Diuretic effect of cilazapril and dopamine system in the spontaneously hypertensive rat.

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Abstract

To assess the role of the kidney dopamine system on the diuretic state induced by angiotensin-converting enzyme (ACE) inhibitors, we examined the changes in urinary excretion and plasma level of dopamine, and kidney dopamine receptors in spontaneously hypertensive rats (SHR) treated with cilazapril, an ACE inhibitor. We administered cilazapril 10 mg/kg orally to 13-week-old SHR daily for 21 days (CILAZA group). Systolic blood pressure was significantly decreased in the CILAZA group on Day 6 compared with that in vehicle-treated SHR (control group). The urine volume was three- to fivefold higher in the CILAZA group, and total urinary dopamine secretion was also increased compared with the control group. There was no significant difference in affinity and number of kidney dopamine receptors between the CILAZA and the control groups. In conclusion, the diuretic effect caused by cilazapril is partly mediated by inhibition of the water reabsorption via the increase of dopamine production in the kidney.

KEYWORDS: dopamine, ACE inhibitor, cilazapril, SHR, kidney

*PMID: 8585395 [PubMed - indexed for MEDLINE]
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Diuretic Effect of Cilazapril and Dopamine System in the Spontaneously Hypertensive Rat

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To assess the role of the kidney dopamine system on the diuretic state induced by angiotensin-converting enzyme (ACE) inhibitors, we examined the changes in urinary excretion and plasma level of dopamine, and kidney dopamine receptors in spontaneously hypertensive rats (SHR) treated with cilazapril, an ACE inhibitor. We administered cilazapril 10 mg/kg orally to 13-week-old SHR daily for 21 days (CILAZA group). Systolic blood pressure was significantly decreased in the CILAZA group on Day 6 compared with that in vehicle-treated SHR (control group). The urine volume was three- to fivefold higher in the CILAZA group, and total urinary dopamine secretion was also increased compared with the control group. There was no significant difference in affinity and number of kidney dopamine receptors between the CILAZA and the control groups. In conclusion, the diuretic effect caused by cilazapril is partly mediated by inhibition of the water reabsorption via the increase of dopamine production in the kidney.

Key words: dopamine, ACE inhibitor, cilazapril, SHR, kidney

It is well known that dopamine has potent natriuretic and vasodilating actions through its specific receptor (DA1 or DA2 receptor) (1-3), but the precise regulation mechanism or role in hypertension of the kidney dopamine system is unknown. We previously reported that the predominant dopamine receptor subtype found in the renal cortex was DA1, and that the major source of dopamine which affected the dopamine receptors in the rat kidney was not located at the nerve ending, but rather in the circulation (4). Recently, we examined the age-related change in the kidney dopamine system and urinary dopamine excretion using 3-, 7-, 12-week-old spontaneously hypertensive rats (SHR). We found that although renal dopamine production was enhanced in SHR in the hypertensive state, dopamine was found not to contribute to natriuresis since the number of dopamine receptor is reduced in SHR. We also found that enhanced dopamine production in response to a salt load was lacking in SHR (5). These findings suggested that dopamine in SHR kidneys has little effect on hypertension and diuresis.

Dopamine was reported to have an effect on the regulation of the aldosterone response to sodium excretion in a sodium-restricted state (6, 7). This report suggested that natriuresis is partly caused by the interaction between aldosterone and the dopamine system in vivo. Therefore, it is important to investigate the alterations in the kidney dopamine system in the natriuretic state induced by inhibiting the renin-angiotensin system.

In this study, we investigated the alterations in the plasma dopamine level, urinary dopamine excretion and kidney dopamine receptors in the controlled hypertensive state of SHR administered with the ACE inhibitor, cilazapril (CILAZA). The aim of this study was to investigate the interaction between the renin-angiotensin system and the kidney dopamine system.

Materials and Methods

Materials. Male 13-week-old SHR (n = 14) were obtained from Charles River Japan (Kanagawa, Japan) and housed in climate-controlled metabolic cages with a 12-h light/dark cycle and food (MF, Oriental Yeast Co., Tokyo, Japan) and water provided ad libitum. [3H]spioperone ([phenyl-4-3H]spioperidol: specific activity 17 Ci/ mmol) was purchased from Radiochemical Center (Amersham, Buckinghamshire, UK). Spioperone

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(unlabeled) and cilazapril were gifts from Eisai Co. (Tokyo, Japan).

**Urinary dopamine excretion.** Urine specimens were collected in cups containing 1 ml of 6 N HCl for dopamine determination using individual metabolic cages. Urine volume and urinary excretion of dopamine were measured in the condition of taking water without additional sodium chloride. Samples of trunical blood were collected on the day when rats were sacrificed by decapitation, and stored at -20°C until assay. Urinary and plasma concentrations of dopamine were measured at Mitsubishi Yuka Bio-Clinical Laboratories, Inc. (Osaka, Japan) by high-performance liquid chromatography. Both the inter-assay and intra-assay coefficients of variation (CV) of dopamine were less than 10%. Serum levels of sodium, potassium, urea nitrogen, creatinine and uric acid were measured using an automatic analyser system. The serum concentration of cilazaprilat, which is the active metabolite of cilazapril, was measured by enzyme immunoassay (EIA) at Mitsubishi Yuka BCL (8). Because this EIA was established only for humans, it is possible that the assay might not be appropriate for rats.

**Membrane preparations.** After the rats were killed by decapitation, the kidneys were quickly removed, decapsulated, and sliced along the corticomedullary axis to separate the cortex from the medulla in ice cold Buffer A (50 mM Tris-HCl buffer containing 250 kallikrein inhibition equivalents [KIE]/ml of aprotinin and 0.3 M saccharose, pH 7.6). The renal cortex was used for the membrane preparation by the method reported previously (9). Briefly, the renal cortex was placed in ice-cold Buffer A. It was mixed with a Polytron PT-10 apparatus (at full speed for 10 s) and then homogenized in a glass homogenizer in ice. The homogenate was centrifuged at 800 × g for 10 min at 4°C and the pellet was discarded. The supernatant was centrifuged at 18,000 × g for 20 min at 4°C. The pellet was washed in Buffer A and again centrifuged at 18,000 × g for 20 min at 4°C. The final pellet, renal cortex membrane preparation, was resuspended in 40 volumes of Buffer A. The protein concentrations of the membrane preparations were determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA).

**Radiolabeled receptor assay.** Radiolabeled receptor assay (RRA) of dopamine was carried out as we described before (4). Membrane preparations were incubated at 37°C for 10 min. In most studies, each assay tube contained 200 μl of membrane preparation (approximately 0.2 mg protein) and 100 μl of various concentrations of [3H]spiperone (0.09-16 nM) with or without an excess (100 μl) of unlabeled 10 μM spiperone, and with RRA buffer B (50 mM Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 1 mM MgCl2, 1 mM CaCl2, and 250 KIE/ml aprotinin, pH 7.6) in a total volume of 500 μl. After the assay tubes were preincubated for 10 min at 37°C, they were cooled in ice. The tubes were then incubated at 37°C for 20 min, and the reaction was terminated by rapid filtration through Whatman GF/C glass fiber filters. The filters were washed twice with 5 ml of ice-cold Buffer C (50 mM Tris-HCl buffer) and placed in scintillation vials with 5 ml of scintillation flour containing methylcellulose. The radioactivity on the filters was then counted in a liquid scintillation spectrometer. Specific binding was measured as the difference between radioactivity bound to the receptor in the presence of excess spiperone and that in its absence. The data were analyzed by Scatchard plots according to Marquardt (10) using a personal computer (NEC 9800). The interassay CV of spiperone RRA was 9.77% (n = 5) and the intra-assay CV was 0.69% (n = 10).

**Experimental protocol of treatment with cilazapril.** Thirteen-week-old SHRs were administered cilazapril orally (10 mg/kg body weight in 0.3 ml of 2% gum arabic solution) every evening for 21 days (CILAZA group). The control group of age-matched male SHR was administered with 0.3 ml of gum arabic solution alone. Systolic blood pressure was measured in conscious, restrained rats by tail-cuff plethysmography (UR-5000, Ueda Seisakusyo, Tokyo, Japan). The measurements were performed periodically in the morning on days 1, 2, 4, 6, 9, 11, 13, 15, 18 and 20. Urine specimens in the CILAZA and control groups were also collected every week. All rats were killed by decapitation on Day 21 and kidneys and kidney blood samples were used for RRA of dopamine and measurement for laboratory data, respectively.

**Statistical analysis.** The results were expressed as mean ± SEM and statistically analyzed by the unpaired Student’s t-test.

**Results**

Fig. 1 shows time course changes in systolic blood pressure of SHR administered cilazapril 10 mg/kg once daily for 3 weeks. Systolic blood pressure in the CILAZA group was significantly lower than that in the
Table 1  Effect of cilazapril (CILAZA) on urine volume, urinary dopamine concentration and excretion in spontaneously hypertensive rats (SHR)

<table>
<thead>
<tr>
<th></th>
<th>Weeks after the beginning of treatment</th>
<th>Control group</th>
<th>CILAZA group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume (ml/100gBW/day)</td>
<td>0</td>
<td>4.0±1.2</td>
<td>3.3±0.4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.8±0.7</td>
<td>13.0±2.4*</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.9±0.8</td>
<td>16.8±2.2**</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7.6±1.7</td>
<td>20.3±2.0**</td>
</tr>
<tr>
<td>Urinary dopamine concentration (µg/l)</td>
<td>0</td>
<td>412.9±83.0</td>
<td>400.1±33.7</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>376.4±54.1</td>
<td>150.2±26.3**</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>308.4±59.0</td>
<td>95.9±14.5*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>250.1±44.6</td>
<td>92.7±7.9*</td>
</tr>
<tr>
<td>Urinary dopamine excretion (µg/day)</td>
<td>0</td>
<td>4.0±0.1</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2.8±0.3</td>
<td>4.5±0.1**</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.5±0.2</td>
<td>4.2±0.5*</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.6±0.1</td>
<td>5.4±0.3**</td>
</tr>
</tbody>
</table>

Time course change of urine volume (ml/100 gBW/day), urinary dopamine concentration (µg/l) and urinary dopamine excretion (µg/day) of SHR treated with cilazapril. Values are expressed as mean ± SEM of 6 rats. *P < 0.05, **P < 0.01 vs the control group. oP < 0.05, ooP < 0.01 vs the value on week 0.

Fig. 1 (Left)  Time course change of systolic blood pressure of the SHR treated with cilazapril (closed circles) and the untreated SHR (open circles). Values are expressed as mean ± SEM of 6 rats. *P < 0.01; **P < 0.005, vs. the untreated spontaneously hypertensive rats (SHR).

controlled SHR after day six. After administration with CILAZA, the urine volume was markedly increased, approximately three- to fivefold compared with the control group. Urinary dopamine concentration was decreased, but total urinary dopamine excretion was increased in the CILAZA group (Table 1). There was no significant change in the serum levels of sodium, potassium, urea nitrogen, creatinine or uric acid between the groups (Table 2). The plasma concentration of cilazaprilat was significantly increased in the CILAZA-treated SHR, but a small amount of CILAZA was detected in the non-treated SHR (the control group). The plasma level of dopamine in the CILAZA group was significantly lower than that in the control group (Table 3). The RRA for dopamine showed that there was no significant difference in renal dopamine receptors between the CILAZA group and the control group (Fig. 2). To exclude the possible direct effects of the administered CILAZA on the RRA for dopamine, various concentrations of CILAZA were added to the incubation medium of RRA for dopamine. No significant change in binding of dopamine to the kidney.
Table 3  Effect of cilazapril (CILAZA) on plasma concentration of dopamine and cilazapril

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>CILAZA group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dopamine (ng/ml)</td>
<td>10.10±0.57</td>
<td>8.17±0.72*</td>
</tr>
<tr>
<td>Cilazapril (ng/ml)</td>
<td>0.58±0.18</td>
<td>2.91±0.22*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM of 6 rats. *P<0.05 vs the control group.

Discussion

We previously reported that the number of dopamine receptors in SHR at the established hypertension state was lower than that in normotensive WKY rats, and that the dopamine production of SHR did not increase under sodium loading (5). We suggested that these two pathological phenomena noted in the renal dopamine system affected the hypertension in SHR. In the present study, we evaluated the effect of an ACE inhibitor, cilazapril, on the kidney dopamine system in SHR.

Cilazapril (11), which is an ACE inhibitor with potent depressor action, was administered to 13-week-old SHR. A significant reduction in blood pressure was observed, and laboratory investigations of blood specimens collected from SHR after administration of the ACE inhibitor revealed no renal dysfunction or electrolyte imbalance. Urinary volume markedly increased as a result of administration of the depressor agent. The diuretic effect observed in cilazapril-treated rats was comparable to that in rats treated by indapamide (an anti-hypertensive diuretic) (12) and rats with diabetes insipidus induced by lithium (13). Chen et al. showed that, in normotensive rats, ACE inhibition enhanced the diuretic and natriuretic effects of fenoldopam (14). The diuretic action of ACE inhibitors is attributable to inhibition of Na+ reabsorption caused by the suppression of aldosterone activity as well as to
increase in renal blood flow caused by dilatation of the afferent and efferent arterioles, namely, ACE inhibitors directly block vascular contraction induced by angiotensin II. This may be due to withdrawal of the stimulatory effect of angiotensin II on renal sodium transport (15), and this increase in renal blood flow and renal sodium excretion may be partly the result of an increase in the local bradykinin level (16). It has been reported that natriuresis in patients with non-modulating hypertension is improved by treatment with ACE inhibitors (17). It has also been shown that dopamine exerts a tonic inhibitory effect on aldosterone secretion resulting in enhanced renal sodium excretion (18). These observations suggest that dopamine is closely associated with the renin-angiotensin system. In this study, the urinary dopamine concentration was decreased, however, total urinary dopamine excretion was markedly increased, and diuresis was enhanced in SHR administered the ACE inhibitor. There are various factors other than dopamine which affect the diuresis of ACE inhibitors. The decrease in the urinary dopamine concentration accounts for the increase in urine volume by the diuretic effect of these factors. If dopamine does not take part in the diuretic effect of ACE inhibitors and if urinary dopamine excretion occurs as a part of the compensatory mechanism against hypertension or is a phenomenon secondary to diuresis, then dopamine excretion should be decreased when blood pressure is lowered. However, what we observed instead was an increase in total urinary dopamine excretion.

Using spiperone RRA, we previously reported that there is a single class of binding sites for dopamine receptors in the rat renal cortex membrane, and showed that DA1 receptors are predominant in the cortex (4). These results are based on the fact that spiperone has affinity for D1, D2 and serotonin receptors, but neither sulpiride, a specific D2 receptor antagonist, nor serotonin inhibited [3H]spiperone binding. The affinity and number of dopamine receptors of the renal tubules were unchanged even under these circumstances. Furthermore, cilazapril did not affect the in vitro assay of dopamine receptors. Marked diuresis, as seen with cilazapril, is not observed with the use of other depressor agents such as Ca++ antagonists and β-blockers (19) and diuretics, as we previously reported (20). Besides, cilazapril increased urinary dopamine secretion in our SHR. These observations suggest that the natriuresis which accompanied the increase in urinary dopamine secretion was most likely due to the pharmacological properties of ACE inhibitors. We suggest that enhancement of kidney dopamine production constitutes one important diuretic mechanism of the ACE inhibitors.

There was no change in the number of dopamine receptors after the administration of cilazapril. The reasons for this phenomenon are, we think, twofold. First, the dopamine level in the renal tubules did not increase because of the increase of urine volume. Second, since renal dopamine production was enhanced and the number of dopamine receptors had already been reduced in the hypertensive state of SHR (5), the increase in dopamine production did not significantly affect the number of dopamine receptors after the administration of cilazapril. Further studies, however, are needed to clarify these phenomena.

A small amount of cilazaprilat was detected also in the non-treated SHR, but the method of measurement of cilazaprilat has not been established for rats. It is possible that there are some substances which cross-react by this method.

In summary, our findings suggested that the diuretic effects of the ACE inhibitors may partly be attributable to the increase in the production of renal dopamine.

Acknowledgments. This work was supported in part by a research grant (No. 04670037) from the Japanese Ministry of Education, Science, and Culture of Japan. The authors thank Dr. T. Yamashita, Dr. T. Oishi, Dr. T. Omiya and Eisai Co. for their helpful advice and encouragement during this study.

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Received November 28, 1994; accepted August 21, 1995.