Role of Kidney Dopamine in the Natriuretic Response to Volume Expansion in Rats

Sharath S. Hegde, Arun L. Jadhav, and Mustafa F. Lokhandwala

It has been postulated that endogenously produced dopamine (DA) may play a role in the regulation of renal sodium excretion. In the present study, experiments were designed to test the hypothesis that acute volume expansion with isotonic sodium chloride stimulates the production of DA within the kidney, which in turn acts on specific DA receptors to promote sodium excretion. In pentobarbital-anesthetized rats, acute volume expansion over a period of 1 hour evoked a pronounced increase in urine output and urinary sodium excretion. These diuretic and natriuretic effects were not accompanied by any significant changes in blood pressure or heart rate. However, there was a significant elevation in central venous pressure and a transient rise in glomerular filtration rate. The natriuretic and diuretic response was accompanied by a significant increase in urinary DA excretion, and this effect was clearly dissociated from the rise in glomerular filtration rate. In a separate group of rats, the effects of acute volume expansion were studied in the presence of selective DA receptor antagonist SCH-23390 (50 μg/kg i.v. bolus; 10 μg/kg/min). During DA receptor blockade, there was a marked attenuation in the diuretic and natriuretic response throughout the period of volume expansion, when compared with that in the control group. The changes in central venous pressure and glomerular filtration rate were identical in the two groups. In another group of rats, the renal effects of exogenously administered DA were studied. DA (0.5 μg/kg/min) produced significant increases in urine output and urinary sodium excretion, without causing any alterations in blood pressure or glomerular filtration rate, suggesting a tubular site of action. SCH-23390, in a dose that had previously attenuated the natriuretic response to volume expansion, blocked the diuretic response and attenuated the natriuretic response to DA. The DA receptor antagonist produced no significant hemodynamic and renal effects by itself in normally hydrated animals. These results suggest that endogenously produced DA contributes, at least in part, to the natriuretic and diuretic response to acute volume expansion by activation of DA receptors located on renal tubules. (Hypertension 1989;13:828–834)

Dopamine (DA) has the ability to produce a variety of cardiovascular and renal effects by acting on specific DA receptors. The renal effects of DA include increases in glomerular filtration rate and renal blood flow as well as diuresis and natriuresis. DA receptors of both the DA₁ and DA₂ subtype have been identified in the nephron using radioligand binding techniques. In addition, DA has been shown to produce an inhibition of Na⁺,K⁺-ATPase in the proximal tubule, a potential mechanism to explain its natriuretic effect. Thus, in addition to an indirect effect resulting from hemodynamic changes, a direct action on the tubular sodium reabsorption process might also be the mechanism underlying the natriuretic effect of DA. However, the renal response to direct activation of these putative tubular DA receptors has not been clearly identified.

It is postulated that endogenously produced DA, arising either from renal dopaminergic nerves or from that produced extraneurally within the kidney, might play an important role in the regulation of sodium excretion by the kidney. This is based on the observation that an increase in dietary sodium intake is accompanied by an increase in sodium and dopamine excretion. However, a cause and effect relation between these two events has not been firmly established.

An acute increase in sodium intake produced by volume expansion with sodium chloride has been shown to evoke a marked diuretic and natriuretic
response.\textsuperscript{10} It has been established that atrial natriuretic factor (ANF) contributes to this phenomenon, but it is not the only substance involved in the natriuretic response to acute volume expansion.\textsuperscript{11,12} In view of the speculated role of DA in regulating sodium excretion, we considered that DA produced endogenously within the kidney might also be contributing to the natriuretic response during volume expansion. The objective of the present study was to test the hypothesis that volume expansion stimulates the production of endogenous DA, which in turn activates DA\textsubscript{1} receptors in the kidney to promote sodium excretion. Therefore, experiments were performed to characterize the renal response to DA\textsubscript{1} receptor activation with DA and to measure urinary DA and sodium excretion during acute volume expansion. The use of selective DA\textsubscript{1} receptor antagonist SCH-23390 allowed us to identify the contribution of endogenous DA to the natriuretic and diuretic response to acute volume expansion.

**Materials and Methods**

**Surgical Procedures**

Male Sprague-Dawley rats (Harlan, Houston, Texas) weighing between 300–350 g were used in the present study. They were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and anesthesia was maintained by administering supplemental doses intraperitoneally throughout the duration of the experiment. After a tracheotomy, the left carotid artery and the right jugular vein were catheterized for measurements of blood pressure and central venous pressure, respectively. In addition, the left jugular vein and the right femoral artery were catheterized for saline and drug administration and blood sampling, respectively. The left ureter was exposed via a midline abdominal incision and cannulated for the collection of urine. After the completion of surgery, an infusion of isotonic sodium chloride at a rate of 0.02 ml/min was begun, and this was maintained throughout the duration of the experiment. Hemodynamic and renal parameters were allowed to stabilize over a period of 30 minutes before the commencement of the experiment.

**Experimental protocol for volume expansion studies.** After the stabilization period, there were two control urine-collection periods, each lasting for 30 minutes and during which the rate of saline infusion was maintained at 0.02 ml/min. Arterial blood samples (0.2 ml) were withdrawn at the midpoint of each control collection period and replaced with an equal volume of saline. The rats were then subjected to volume expansion by infusing isotonic sodium chloride at a rate of 0.34 ml/min over a 60-minute period. During this period, four consecutive 15-minute urine collections were made. After 1 hour of volume expansion, the rate of saline infusion was reduced back to 0.02 ml/min and maintained for 60 minutes. During this recovery period, four consecutive 15-minute urine collections were made. Blood samples were taken at an interval of every 30 minutes after the onset of volume expansion and up to the conclusion of the experimental protocol.

In a second group of rats, the influence of DA\textsubscript{1} receptor blockade on the effects of volume expansion were studied. SCH-23390, a selective DA\textsubscript{1} receptor antagonist, was administered as a bolus of 50 µg/kg i.v. and infused at a dose of 10 µg/kg/min from the onset of the second control urine-collection period until the end of the experiment. The rest of the protocol was identical to that in the control group.

In a third group of six rats, the effects of SCH-23390 (50 µg/kg i.v.; 10 µg/kg/min) alone, in the absence of any volume expansion, was investigated. In this group, there were five consecutive 30-minute urine-collection periods. The first period served as control during which saline alone was infused. SCH-23390 was administered during the subsequent four periods.

**Experimental protocol for studies with dopamine.** The protocol consisted of four consecutive 30-minute urine-collection periods. The initial two periods served as control during which saline alone was infused at a rate of 0.02 ml/min. Because values for the two control periods were quite similar, the average of the two control periods is reported in the Results section. During the third period, DA was infused at a dose of 0.5 µg/kg/min. After the termination of the DA infusion, saline alone was infused during the last postdopamine 30-minute period. Arterial blood samples were collected at the midpoint of each collection period. In a separate group of rats, the effects of DA were studied in the presence of DA\textsubscript{1} receptor antagonist SCH-23390 (50 µg/kg bolus; 10 µg/kg/min). SCH-23390 was administered as a bolus at the onset of the second period and also infused during this and the subsequent periods. In another group of rats (n=6), saline time controls were performed by infusing saline alone during the five periods. The rest of the protocol was the same.

**Analytical procedures.** Sodium and potassium concentrations in the plasma and urine were measured using a Perkin-Elmer flame photometer (Perkin-Elmer, Oakbrook, Illinois). Plasma and urine creatinine concentrations were measured using a Beckman Creatinine Analyzer 2 (Beckman Instr., Inc., Fullerton, California). The urine samples used for DA measurement were added to a solution containing 0.1N HCl, 0.2% Na\textsubscript{2}S\textsubscript{2}O\textsubscript{5}, and 1 mM EDTA Na\textsubscript{3}, and frozen at −70°C until ready for analysis. DA was extracted using alumina columns, which were previously equilibrated to pH 8.6 with 1.5 M Tris buffer containing 0.05M EDTA. The samples, which were also equilibrated to pH 8.6, were loaded into the alumina columns. DA was eluted with 0.1 M perchloric acid (2.5 ml) and analyzed using high-performance liquid chromatography (Waters Chromatography Div., Millipore...
TABLE 1. Effect of Saline Infusion and SCH-23390 Infusion on Urine Output, Urinary Sodium Excretion, Mean Blood Pressure, and Glomerular Filtration Rate

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV</td>
<td>Saline</td>
<td>65.1±9.4</td>
<td>69.0±11.1</td>
<td>74.5±14.6</td>
<td>68.6±11.1</td>
<td>64.9±5.5</td>
</tr>
<tr>
<td>(μl/30 min)</td>
<td>SCH-23390</td>
<td>51.7±10.1</td>
<td>52.2±7.2</td>
<td>49.9±7.6</td>
<td>51.4±9.1</td>
<td>60.9±13.5</td>
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<tr>
<td>U^NaV</td>
<td>Saline</td>
<td>2.5±0.6</td>
<td>2.7±0.6</td>
<td>2.9±0.7</td>
<td>2.8±0.6</td>
<td>2.7±0.6</td>
</tr>
<tr>
<td>(μeq/30 min)</td>
<td>SCH-23390</td>
<td>3.6±0.7</td>
<td>3.5±0.6</td>
<td>3.5±0.5</td>
<td>3.5±0.6</td>
<td>4.1±0.7</td>
</tr>
<tr>
<td>MBP</td>
<td>Saline</td>
<td>116.0±4.2</td>
<td>115.3±3.2</td>
<td>116.4±6.3</td>
<td>118.5±4.8</td>
<td>116.7±7.1</td>
</tr>
<tr>
<td>(mm Hg)</td>
<td>SCH-23390</td>
<td>103.7±2.1</td>
<td>100.5±3.1</td>
<td>100.1±1.7</td>
<td>104.3±2.3</td>
<td>105.4±1.3</td>
</tr>
<tr>
<td>GFR</td>
<td>Saline</td>
<td>1.05±0.18</td>
<td>1.08±0.25</td>
<td>0.92±0.3</td>
<td>0.95±0.18</td>
<td>1.04±0.23</td>
</tr>
<tr>
<td>(ml/min)</td>
<td>SCH-23390</td>
<td>0.78±0.09</td>
<td>0.79±0.13</td>
<td>0.86±0.11</td>
<td>0.86±0.11</td>
<td>0.88±0.12</td>
</tr>
</tbody>
</table>

Each collection period consisted of 30 minutes. SCH-23390 was administered as a bolus at the onset of period 2 and also infused during this and the subsequent periods. There were no significant differences in any of these parameters within the same group over time. n=6 per group. UV, urine output; U^NaV, urinary sodium excretion; MBP, mean blood pressure; GFR, glomerular filtration rate; saline infusion, 0.02 ml/min; SCH-23390, 50 μg/kg, 10 μg/kg/min.

Drugs Used
DA hydrochloride was obtained from Sigma Chemical Co., St. Louis, Missouri. SCH-23390 was a gift from Dr. Allen Barnett, Schering-Plough Corporation, Bloomfield, New Jersey.

Statistical Analysis
All data are presented as mean±SEM. Student’s paired and unpaired t tests were used for comparison within and between groups, respectively. A p<0.05 was considered to be statistically significant.

Results
Control experiments showed that when saline alone was infused (0.02 ml/min), all the hemodynamic and renal parameters were stable throughout the duration of the experiment (Table 1). This showed that the preparation was suitable for carrying out the designed experimental protocol.

Volume Expansion Studies
In a group of rats, acute volume expansion resulted in a significant increase in urine output (UV) and urinary sodium excretion (U^NaV), the peak effects being observed toward the end of the 1-hour period (Figure 1). After the termination of the volume expansion, both UV and U^NaV tended to decrease, although they were still significantly greater than basal values even at 60 minutes after stopping volume expansion (Figure 1). These diuretic and natriuretic responses were not accompanied by any significant changes in mean blood pressure or heart rate (Figure 2); however, there was a transient increase in glomerular filtration rate (GFR) (67%) and a significant increase in central venous pressure (2 mm Hg), which had returned to control values at the end of the volume expansion period (Figure 3). Acute volume expansion led to a significant increase in urinary DA excretion, which closely paralleled the increase in U^NaV (Figure 4). Urinary DA excretion decreased in the period after volume expansion, although the values were still significantly higher than the basal DA excretion (Figure 4).

FIGURE 1. Graphs of diuretic and natriuretic response to acute volume expansion in two separate groups of rats with and without dopamine-1 (DA1) receptor blockade with SCH-23390 (50 μg/kg, 10 μg/kg/min). *Statistically significant difference (p<0.05) from prevolume expansion values in same group. **Statistically significant difference (p<0.05) from corresponding values between SCH-23390-treated and control groups (n=6 in each group).
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Dopamine in Volume Expansion Natriuresis

In the group treated with SCH-23390, volume expansion still evoked a significant increase in UV and U$_{NaV}$ (Figure 1). However, the magnitude of the increases in UV and U$_{NaV}$ were significantly lower during the volume expansion period, when compared with that in the control group. The magnitude of the changes in GFR and central venous pressure were similar to that observed in the control group (Figure 3).

In a separate group of rats SCH-23390 by itself, in the dose employed in these experiments, produced no significant changes in mean blood pressure, GFR, UV, and U$_{NaV}$ (Table 1).

Studies With Dopamine

DA (0.5 µg/kg/min) produced a significant increase in UV (34%) and U$_{NaV}$ (57%) (Table 2). This diuretic and natriuretic response was not accompanied by any changes in blood pressure or GFR. In the presence of SCH-23390, DA did not produce significant diuresis, whereas the natriuretic response was attenuated by approximately 50% (57% vs. 29%) (Table 2).

Discussion

The results of our study show that DA is released from the kidney during acute volume expansion, and it contributes to the natriuresis and diuresis via activation of DA$_1$ receptors.

Acute volume expansion produced a significant increase in urinary DA excretion concomitant with the natriuretic and diuretic response. It may be argued that the rise in GFR may have been the cause of the increased DA excretion. However, this

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flupentixol, a nonselective DA receptor antagonist, loading in rats. It is likely that the attenuation was able to attenuate the natriuresis following Ringer In a previous study, it was reported that cis-DA excretion and a part of the accompanying between the volume expansion-induced increase in natriuresis that is mediated through DA, receptors. The data suggest that a causal relation does exist also be ruled out since SCH-23390 did not produce a physiological antagonism of the natriuretic volume expansion (which is a good index of the extent of pressure (which is a good index of the extent of degree of volume expansion in the SCH-23390-attenuation could not have been due to a lesser produced extraneural DA in the kidney might rep- resent an important source of endogenous DA. Although the results of our study do not allow us to distinguish between these two sources, it is clear that the increase in U NaV during volume expansion was accompanied by the increased urinary DA excretion and that this DA was of intrarenal origin. Pharmacological evidence showing the involvement of DA in this natriuretic response was obtained by using the selective DA1 receptor antagonist SCH-23390. Blockade of DA1 receptors with SCH-23390 resulted in an attenuation of the diuretic and natriuretic response during volume expansion. The attenuation could not have been due to a lesser degree of volume expansion in the SCH-23390-treated group since the increase in central venous pressure (which is a good index of the extent of volume expansion) was identical in the two groups. A physiological antagonism of the natriuretic response to volume expansion by SCH-23390 can also be ruled out since SCH-23390 did not produce any changes in UV and U NaV by itself. Therefore, the data suggest that a causal relation does exist between the volume expansion–induced increase in DA excretion and a part of the accompanying natriuresis that is mediated through DA1 receptors. In a previous study, it was reported that cis-flupentixol, a nonselective DA receptor antagonist, was able to attenuate the natriuresis following Ringer loading in rats. It is likely that the attenuation produced by cis-flupentixol was due, at least in part, to its DA1 receptor blocking activity. The present study further extends these findings by showing specifically the involvement of DA1 receptors and more importantly that of endogenous DA in the natriuretic response. Interestingly, studies in the dog have shown that selective or nonselective DA receptor antagonists, or both, have no effect on the natriuresis occurring during saline loading. It is important to note that the studies that have identified DA1 receptors in the renal tubules have been carried out in the rat and rabbit. There is yet no firm evidence for the presence of specific tubular DA1 receptors in the dog. Thus, the observed discrepancies between the studies in the rat and dog might merely be due to species differences. The mechanism by which DA produces its diuretic and natriuretic response is still debatable. Both indirect hemodynamic changes and a direct effect on tubular sodium reabsorption have been suggested as possible mechanisms. In the present study, an attenuation of the natriuresis during volume expansion in the SCH-23390-treated group was observed despite identical increases in GFR in the two groups. These results suggest that the natriuresis caused by endogenous DA during volume expansion involved a mechanism other than an increase in GFR; perhaps it resulted from the direct activation of tubular DA1 receptors. This, however, does not rule out the possibility that endogenously produced DA could have altered renal blood flow by activation of vascular DA receptors. However, inasmuch as most of the urinary DA is produced in the tubule and released into the lumen, it is rather unlikely that plasma DA levels could have been elevated high enough for vascular DA receptor activation to occur. Further evidence for a tubular action of DA was obtained from our experiments showing that exogenous DA, at low doses, produced its diuretic and natriuretic response without

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Treatment</th>
<th>Control</th>
<th>Dopamine (0.5 µg/kg/min)</th>
<th>Postdopamine</th>
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<tbody>
<tr>
<td>UV (µl/30 min)</td>
<td>None</td>
<td>44.6±4.7</td>
<td>59.9±6.7* (34%)</td>
<td>63.1±5.9* (41%)</td>
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<tr>
<td></td>
<td>SCH-23390</td>
<td>31.9±2.5</td>
<td>38.9±3.9</td>
<td>40.9±5.5</td>
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<tr>
<td>U NaV (µeq/30 min)</td>
<td>None</td>
<td>2.2±0.3</td>
<td>3.4±0.5* (57%)</td>
<td>4.0±0.5* (84%)</td>
</tr>
<tr>
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<td>SCH-23390</td>
<td>2.2±0.3</td>
<td>2.8±0.3* (29%)</td>
<td>3.4±0.4* (57%)</td>
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<tr>
<td>MBP (mm Hg)</td>
<td>None</td>
<td>116.1±10.9</td>
<td>109.5±10.1</td>
<td>106.1±11.4</td>
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<tr>
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<td>109.0±5.3</td>
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<td>GFR (ml/min)</td>
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<td>SCH-23390</td>
<td>0.62±0.09</td>
<td>0.66±0.11</td>
<td>0.62±0.11</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent percent change from control. The control value given is the mean of the two control periods. n=7 per group.

UV, urine volume; U NaV, urinary sodium excretion; MBP, mean blood pressure; GFR, glomerular filtration rate; dopamine infusion, 0.5 µg/kg/min; SCH-23390, 50 µg/kg, 10 µg/kg/min.

*Statistically significant difference from control value in same group.

†Statistically significant difference from control value in same group.
any changes in blood pressure or GFR. This result is also in agreement with similar conclusions reached from a previous study in the isolated perfused rat kidney. The apparent lack of recovery of the natriuretic and diuretic response in the 30 minutes after termination of the DA infusion might be due to the slow rate of dissociation of DA from its receptor. In the present study, DA-mediated natriuresis was blunted but not entirely blocked by SCH-23390. This indicates that DA-mediated natriuresis might involve DA₂ receptors, whose existence in the kidney has been demonstrated in receptor-ligand binding studies. However, the observations that SCH-23390 attenuated the natriuretic response to volume expansion that was associated with increased urinary DA excretion and that it also attenuated the response to exogenous DA both suggest that DA₂ receptor activation is one of the important factors contributing to the increase in sodium excretion caused by either endogenously released or exogenously administered DA.

It is now known that acute volume expansion is also a powerful stimulus for the release of ANF from the atria. It has also been established that ANF released during volume expansion contributes to the natriuresis since administration of ANF antibodies or atrial appendectomy greatly attenuates the natriuretic response during volume expansion. However, a recent study showed that infusion of synthetic ANF at a dose that produces a similar concentration of plasma ANF as that produced by acute volume expansion can induce only 40% of the natriuresis and diuresis of volume expansion. This implies that factors other than ANF, one of which might be endogenous DA, might also be mediating the natriuresis seen during volume expansion. Interestingly, several investigators have reported an antagonism of the natriuretic effect of ANF by DA receptor antagonists in the rat. This raises the possibility that either ANF could be stimulating the production of endogenous DA or it activates DA receptors directly. Unlike the observations made in the rat, DA receptor antagonist failed to attenuate the natriuretic response to ANF in the dog, a finding suggestive of possible species differences between the renal dopaminergic system as well as its interaction with ANF between the rat and the dog. It is tempting to speculate that both ANF and DA might be acting interdependently and in a positive feedback manner in an attempt to maximize the natriuretic response during volume expansion.

In conclusion, the findings of our study strongly suggest that endogenously produced DA contributes to the natriuresis during volume expansion and it does so by activation of DA₁ receptors that are perhaps situated in the renal tubules. Future studies would be aimed at elucidating the possible involvement of DA₂ receptors and also the interdependence between DA and ANF in the natriuretic response to volume expansion.

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References

5. Felder RA, Jose PA: Dopamine receptors in rat kidneys identified with 125I-SCH 23982. Am J Physiol 1988;255:F970–F976


KEY WORDS • natriuresis • dopamine-receptor antagonists • dopamine
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