Diminished Phospholipase C Activation by Dopamine in Spontaneously Hypertensive Rats

Chang-Jian Chen, Subhash J. Vyas, Joseph Eichberg, and Mustafa F. Lokhandwala

It is reported that a defect in dopamine-1 (DA-1) receptor adenylate cyclase coupling in the proximal convoluted tubule in the spontaneously hypertensive rat may contribute to the diminished natriuretic response to DA-1 receptor agonists. Since the tubular DA-1 receptor is also coupled to phospholipase C, and both of these cellular signaling processes are involved in DA-1 receptor-mediated diuresis and natriuresis, it is important to know whether a similar defect is also present in DA-1 receptor-coupled phospholipase C pathway. The present study was therefore designed to determine the functional status of DA-1 receptor–phospholipase C coupling system of adult spontaneously hypertensive rats using a renal cortical slice preparation. In addition, the renal response to exogenously administered dopamine (1 μg/kg/min i.v.) was also determined. We found that basal phospholipase C activity was significantly higher in hypertensive rats than in age-matched Wistar-Kyoto rats (7.6±0.2% versus 5.6±0.27%, p<0.05). However, compared with the normotensive controls, dopamine-induced increases in phospholipase C activity were significantly attenuated in the preparations of hypertensive rats in a concentration-dependent manner (13±6% versus 38±6% for 1 mM dopamine, p<0.05; 49±6% versus 71±9% for 3 mM dopamine, p<0.05; 50±16% versus 106±22%, p<0.05 for 10 mM dopamine). The diminished dopamine-induced phospholipase C activation was due to a deficiency in dopamine receptor–phospholipase coupling since the DA-1 receptor antagonist SCH 23390 (30 μM), which blocked 50% of dopamine-induced inositol phosphate production in the Wistar-Kyoto rats, did not exert such an effect in the spontaneously hypertensive rats. The in vivo functional study showed that the diuretic and natriuretic responses to intravenous administration of dopamine were significantly diminished in spontaneously hypertensive rats compared with Wistar-Kyoto rats (urine output, 34±1 versus 63±10 μl/min, p<0.05; urinary sodium excretion, 1.8±0.1 versus 3.2±0.2 meq/30 min, p<0.05). These results suggest that there is a defect in the tubular DA-1 receptor–phospholipase C coupling process in spontaneously hypertensive rats that may contribute to a diminished natriuretic response to DA-1 receptor activation. (Hypertension 1992;19:102–108)

It is generally accepted that an abnormality in renal handling of sodium is one of the factors in the pathogenesis and/or maintenance of high blood pressure in genetic hypertension. Central to this assumption are the observations that spontaneously hypertensive rats (SHR), as opposed to their normotensive Wistar-Kyoto (WKY) counterparts, retain sodium avidly before the development of hypertension and that urinary sodium excretion (U\textsubscript{Na},V) in SHR is normal or subnormal despite the presence of elevated blood pressure. Furthermore, dietary sodium restriction retards the development of hypertension in SHR. Inasmuch as intrarenally produced dopamine has a physiological role in regulation of sodium excretion, a malfunction of the renal dopaminergic system has been proposed to be partly responsible for the abnormal renal sodium handling in SHR. It is reported that in young SHR, renal tissue or urinary dopamine concentrations are elevated, yet renal sodium excretion is less in the SHR than in WKY rats. The renal response to exogenously administered dopamine receptor agonists as well as antagonists is diminished in the SHR.
was also reported that inhibition of peripheral dopamine production by carbidopa reduced renal sodium excretion and accelerated the development of hypertension in the SHR.12

It has been demonstrated that the major renal action of kidney dopamine, which is to increase renal sodium excretion, is mediated via a tubular dopamine-1 (DA-1) receptor3,7 coupled to both adenylate cyclase13,14 and phospholipase C.14–16 Therefore, it is reasonable to speculate that a defect in either one or both of the cellular signaling processes may contribute to the abnormal renal response to DA-1 receptor activation. Indeed, a defect in DA-1 receptor adenylate cyclase coupling in the proximal convoluted tubules from the SHR has recently been reported.8,17 However, there is so far no report on the functional status of DA-1 receptor–phospholipase C coupling system in SHR. In the present study, we determined dopamine receptor–mediated increases in phospholipase C activity in WKY rats and SHR and further studied the renal response to exogenously administered dopamine in these two strains to identify a potential defect in this signal transduction pathway that may be a contributing factor in the diminished natriuretic response to DA-1 receptor agonists.

Methods
Measurement of Phospholipase C Activity

Kidney slice preparation. Male WKY rats and SHR Okamoto strain (Harlan Sprague Dawley, Indianapolis, Ind.) of 10–12 weeks of age were used in the present study. Rats were anesthetized with pentobarbital (50 mg/kg i.p.), and the kidneys were removed immediately and immersed in Krebs-Ringer bicarbonate buffer containing glucose (KRB). The outer renal cortex was carefully removed and chopped on ice-cold KRB to remove unbound activity and were incubated for 20 minutes in fresh nonradioactive KRB containing 10 mM Li+ to prevent breakdown of inositol monophosphate.

Experimental protocol. The slices were then distributed among tubes, to which various concentrations of dopamine (1, 3, and 10 mM) were added, and then were incubated for 60 minutes. Ascobic acid (0.1% wt/vol) was present in each tube to prevent oxidation of dopamine. Preliminary study in Sprague-Dawley rats showed that a linear concentration–response relation was present with the range of dopamine concentrations from 0.3 mM to 10 mM and that 0.3 mM was the lowest concentration to produce significant increase in fractional release (unpublished results from our laboratory). In a separate group of experiments, the relative involvement of DA-1 receptor and α-adrenergic receptor (namely, α1-adrenergic receptor) in dopamine-induced activation of phospholipase C was examined by incubating slices with the selective DA-1 receptor antagonist SCH 23390 (30 μM) and the nonselective α-adrenergic receptor antagonist phentolamine (10 μM), respectively, for 20 minutes before addition of dopamine. The incubation was terminated by adding 300 μl ice-cold 5% trichloroacetic acid (TCA) to each tube.

**Extraction of inositol phosphates.** The slices were homogenized in TCA, and the homogenate was centrifuged. The supernatant was collected, and the TCA was removed by extraction with ether (4 × 1 ml) presaturated with water. The supernatant containing the inositol phosphates was then neutralized with 100 μl NaHCO3 (1.4%). The volume was then made up to 2 ml with distilled water containing hydrolysate phytic acid so as to give a total phosphorus content of 25 μg per tube. The inositol (mono-, bis-, and tris-) phosphates in the tubes were then separated by ion exchange chromatography using anion exchange SepPaks (ACCELL QMA, Waters Associates, Milford, Mass.) with ammonium formate–based solutions as described by Wreggett and Irvine.18 The activity in the fractions was measured using a scintillation counter (Beckman LS 7500, Irvine, Calif.) and expressed as disintegrations per minute. The lipids (total) in the cortical slices were extracted from the pellet obtained from the homogenate. Lipids were extracted once with 1 ml chloroform/methanol (1:1, vol/vol) and then twice with chloroform/methanol (2:1, vol/vol) containing 1 part in 400 of 12 M HC1 for 30 minutes at 37°C. The residue was rinsed with 1 ml acidified chloroform/methanol, and 0.5 ml chloroform was added to the combined chloroform/methanol extracts. The extract was shaken with one fifth volume of 100 mM HCl. The upper phase was discarded, and the lower phase was washed with two thirds volume of chloroform/methanol/100 mM HCl (3:48:47, vol/vol) containing 1 mM inositol, followed by chloroform/methanol/100 mM HCl (3:48:47, vol/vol). The mixture was neutralized with 1 drop of NH4OH (1 M) and changed to one phase by dropwise addition of methanol. The mixture was dried under nitrogen, and the residue was resuspended in 0.6 ml chloroform/methanol/water (75:25:2, vol/vol). Aliquots (20 μl) of these extracts were counted in triplicate on a scintillation counter, and the total lipid content in each sample expressed as disintegrations per minute (dpm).

The phospholipase C activity has been expressed in terms of fractional release of combined inositol (mono-, bis-, and tris-) phosphates (IPs) described as follows:

\[
\text{fractional release} = \left( \frac{\text{dpm IPs}}{\text{dpm IPs+dpm TL}} \right) \times 100
\]

where IPs is IP1+IP2+IP3, and TL is total lipids.
**In Vivo Functional Studies**

**Surgical procedures.** Both SHR and WKY rats were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and anesthesia was maintained by continuous intravenous infusion of sodium pentobarbital (6 mg/hr). After a tracheotomy, the left carotid and right femoral arteries were catheterized for monitoring blood pressure and collecting blood samples, respectively. In addition, both jugular veins were catheterized for anesthetic and saline or dopamine infusions, respectively. The left ureter was exposed via a midline abdominal incision and cannulated for the collection of urine. After completion of surgery, an infusion of isotonic NaCl at a rate of 0.02 ml/min was begun to maintain animals under euolemic conditions. Hemodynamic and renal parameters were allowed to stabilize over a period of 45 minutes before beginning the experiment.

**Experimental protocol.** The protocol consisted of five 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. During the third period, saline was replaced by dopamine (1 µg/kg/min i.v.), which was infused for a period of 30 minutes at the rate of 0.02 ml/min. After the termination of dopamine infusion, saline alone (0.02 ml/min) was infused during the last two periods. Arterial blood samples (0.2 ml) were collected at the midpoint of each period, and the volume was replaced with an equal amount of saline.

**Analytical measurements.** Sodium and potassium concentrations in the plasma and urine were measured using a Perkin-Elmer flame photometer (Perkin-Elmer, Oakbrook, Ill.). Plasma and urine creatinine concentrations were measured using a creatinine analyzer (model 2, Beckman Instruments, Inc., Fullerton, Calif.). Glomerular filtration rate (GFR) was calculated from the clearance of creatinine.

**Statistical analysis.** All data are presented as mean±SEM. Data were analyzed with Student's paired and unpaired t test and, wherever appropriate, analysis of variance. A value of p<0.05 was considered statistically significant.

**Results**

**Dopamine-Induced Activation of Phospholipase C**

Dopamine (1, 3, and 10 mM) produced concentration-related increases in inositol phosphates released in renal cortical slices obtained from both WKY rats and SHR. In WKY rats, dopamine produced 38±6%, 71±9%, and 106±22% increases over control at the respective concentrations. In SHR, dopamine produced 13±6%, 49±6%, and 50±16% increases over the control at the respective concentrations, which was significantly less than that seen in WKY rats (Figure 1). It was found that in SHR, although there was an increase in inositol phosphates released at 1 and 3 mM dopamine concentrations, unlike the WKY rats at the highest concentration of dopamine (10 mM), no further increase in the inositol phosphate release was seen in SHR (Figure 1). It was interesting that the basal inositol phosphates release in SHR (7.36±0.32%) was significantly higher than that from WKY rats (5.61±0.27%). To explore whether the reduced dopamine-induced activation of phospholipase C in the SHR was due to an impaired α1-adrenergic receptor or DA-1 receptor function, we performed experiments with selective DA-1 receptor antagonist SCH 23390 and nonselective α-adrenergic receptor antagonist phentolamine. The results from WKY rats showed that approximately 50% of dopamine-stimulated inositol phosphate production was blocked by the DA-1 receptor blocker SCH 23390, and 50% was blocked by α-adrenergic receptor antagonist phentolamine (Figure 2), which is consistent with our earlier studies in Sprague-Dawley rats. In contrast, in the SHR, DA-1 receptor antagonist SCH 23390 failed to produce blockade of dopamine-induced activation of phospholipase C, and furthermore, approximately 70% of dopamine-induced activation was blocked by phentolamine in SHR.
stimulated inositol phosphate production in the SHR was blocked by phentolamine (Figure 3). DA-2 receptor antagonist was not included in these experiments since it was demonstrated in our earlier study with Sprague-Dawley rats that DA-2 receptors are not involved in the dopamine-induced activation of phospholipase C.

Effect of Dopamine on Renal Function

In preliminary studies, it was established that all renal and hemodynamic parameters remained quite stable throughout the duration of the experiment. As shown in Figure 4 and Table 1, urine output (UV), \( U_{Na} \), fractional sodium excretion (\( FE_{\text{Na}} \)), urinary potassium excretion (\( U_{K} \)), and GFR during control periods were not statistically different between SHR and WKY rats, although mean arterial blood pressure was significantly higher in the SHR. Dopamine infusion (1 \( \mu \)g/kg/min i.v.) produced a significant increase in UV, \( U_{Na} \), \( FE_{\text{Na}} \), and \( U_{K} \) without causing any changes in mean arterial blood pressure, heart rate, or GFR in either of these two groups. However, the diuretic and natriuretic responses to dopamine administration were significantly attenuated in SHR as compared with the WKY rats (UV, 34±1 versus 63±10 \( \mu \)l/30 min, \( p<0.05; \) \( U_{Na} \), 1.8±0.1 versus 3.2±0.2 \( \mu \)eq/30 min, \( p<0.05; \) \( FE_{\text{Na}} \), 0.05±0.01 versus 0.10±0.01%, \( p<0.05 \), respectively, Figure 4 and Table 1).

Discussion

The present study clearly showed that the natriuretic response to exogenously administered dopamine, which we have recently reported to be mediated via the activation of tubular DA-1 receptors, was significantly diminished in SHR compared with normotensive WKY rats. These observations are consistent with previous reports that demonstrated a blunting of renal response to intrarenally administered DA-1 receptor agonists as well as antagonists in this strain of animals. Although the possibility cannot be totally excluded that subtle changes in renal hemodynamics may contribute to the different
natriuretic response to exogenous dopamine, it cannot be a major reason since arterial blood pressure and GFR did not change during dopamine administration in both groups of animals. Therefore, it is appropriate to assume that there are some alterations at the level of renal tubules that may account for the diminished natriuretic response to exogenous dopamine seen in SHR.

It is proposed that intrarenally produced dopamine increases renal sodium excretion via activation of tubular DA-1 receptor.  Although the mechanism by which DA-1 receptor activation leads to decreased tubular transport of sodium is not yet clear, it is interesting to note that activation of both adenylate cyclase and phospholipase C is seen after DA-1 receptor stimulation. It is reported that activation of adenylate cyclase after occupation of DA-1 receptor is linked to an inhibition of Na⁺-H⁺ exchange in brush border membrane preparation, whereas DA-1 receptor stimulation at the level of basolateral membrane activates phospholipase C, which is followed by protein kinase C activation and subsequent inhibition of Na⁺,K⁺-ATPase located on the basolateral membrane of renal tubule. Therefore, it is likely that both pathways may be involved in overall DA-1 receptor-mediated inhibition of sodium reabsorption. It can be suggested that a defect in either one or both of these pathways might contribute to the diminished natriuretic effect of dopamine and selective DA-1 receptor agonists seen in SHR. Indeed, it has been recently reported that DA-1 receptor adenylate cyclase coupling is defective in the proximal convoluted tubule from the SHR, whereas there were no differences in DA-1 receptor number or affinity between WKY rats and SHR.

It was clearly demonstrated in the present study that dopamine-induced activation of phospholipase C, which is linked to both a-adrenergic receptor and DA-1 receptor activation was significantly diminished in renal cortical slices of SHR as compared with WKY rats. More importantly, the diminished dopamine-induced activation of phospholipase C in the SHR was due, mainly if not entirely, to an impaired DA-1 receptor function as evidenced by the observation that the nonselective a-adrenergic receptor antagonist phentolamine blocked most (approximately 70%) of dopamine-induced activation of phospholipase C and that the selective DA-1 receptor antagonist SCH 23390, which blocked 50% of dopamine-stimulated inositol phosphate production in the WKY rats, and did not have any significant effect on dopamine-induced inositol phosphate production in the SHR. The high basal level of phospholipase C seen in the SHR could be attributed to hypernoradrenergic state and increased urinary dopamine excretion in this strain of animals. However, reports from literature are conflicting with regard to the basal level of phospholipase C and a-adrenergic receptor phospholipase C coupling status. For example, reports of Jeffries et al showed no difference in the basal level of phospholipase C activity in kidney slices between SHR and WKY rats and reduced efficiency of a-adrenergic receptor phospholipase C coupling. However, consistent with our findings, there is a report of increased phospholipase C activity in SHR compared with WKY rats.

The result of this study cannot identify the specific sites of renal DA-1 receptor phospholipase C signal transduction pathway that are affected in the adult SHR. However, it is possible that the sites involved are distal to the dopamine receptors, since there are reports that showed no differences in renal tubular DA-1 receptor number, distribution, or affinity between WKY rats and SHR of this age. Although in 32-week-old SHR, the renal tubular DA-1 receptors were reported to be decreased.

The mechanism for diminished DA-1 receptor-mediated phospholipase C activation remains to be clarified. It could be a normal physiological response to the elevated urinary dopamine excretion that potentially downregulates certain parts of dopamine signal transduction pathways in the kidney as proposed. It is likely that increased kidney dopamine production may be a compensatory response to the increased antinatriuretic factors present in the animal model of hypertension (e.g., hypernoradrenergic state). More studies are needed to determine if the reduced DA-1 receptor-mediated phospholipase C

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**Table 1. Fractional Sodium Excretion, Urinary Potassium Excretion, and Heart Rate in Spontaneously Hypertensive Rats and Wistar-Kyoto Rats Receiving Exogenous Dopamine**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WKY</th>
<th>SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEₘ (mL/g/min)</td>
<td>0.03±0.005</td>
<td>0.03±0.005</td>
</tr>
<tr>
<td>UᵦV (µg/30 min)</td>
<td>5.5±1.3</td>
<td>9.7±1.7 *</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>388±15</td>
<td>395±22</td>
</tr>
</tbody>
</table>

**Values are mean±SEM. n=6 per group.** C₁ and C₂, control periods; DA, periods of dopamine (1 µg/kg/min i.v.) infusion; R₁ and R₂, recovery periods; FEₘ, fractional sodium excretion; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; UᵦV, urinary potassium excretion; HR, heart rate; bpm, beats per minute.

*Significantly different within the group as compared with the corresponding control.
†Significant difference between SHR and WKY group.
activation in SHR is a fundamental abnormality or a consequence of sustained hypertension.

The functional significance of diminished DA-1 receptor–mediated phospholipase C activation in terms of its relation to abnormal renal sodium handling in the SHR deserves to be further explored. It is important to note that accompanied with the high urinary dopamine excretion in the SHR was the reduced renal sodium excretion under normal sodium diet at prehypertensive stage. Furthermore, we have recently reported that in SHR, there is a diminished natriuresis to acute volume expansion as compared with WKY rats. Therefore, these findings strongly suggest that there is a decrease in the ability of endogenous kidney dopamine to regulate renal sodium excretion in the SHR. The present study shows that such a defect may result at least in part from the decreased responsiveness of renal DA-1 receptor–coupled phospholipase C pathways. Although it is not clear as to the mechanism by which the same enzyme (phospholipase C) and the same second messenger systems (IP3, diacylglycerol) are able to mediate the totally different responses according to the type of receptors, it has been observed that phospholipase C activation following occupation of α1-adrenergic receptor leads to increased tubular transport, possibly via enhancement of Na+H+ exchanger activity induced by protein kinase C–mediated phosphorylation. However, phospholipase C activation after occupation of DA-1 receptor leads to decreased tubular transport via inhibition of Na+,K+-ATPase induced by protein kinase C–mediated phosphorylation. One explanation may be that different isomeric forms of phospholipase C are coupled to α1-adrenergic receptor and DA-1 receptor. A deficiency in renal DA-1 receptor phospholipase C coupling with relatively intact α1-adrenergic receptor phospholipase C pathway may be one of the reasons for increased tubular transport in the SHR. Therefore, a deficiency in renal DA-1 receptor phospholipase C coupling system may potentially have a pathophysiological impact on the development as well as maintenance of hypertension in SHR, since endogenous kidney dopamine plays an important role in regulation of renal sodium excretion and abnormal renal sodium metabolism is recognized to be one of the major factors involved in the initiation and maintenance of high blood pressure in the SHR.

In summary, these results have shown, for the first time, that DA-1 receptor phospholipase C coupling system in the kidney is less efficient in adult SHR than in WKY rats. This is an additional mechanism that may be contributing to the diminished natriuretic response to dopamine and DA-1 receptor agonists seen in SHR. Further studies will be focused on the mechanisms for and functional significance of impaired DA-1 receptor phospholipase C–mediated cellular signaling processes in models of hypertension.

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