Original Article

Suppression of Catechol-*O*-Methyltransferase Activity through Blunting of α_2 -Adrenoceptor Can Explain Hypertension in Dahl Salt-Sensitive Rats

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Catecholamines have been reported to be involved in the development of salt-sensitive hypertension. We investigated the relation between catechol-*O*-methyltransferase (COMT) and salt-sensitivity. In the first experiment, Dahl salt-sensitive (DS) rats were given a normal-salt (NS), high-salt (HS), or HS+hydralazine (80 mg/l water) diet for 4 or 13 weeks, and Dahl salt-resistant (DR) rats were given a NS or HS diet. COMT activities in both the kidneys and liver and urinary norepinephrine (NE) and dopamine (DA) excretion were measured. In the second experiment, HepG2 cells were used to investigate the role of NE in regulating COMT activity. In the third experiment, we investigated the reactivity of pre- and postsynaptic α_2 -adrenoceptor (AR) in DS rats. HS loading significantly suppressed the activities of membrane-bound COMT (MB-COMT) and, consistent with this finding, increased the urinary NE level in DS rats, but not in DR rats. Hydralazine did not restore the MB-COMT activities, which suggested that HS loading rather than elevated blood pressure suppressed the MB-COMT activities. The *in vitro* experiment using HepG2 cells revealed that NE increased the MB-COMT activity *via* the α_2 -AR. However, both the pre- and postsynaptic α_2 -AR reactivity was decreased by HS loading in DS rats. In conclusion, HS intake suppresses the MB-COMT activities in DS rats, presumably by blunting α_2 -AR signaling. The suppression of MB-COMT activities, consequent decrease in degradation of NE, and increase in NE release by blunting of α_2 -AR function may be involved in the development of salt-sensitive hypertension in DS rats, in whom DA-dependent natriuresis may be suppressed. (*Hypertens Res* 2007; 30: 269–278)

Key Words: catechol-*O*-methyltransferase, catecholamines, norepinephrine, Dahl, pithed rat

Introduction

Excessive salt intake induces the development of hypertension, and this effect may be mediated by various mechanisms. Dahl salt-sensitive (DS) and salt-resistant (DR) rats are commonly used models to study salt-sensitive hypertension (1).

Catecholamines (CAs) have been reported to be involved in

the development of salt-sensitive hypertension. For example, increase in the adrenal (2) and plasma (3, 4) levels of norepinephrine (NE) have been reported following high-salt (HS) loading in DS rats. Increase in sympathetic nervous activity in the kidneys has been shown to induce sodium re-absorption and suppress natriuresis *via* the α_1 -adrenoceptor (AR) (5). On the other hand, dopamine (DA) is an intrarenal natriuretic hormone that acts by inhibiting the sodium transporter pro-

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teins (6, 7). DS rats have been reported to exhibit a defect in the D₁ receptor, which blunts natriuresis (8, 9).

The CAs are inactivated by catechol-*O*-methyltransferase (COMT; EC2.1.1.6) (10). Two isoforms of COMT have been described: the membrane-bound COMT (MB-COMT) and the soluble COMT (S-COMT) (10). COMT is distributed in various tissues, and especially high activities have been reported in the kidneys and liver.

In our previous studies we found that both the activity and protein expression of MB-COMT were lower in the liver of spontaneously hypertensive rats (SHR) than in the liver of Wistar-Kyoto (WKY) rats (11). We hypothesized that this reduced activity of MB-COMT is involved in the development of hypertension by increasing the local NE content. Inhibition of COMT, on the other hand, increases DA content. In fact, when COMT is pharmacologically inhibited in normotensive rats, DA-dependent natriuresis is observed by stimulating the D₁ receptor (12, 13). Furthermore, COMT-gene-disrupted mice have been reported to be resistant to salt-induced hypertension (14).

Thus, COMT could play a role in the regulation of blood pressure by balancing NE and DA. In this study, we investigated the relation between COMT and salt-sensitivity in DS rats. We also investigated whether HS intake or the associated increase in blood pressure would affect the various rat models by using an HS + hydralazine (HS+H) group (an HS-loaded normotensive model) and a normal-salt + angiotensin II (NS+AI) group (a salt-independent hypertensive model).

Methods

The Role of COMT in Salt-Sensitive Hypertension *In Vivo*

Animals

Male DS and DR rats, 6–7 weeks of age, were purchased from the Shizuoka Laboratory Animal Center (Shizuoka, Japan). The DS rats were randomly assigned to a normal-salt (NS; 0.3% NaCl), HS (8% NaCl), or HS+H group, and the DR rats were randomly assigned to the NS or HS group. Hydralazine was given in drinking water (80 mg/l) for 13 weeks. Throughout the study period, the animals were maintained at 26±2°C, with a 12 h/12 h light/dark cycle. Systolic blood pressure (SBP) was measured by the tail-cuff method every 2 weeks. For each rat, 24-h urinary specimens were collected after 4 or 13 weeks of the loading, by housing the rats in individual metabolic cages. The urine samples were analyzed for the levels of NE, DA, and creatinine. The entire protocol was devised in accordance with our institutional guidelines, and the study was approved by the ethics committee of our institute.

Evaluation of the Tissue COMT Activities

The COMT activities in the tissues were measured using a previously described method (15). Protein concentrations of

the samples were determined according to the method of Bradford (16).

Measurement of the K_m Values of COMT for NE

The K_m values of COMT for NE in the tissues were determined by varying the concentrations of NE (0.050–2.0 mmol/l and 0.5–200 µmol/l for S- and MB-COMT, respectively) (15), using the Lineweaver-Burk plot (17).

Quantification of the Urinary NE and DA Excretion

The urinary NE and DA concentrations were determined by a high-performance liquid chromatography (HPLC)–chemiluminescence detection system (18). In brief, 6 mol/l HCl (150 µl) was added to the 24-h urinary collections to prevent decomposition of the CAs. The urinary collections were centrifuged at 4,000 × *g* at 4°C for 5 min and the supernatants were diluted 1:10 with water. Then, 0.1% 50 µl trifluoroacetic acid was added to 10 µl aliquots of the supernatants, and 45 µl of 60 µl aliquots was injected into the HPLC system.

Evaluation of COMT *In Vitro*

HepG2 Cell Culture

HepG2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS) and the antibiotics penicillin and streptomycin, in a humidified incubator with 5% CO₂ at 37°C. The culture medium was changed three times every week, and the cells were subcultured every 5–6 days. Semi-confluent cells were used for further experiments.

Effects of NE and AR Antagonists on the COMT Activities *In Vitro*

To determine the regulation of the COMT activities *in vitro*, we applied NE and AR antagonists. The COMT activities were measured in the HepG2 cells 24 h after stimulation by NE (10 µmol/l) and/or clonidine, an α₂-AR agonist (3 µmol/l), prazosin, an α₁-AR antagonist (1 µmol/l), and yohimbine, an α₂-AR antagonist (1 µmol/l). The cells were collected and washed twice with phosphate-buffered saline (PBS). Then, the cells were resuspended in 1 ml of homogenizing buffer (50 mmol/l sodium phosphate buffer [pH 7.5] with 0.5 mmol/l dithiothreitol), and the COMT activity was measured as described previously (15).

RNA Isolation and Reverse Transcription–Polymerase Chain Reaction of the HepG2 Cell Lines

The expression levels of MB-COMT mRNA were measured by reverse transcription (RT)–polymerase chain reaction (PCR). Total RNAs were extracted from the HepG2 cells and cDNAs were synthesized using random hexamer primers. PCR was performed using the primers 5'-TTCTTGTCGCCCACGTTTCATG-3' (downstream; +267 to +287) and 5'-GCCGCCATCGTCGTGG-3' (upstream; +11 to +26) (19). The protocol consisted of 30 cycles of 95°C for 1 min, 54°C

Table 1. Systolic Blood Pressure, Urinary NE and DA Excretion and Plasma NE Concentrations in All the Groups

Parameter	DS (NS)	DS (HS)	DS (HS+H)	DR (NS)	DR (HS)	DS (NS+AII)
Systolic blood pressure (mmHg)						
0 week	124±1.4	113±4.8	118±2.7	112±5.2	113±3.7	119±3.4
3–4 week	134±1.4	157±2.8**	128±5.2	123±2.8	126±2.8	159±10*
13 week	129±2.8	217±8.1**	141±1.8	116±3.4	119±2.8	—
Urinary NE excretion (nmol/mmol creatinine)						
3–4 week	121±8.8	196±14*	176±25	151±12	152±4.8	142±6.1
13 week	128±25	312±46*	271±72	133±25	135±20	—
Urinary DA excretion (nmol/mmol creatinine)						
3–4 week	305±27	657±240*	521±97*	228±54	247±41	337±54
13 week	318±34	841±400*	563±50	331±15	288±54	—
Plasma NE concentration (pmol/ml)						
3–4 week	3.47±0.34	6.00±1.6	—	—	—	3.83±1.1

Data are the means±SEM of groups ($n=6$ for each group). NE, norepinephrine; DA, dopamine; DS, Dahl salt-sensitive; DR, Dahl salt-resistant; NS, normal salt (0.3% NaCl); HS, high salt (8% NaCl); HS+H, high salt (8% NaCl) plus hydralazine; NS+AII, normal salt (0.3% NaCl) plus angiotensin II. The data of systolic blood pressure and urinary NE excretion were analyzed by 2-way analysis of variance (ANOVA). The data of plasma NE concentration were analyzed by ANOVA. * $p<0.05$, ** $p<0.01$ vs. DS (NS) group at the same-week loading.

for 1 min, and 72°C for 1.5 min. The PCR products were examined by polyacrylamide gel electrophoresis and standardized by β -actin.

Quantitative Assessment of the Pre- and Postsynaptic α_2 -AR Reactivity in Salt-Sensitive Hypertension *In Vivo*

Animal

The DS rats were randomly assigned to the NS, HS, or NS+AII group. The NS+AII group was fed an NS diet and administered angiotensin II (AII) intraperitoneally (150 ng/min/kg; model 1002 micro-osmotic pump, Durect Co., Cupertino, USA) until the blood pressure reached the mean level in the HS group. Various parameters were measured as described above and plasma NE concentrations were determined by an HPLC-chemiluminescence detection system using 50 μ l of rat plasma (20). After these measurements, the SBP was confirmed by direct intra-arterial measurement as described previously (21). After the loading, the rats were pithed as described previously (22, 23).

Measurement of the Pre- and Postsynaptic α_2 -AR Reactivity

Presynaptic α_2 -AR reactivity was assessed by the effect of clonidine (10 μ g/kg, i.v.) on the inhibition of clonidine tachycardia induced by direct cardiac nerve stimulation (1 Hz, 50 V, 10 ms for 60 s) (24, 25). Postsynaptic α_2 -AR reactivity was assessed by measuring the pressor responses to clonidine (10 μ g/kg, i.v.) (24, 25). Sufficient time was allowed between each operation for the blood pressure to return to its pre-stimulation or pre-injection levels.

Statistics

Values were expressed as the mean±SEM. Statistical analysis was performed with analysis of variance (ANOVA) or 2-way ANOVA followed by Fisher's least significant difference test. A p value of <0.05 was considered to be significant.

Results

The Role of COMT in Salt-Sensitive Hypertension *In Vivo*

Blood Pressure

The mean SBP in the rats is shown in Table 1. HS loading elevated the SBP in the DS rats but not the DR rats. Furthermore, hydralazine suppressed the salt-induced hypertension in the DS rats.

Change of Tissue COMT Activities Following HS Loading

As shown in Fig. 1a, b, both the MB-COMT activities in the kidneys and liver were significantly suppressed by HS loading in the DS rats. On the other hand, the S-COMT activities in both tissues were higher in the DS (HS) group than in the DS (NS) group (Fig. 1c, d). Moreover, hydralazine treatment did not restore the COMT activities in the HS-loaded DS rats, except for the S-COMT activity in the kidneys (Fig. 2). There were no significant changes in either the MB- or S-COMT activities in the DR rats following HS loading; however, the S-COMT activities in both tissues were higher in the DR rats than in the DS rats irrespective of whether NS or HS was loaded (Fig. 1).

Fig. 1. Comparison and change of the MB-COMT activities in the kidneys (a) and liver (b), and of the S-COMT activities in the kidneys (c) and liver (d) in the DS (NS) (bold broken line), DS (HS) (bold solid line), DR (NS) (thin broken line), and DR (HS) (thin solid line) groups ($n=6$ for each group). The data were analyzed by 2-way ANOVA. * $p<0.05$, ** $p<0.01$ between the DS (NS) and DS (HS) groups. † $p<0.05$, †† $p<0.01$ between the rats of the same group subjected to the loading for 4 weeks and 13 weeks.

K_m Values of COMT for NE

The K_m values of both MB- and S-COMT for NE in the kidneys and liver are shown in Table 2. The K_m values of S-COMT were 20–30 times higher than those of MB-COMT in both tissues in all groups. Neither hydralazine administration nor HS loading significantly changed the K_m values of either MB- or S-COMT.

Change of Urinary Excretion of NE and DA

The changes in the urinary excretion of NE and DA are shown in Table 1. The urinary NE and DA excretion increased in a time-dependent manner in the DS rats after HS loading. Hydralazine treatment did not reverse these changes.

Evaluation of COMT *In Vitro*

Effects of NE and AR Antagonists on the COMT Activities and mRNA Expression Levels *In Vitro*

The effects of NE and selective α -AR antagonists on the MB-COMT activity are shown in Fig. 3. The MB-COMT activity in the cells was increased by NE treatment. The increase of MB-COMT activity by NE was inhibited by yohimbine but not by prazosin. Moreover, clonidine elevated the MB-COMT activity that was suppressed by yohimbine. The effects of NE and selective α -AR antagonists on the expression levels of MB-COMT mRNA are shown in Fig. 4. There was no significant increase in the MB-COMT mRNA expression in the cells following NE treatment.

Fig. 2. Effect of hydralazine on the MB-COMT (a, b) and S-COMT (c, d) activities in the kidneys (a, c) and liver (b, d) of DS rats ($n = 6$ for each group). The data were analyzed by ANOVA. * $p < 0.05$, ** $p < 0.01$.

Pre- and Postsynaptic α_2 -AR Reactivity in Salt-Sensitive Hypertension *In Vivo*

Blood Pressure

The SBP confirmed by direct measurement in the NS and HS group was 139 ± 3.1 and 162 ± 6.0 mmHg ($p < 0.01$), respectively, after 4 weeks. The SBP in the NS+AII group increased to 168 ± 1.9 mmHg ($p < 0.01$ vs. that in the NS group) after 3 weeks.

Changes in the NS+AII Group

The MB-COMT activities in both the kidneys and liver of the NS+AII group were similar to those in the NS group after the loading (121 ± 12 pmol/min/mg protein in the kidneys for NS+AII vs. 121 ± 6.7 for NS; 279 ± 8.8 pmol/min/mg protein in the liver for NS+AII vs. 262 ± 10 for NS). The urinary excretion of NE and DA and plasma concentrations of NE after the loading were also similar in the NS and NS+AII groups (Table 1). The plasma NE concentration in the HS group tended to be higher than those in the NS and NS+AII groups, though the difference did not reach the level of statis-

Table 2. K_m Values of Both MB- and S-COMT for NE in All the Groups

	DS (NS)	DS (HS)	DS (HS+H)	DR (NS)	DR (HS)	DS (NS+AII)
MB-COMT (kidney)						
3–4 week	8.14±0.70	10.4±1.5	—	7.76±0.88	6.75±0.99	5.83±0.11
13 week	8.62±0.71	7.97±1.1	7.62±1.1	9.90±1.3	8.75±1.3	—
S-COMT (kidney)						
3–4 week	219±13	236±4.3	—	250±11	242±9.3	240±15
13 week	234±19	205±33	242±16	272±18	255±9.6	—
MB-COMT (liver)						
3–4 week	8.52±1.2	9.77±0.51	—	9.65±0.76	11.4±1.5	5.57±0.33
13 week	11.6±0.37	11.6±0.37	12.3±1.0	12.4±1.1	12.9±1.5	—
S-COMT (liver)						
3–4 week	189±14	163±7.5	—	192±4.8	203±23	304±21
13 week	178±3.8	176±22	173±12	191±21	170±18	—

Data are the means±SEM ($\mu\text{mol/l}$) of groups ($n=3$ for each group). MB, membrane-bound; S, soluble; COMT, catechol-*O*-methyltransferase; NE, norepinephrine; DS, Dahl salt-sensitive; DR, Dahl salt-resistant; NS, normal salt (0.3% NaCl); HS, high salt (8% NaCl); HS+H, high salt (8% NaCl) plus hydralazine; NS+AII, normal salt (0.3% NaCl) plus angiotensin II.

Fig. 3. MB-COMT activities in HepG2 cells after the addition of NE or clonidine and selective α -AR antagonists ($n=9$ for each group). The data were analyzed by ANOVA. * $p < 0.05$, ** $p < 0.01$. n.s., not significant.

tical significance. The K_m values of COMT for NE were also similar to those in the other groups (Table 2).

Pre- and Postsynaptic α_2 -AR Reactivity

The presynaptic α_2 -AR reactivity was assessed by the heart rate response to clonidine. Peripheral sympathetic nerves were exogenously stimulated using a pithing rod as an electrode. Baseline values for the heart rate were not significantly different among the three groups (Table 3). The heart rate was increased by electrical stimulation to the same degree in all three groups (Table 3), and stimulation of the presynaptic α_2 -

AR by clonidine decreased the heart rate. This decrease is a marker for the presynaptic α_2 -AR reactivity and is shown in Fig. 5a. In the HS-loaded rats, however, the heart rate was not significantly lower than those in the AII-loaded and normotensive rats. The postsynaptic α_2 -AR reactivity was monitored by clonidine-induced vasoconstriction in pithed rats. Baseline values for the SBP and diastolic blood pressure (DBP) were higher in the HS- and/or AII-loaded rats than in the normotensive rats (Table 3). The clonidine-induced increases of SBP and DBP are shown in Fig. 5b and c, respectively. In the HS-loaded rats, the blood pressure response to

Fig. 4. Relative MB-COMT mRNA expression in HepG2 cells after the addition of NE and selective α -AR antagonists, using β -actin as a control ($n=5$ for each group). The data were analyzed by ANOVA.

Table 3. Absolute Values for Increase in Heart Rate by Electrical Stimulation as well as Baseline Values for Heart Rate and Systolic and Diastolic Blood Pressures in Three Groups of Pithed DS Rats

Parameter	NS	HS	NS+AII
Absolute values for increase in heart rate by electrical stimulation (beats/min)	30.7 $\pm$ 6.4	32.3 $\pm$ 13	26.7 $\pm$ 3.5
Baseline values for heart rate (beats/min)	234 $\pm$ 4.8	233 $\pm$ 6.2	241 $\pm$ 7.1
Baseline values for systolic blood pressure (mmHg)	49.5 $\pm$ 4.1	59.8 $\pm$ 3.2	62.0 $\pm$ 3.0*
Baseline values for diastolic blood pressure (mmHg)	25.8 $\pm$ 2.8	34.6 $\pm$ 2.6*	37.8 $\pm$ 2.6*

Data are the means $\pm$ SEM of groups ($n=5-6$ for each group). NS, normal salt (0.3% NaCl); HS, high salt (8% NaCl); NS+AII, normal salt (0.3% NaCl) plus angiotensin II. The data were analyzed by ANOVA. * $p<0.05$ vs. NS group.

clonidine was smaller than in the AII-loaded and control rats.

Discussion

The activities of MB-COMT in both the kidneys and liver were significantly suppressed by HS loading in DS rats, irrespective of whether or not hydralazine was administered. This result suggests that HS loading rather than the associated increase of blood pressure suppresses MB-COMT activities in DS rats. In contrast, the regulation of S-COMT activities is partly dependent on the blood pressure. While hydralazine treatment did not decrease the S-COMT activity in the liver of DS rats, the S-COMT activity in the kidneys was decreased, suggesting that it was regulated by blood pressure. The S-COMT activities in both tissues were lower in the normotensive DS rats than in DR rats fed a NS diet, which suggests that the S-COMT activities in those tissues are lower in DS rats

than in DR rats, and that this difference is genetic. The K_m values of S-COMT for NE in both tissues of all groups were 20–30 times higher than those of MB-COMT, consistent with the findings reported in a previous study (26). This result indicates that MB-COMT has higher affinity for NE than S-COMT and plays a more important role in metabolizing NE even considering that the activities of S-COMT are 5–10 times higher than those of MB-COMT. The results of our study suggest that HS suppresses the MB-COMT activities, thereby suppressing NE degradation and increasing the blood pressure. By contrast, previous reports have suggested that the suppression of COMT causes DA-dependent natriuresis, leading to salt-resistance (12–14), and that natriuresis is suppressed in DS rats because of a defect in the D_1 receptor (8, 9). We investigated whether the genetic background of DS rats or salt intake is important to the regulation of COMT and salt-sensitive hypertension.

Fig. 5. Comparison of pre- (a) and postsynaptic (b, c) α_2 -AR reactivity in DS rats; increase of systolic blood pressure (SBP) (b) and diastolic blood pressure (DBP) (c) ($n = 5-6$ for each group). The data were analyzed by ANOVA. * $p < 0.05$, ** $p < 0.01$.

We first investigated the NE feedback mechanism on MB-COMT in an *in vitro* study. NE has been found to activate α -AR more strongly than β -AR, and HepG2 cell has been reported to express both α_1 - (27) and α_2 -AR (28, 29). In the present study, exposure to NE increased MB-COMT activity in HepG2 cells, and the increase was suppressed by yohimbine, an α_2 -AR antagonist, but not by prazosin, an α_1 -AR antagonist. Furthermore, the MB-COMT activity in the cells was increased by the addition of clonidine, an α_2 -AR agonist, and the increase was also inhibited by yohimbine. However, the exposure to NE did not affect the expression level of MB-COMT mRNA. These results suggest that NE stimulated α_2 -AR of the cells to increase the MB-COMT activity, but not

the level of MB-COMT mRNA transcription. In DS rats fed an HS diet, the MB-COMT activities were suppressed despite the increase of NE release. This result suggests that the α_2 -AR reactivity in DS rats might be blunted genetically or by HS intake.

We therefore tried to determine whether the α_2 -AR in DS rats was blunted by either a genetic factor or by HS loading. We compared the α_2 -AR reactivity of DS rats subjected to HS loading with that in a model of salt-independent hypertension (AII-loaded) and that in normotensive DS rats. First, to investigate the presynaptic α_2 -AR reactivity, we assessed the inhibition of electrical stimulation-induced tachycardia when the presynaptic α_2 -AR was stimulated by clonidine. Second, to

compare the postsynaptic α_2 -AR reactivity, we monitored the clonidine-induced vasoconstriction. In pithed rats, it is possible to examine the peripheral α_2 -AR reactivity in isolation, and clonidine stimulates the postsynaptic α_2 -AR to cause vasoconstriction. The results revealed a decrease of both pre- and postsynaptic α_2 -AR reactivity following HS loading in DS rats as compared with those in the AII-loaded and control DS rats. These findings suggest that HS loading rather than its effect on the blood pressure may blunt α_2 -AR reactivity in DS rats. Previous studies reported that the density of renal α_2 -AR in DS rats increased in response to HS loading (3). In the present study, however, the reactivity of α_2 -AR was investigated in peripheral sympathetic nerve endings throughout the body, and the results revealed that the function of α_2 -AR was decreased by HS loading. It is probable that HS loading blunted α_2 -AR signaling, even if the density of renal α_2 -AR was increased. The precise mechanism of the blunting of α_2 -AR signaling by HS intake and its relation to the genetic background of DS rats are still unclear and will require further study.

In the present study, the urinary excretion of NE was increased in HS-loaded DS rats. Two mechanisms have been proposed to explain this finding: 1) a suppression of the MB-COMT activities was responsible for the higher urinary NE level; 2) a defect in the presynaptic α_2 -AR reactivity induced a high level of NE release, which resulted in an increase in its urinary excretion.

In summary, HS intake, rather than the consequent increase in blood pressure, suppressed the MB-COMT activities in DS rats by a feedback mechanism to induce DA-dependent natriuresis, consistent with the increase in urinary DA excretion. The mechanism responsible for the reduction in MB-COMT activities could involve blunting of the α_2 -AR signaling. Considering that the urinary DA excretion did not significantly increase in DR rats by HS intake, the salt-resistance of DR rats might not always depend on DA-dependent natriuresis. Rat models of genetic salt-sensitivity respond to salt loading, which may alter several signals. This is the first report that salt loading alters α_2 -AR function and MB-COMT activities. The decrease in MB-COMT activities, and the resultant decrease in the degradation of NE and increase in NE release may be involved in the development of salt-sensitive hypertension in DS rats, in whom DA-dependent natriuresis may be suppressed because of a defect in the D_1 receptor. This study could be an important step towards clarifying the association between COMT activity and hypertensive disorders, including salt-sensitive hypertension.

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