Pathophysiological Role of Dopamine on the Development of Hypertension in Rats

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The aim of the study is to investigate the pathophysiological role of dopamine (DA) in the development of hypertension in DOCA-salt hypertensive rats and spontaneously hypertensive rats (SHRs). The augmentation of dopaminergic activity by chronic administration of bromocriptine, a DA agonist, suppressed the increase of blood pressure in DOCA-salt hypertensive rats. In contrast, suppression of dopaminergic activity by chronic administration of carbidopa, an inhibitor of dopa decarboxylase, accelerated the development of hypertension in SHRs, and this acceleration was also increased by salt loading. Increased urinary excretion of norepinephrine (NE) by DOCA-salt treatment was suppressed by the treatment of bromocriptine. In contrast, administration of carbidopa and salt loading in SHRs resulted in an increase in renal NE content and in urinary NE and epinephrine (E) excretion and a decrease in urinary sodium excretion. These results suggest that dopaminergic activity participate in the development of hypertension and decreased dopaminergic activity accelerates the development of hypertension in hypertensive rats mainly through the enhancement of peripheral sympathetic nerve activity.

The role of dopamine (DA) in blood pressure regulation, mediated through central and/or peripheral dopaminergic mechanisms, has been accepted in literature. Stimulation of DA neuron in the brain or the intracerebroventricular injection of DA lowers blood pressure in rats, while these depressor effects are reduced by treatment with DA antagonist, haloperidol or metoclopramide. These results suggest that DA in the central nervous system is involved in blood pressure control. Moreover, the activation of peripheral DA-2 presynaptic receptors lowers blood pressure and heart rate through the decreased sympathetic neuronal release of norepinephrine (NE), and the depressor effect is antagonized by domperidone, a peripheral DA-2 receptor antagonist. These results suggest that depressor effect of DA is mediated through peripheral dopaminergic mechanisms.

However, the role of DA in the development of hypertension remains unclear. While a number of studies found an increase in plasma or urinary excretion of DA in patients or rats with hypertension others described a decrease in plasma or urinary DA in patients with hypertension. The present experiments were designed to investigate whether the enhanced dopaminergic activity resulting from a chronic administration of bromocriptine, a DA agonist, or the suppressed dopaminergic activity caused by the chronic administration of carbidopa, an inhibitor of dopa decarboxylase, affects the blood pressure, urinary excretion of sodium and catecholamines in hypertensive rats.

Key words:
- Dopamine
- Hypertension
- Bromocriptine
- Carbidopa
- Dopaminergic mechanism
- DOCA-salt hypertensive rat
- SHR

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TABLE 1  EFFECTS OF BROMOCRIPTINE, CARBIDOPA OR SALT ON BLOOD PRESSURE IN DOCA-SALT HYPERTENSIVE RATS (DSHRs) OR SPONTANEOUSLY HYPERTENSIVE RATS (SHRs)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animal (n)</th>
<th>Blood pressure (mmHg)</th>
<th>Before</th>
<th>After (3W or 4W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromocriptine</td>
<td>DSHRs (7)</td>
<td></td>
<td>108 ± 2</td>
<td>138 ± 4*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>DSHRs (7)</td>
<td></td>
<td>107 ± 2</td>
<td>163 ± 3</td>
</tr>
<tr>
<td>Carbidopa</td>
<td>SHRs (6)</td>
<td></td>
<td>124 ± 2</td>
<td>170 ± 2**</td>
</tr>
<tr>
<td>Vehicle</td>
<td>SHRs (6)</td>
<td></td>
<td>125 ± 2</td>
<td>162 ± 2</td>
</tr>
<tr>
<td>Carbidopa and salt</td>
<td>SHRs (6)</td>
<td></td>
<td>123 ± 3</td>
<td>195 ± 2*</td>
</tr>
<tr>
<td>Vehicle and salt</td>
<td>SHRs (6)</td>
<td></td>
<td>123 ± 2</td>
<td>186 ± 2</td>
</tr>
</tbody>
</table>

*significant difference from the control  p < 0.01
**significant difference from the control  p < 0.05

MATERIALS AND METHODS

Animals
DOCA-salt hypertensive rats and spontaneously hypertensive rats (SHRs) were used in experiments. Male Wistar rats 3-weeks-old, were unilaterally nephrectomized in the left kidney. A week later, they were treated weekly with subcutaneous injections of 10 mg DOCA with 1% methyl cellulose solution and fed a high sodium diet (Oriental Yeast) containing 3.14% sodium for 3 weeks. Water and feed were provided ad libitum. These rats were subcutaneously injected daily with 1 mg/kg/day of bromocriptine (Sandoz) solution or the vehicle as the control.

Male SHRs at 4-weeks-old were fed a basal sodium diet (Oriental Yeast) containing 0.26% sodium or a high sodium diet for 4 weeks. These SHRs were given orally an inhibitor or DA decarboxylase, carbidopa (Merk) solution, 40 mg /kg/day (in two doses, 20 mg/kg in the morning and again the evening) or a vehicle of 1% methyl cellulose as the control for 4 weeks.

Experimental design
Blood pressure was measured weekly using the tail-cuff method and a programmed electrophysmomanometer (Narco) before administration of the vehicle, bromocriptine or carbidopa. The rats were housed in individual metabolic cages and a 24-hour urine collection was made in flasks containing 1 ml of 6N-HCl for determination of catecholamines content after a 24-hour period of acclimatization. Urine collection for sodium followed the urine collection for catecholamines.

Urinary free NE, epinephrine (E) and DA contents were determined using high performance liquid chromatography with electrochemical detection (HPLC-ECD: Bioanalytical System Inc.). Dowex AG ion exchange resin, 50–100 mesh (Muromac, MWC-1, Muromachi Chemical Co.), were used for extraction of catecholamines from the urine.

After measurements of blood pressure and urinary excretions for catecholamine and sodium, the rats were killed. The kidneys were rapidly removed, then frozen on dry-ice and stored at −70°C for later determination of catecholamines using HPLC-ECD. Sodium content in urine was determined by flame photometry (Corning, type 480).

Statistical analysis
Statistical analysis of the data was performed using Student’s paired and unpaired t comparisons test. Values were expressed as means ± SEM.

RESULTS

Body weights of DOCA-salt hypertensive rats and SHRs showed no difference between groups treated with bromocriptine or carbidopa and the control group treated with the vehicle. Blood pressure of these animals increased during the following weeks. Blood pressure of rats with bromocriptine was significantly lower than that of the control, as shown in Table I. In contrast, carbidopa administration to SHRs resulted in a significant increase in blood pressure in basal sodium diet as compared to the control SHRs with vehicle administration. Enhanced develop-
TABLE II  EFFECT OF BROMOCRIPTINE, CARBIDOPA OR SALT ON URINARY EXCRETION OF SODIUM (U-Na) IN DOCA-SALT HYPERTENSIVE RATS (DSHRs) OR SPONTANEOUSLY HYPERTENSIVE RATS (SHRs)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Animals (n)</th>
<th>U-Na (mEq/day) Before</th>
<th>U-Na (mEq/day) After (3W or 4W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromocriptine</td>
<td>DSHRs (7)</td>
<td>0.43 ± 0.07</td>
<td>20.0 ± 0.9**</td>
</tr>
<tr>
<td>Vehicle</td>
<td>DSHRs (7)</td>
<td>0.41 ± 0.04</td>
<td>17.9 ± 1.1</td>
</tr>
<tr>
<td>Carbidopa</td>
<td>SHRs (6)</td>
<td>0.74 ± 0.06</td>
<td>1.00 ± 0.06*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>SHRs (6)</td>
<td>0.75 ± 0.07</td>
<td>1.18 ± 0.06</td>
</tr>
<tr>
<td>Carbidopa and salt</td>
<td>SHRs (6)</td>
<td>0.76 ± 0.06</td>
<td>17.82 ± 0.88**</td>
</tr>
<tr>
<td>Vehicle and salt</td>
<td>SHRs (6)</td>
<td>0.77 ± 0.05</td>
<td>20.01 ± 0.91</td>
</tr>
</tbody>
</table>

*significant difference from the control p < 0.01  
**significant difference from the control p < 0.05

TABLE III  EFFECT OF BROMOCRIPTINE, CARBIDOPA OR SALT ON URINARY EXCRETION OF NOREPINEPHRINE (U-NE), EPINEPHRINE (U-E) AND DOPAMINE (U-DA) IN DOCA-SALT HYPERTENSIVE RATS (DSHRs) OR SPONTANEOUSLY HYPERTENSIVE RATS (SHRs)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Animals (n)</th>
<th>U-NE (ng/day) Before</th>
<th>U-NE (ng/day) After (3W or 4W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromocriptine</td>
<td>DSHRs (7)</td>
<td>228 ± 10</td>
<td>302 ± 9*</td>
</tr>
<tr>
<td>Vehicle</td>
<td>DSHRs (7)</td>
<td>214 ± 12</td>
<td>418 ± 15</td>
</tr>
<tr>
<td>Carbidopa and salt</td>
<td>SHRs (6)</td>
<td>452 ± 34</td>
<td>2126 ± 174**</td>
</tr>
<tr>
<td>Vehicle and salt</td>
<td>SHRs (6)</td>
<td>456 ± 51</td>
<td>1652 ± 103</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Animals (n)</th>
<th>U-E (ng/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromocriptine</td>
<td>DSHRs (7)</td>
<td>62 ± 3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>DSHRs (7)</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>Carbidopa and salt</td>
<td>SHRs (6)</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>Vehicle and salt</td>
<td>SHRs (6)</td>
<td>42 ± 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Animals (n)</th>
<th>U-DA (ng/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromocriptine</td>
<td>DSHRs (7)</td>
<td>1.61 ± 0.05</td>
</tr>
<tr>
<td>Vehicle</td>
<td>DSHRs (7)</td>
<td>1.56 ± 0.03</td>
</tr>
<tr>
<td>Carbidopa and salt</td>
<td>SHRs (6)</td>
<td>2.64 ± 0.33</td>
</tr>
<tr>
<td>Vehicle and salt</td>
<td>SHRs (6)</td>
<td>2.49 ± 0.26</td>
</tr>
</tbody>
</table>

*significant difference from the control p < 0.01  
**significant difference from the control p < 0.05

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The administration of bromocriptine by carbidopa administration was accelerated by salt loading as compared to SHRs with carbidopa in a basal sodium diet.

Although the DOCA-salt treatment resulted in an increased urinary excretion of sodium, DOCA-salt rats with bromocriptine excreted significantly more sodium than that of the control as shown in Table II. In contrast, the SHRs receiving carbidopa showed significantly less urinary excretion of sodium than that of the control. Although the salt loading increased the
urinary excretion of sodium in SHRs with and without carbidopa, SHRs receiving carbidopa and a high sodium diet excreted significantly less urinary sodium than that of control rats.

Urinary excretion of NE increased with the treatment of DOCA-salt or a high salt diet in the following weeks. Urinary excretion of NE in bromocriptine rats decreased significantly as compared to that of control rats as shown in Table III. In contrast, SHRs receiving carbidopa with a high sodium diet showed significantly greater amounts of urinary excretion of NE than that of control SHRs.

Urinary excretion of E increased with the treatment of DOCA-salt or only with a high sodium diet in the following weeks. There was no significant reduction in urinary excretion of E by the treatment with bromocriptine. However, SHRs treated both with carbidopa and a high sodium diet showed a significant increase in urinary excretion of E as compared with that of control SHRs.

Renal content of DA was significantly lower in both SHRs receiving carbidopa with a basal sodium diet (10.2 ± 0.4 vs 12.1 ± 0.4 ng/g tissue, p < 0.02) and with a high sodium diet (7.1 ± 0.9 vs 11.1 ± 0.9 ng/g tissue, p < 0.01) than those of the control SHRs. In contrast, renal content of NE was significantly higher in both SHRs receiving carbidopa with a basal sodium diet (384 ± 14 vs 232 ± 7 ng/g tissue, p < 0.01) and with a high sodium diet (330 ± 21 vs 213 ± 8 ng/g tissue, p < 0.01) than those of the control SHRs.

**DISCUSSION**

The enhanced dopaminergic activity caused by chronic administration of bromocriptine attenuated the development of hypertension in rats treated with DOCA-salt. In contrast, the suppression of the dopaminergic activity by chronic administration of carbidopa enhanced the development of hypertension in SHRs. These results suggest that endogenous DA participates in the regulation of blood pressure and plays an important role in the pathogenesis of hypertension.

In patients or rats with hypertension, either a higher or a lower level of DA concentration in plasma or urine than normotensive subjects has been reported in literature. These conflicting data may be caused by the DA levels in plasma or urine at different stages of hypertension and/or the different subtypes of hypertension. In SHRs, urinary free DA excretion and tissue DA content were higher in the developing stage of hypertension as compared to those of normotensive animals, but these increases of the DA level or content were absent in the established stage of hypertension. In patients with essential hypertension, urinary excretion of DA and homovanillic acid, main metabolite of DA, were higher in patients with labile hypertension than those with stable hypertension. These results suggest that for patients or animals in the developing stage of hypertension the release of DA from sympathetic nerve terminals, adrenal glands and renal tubules is enhanced. Moreover, younger patients with essential hypertension show an increase of sympathetic and adrenomedullary activities than do the older patients. Therefore, it appears that DA is released in response to stimulus of an augmentation of NE release as a compensatory mechanism to maintain blood pressure.

So far as the subtypes of essential hypertension are concerned, decreased dopaminergic activity in adrenal cortex and kidney has been reported in both patients with low-renin hypertension and in salt-sensitive patients. It is suggested that the decreased dopaminergic activity may be involved in the pathogenesis of hypertension. These patients are believed to be volume-dependent hypertension, accompanied by a high plasma aldosterone level in proportion to a low plasma renin activity and decreased urinary excretion of DA in response to salt loading. As these patients may show decreased DA release into plasma or urine as compared with patients with normo- and high-renin hypertension or nonsalt-sensitive patients the experiments from heterogenous groups of essential hypertension may yield different results.

Chronic subcutaneous administration of bromocriptine attenuated the development of hypertension by DOCA-salt treatment, and the depressor effects of bromocriptine were accompanied by both the inhibition of NE release and augmentation of natriuresis. These hypertensive effects of bromocriptine were inhibited by domperidone, DA-2 receptor antagonist, which does not cross the blood brain barrier. These results suggest that the depressor action of bromocriptine is mediated mainly through a peripheral dopaminergic mechanism, probably due to the presynaptic inhibition against NE release of the sympathetic nerve terminals.

On the contrary, chronic oral administration
of carbidopa augmented the development of hypertension in SHRs, and the pressor action of carbidopa was associated with the enhancement of renal sympathetic and adrenomedullary activities, which were suggested by an increased urinary excretion of norepinephrine and epinephrine and an increased renal content of norepinephrine. The urinary excretion of sodium was also suppressed by reduction of DA synthesis caused by carbidopa, which suggested an increase of sodium retention in body fluid in SHRs. Such enhancement of renal sympathetic and adrenomedullary activities and increase of sodium retention may accelerate the development of hypertension by carbidopa treatment.

Decreased DA content in kidneys by carbidopa suggests decreased DA content in the other peripheral tissues. Decreased DA content in the peripheral tissue may accelerate the NE and E release from the sympathetic nerve terminals and the adrenal medulla by inhibiting presynaptic and adrenal DA-2 receptors.\textsuperscript{2,3}

Inhibition of DA biosynthesis in kidneys by carbidopa caused the reduction of urinary sodium excretion. It has been accepted that DA increases the renal blood flow by the vasodilation through DA-1 receptors at renal arteries\textsuperscript{22} and the inhibited release of NE via presynaptic DA-2 receptors at the renal sympathetic nerve terminals\textsuperscript{3} and increases the glomerular filtration rate\textsuperscript{23}. And also DA diminishes the sodium and water reabsorption through DA-1 receptors at the renal tubules\textsuperscript{24}. These evidence strongly support the idea that the inhibition of DA biosynthesis in kidneys by carbidopa reduces natriuresis.

Carbidopa is a peripheral decarboxylase inhibitor, which does not cross the blood brain barrier.\textsuperscript{25} It inhibits catecholamine biosynthesis in the peripheral organs but not in the brain and adrenal gland.\textsuperscript{26} Low doses of carbidopa do not affect the NE biosynthesis in the peripheral organ.\textsuperscript{27} Therefore, the combined use of carbidopa and dopa has been applied clinically for the treatment of parkinsonism. In the present study, carbidopa caused both the increase of NE content in kidneys and of urinary excreted NE, which suggests enhanced renal sympathetic nerve activity. These results suggest that carbidopa in a low dose mainly inhibits extraneuronal dopa decarboxylase.

In summary, the present study demonstrates that enhanced dopaminergic activity by chronic administration of bromocriptine, DA agonist, suppressed the development of hypertension in DOCA-salt hypertensive rats; in contrast, decreased dopaminergic activity by chronic administration of carbidopa, an inhibitor of dopa decarboxylase, accelerated development of hypertension in SHRs. Suppression or acceleration in development of hypertension was thought to be mediated through a decrease or increase in the renal sympathetic nerve tone and the resulting sodium balance. These results suggest that DA plays an important role in the regulation of blood pressure by affecting depressor action against the enhancement of renal sympathetic nerve activity and sodium retention.

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