Dopamine Fails to Inhibit Renal Tubular Sodium Pump in Hypertensive Rats

Changjian Chen, Robert E. Beach, and Mustafa F. Lokhandwala

We have previously reported that dopamine-1 receptor-mediated activation of phospholipase C is diminished in renal cortical slices of adult spontaneously hypertensive rats. To determine the potential consequences of this phenomenon, we performed the present studies in which renal proximal tubule suspensions obtained from spontaneously hypertensive and Wistar-Kyoto rats of 10–12 weeks of age were used. The tubule suspensions were incubated with dopamine in the presence or absence of dopamine receptor antagonists, and sodium, potassium adenosine trisphosphatase (sodium pump) activity was measured as the ouabain-sensitive adenosine trisphosphate hydrolysis. We found that dopamine produced a concentration-related inhibition of sodium pump activity in the normotensive rats but not in the hypertensive rats. Dopamine-induced inhibition of sodium pump activity in the normotensive rats was abolished by the phospholipase C inhibitor U-73122 or the protein kinase C inhibitor sphingosine, suggesting the involvement of a phospholipase C-coupled protein kinase C pathway in this response. Dopamine-induced inhibition in the normotensive rats was attenuated by the dopamine-1 receptor antagonist SCH 23390 but not by the dopamine-2 receptor antagonist domperidone. To identify possible sites of defect in dopamine-1 receptor-coupled signaling pathways in the hypertensive rats, we incubated the proximal tubules with phorbol 12,13-dibutyrate or the synthetic diacylglycerol analogue l-oleoyl-2-acetyl-rac-glycerol. The results showed that both compounds inhibited sodium pump activity as effectively in the hypertensive as in the normotensive rats, suggesting that the protein kinase C-coupled sodium pump pathway was not defective in the hypertensive animals. Failure of dopamine to inhibit sodium pump activity in the hypertensive rats could not be due to a defective dopamine-1 receptor adenylate cyclase coupling, because dopamine was still unable to inhibit sodium pump activity in the presence of dibutyryl cyclic adenosine monophosphate or forskolin. These results show that dopamine failed to inhibit sodium pump activity in the proximal tubules of adult hypertensive rats, which may be mainly due to a defect in the dopamine-1 receptor phospholipase C coupling process. (Hypertension 1993;21:364–372)

KEY WORDS • receptors, dopamine • dopamine • sodium • phospholipase C • adenosine trisphosphatase, sodium, potassium

Abnormal renal sodium handling has been known to be one of the major factors involved in the initiation and maintenance of high blood pressure in several models of hypertension, including genetic hypertension. Endogenous kidney dopamine plays an important role in regulation of renal sodium excretion, so it has been proposed that impaired renal sodium handling in spontaneously hypertensive rats (SHR) may partly be due to a malfunction or a defect in the renal dopaminergic system. It has been reported that endogenous kidney dopamine synthesis/secretion is either normal or even supernormal in the SHR, and that no differences in renal tubular dopamine-1 receptor number, distribution, or affinity are present between SHR and Wistar-Kyoto (WKY) rats. Increasing evidence suggests that diminished involvement of endogenous kidney dopamine in the regulation of renal sodium excretion may be at least in part attributed to defective dopaminergic cellular signaling mechanisms. It is reported that dopamine-1 receptor adenylate cyclase coupling is defective in renal proximal tubules and dopamine-1 receptor-mediated activation of phospholipase C (PLC) is diminished in renal cortical slices of SHR. Such a malfunction of the renal dopaminergic system, which involves a defect in dopamine-1 receptor-mediated signal transduction pathways in the renal proximal tubule of the SHR, might contribute to the diminished natriuretic response to dopamine and dopamine receptor agonists previously demonstrated in this model of hypertension.

It has recently been reported that a defect in dopamine-1 receptor adenylate cyclase coupling may be related to diminished dopamine-induced inhibition of the activity of the Na⁺-K⁺ exchanger in the brush border membrane preparation of the SHR. The functional consequences of reduced dopamine-1 receptor-mediated activation of PLC remain to be determined. Inas-
much as protein kinase C (PKC) is clearly demonstrated to be involved in dopamine-induced inhibition of Na⁺,K⁺-ATPase and activation of PKC is known to be mediated by diacylglycerol, a product of PLC activation, diminished dopamine-induced activation of PLC could lead to an altered response of tubular Na⁺,K⁺-ATPase to dopamine in the SHR. In a preliminary study, we found that dopamine-induced inhibition of Na⁺,K⁺-ATPase was abolished in the basolateral membrane of the renal proximal tubule of adult SHR. The present study was performed in renal proximal tubule suspensions to determine the significance of diminished dopamine-1 receptor-mediated activation of PLC as it relates to the inhibition of Na⁺,K⁺-ATPase activity and to identify potential alterations in the dopaminergic cellular signaling pathways responsible for this phenomenon.

Methods

Male SHR and WKY rats of 10–12 weeks of age (Harlan Sprague Dawley, Inc., Indianapolis, Ind.) were used in all experiments. All rats were fed ad libitum with ordinary rat chow containing 0.4% sodium (Purina Mills, St. Louis, Mo.) and were supplied with tap water. Rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and the right carotid artery was cannulated to measure arterial blood pressure and heart rate. The mean arterial blood pressure in the SHR (156±10 mm Hg, n=12) was significantly higher than in WKY rats (123±8 mm Hg, n=12). For preparation of proximal tubular suspensions, kidneys from two rats were used for each experiment.

Isolation of Renal Cortical Tubules

Renal cortical tubular suspensions were obtained according to the method described by Gesek et al13 with slight modification. After a midline incision, the aorta was cannulated with PE-160 tubing below the kidney, and both mesenteric and celiac arteries were tied tightly according to the method described by Gesek et al13 with slight modification. After a midline incision, the aorta was cannulated with PE-160 tubing below the kidney, and both mesenteric and celiac arteries were tied tightly according to the method described by Gesek et al13 with slight modification. After a midline incision, the aorta was cannulated with PE-160 tubing below the kidney, and both mesenteric and celiac arteries were tied tightly according to the method described by Gesek et al13 with slight modification. After a midline incision, the aorta was cannulated with PE-160 tubing below the kidney, and both mesenteric and celiac arteries were tied tightly according to the method described by Gesek et al13 with slight modification. After a midline incision, the aorta was cannulated with PE-160 tubing below the kidney, and both mesenteric and celiac arteries were tied tightly according to the method described by Gesek et al.13

The inhibition became significant at 5 minutes of incubation with various concentrations of dopamine (10⁻⁴ to 10⁻⁸ M) were added. The incubation was initiated by replacement of KHB with incubation flow rate of 6 mL/min was maintained with a peristaltic pump (Cole-Parmer Instrument Co., Chicago). After both kidneys turned completely pale in color (usually taking about 5–10 minutes), the in situ digestion period was initiated by replacement of KHB with the enzyme perfusion solution. The enzyme perfusion solution consisted of collagenase (type IV, 230 units/mL) and hyaluronidase (type III, 250 units/mL) in 30 mL KHB. After enzyme perfusion (usually taking about 5 minutes), the kidneys were excised, the capsule removed, and immediately placed in ice-cold KHB. The kidneys were cut into halves sagittally in a Pyrex culture dish placed on ice, and the outer cortex was removed above the corticomedullary junction with a scalpel. The cortex was then chopped into pieces of 0.5×2 mm, which were washed into a 25-mL Eppendorf tube to which 20 mL KHB-enzyme solution (type IV collagenase, 460 units/mL; type III hyaluronidase, 500 units/mL; 0.8–1.2 mg protein/mL) was added. The flask was placed in a water bath (Blue M Electric Co.), aerated constantly with 95% O₂–5% CO₂, and maintained at 37°C for 25 minutes. Afterward, the tubes were pelleted by centrifugation in an IEC clinical centrifuge (Needham HTS) at 50g for 2 minutes. The enzyme-KHB solution was decanted from the pellet. The pellet was resuspended in ice-cold KHB containing no calcium, 0.12 mM magnesium, and 10% dialyzed calf serum; was filtered through a nylon net (mesh opening, 105 µm; Spectrum Medical Industries Inc., Los Angeles); and was washed by centrifugation and resuspension three times to remove residual enzyme.

Enrichment of Renal Proximal Tubules by Percoll Density-Gradient Centrifugation

Renal proximal tubules were enriched according to the method of Vinay et al14 with slight modification. Isosmotic Percoll (90%, vol/vol) was prepared by combining nine parts stock Percoll with one part buffer concentrate (100 x Krebs-Henseleit bicarbonate containing no calcium and 2.4 mM sodium and 10% dialyzed calf serum) was layered on 35 mL of 50% (vol/vol) Percoll and 50 mL polycarbonate centrifuge tubes and was centrifuged at 4°C for 30 minutes at 13,000 rpm (20,000g) in a refrigerated centrifuge (Sorval RC-5B, Du Pont Instruments, Irving, Tex.). The bottom layer (F₁) was collected and washed three times with modified KHB. The preparation was verified morphologically and biochemically as enriched proximal tubules (unpublished observations from our laboratory). The tubule viability was determined by the trypan blue exclusion test, which showed that more than 95% of tubules excluded trypan blue. The final proximal tubule suspension was adjusted to 0.8–1.2 mg protein/mL.

Experimental Protocol

First we examined the possible alterations in dopamine-induced inhibition of Na⁺,K⁺-ATPase activity in renal proximal tubules of SHR. Samples were distributed among tubes (1 mL for each tube) to which various concentrations of dopamine (10⁻⁴ to 10⁻⁸ M) were added. Ascorbic acid (0.1%, wt/vol) was present to prevent rapid oxidation of dopamine. For identification of the subtype of dopamine receptors involved in dopamine-induced inhibition of Na⁺,K⁺-ATPase, the samples were incubated with 10⁻⁵ M dopamine in the presence or absence of various concentrations of the selective dopamine-1 receptor antagonist SCH 23390, the selective dopamine-2 antagonist domperidone, or vehicles, respectively. In these experiments, the samples were incubated with antagonists at room temperature for 10 minutes before addition of dopamine. The tubes were incubated in the water bath at 37°C for 20 minutes when transferred on ice. We incubated samples for 20 minutes because in our preliminary study we found that dopamine (10⁻⁴ M) inhibited Na⁺,K⁺-ATPase in a time-dependent manner. The inhibition became significant at 5 minutes of incubation, maximal at 20 minutes, and tended to be less thereafter (unpublished observations). To determine the possible contribution of α-adrenergic receptor activation to the altered response of α-adrenergic receptor activation to the altered response of Na⁺,K⁺-ATPase, the samples were incubated with various concentrations of the selective α1-adrenergic receptor antagonist prazosin. The inhibition of Na⁺,K⁺-ATPase by prazosin became significant at 5 minutes of incubation, maximal at 20 minutes, and tended to be less thereafter (unpublished observations). To determine the possible contribution of α-adrenergic receptor activation to the altered response of α-adrenergic receptor activation to the altered response of Na⁺,K⁺-ATPase, the samples were incubated with various concentrations of the selective α1-adrenergic receptor antagonist prazosin. The inhibition of Na⁺,K⁺-ATPase by prazosin became significant at 5 minutes of incubation, maximal at 20 minutes, and tended to be less thereafter (unpublished observations).
Na⁺,K⁺-ATPase to dopamine, we incubated the suspensions with dopamine (10⁻⁵ to 10⁻⁸ M) in the presence of phenolamine (10⁻⁵ M) or vehicle. The samples were incubated with phenolamine at room temperature 10 minutes before addition of dopamine.

It has been reported that dopamine-induced inhibition of renal tubular Na⁺,K⁺-ATPase activity involves activation of PKC. However, the direct evidence for dopamine-induced inhibition of Na⁺,K⁺-ATPase, we incubated the proximal tubule suspension with dopamine in the presence of either the PLC inhibitor U-73122 (10⁻⁶ M) or the inert analogue U-73343 (10⁻⁶ M). In addition, the involvement of PKC activation in this response was examined by incubating samples with dopamine in the presence of the PKC inhibitor sphingosine (10⁻⁵ M).

To assess whether altered response of Na⁺,K⁺-ATPase in the SHR to dopamine is due to a deficient PKC activation or an alteration in the Na⁺,K⁺-ATPase itself, we incubated the tubule suspensions with the PKC activator phorbol 12,13-dibutyrate (PDBu) or the synthetic diacylglycerol analogue 1-oleoyl-2-acyl-rac-glycerol (OAG) for 20 minutes at 37°C in the presence or absence of sphingosine or vehicle. PDBu, OAG, and sphingosine were dissolved in 10% dimethyl sulfoxide. The final concentration of dimethyl sulfoxide in the incubation media was less than 0.2%.

A defective dopamine-1 receptor adenylate cyclase coupling was recently reported to contribute to the loss of dopamine-induced inhibition of Na⁺,K⁺-ATPase activity in the renal proximal tubule of Dahl salt-sensitive rats. Because a defect in dopamine-1 receptor adenylate cyclase coupling was also shown in the SHR, which possibly could also contribute to the altered response of Na⁺,K⁺-ATPase to dopamine in this rat strain, we examined this possibility by incubating samples with dopamine (10⁻⁵ M) in the presence of either dibutyryl cyclic AMP (cAMP, 10⁻⁶ M), forskolin (10⁻⁶ M), or vehicles. In addition, the proximal tubular suspensions from both SHR and WKY rats were incubated with dopamine (10⁻⁵ M) in the presence of nystatin (5×10⁻⁵ M) or vehicle.

**Measurement of Na⁺,K⁺-ATPase Activity**

Na⁺,K⁺-ATPase activity was determined by the method of Quigley and Gotterer with slight modification on 100 μL of tubular suspensions, after permeabilization by rapid freezing in dry ice/acetone and thawing, with absorbance determined at 740 nm (Spectronic 20D, Milton Roy Co., Rochester, N.Y.). The reaction mixture medium contained (mM) 37.5 imidazole, 75 NaCl, 5.0 KCl, 1.0 NaEGTA, 5.0 MgCl₂, 6.0 sodium azide, and 75 Tris-HCl. The reaction was initiated by the addition of 4 mM Tris-ATP. For determination of ouabain-insensitive ATPase, NaCl and KCl were omitted and Tris-HCl (150 mM) and ouabain (1 mM) were added. It was demonstrated in our preliminary study that when NaCl and KCl were omitted from the medium, only 30% of Na⁺,K⁺-ATPase activity was present compared with the control, and 1 mM ouabain produced maximal inhibition. However, when 75 mM NaCl and 5 mM KCl were present, a much higher concentration of ouabain (4 mM) was needed to produce the maximal inhibition. In addition, omission of NaCl and KCl had no detectable effect on ouabain-resistant ATPase activity (unpublished observations). After incubation at 37°C for 15 minutes, the reaction was terminated by rapid shifting on ice and addition of 50 μL cold 50% trichloroacetic acid into each tube. Total ATPase activity was estimated from the amount of inorganic phosphate (Pₗ) in the supernatant after centrifugation at 3,000 rpm for 10 minutes (model TJ-60 centrifuge, Beckman Instruments, Inc., Palo Alto, Calif.). Na⁺,K⁺-ATPase activity was measured as the difference between total ATPase activities and ouabain-insensitive ATPase activities and was expressed as nanomoles Pₗ per milligram of protein per minute. Protein content was determined by the method of Lowry et al 18 with bovine serum albumin as standard.

**Drugs and Chemicals**

SCH 23390 was a gift from Schering-Plough Corp., Bloomfield, N.J.; domperidone was from Janssen Pharmaceutica, New Brunswick, N.J.; and U-73122 and U-73343 were from the Upjohn Co., Kalamazoo, Mich. All other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.

**Statistical Analysis**

All data are presented as mean±SEM. The repeated measurements followed by protected least significant difference multiple range test were used for comparison between groups, and two-way analysis of variance was used for comparison between groups. A value of p<0.05 was considered statistically significant.

**Results**

There were no significant differences in basal Na⁺,K⁺-ATPase activity between SHR (319±19 nmol P/mg protein per minute) and WKY rats (327±17 nmol P/mg protein per minute). Dopamine produced concentration-dependent inhibition of Na⁺,K⁺-ATPase activity in WKY rats but not in SHR (Figure 1). The minimum concentration of dopamine required to cause a significant inhibition in WKY rats was 10⁻⁷ M. The inhibition was maximal at 10⁻⁵ M and tended to be less at 10⁻⁴ M. In addition, dopamine did not have any significant effect on ouabain-resistant ATPase activity in both the SHR and WKY rats (data not shown).

The potential significance of α-adrenergic receptor activation on dopamine-induced inhibition of Na⁺,K⁺-ATPase was investigated by incubating samples with dopamine in the presence of the α-adrenergic receptor antagonist phentolamine. As shown in Figure 2, dopamine at the lower concentration range (10⁻⁵ to 10⁻² M) produced a similar degree of inhibition in the presence of phentolamine as in the presence of vehicle in WKY rats. With the same concentration range of dopamine, no significant inhibition of Na⁺,K⁺-ATPase activity was observed in the SHR in the presence of phentolamine. However, at the highest concentration used, dopamine (10⁻⁴ M) produced significantly greater inhibition of Na⁺,K⁺-ATPase in the presence of phentolamine than in the absence of phentolamine in WKY rats (compare Figure 1 and Figure 2). Interestingly, with such a high concentration of dopamine (10⁻⁴ M), statistically significant, although marginal, inhibition of Na⁺,K⁺-ATPase...
was observed in the SHR when phentolamine was present in the media (Figure 2).

Dopamine-induced inhibition of Na⁺,K⁺-ATPase activity in WKY rats was attenuated by the selective dopamine-1 receptor antagonist SCH 23390 but not by the dopamine-2 receptor antagonist domperidone, indicating the involvement of dopamine-1 but not dopamine-2 receptors in dopamine-induced inhibition of Na⁺,K⁺-ATPase (Table 1).

PKC activation is shown to be involved in the dopaminergic cellular signaling pathway leading to an inhibition of Na⁺,K⁺-ATPase in the proximal tubule.11 In the present study, we found that PLC activation is also involved in dopamine-induced inhibition of Na⁺,K⁺-ATPase. As shown in Figure 3, although dopamine-induced inhibition in WKY rats was attenuated by the PLC inhibitor U-73122 in a concentration-related manner, U-73343, an inert analogue, had no observable effect. Furthermore, dopamine-induced inhibition in WKY rats was also abolished by the PKC inhibitor sphingosine (Figure 3), consistent with the findings of Bertorello and Aperia.11 No significant effect on ouabain-resistant ATPase activity was observed with U-73122, U-73343, or sphingosine in either SHR or WKY rats (data not shown).

The absence of dopaminergic inhibition of Na⁺,K⁺-ATPase activity in the SHR could be due to diminished dopamine-induced activation of PLC via the dopamine-1 receptor, which subsequently fails to activate PKC efficiently, or due to a certain alteration in the Na⁺,K⁺-ATPase itself that somehow leads to its unresponsiveness to the PKC-mediated inhibitory effect. As

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Table 1. Effects of SCH 23390 and Domperidone on Dopamine-Induced Inhibition of Na⁺, K⁺-ATPase Activities in the Renal Proximal Tubule of Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>0</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCH 23390</td>
<td>337±15</td>
<td>275±15*</td>
<td>325±18</td>
<td>309±11</td>
<td>292±13*</td>
</tr>
<tr>
<td>Domperidone</td>
<td>342±23</td>
<td>282±16*</td>
<td>285±12*</td>
<td>280±14*</td>
<td>275±17*</td>
</tr>
</tbody>
</table>

Values are mean±SEM of Na⁺,K⁺-ATPase activity in nanomoles P, per milligram of protein per minute. Dopamine (10⁻⁵ M) was present in all samples except in control. n=6 animals. Two rats were used for preparation of renal proximal tubule suspensions for each experiment; incubations were performed in triplicate.

*Significantly different compared with corresponding control within the group (p<0.05; analysis of variance).
†Significant difference between the two groups (p<0.05; analysis of variance).
shown in Figure 4, both the phorbol ester PDBu and the synthetic diacylglycerol analogue OAG, direct activators of PKC, produced a similar degree of inhibition of Na⁺,K⁺-ATPase activities in SHR and WKY rats. The inert analogue of PDBu, 4-α-12,13-didecanoate (PDD), had no significant effect on Na⁺,K⁺-ATPase activities (data not shown). In addition, PDBu-induced inhibition of Na⁺,K⁺-ATPase activities in both SHR and WKY rats was attenuated by the PKC inhibitor sphingosine, and neither PDBu nor OAG had a significant effect on ouabain-resistant ATPase activity (data not shown).

Inasmuch as activation of adenylate cyclase is linked to an inhibition of the activity of the Na⁺-H⁺ exchanger, a defective dopamine-1 receptor adenylate cyclase coupling in the SHR could result in the failure of dopamine to inhibit this antiporter and eventual increase in intracellular Na⁺ levels, which consequently would lead to a diminished dopamine-induced inhibition of Na⁺,K⁺-ATPase. To examine this possibility, we included the cAMP analogue dibutyryl cAMP or the adenylate cyclase activator forskolin in the incubation.

As shown in Table 2, dibutyryl cAMP or forskolin alone had no significant effect on Na⁺,K⁺-ATPase activity in both SHR and WKY rats. Neither compound had any significant effect on dopamine-induced inhibition in WKY rats, and more importantly, dopamine was still unable to inhibit Na⁺,K⁺-ATPase activity in the SHR in the presence of these compounds. In a separate set of experiments, it was found that the presence of nystatin, a cation ionophore, did not affect dopamine-induced inhibition of Na⁺,K⁺-ATPase activity in WKY rats, and, as expected, dopamine did not inhibit Na⁺,K⁺-ATPase in the SHR in the presence of nystatin (Figure 5). In addition, the presence of nystatin in the media did not significantly affect either Na⁺,K⁺-ATPase activities or ouabain-resistant ATPase activities in both SHR and WKY groups (Figure 5).

Discussion

Although a substantial body of evidence indicates the involvement of endogenous kidney dopamine in main-
taining body sodium and volume homeostasis, the dopaminergic signal transduction pathways mediating dopamine-induced renal responses are just beginning to be clarified. It is proposed that endogenous kidney dopamine produces diuresis and natriuresis mainly, if not solely, through activation of the tubular dopamine-1 receptor, which is coupled to both PLC and adenylyl cyclase. Activation of adenylyl cyclase after occupation of the dopamine-1 receptor is linked to an inhibition of Na⁺-H⁺ exchange in brush border membrane possibly induced by cAMP-dependent protein kinase-mediated phosphorylation. The functional significance of activation of PLC in terms of overall dopamine-induced renal response at the level of the basolateral membrane, however, remains to be determined. Although dopamine is known to inhibit Na⁺,K⁺-ATPase activity in the renal proximal tubule, the subtype of dopamine receptors involved in this phenomenon is not clear. The results of the present study indicate that only dopamine-1 and not dopamine-2 receptors are involved in dopamine-induced inhibition of Na⁺,K⁺-ATPase in normotensive WKY rats. This is shown by the observation that dopamine-induced inhibition was antagonized by the selective dopamine-1 receptor antagonist SCH 23390 but not by the dopamine-2 receptor antagonist domperidone. This finding does not support the observations of Bertorello and Aperia, who reported that activation of both dopamine-1 and dopamine-2 receptors was involved in dopamine-induced inhibition of Na⁺,K⁺-ATPase. The reason for these discrepant results is not known. It is unlikely due to species difference, because we recently obtained similar results with Sprague-Dawley rats. Based on the observation that activation of PKC is involved in dopamine-induced inhibition of Na⁺,K⁺-ATPase, it is speculative that activation of PKC might be an intermediary event linking PLC activation to an inhibition of Na⁺,K⁺-ATPase in the proximal tubule, because diacylglycerol, a well-established endogenous PKC activator, is produced on PLC activation, and the dopamine-1 receptor is positively coupled to PLC. The results of the present study provide the first direct evidence for such a postulation. This is based on the observations that dopamine-induced inhibition of Na⁺,K⁺-ATPase activity was effectively antagonized by the PLC inhibitor U-73122, whereas the inert analogue U-73343 had no observable effect.

Increasing evidence suggests a relation between a malfunction of the renal dopaminergic system and impaired renal sodium excretion in the SHR. It has been

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### TABLE 2. Effects of Dibutyryl Cyclic AMP or Forskolin on Dopamine-Induced Changes in Na⁺, K⁺-ATPase Activities in the Renal Proximal Tubule of Spontaneously Hypertensive and Wistar-Kyoto Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Blank (vehicle)</th>
<th>DBcAMP (10⁻⁶ M)</th>
<th>Forskolin (10⁻⁶ M)</th>
<th>DA (10⁻⁵ M)</th>
<th>DA+DBcAMP (10⁻⁴+10⁻⁶ M)</th>
<th>DA+forskolin (10⁻⁵+10⁻⁶ M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY</td>
<td></td>
<td>335±18</td>
<td>313±20</td>
<td>344±22</td>
<td>261±17*</td>
<td>255±17*†</td>
<td>262±19†</td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td>343±21</td>
<td>325±19</td>
<td>366±15</td>
<td>332±18</td>
<td>345±19†</td>
<td>321±30</td>
</tr>
</tbody>
</table>

DBcAMP, dibutyryl cyclic AMP; DA, dopamine; WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats. n=6 rats in each group. Two rats in each group were used for preparation of renal proximal tubule suspensions for each experiment; incubations were performed in triplicate. Values are mean±SEM.

*Significantly different compared with corresponding control (p<0.05; analysis of variance).
†Significant difference between WKY and SHR groups (p<0.05; analysis of variance).

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### Figure 5

Bar graph shows effects of nystatin (5x10⁻⁵ M) on dopamine (DA) (10⁻⁵ M)-induced changes in Na⁺, K⁺-ATPase activities in the renal proximal tubules of spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats. Control values of ouabain-resistant ATPase activities (nanomoles P₄ per milligram protein per minute) were 283±24 in the WKY and 291±23 in the SHR group. n=6 animals per group; two animals were used for preparation of renal proximal tubule suspensions for each experiment; incubation was performed in triplicate. Data are mean±SEM. *Significantly different compared with corresponding control within the group (p<0.05; analysis of variance); †Significant difference between the two groups (p<0.05; analysis of variance).
shown that, although the kidney dopamine content and urinary dopamine excretion are markedly elevated in young SHR, renal sodium excretion is paradoxically less in SHR than in WKY rats. The renal responses to both endogenous and exogenously administered dopamine as well as dopamine receptor agonists are diminished in the adult SHR. It is also demonstrated that dopamine receptor adenylate cyclase coupling is less efficient in the renal proximal tubule of adult SHR compared with WKY rats.

Dopamine-1 receptor-mediated activation of adenylate cyclase at the level of the brush border membrane of the proximal tubule is linked to an inhibition of the activity of the Na\(^+\)-H\(^+\) exchanger, so a defective dopamine-1 receptor adenylate cyclase coupling in the SHR may be one of the factors responsible for diminished renal response to dopamine and dopamine receptor agonists due to loss of inhibition of the activity of the Na\(^+\)-H\(^+\) exchanger. We have recently shown that dopamine-induced PLC activation in renal cortical slices was reduced in the adult SHR, almost entirely because of an impaired tubular dopamine-1 receptor function. The functional significance of diminished dopamine-1 receptor-mediated activation of PLC in terms of its possible relation to impaired renal sodium excretion in this model of hypertension remains to be determined. Recently, we observed that the basal PLC activity in renal cortex was significantly elevated in normotensive rats placed on a high sodium diet, which was associated with increased urinary dopamine excretion.

In addition, pretreatment with SCH 23390 significantly reduced the elevated basal PLC activity in these animals. These observations indicate that PLC activation mediated by the tubular dopamine-1 receptor is involved in the natriuretic response to an increase in sodium intake. Therefore, it is likely that diminished dopamine-1 receptor–mediated activation of PLC in the SHR could contribute to reduced natriuretic response to acute volume expansion and exogenously administered dopamine-1 receptor agonists observed in these hypertensive animals. Because one of the mechanisms by which dopamine produces natriuresis is by PKC-mediated inhibition of Na\(^+\),K\(^+\)-ATPase subsequent to dopamine-1 receptor–mediated activation of PLC on the basolateral membrane, diminished dopamine-1 receptor–coupled PLC activation may directly lead to reduced inhibition of renal tubular Na\(^+\),K\(^+\)-ATPase activity. In the present study, we found that dopamine-induced inhibition of Na\(^+\),K\(^+\)-ATPase activity was abolished in the renal proximal tubule of the SHR. This observation is consistent with our earlier study with a basolateral membrane preparation in which we showed that dopamine failed to inhibit Na\(^+\),K\(^+\)-ATPase in the SHR. Although the abolished dopamine-induced inhibition of Na\(^+\),K\(^+\)-ATPase activity could be caused by the failure of dopamine to inhibit the activity of the Na\(^+\)-H\(^+\) exchanger because of defective dopamine-1 receptor adenylate cyclase coupling, it appears that the abolished dopamine-induced inhibition in the SHR involves a defective dopaminergic cellular signaling mechanism coupled with the PLC pathway. This is suggested by the observation that dopamine was still unable to inhibit Na\(^+\),K\(^+\)-ATPase activity in the SHR even in the presence of the cAMP analogue dibutyryl cAMP or forskolin, which directly activates adenylate cyclase. In addition, presence of nystatin, which eliminated cation gradients across the cell membrane, did not significantly affect dopamine-induced inhibition of Na\(^+\),K\(^+\)-ATPase activity in WKY rats, suggesting that a direct signaling mechanism, rather than intracellular sodium ion changes, was responsible for this inhibition seen in the normotensive rats. As expected, in the SHR, dopamine did not inhibit Na\(^+\),K\(^+\)-ATPase activity in the presence of nystatin as observed in the absence of nystatin.

Some reports indicate that norepinephrine and epinephrine increase Na\(^+\),K\(^+\)-ATPase activity in the proximal tubule possibly through \(\alpha\)-adrenergic receptor activation. Because dopamine activates \(\alpha\)-adrenergic receptors at a concentration higher than that required to activate dopamine-1 receptors, it is reasonable to speculate that dopamine-induced inhibition of Na\(^+\),K\(^+\)-ATPase may be opposed by simultaneous \(\alpha\)-adrenergic receptor activation, especially when dopamine concentration is high. The results of the present study support this proposal. It was demonstrated that under a relatively low concentration range, dopamine mainly inhibited tubular Na\(^+\),K\(^+\)-ATPase activity by dopamine-1 receptor activation in WKY rats and that the opposing effect from \(\alpha\)-adrenergic receptor activation was minimal, because dopamine under these concentrations in the presence of \(\alpha\)-adrenergic receptor blockade produced a similar degree of inhibition as dopamine alone. However, with the highest concentration of dopamine (10\(^{-4}\) M) used in this study, the opposing effect from \(\alpha\)-adrenergic receptor activation was observed. This is shown by the finding that dopamine at this concentration produced a significantly lower degree of inhibition compared with that at 10\(^{-5}\) M, and this inhibition was reversed by phentolamine. The possible explanation for this phenomenon is that dopamine at a relatively low concentration may preferentially activate the dopamine-1 receptor, which leads to an inhibition of Na\(^+\),K\(^+\)-ATPase activity.

Because \(\alpha\)-adrenergic receptor activation stimulates Na\(^+\),K\(^+\)-ATPase activity and some reports suggest that \(\alpha\)-adrenergic receptor is upregulated in the kidney of SHR, it is possible that \(\alpha\)-adrenergic receptor activation may contribute to the abolished dopamine-induced inhibition of Na\(^+\),K\(^+\)-ATPase activity in the SHR. Indeed, it has been reported that exogenous norepinephrine produced a more pronounced stimulation of Na\(^+\),K\(^+\)-ATPase activity in basolateral membranes from 5-week-old SHR compared with WKY controls. The results of the present study, however, suggest that the role of \(\alpha\)-adrenergic receptor activation in opposing dopamine receptor–mediated inhibition of Na\(^+\),K\(^+\)-ATPase activity under the present experimental conditions seems minimal in the renal proximal tubule of adult SHR. First, in the presence of \(\alpha\)-adrenergic receptor blockade, dopamine, in the wide range of concentrations that produced an inhibition of Na\(^+\),K\(^+\)-ATPase in normotensive rats, failed to inhibit Na\(^+\),K\(^+\)-ATPase in hypertensive rats. Second, although dopamine at 10\(^{-4}\) M induced statistically significant inhibition in the presence of phentolamine, the inhibition was marginal, and such a high concentration of dopamine may be well beyond the physiological range.

Inasmuch as PKC activation appears to be an intermediate step linking dopamine-1 receptor activation to
an inhibition of Na\(^+\),K\(^+\)-ATPase activity in the renal proximal tubule,\(^{20}\) it is interesting to know whether failure of dopamine to inhibit this enzyme is solely due to a defective dopamine-1 receptor PLC coupling process or involves additional mechanisms (e.g., an alteration of other parts of the signal transduction pathway or an alteration of Na\(^+\),K\(^+\)-ATPase itself). In the present study, we found that both the phorbol ester PDBu and the diacylglycerol analogue OAG produced a similar degree of inhibition in both SHR and WKY rats. Therefore, it is reasonable to speculate that the possible site of a defective dopaminergic signaling pathway is proximal to PKC activation.

Although cAMP-dependent protein kinase activation is shown to be involved in dopamine-induced inhibition of Na\(^+\),K\(^+\)-ATPase in the renal cortical collecting duct\(^{21}\) and medullary thick ascending limb of Henle,\(^{22}\) its role in the proximal tubule in this phenomenon is still not clear. One report suggests that cAMP-dependent protein kinase may play a permissive role in dopamine-induced inhibition of Na\(^+\),K\(^+\)-ATPase in the proximal tubule.\(^{23}\) According to this proposal, the defective dopamine-1 receptor adenylate cyclase coupling present in the proximal tubule of SHR could also lead to the failure of dopamine to inhibit Na\(^+\),K\(^+\)-ATPase activity in this segment of nephron. However, the result of the present study indicates that the defective dopamine-1 receptor adenylate cyclase coupling in this rat strain contributes little, if any, to this phenomenon, because in the presence of dibutyryl cAMP or forskolin, dopamine still failed to inhibit Na\(^+\),K\(^+\)-ATPase activity.

The results of the present study do not allow us to ascertain whether the deficiency of dopamine-1 receptor-coupled signaling pathways in the proximal tubule of SHR is part of a desensitization process as a result of the chronic elevated renal dopamine level\(^{23}\) or is a fundamental abnormality in this hypertensive model. However, a recent preliminary report suggests that such a defect may exist in the prehypertensive stage of the SHR.\(^{24}\) This preliminary study showed that dopamine failed to inhibit Na\(^+\),K\(^+\)-ATPase activity in the renal proximal tubule of the 4-5-week-old SHR, whose arterial blood pressure was not different from that of normotensive age-matched WKY rats.\(^{24}\) Therefore, it is likely that the inefficiency of this signaling pathway leading to failure of dopamine to inhibit Na\(^+\),K\(^+\)-ATPase activity in the SHR is not a consequence of elevated blood pressure and may have pathogenic significance, because an inhibition of Na\(^+\),K\(^+\)-ATPase activity is one of the major mechanisms by which dopamine promotes renal sodium excretion,\(^{20,21}\) and impaired renal sodium excretion is one of the major factors involved in both the pathogenesis and maintenance of high blood pressure in the genetic model of hypertension.\(^{1,2}\)

In summary, our results show that dopamine failed to inhibit Na\(^+\),K\(^+\)-ATPase activity in the proximal tubule of the SHR. The failure of dopamine to inhibit this enzyme involves a defect in the dopamine-1 receptor-coupled PLC cellular signaling mechanism, which is located most probably proximal to the activation of PKC. The impaired dopamine-1 receptor-coupled PLC signaling pathway may contribute, at least in part, to the diminished involvement of endogenous kidney dopamine in the maintenance of body volume and sodium homeostasis in the SHR.


Dopamine fails to inhibit renal tubular sodium pump in hypertensive rats.
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