, for the supply of SKF-38393 and SCH-23390, and we express our appreciation for the secretarial help of Mrs. Carolyn K. Patterson and the technical assistance of Mark Canada.

References


10. Frederickson ED, Bradley T, Goldberg LI: Blockade of renal effects of dopamine in the dog by the DA1 antagonist SCH 23390, and we express our appreciation for the secretarial help of Mrs. Carolyn K. Patterson and the technical assistance of Mark Canada.


43. van den Buijse M, Versteeg DHG, de Jong W: Role of dopamine in the development of spontaneous hypertension. *Hypertension* 1984;6:899-905


45. Yoshimura M, Kambura S, Okabayashi H, Takahashi H, Ijichi H: Effect of decreased dopamine synthesis on the develop-
Felder et al. Renal DA, Defect in Hypertension


KEY WORDS • dopamine • renal function • dopamine receptors
• essential hypertension • spontaneously hypertensive rats
Attenuated renal response to dopaminergic drugs in spontaneously hypertensive rats.
R A Felder, M G Seikaly, P Cody, G M Eisner and P A Jose

Hypertension. 1990;15:560-569
doi: 10.1161/01.HYP.15.6.560

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/15/6_Pt_1/560

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located, click
Request Permissions in the middle column of the Web page under Services. Further information about this
process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/