Dopamine$_1$ Receptor, $G_{s\alpha}$, and Na$^+$.H$^+$ Exchanger Interactions in the Kidney in Hypertension

Jing Xu, Xiao Xi Li, Frederick E. Albrecht, Ulrich Hopfer, Robert M. Carey, Pedro A. Jose

Abstract—The ability of dopamine$_1$ (D$_1$) receptors to inhibit luminal Na$^+$.H$^+$ exchanger (NHE) activity in renal proximal tubules and induce a natriuresis is impaired in spontaneously hypertensive rats (SHR). However, it is not clear whether the defect is at the level of the D$_1$ receptor, $G_{s\alpha}$, or effector proteins. The coupling of the D$_1$ receptor to $G_{s\alpha}$ and NHE3 was studied in renal brush border membranes (BBM), devoid of cytoplasmic second messengers. D$_1$ receptor, $G_{s\alpha}$, and NHE3 expressions were similar in SHR and their normotensive controls, Wistar-Kyoto rats (WKY). Guanosine-5’-O-(3-thiotriphosphate) (GTPyS) decreased NHE activity and increased NHE3 linked with $G_{s\alpha}$ similarly in WKY and SHR, indicating normal $G_{s\alpha}$ and NHE3 regulation in SHR. However, D$_1$ agonists increased NHE3 linked with $G_{s\alpha}$ in WKY but not in SHR, and the inhibitory effects of D$_1$ agonists on NHE activity were less in SHR than in WKY. Moreover, GTPyS enhanced the inhibitory effect of D$_1$ agonist on NHE activity in WKY but not in SHR, suggesting an uncoupling of the D$_1$ receptor from $G_{s\alpha}$/NHE3 in SHR. Similar results were obtained with the use of immortalized renal proximal tubule cells from WKY and SHR. We conclude that the defective D$_1$ receptor function in renal proximal tubules in SHR is proximal to $G_{s\alpha}$/effectors and presumably at the receptor level. The mechanism(s) responsible for the uncoupling of the D$_1$ receptor from G proteins remains to be determined. Because the primary structure of the D$_1$ receptor is not different between normotensive and hypertensive rats, differences in D$_1$ receptor posttranslational modification are possible. (Hypertension. 2000;36:395-399.)

Key Words: dopamine □ receptors, dopamine □ G protein □ rats, inbred SHR

Renal dopamine production, dopamine receptors, and dopamine receptor regulation are important in the pathogenesis of hypertension.1 Dopamine, produced by renal proximal tubules, is an important paracrine/autocrine inhibitor of renal sodium transport under conditions of sodium loading.1 The inhibition of sodium transport in renal proximal tubules by dopamine is exerted via the Na$^+$.H$^+$ exchanger (NHE) at the luminal or brush border membrane (BBM) and via Na$^+$.K$^+$.ATPase at the basolateral membrane.1 The major transport of sodium across the luminal membrane of renal proximal tubules is caused by NHE activity.2 Five of the 6 isoforms of NHE are expressed in the kidney.3,4 However, the NHE3 isofrom predominates in the BBM of rat renal proximal tubules.5,5 There is an impaired ability of dopamine, (D$_1$)-like receptors to inhibit NHE activity in BBM of the spontaneously hypertensive rat (SHR).6 The decreased inhibitory effect of D$_1$-like receptors on NHE activity in renal proximal tubules and failure to induce a natriuresis cosegregate with hypertension in SHR and normotensive Wistar-Kyoto rat (WKY) crossbreeds.6 However, it is not clear whether the impaired inhibitory action of D$_1$-like receptors on NHE activity is at the receptors, G proteins, signal transducers, or effectors. G proteins can regulate NHE activity in renal BBM,7 and there are reports of decreased expression and activity of $G_{s\alpha}$,4 increased activity and expression of $G_{i\alpha}$,8 and increased activity of NHE in renal proximal tubules in SHR.10,11 We therefore designed experiments to determine the regulation of NHE activity in renal BBM by D$_1$-like receptors, via $G_{s\alpha}$, independent of cytoplasmic second messengers in WKY and SHR. However, a decreased generation of cAMP by D$_1$-like receptors12,13 contributes to the decreased ability of D$_1$-like agonist to inhibit NHE activity in renal proximal tubules in SHR.10,11 Moreover, post-cAMP sodium transport defect may develop with the establishment of hypertension.12 Therefore, in additional studies, we determined the coupling of D$_1$-like receptors, $G_{s\alpha}$, and NHE3 in immortalized renal proximal tubule cells (PTC) from WKY and SHR.14

Methods

Preparation of BBM
Male WKY and SHR (Taconic Farms, Germantown, NY) were anesthetized with pentobarbital (50 mg/kg body wt IP). The mean arterial pressures were greater in SHR (>160 mm Hg) than in WKY (<100 mm Hg). The kidneys were harvested and the cortex was

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separated from the medulla before the animals were killed with an injection of pentobarbital (100 mg/kg body wt IV). Renal BBM were prepared by MnCl₂ precipitation and differential centrifugation as previously described.6,7,12

Cell Culture
Immortalized renal PTC from microdissected S1 segments of proximal tubules from WKY and SHR were cultured in DMEM/F-12 with pyridoxide HCl, l-glutamine, and HEPES (15 mmol/L) buffer plus penicillin (100 U/mL), streptomycin (100 μg/mL), transferrin (5 μg/mL), insulin (5 μg/mL), epidermal growth factor (10 ng/mL), dexamethasone (4 μg/mL), fetal bovine serum 5%, and NaHCO₃ (7 mmol/L) on a 100-mm Petri dish.14

Measurement of NHE Activity
NHE activity was determined by the 100 μmol/L 5-(N-methyl-N-isobutyl)-amiloride-sensitive uptake of ²²Na at room temperature by rapid filtration technique with the use of 0.65-μm nitrocellulose filters.6,7,12 The BBM vesicles (BBMV) were preincubated with the indicated drugs for 30 minutes. Since amiloride-sensitive ²²Na uptake at 3 seconds is due mainly to NHE activity, comparisons were made at this time.6,7,12,15 Uptake of ²²Na⁺ at 1 to 2 hours was assumed to represent equilibrium values and also served as an index of vesicle size.6,7,12,15 Incubation of the BBM with drug or antibodies, which required access into the interior of the vesicle, was added during vesicle formation.6,7,12,15

Immunoprecipitation Studies
BBM and immortalized PTC were incubated with vehicle or a D₁-like agonist (fenoldopam, 5×10⁻⁶ mol/L) for 10 minutes and guanosine-5’-O-(2-thiodiphosphate) (GDPβS) (3×10⁻⁴ mol/L), guanosine-5’-O-(3-thiotriphosphate) (GTPγS) (3×10⁻³ mol/L), or vehicle for 30 minutes. The membranes were lysed with ice-cold lysis buffer (PBS with 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L sodium vanadate, 1 mmol/L PMSF, 10 μg/mL aprotinin, and 10 μg/mL leupetin) for 1 hour and centrifuged at 14 000 rpm for 30 minutes. The lysates (supernatant) were then incubated with affinity purified leupeptin) for 1 hour and centrifuged at 14 000 rpm for 30 minutes. The membranes were lysed with ice-cold lysis buffer (PBS with 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mmol/L EDTA, 1 mmol/L EGTA, 1 mmol/L sodium vanadate, 1 mmol/L PMSF, 10 μg/mL aprotinin, and 10 μg/mL leupetin) for 1 hour and centrifuged at 14 000 rpm for 30 minutes. The lysates (supernatant) were then incubated with affinity purified anti-NHE3 antibody or anti-Gₛα antisera (NEN) at 4°C for 1 hour. The membranes were then centrifuged at 14 000 rpm for 30 minutes. The supernatant was added to the preimmune sera or preadsorbed antiserum (NEN) at 4°C for 1 hour. The membranes were then centrifuged at 14 000 rpm for 30 minutes. The supernatant was added to the preimmune sera or preadsorbed antiserum (NEN) at 4°C for 1 hour. The membranes were then centrifuged at 14 000 rpm for 30 minutes. The supernatant was added to the preimmune sera or preadsorbed antiserum (NEN) at 4°C for 1 hour.

Immunoblotting Studies
The proteins were separated by electrophoresis (7.5% or 15% SDS–polyacrylamide gel) and then electrophoretically transferred onto nitrocellulose membranes. The trans-blots were probed with the secondary antibody and an enhanced chemiluminescence system (Amersham Life). The densities of the appropriate bands were determined with the use of Quantscan (Biosoft).16

Materials
Rabbit polyclonal anti-NHE3 and anti-D₁ receptor antibodies were produced against a synthetic oligopeptide from the amino acid sequence of rat NHE3 (amino acids 633 to 646)17 or rat D₁ receptor (amino acids 299 to 307) (Research Genetics). The antisera were affinity purified with immunizing peptide or protein A sepharose (Pharmacia). The antibodies are specific to their respective proteins determined by Western blotting with preimmune sera or preadsorbed antibody and immunoprecipitation.17,18

Other materials included the following: GTPγS and GDPβS (Calbiochem); 5-(N-methyl-N-isobutyl)-amiloride, 5-(N-ethyl-N-isopropyl)-amiloride, dopamine, and SKF81297 (Research Biochemicals); fenoldopam (Smith Kline Beecham); and G protein subunit antibodies (NEN Life Science Products). All other reagents were from Sigma Chemical Co.

Figure 1. Expression of NHE3, D₁ receptor, Gₛα, and Gₛβ in BBM and immortalized PTC. BBM (top) and immortalized PTC (bottom) from WKY and SHR were immunoblotted with antibodies against NHE3, D₁ receptor, Gₛα, and Gₛβ. The expressions of NHE3, D₁ receptor, Gₛα, and Gₛβ were similar in WKY and SHR (f test). Representative immunoblots are depicted on top of the bar graphs.

Statistical Analysis
Data are expressed as mean±SE. Differences within groups were analyzed by ANOVA for repeated measures, followed by Scheffe’s test; the paired t test was used when only 2 groups were compared. Differences among groups were analyzed by 1-way ANOVA, followed by Duncan’s or Scheffe’s test.

Results
D₁ Receptors, Gₛα, Gₛβ, and NHE3 Are Expressed in Renal BBM
The expressions of D₁ receptors, Gₛα, Gₛβ, and NHE3 in BBM and immortalized PTCs were similar in WKY and SHR (Figure 1). Although there are 2 D₁-like receptors (D₁ and D₅), D₁ receptor function seems to predominate over the D₅ receptor1,6; thus, only the D₁ receptor was studied in these experiments.

Dopamine and D₁-Like Agonists Inhibit NHE Activity in WKY to a Greater Extent Than in SHR
In BBMV, a system devoid of cytoplasmic components and second messenger, basal NHE activity was slightly but not significantly greater in SHR (3.0±0.7 mmol Na per milligram protein per minute; n=11) than in WKY (2.1±0.3; n=12) (P>0.05, t test). Dopamine and the D₁-like agonists fenoldopam and SKF81297 inhibited NHE activity to a greater extent in WKY than in SHR (Table ). In a separate group of studies, the effect of varying concentrations of SKF81297 (5×10⁻⁸ to 5×10⁻⁵ mol/L; 4 concentrations; n=5 to 7/concentration) on NHE activity was determined. The maximum effect (calcu-
Percent Inhibition From Control of NHE Activity in BBMV by Dopamine and D₁ Agonists

<table>
<thead>
<tr>
<th>Drug</th>
<th>WKY</th>
<th>SHR</th>
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<tbody>
<tr>
<td>Dopamine (5×10⁻⁷ mol/L)</td>
<td>-21.2±2.6 (n=7)†</td>
<td>-11.9±1.1 (n=5)</td>
</tr>
<tr>
<td>Fenoldopam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1×10⁻⁸ mol/L)</td>
<td>-26.1±4.1 (n=5)†</td>
<td>-8.9±6.5 (n=5)</td>
</tr>
<tr>
<td>(5×10⁻⁶ mol/L)</td>
<td>-45.4±3.4 (n=5)†</td>
<td>-32.5±4.0 (n=5)</td>
</tr>
<tr>
<td>SKF81297 (5×10⁻⁶ mol/L)</td>
<td>-58.7±4.6 (n=12)†</td>
<td>-25.1±3.2 (n=11)</td>
</tr>
</tbody>
</table>

*P<0.05 vs control.
†P<0.05, WKY vs SHR, t test.

GTPγS Inhibits NHE Activity in Both WKY and SHR

The decreased inhibitory effect of fenoldopam on NHE activity in SHR was not due to impaired Gₛ receptor function, because the nonhydrolyzable GTP analogue GTPγS inhibited the NHE activity in BBMV to a similar extent in adult WKY and SHR (Figure 2). However, GTPγS increased the inhibitory effect of fenoldopam (5×10⁻⁶ mol/L) on NHE activity in WKY (33.2±14.1%; n=5; P<0.05 versus fenoldopam alone) but not in SHR (6.9±5.67%; n=12; P>0.05 versus fenoldopam alone), indicating impaired coupling between D₁-like receptors and Gₛ in SHR. The effect of GTP on NHE activity was specific because ATPγS had no effect on NHE activity in either WKY or SHR (data not shown).

Fenoldopam Increases the Amount of NHE3 Coimmunoprecipitated With Gₛ in WKY but not in SHR

Because the inhibitory effect of G proteins on NHE activity in BBM is exerted mainly by Gₛ, we studied the interaction between D₁-like receptors and Gₛ. Under basal conditions, more Gₛ was bound to NHE3 in immortalized PTC in SHR than in WKY. Fenoldopam (5×10⁻⁶ mol/L) increased NHE3 coimmunoprecipitated with Gₛ in BBM and immortalized PTC in WKY but not in SHR (Figure 3). However, GTPγS, but not GDPβS, increased NHE3 coimmunoprecipitated with Gₛ in both WKY and SHR (Figure 4), indicating normal coupling between Gₛ and NHE3.

Discussion

These studies confirm previous reports that dopamine and D₁-like agonists inhibit NHE activity in renal brush border to a lesser extent in hypertensive than in normotensive rats. Two D₁-like receptors, D₁ and D₅, are expressed in BBM; however, D₁ receptor function seems to predominate over D₅ receptor function. We now report that the decreased D₁-like action in BBM in hypertension is not due to decreased expression of D₁ receptors in renal BBM. Previous reports have also found that the expression of D₁-like receptors (determined by radioligand binding) is not different in renal proximal tubules in WKY and SHR. However, the D₁ receptor in renal proximal tubules is expressed to a greater extent in cytosol than in surface membranes. A comparison of D₁ expression in BBM of WKY and SHR has not been reported; NHE3 is expressed at BBM and is responsible for the majority of sodium transport into the proximal tubule. NHE activity in renal proximal tubules has been reported not to be regulated in hypertensive rats. In genetically hypertensive rats, the inhibitory effects on renal proximal tubular NHE activity and natriuretic action of dopamine and D₁-agonists are impaired. NHE3 expression and activity have also been reported to be variably increased in renal proximal tubules of SHR; the NHE isoform expressed in BBMV to a similar extent in adult WKY and SHR.

Figure 2. Effect of GTPγS on NHE activity in BBMV. Vehicle or GTPγS was introduced inside BBMV from WKY and SHR and allowed to incubate for 30 minutes. *P<0.05, ANOVA for repeated measures, Scheffé’s test, vs control (0) in both WKY and SHR.

Figure 3. Effect of the D₁-like agonist fenoldopam on NHE3 and Gₛ in BBMV and immortalized PTC. After BBMV (top) or immortalized PTC (bottom) were incubated with vehicle (control) or fenoldopam (5 μmol/L) for 10 minutes, the samples were immunoprecipitated with anti-Gₛ antibodies and immunoblotted with anti-NHE3 antibodies. *P<0.05 vs control WKY, #P<0.05, SHR vs WKY, ANOVA, Scheffé’s test. Representative immunoblots are depicted on top of the bar graphs.
BBM of renal proximal tubules is NHE3. In the present study, NHE expression tended to be higher in SHR than in WKY in BBMV but not in immortalized PTC. However, NHE activity was not different between normotensive and hypertensive rats.

In the present study dopamine and 2 different D1-like agonists (fenoldopam and SKF82917) had an attenuated inhibitory effect on NHE activity. The mechanism for the decreased ability of dopamine and D1 agonists to inhibit NHE activity in renal proximal tubules in genetic hypertension has been related to decreased cAMP production following dopaminergic stimulation. However, in the present study, which used BBMV, D1 agonists inhibited NHE activity via Gs, independent of cytoplasmic second messengers. GTPγS inhibited NHE activity in BBMV to a similar extent in WKY and SHR. The ability of GTPγS and cAMP to inhibit NHE activity to a similar degree in WKY and SHR suggests that the impaired D1-like action on NHE activity is caused by a defective coupling of D1-like receptors with Gs, G proteins, which are composed of heterotrimer subunits Ga, Gβ, and Gγ, are expressed in renal proximal tubules. G protein subunit abundance in kidneys of WKY and SHR has not been shown to be consistently different between these 2 rat strains. In the present study we find no differences in the abundance of Ga, Gr, or Gs in BBM or immortalized PTC from WKY and SHR. NHE activity in BBMV can be inhibited by GTPγS and can be stimulated by Ga and Gβγ dimers. Ga does not influence D1 action on NHE activity in BBMV in WKY. However, in renal proximal tubules of SHR, pertussis toxin normalized the inhibitory effect of dopamine on sodium pump activity. Increased Ga activity is probably not involved in the diminished inhibitory effect of D1-like agonists because pertussis toxin did not affect the ability of fenoldopam to inhibit NHE activity (data not shown). Previous studies have shown that Ga can directly mediate the inhibitory effect of D1 agonists and GTPγS on NHE activity. In the present report the inhibition of NHE activity by GTPγS in BBMV in WKY and SHR is associated with an increase in binding of Ga to NHE3 to a similar degree in both rat strains. In contrast, the D1-like agonist fenoldopam increased the quantity of Ga bound to NHE3 in BBM of WKY but not in SHR. These studies support the notion that the failure of D1 receptors to inhibit NHE3 activity in BBM is caused by an impaired coupling of the D1 receptor to Ga and not caused by abnormalities in NHE3 or Ga per se. Although the other D1-like receptor, the D3 receptor, is also expressed in renal proximal tubules, its influence on renal tubular function is minimal relative to the D1 receptor. The difference in inhibition constant but not in maximum effect of SKF81297 and previous studies that showed similar maximum receptor density but a lower D1-like agonist affinity in renal proximal tubules in SHR than in WKY support the concept of an inefficient coupling between D1-like receptors and Ga in SHR. The mechanism of the inefficient coupling of the D1 receptor with G proteins in the kidney is not known. Increased renal and urinary dopamine levels in SHR could be taken to suggest homologous desensitization as a mechanism. In the present study we show that immortalized PTC carry a phenotype (D1-like receptor function) similar to that of the native kidney. In immortalized PTC from SHR, fenoldopam also failed to increase NHE3 binding to Ga but as in the native kidney, GTPγS increased the quantity of Ga bound to NHE3 to a similar extent in WKY and SHR. Homologous desensitization cannot be invoked in these cells because exogenous L-dopa is necessary for renal PTC to synthesize dopamine. It is not due to an abnormality in the primary structure of the D1 receptor. However, abnormal posttranslational modification is possible. Thus, we have reported that the serine-phosphorylation of the D1 receptor in renal proximal tubules is increased in SHR and in essential hypertension. G protein–coupled receptor kinases GRK2, GRK3, and GRK5 desensitize the D1 receptor, in part, by serine-phosphorylation. GRK activity and GRK2 expression are increased in lymphocytes of patients with essential hypertension and in rats with genetic hypertension. The increase in GRK2 expression occurs after the establishment of hypertension. However, hypertension seems to increase the activity and expression of GRK5 in rat aortic smooth muscles. The ubiquitous expression of these G protein–coupled receptor kinases also does not explain the organ and nephron segment specificity of the uncoupling of the D1 receptor in genetic hypertension. We have suggested that the uncoupling of the D1 receptor in genetic hypertension may be due to a kinase but not necessarily due to GRK2. In conclusion, we have shown that in renal PTC and specifically in BBM, D1 receptor, Ga, Ga, and NHE3 expressions are similar in WKY and SHR. GTPγS inhibits NHE activity and increases binding of Ga and NHE3 to a similar extent in WKY and SHR. However, D1-like agonist inhibition of NHE3 activity and increase of the binding of Ga
and NHE3 are impaired in SHR compared with WKY. These studies suggest that a defective D1 receptor/Gs coupling may be responsible, in part, for the defective inhibitory action of dopamine and D1-like agonists on NHE activity in renal proximal tubular BBM.

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References

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