Original Article

Exclusion of the Catechol-*O*-Methyltransferase Gene from Genes Contributing to Salt-Sensitive Hypertension in Dahl Salt-Sensitive Rats

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Catechol-*O*-methyltransferase (COMT) is an enzyme that inactivates catecholamines. Several studies have suggested that this enzyme may play a role in blood pressure regulation. We previously reported that the expression levels of *Comt* mRNA in Dahl salt-sensitive (DS) rats were lower than those in Lewis (LEW) rats. However, the physiological significance of this phenomenon has not been investigated. The purpose of the present study was to evaluate the significance of lower expression of *Comt* in Dahl salt-sensitive hypertension. The *Comt* gene in DS rats has a palindromic insertion in 3′-untranslated region, which appears to be responsible for reduced mRNA stability. A genome-wide quantitative trait loci (QTL) analysis of blood pressure using 107 F2 rats indicated that a statistically significant QTL for pulse pressure was located at the *Comt* locus in chromosome 11. Microarray analysis confirmed that *Comt* was the only gene differentially expressed between DS and LEW rats in this chromosomal region. However, COMT inhibitors had no significant effects on blood pressure in either DS or LEW rats. Thus, *Comt* was excluded from the candidate genes contributing to salt-sensitive hypertension in DS rats. A true gene responsible for pulse pressure in this chromosome 11 region remains to be determined. (*Hypertens Res* 2007; 30: 459–467)

***Key Words*: *Comt*, quantitative trait loci, genetics, hypertension, salt**

Introduction

Dahl salt-sensitive (DS) rats have been widely used for investigation of salt-sensitive hypertension. More than 16 genomic regions for blood pressure (BP) regulation have been reported in this strain. However, most of the genes responsible for this salt-sensitive hypertension have not yet been confirmed (*1*–*3*).

We previously found that the expression levels of *Comt* mRNA in the kidneys of DS rats were lower than those in Lewis (LEW) rats (*4*). However, the physiological significance of this phenomenon has not been investigated.

Catechol-*O*-methyltransferase (COMT) is an enzyme which inactivates catecholamines, including dopamine (DA) and norepinephrine (NE). This enzyme is expressed in various tissues of the rat (*5*), with the liver showing the highest enzyme activity (*6*). Several studies have suggested that this enzyme may play a role in BP regulation. Spontaneously hypertensive rats (SHR) have been reported to have lower activities and less membrane-bound COMT (MB-COMT) than Wistar-Kyoto (WKY) rats (*7*, *8*). In humans, entacapone, a selective COMT inhibitor, has been reported to increase BP dose-dependently (*9*). Although these reports suggest that the

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Table 1. Primers for Sequence Analysis of the *Comt* Gene

Forward primer		Reverse primer	
P1S	5′-tgcacagcctggctgaagaaacag	P1A	5′-aacaggagacccaatgagactgca
E1S	5′-gagtcatgtggatgcattctctga	E1A	5′-ttctaacaggctctcctacaagga
E2S	5′-tcagggacatgagaggagtgtgat	E2A	5′-tctaccggatagatcagtaagacc
E3S	5′-tccagagccccaaagagatgtaga	E3A	5′-cctttcacctctacctaccatagg
E4S1	5′-atagatgctagaagtggctccacg	E4A1	5′-ctaactggtgatgcagtgcctagg
E4S2	5′-ggatgaaaggctgaattgaggcca	E4A2	5′-tgcagagtaacagcagtgtggtag
E5S	5′-tcgcataggcagctgggtccaagaa	E5A	5′-agccctggctgataactcactaca
E6S	5′-gaatggttgcaaaccatcgtatgg	E6A	5′-agccctggctgataactcactaca
E7S	5′-tgtgaaatgttcactgagcagacc	E7A	5′- acgaattggtcagtcccagactca

Primers used for sequence analysis of a promoter region and all of the exon regions including non-coding regions in the *Comt* gene are shown.

inhibition of COMT may induce higher BP, mice in which the *Comt* gene has been disrupted have been reported to be resistant to salt-induced hypertension (*10*). A natriuretic effect of COMT inhibition has also been demonstrated in rats treated with nitecapone (*11*) or entacapone (*12*). Indeed, in our previous F2 analysis, a Dahl-type *Comt* locus genotype was nominally associated with lower BP levels (*4*). The purpose of the present study was to extend our previous study and more precisely evaluate the significance of *Comt* in Dahl salt-sensitive hypertension.

Methods

Northern Blotting

Twelve-week-old DS and LEW rats ($n=4$ each) were obtained from Japan SLC (Shizuoka, Japan) and Oriental Yeast (Tokyo, Japan), respectively. Total RNA samples were prepared from various tissues including the kidneys, adrenal glands, livers and brains, using TRIzol reagent (Invitrogen, Carlsbad, USA). Poly(A)$^+$ RNA was purified from total RNA using oligotex-dT30<Super> (TaKaRa Shuzo, Shiga, Japan). Aliquots of poly(A)$^+$ RNA (1 μg) were separated by 1% denatured agarose gel electrophoresis and transferred to a Zeta-Probe membrane (Bio-Rad, Hercules, USA). After prehybridization for 1 h at 65°C, the membranes were subjected to hybridization for 16 h at the same temperature and in the same solution containing ^{32}P-labeled *Comt* or *Gapdh* cDNA fragments as probes. The cDNA fragment was prepared by reverse transcription (RT)–polymerase chain reaction (PCR) with the following amplimers: *Comt*-S, 5′-tggtttgagtacgtgctg ca; *Comt*-A, 5′-atctccatggtgagaagcct; *Gapdh*-S, 5′-ccactcaga agactgtgga; *Gapdh*-A, 5′-cctctctcttgctctcagta; and then labeled using a Prime-It II Random Primer Labeling Kit (Stratagene, La Jolla, USA). After hybridization, the membranes were washed twice (5 min each time) at room temperature with 2× SSC and 0.1% SDS, and then twice (30 min each time) at 60°C with 1× SSC and 0.1% SDS. Hybridized signals were detected and quantified by a BAS-2500 imaging system (Fuji Film, Tokyo, Japan).

Western Blotting

Anti-COMT monoclonal antibody and anti-actin polyclonal antibody were obtained from BD Transduction Laboratories (San Diego, USA) and Santa Cruz Biotech (Santa Cruz, USA), respectively. Kidneys from LEW and DS rats (12 weeks old; $n=4$) were homogenized in 250 mmol/L Tris-HCl (pH 7.4) and centrifuged at 1,000 rpm and 4°C for 10 min. The protein concentration of the post nuclear fraction was determined using a BCA Protein Assay Kit (Pierce, Rockford, USA). Equal amounts of proteins (20 μg) were separated by SDS-PAGE (10%) and transferred to a nitrocellulose membrane (Pall Corporation, East Hills, USA). After being blocked with 5% BSA, membranes were incubated with primary antibody (1:10,000 dilution) at room temperature for 1 h, and membrane-bound antibodies were visualized by horseradish peroxidase–conjugated secondary antibodies (1:10,000 dilution; 1 h) and an ECL system (Amersham Biosciences, Piscataway, USA). The expression levels were determined by densitometry.

Sequencing Analysis of the Rat *Comt* Gene

Genomic DNAs were extracted from the livers of DS and LEW rats using proteinase K and phenol (*13*). The entire sequences of the promoter region and all of the 7 exon regions, including the non-coding regions (exons 5–7), of *Comt* of DS and LEW rats were determined. The primers used for the sequencing are shown in Table 1.

Sequence analyses revealed that an insertion (212 bps) was present in exon 4 in DS rats. The sequence of this insertion was palindromic. The secondary structure was predicted using Genetyx software version 8 (Genetyx, Tokyo, Japan).

Construction of Reporter Plasmids and Reporter Analysis

To evaluate the possible effect of the 3′-untranslated region (3′-UTR) of the *Comt* mRNA on its mRNA stability, the 3′-UTRs of the *Comt* mRNA from DS and LEW rats were subcloned into the 3′-UTR region of the luciferase expression vector. The 3′-ends of *Comt* mRNA in the kidneys of DS and LEW rats were determined using the 3′RACE System for the Rapid Amplification of cDNA Ends Kit (Invitrogen) and the following primers: *Comt*-3RACE-F1, 5′-gtagacggcttggagaaggc; and *Comt*-3RACE-F2, 5′-gtcttgatccctcagcctgc. We subcloned the 3′-UTRs of *Comt* mRNA from DS and LEW rats into the *Eco*RI site of the pAcGFP1-C1 vector (BD Biosciences Clontech, Palo Alto, USA) in a forward direction. The 3′-UTR region was amplified by *Comt*-3UTR-A (5′-atc caaatatgtagatctttaatttct), which corresponded to the 3′-terminal region of *Comt* mRNA, and by *Comt*-3RACE-F2.

Madin-Darby canine kidney (MDCK) cells were cultured in Dulbecco's modified Eagle medium (DMEM) with 10% fetal calf serum and transiently cotransfected with the above reporter plasmids, pGL3-control (Promega, Madison, USA) and phRL-SV40 vector (Promega) by the Lipofectamine2000 reagent (Invitrogen). After being incubated for 24 h, the cells were washed with 1× PBS and then used for the following experiments. First, to compare the amounts of AcGFP expression constructs in transfected cells, total DNA samples were prepared using a DNeasy Tissue Kit (QIAGEN, Hilden, Germany). The DNA level of AcGFP was analyzed by PCR analysis using the primers AcGFP-S (5′-cctgatcgagctgaatggcg) and AcGFP-A (5′-tgctggtagtggtcggccag), with 18S ribosomal DNA as a control (QuantumRNA 18S Internal Standards Kit; Ambion, Austin, USA). Second, to compare the mRNA decay rate among the transfectants, actinomycin D (Nacalai Tesque, Kyoto, Japan) (5 μg/mL) was added to the culture medium and cells were incubated for varying lengths of time (up to 6 h). Total RNA samples were isolated from these cells using an RNeasy Mini Kit (QIAGEN) according to the manufacturer's protocol. RT-PCR was carried out with the following amplimers: the primer pair for AcGFP described above and Luc-S (5′-cgttatttatcggagttgcagttg) and Luc-A (5′-aagaag gagaatagggttggcac). The intensity of the PCR product from the AcGFP reporter constructs relative to that of the PCR product from the pGL3-control vector was assessed by a densitometer. Third, to measure the fluorescence intensity of AcGFP expressed in the transfectants, cells were lysed using Triton-based lysis buffer (1% Triton X-100, 20 mmol/L Tris, pH 7.4, 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L EGTA, 2.5 mmol/L sodium pyrophosphate, 1 mmol/L β-glycerol phosphate, 1 mmol/L Na_3VO_4, 1 μg/mL leupeptin, and a 0.1% protease inhibitor mixture [Nacalai Tesque]). The fluorescence intensity of AcGFP was then measured using a 1420 ARVO-HTS system (PerkinElmer, Wellesley, USA) (excitation: 485 nm; emission: 535 nm). Fourth, luciferase activity in these transfectants was also determined using a Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's protocol.

Genotyping of F2 Rats

The details of F2 analysis have been described previously (*1*). Briefly, male DS rats were mated with female LEW rats to produce F1 rats, and F1 rats were then intercrossed to produce an F2 population consisting of 107 male rats. F2 rats were started on an 8% NaCl diet (Oriental Yeast, Tokyo, Japan) at 5 weeks of age according to the protocol described by Rapp *et al*. (*3*). Radiotelemetry devices (Data Sciences International, Arden Hills, USA) were implanted into the lower abdominal aorta of F2 rats at 9 weeks of age using sodium pentobarbital as an anesthetic agent (25 mg/kg, i.p.). At 14 weeks of age, BP and heart rate were continuously measured for 44 h (daytime 7:00 AM to 7:00 PM; nighttime 7:00 PM to 7:00 AM), and the data obtained during the latter 24 h were used for analyses. The results were analyzed using Fluclet TM software (Dainippon Pharmaceutical, Osaka, Japan) (*14*).

Genotyping was performed by PCR using appropriate PCR primer pairs (custom-made by Amersham Pharmacia Biotech, Piscataway, USA), based on information from the Rat Genome Database (http://rgd.mcw.edu/) and Ratmap (http://ratmap.gen.gu.se/). We found sequence variations between DS and LEW rats in several genes, and these polymorphisms were also included in the genotyping data. Genotyping of *Comt* was performed by PCR-electrophoresis using the primer pair E4S2 and E4A2 (Table 1). We separately analyzed quantitative trait loci (QTLs) for daytime systolic BP (D-SBP), daytime diastolic BP (D-DBP), nighttime systolic BP (N-SBP), nighttime diastolic BP (N-DBP), and pulse pressure (PP) levels using MapManager QTLb20.6 (*15*). We performed Quick Test to obtain significant thresholds for the BP and PP values. The likelihood ratio statistics (LRSs) for suggestive, significant, and highly significant loci were calculated to be 12.2, 17.7, and 25.9, respectively.

Microarray Analysis

Male DS and LEW rats ($n=3$ each) were fed an 8% NaCl diet starting at 5 weeks of age for 8 weeks. Total RNA was isolated from DS and LEW kidneys with TRIzol reagent (Invitrogen), and subjected to microarray analysis. Procedures for the microarray analysis were essentially identical to those reported previously (*16*). The rat genome GeneChip® Rat Genome 230 2.0 arrays were used in the present study. The resulting data were analyzed with GeneChip Operating Software (Affymetrix, Santa Clara, USA). Three independent experiments were performed to compare expression levels of mRNAs between the kidneys of salt-loaded DS and salt-loaded LEW rats (the raw data have been submitted to NCBI http://www.ncbi.nih.gov/geo/; GEO accession number: GSE4800). We selected genes whose expression levels were modulated more than 2-fold or less than 0.5-fold in all three

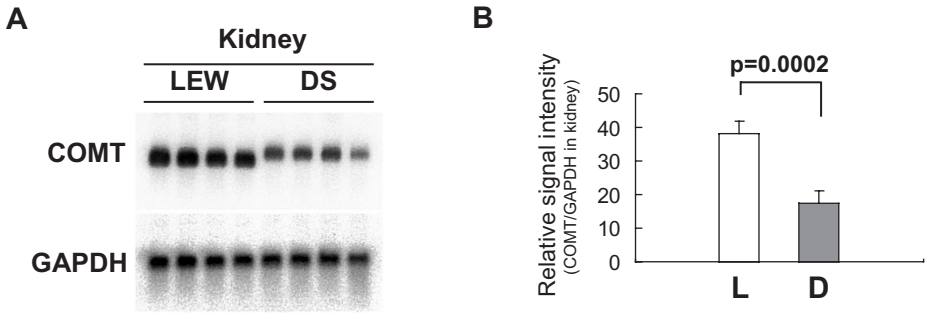

Fig. 1. *Differential expression of Comt mRNA in LEW and DS tissues. A: RNA samples were prepared from kidneys from 12-week-old LEW and DS rats (n=4 each). Poly(A)⁺ RNA (1 μg) was subjected to Northern blotting. B: Hybridized signals were detected and quantified by a BAS-2500 imaging system. The signal intensities of Comt mRNA relative to Gapdh mRNA are indicated. Data are the mean±SD.*

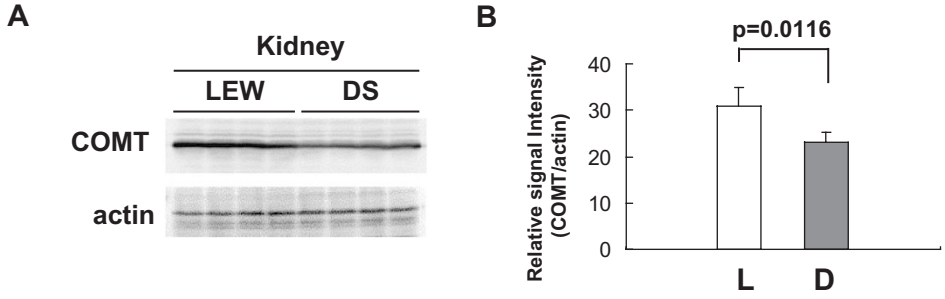

Fig. 2. *Protein levels of COMT in LEW and DS kidneys. A: Tissue lysates were prepared from 12-week-old LEW and DS kidneys (n=4). Equal amounts (20 μg) of post nuclear fraction were subjected to Western blotting. B: Detected signals were quantified by densitometry and the signal intensities of COMT relative to actin are indicated. Data are the mean±SD.*

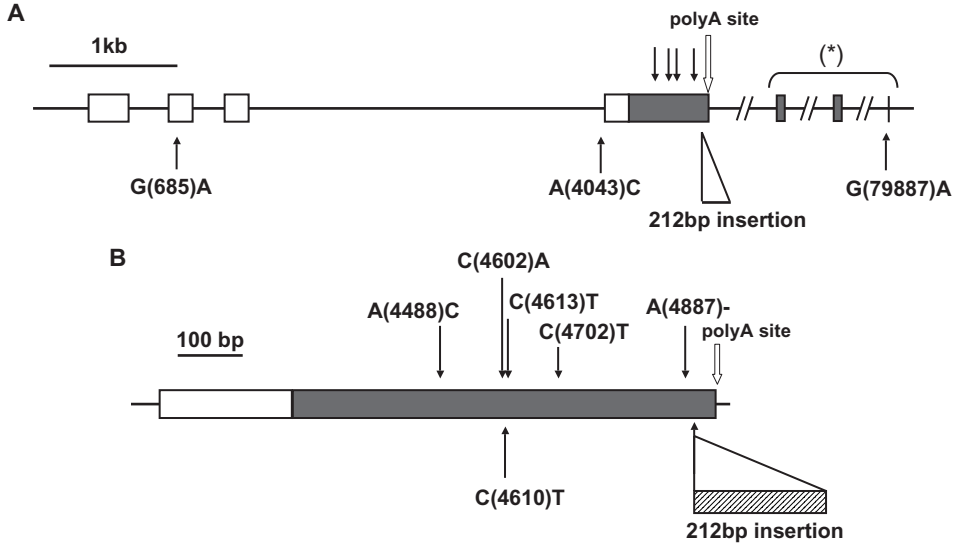

Fig. 3. *Scheme of the Comt gene and the positions of the determined polymorphisms. A: A promoter region and all of the 7 exon regions are shown. B: The region of exon 4 is expanded. The 5′- and 3′-UTR regions are indicated by gray boxes, and coding regions are indicated by white boxes. The 10 polymorphisms found are indicated by arrows. *According to 3′RACE, a polyA site exists at the end of exon 4, and therefore exons 5–7 are not used in DS and LEW kidneys.*

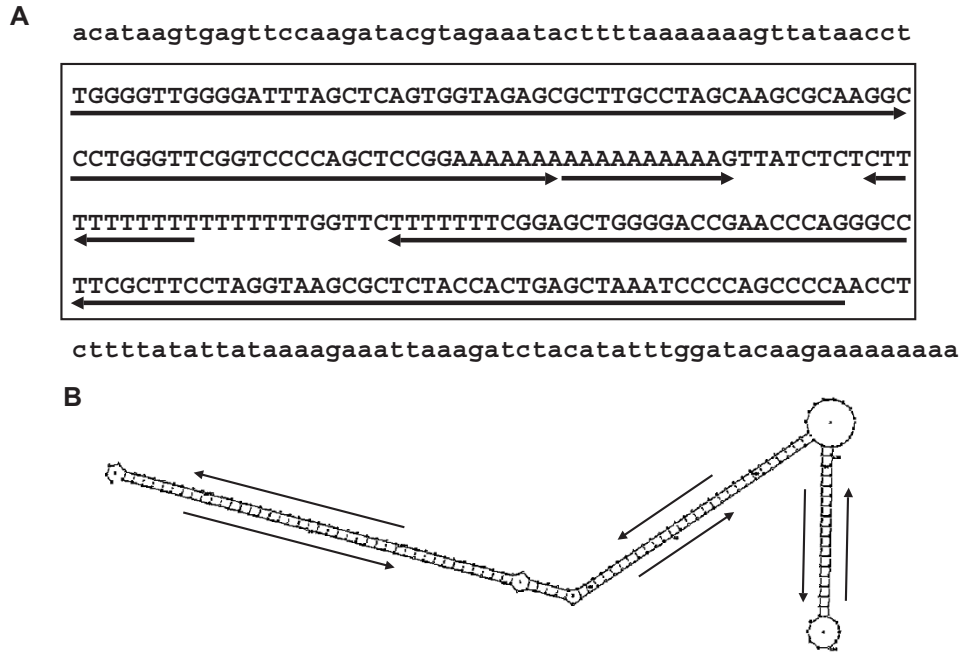

Fig. 4. *Entire sequences of a 212-bp insertion and the secondary structure prediction of its transcript. A 212-bp palindromic fragment was inserted in the 3′ end of exon 4 in the DS Comt gene. A: The entire sequences of this insertion are shown. B: The secondary structure of its transcript was predicted using Genetyx software version 8. The computational prediction indicated the existence of a stable and tight stem-loop structure.*

independent assessments. Genes with expression levels below 100 in both strains were excluded due to unreliability. We then selected genes that were localized to the chromosome (Ch)11 QTL region.

Effects of COMT Inhibitors on BP Levels

The effect of oral administration of COMT inhibitors on BP levels was studied in the DS and LEW rats on a high salt diet (8% NaCl). Male DS rats at 8 weeks of age were randomly divided into two groups: the COMT inhibitor–treated group (n=3, a high salt diet and the COMT inhibitor) and the control group (n=3, a high salt diet only). Radiotelemtery devices were implanted into the lower abdominal aorta of DS rats at 9 weeks of age. Rats were allowed 1 week of recovery from surgery. Both oral administration of COMT inhibitor and salt loading were started at 11 weeks of age. The COMT inhibitor–treated group received 3,5-dinitrocatechol (*17*) suspended in water (30 mg/kg body weight) by gastric gavage every 48 h for 18 days. The control group was given the same amount of water as vehicle (0.5 mL/100 g body weight). The identical protocol was applied to the LEW rats (n=3 in the COMT-treated group, n=4 in the control group) under a high salt diet (8% NaCl) using the same dose (30 mg/kg body weight/48 h).

All the statistical analyses were performed using the JMP statistical package (SAS Institute, Cary, USA). Differences in BP over time between the control and treatment groups were evaluated by MANOVA.

The present study was conducted in accordance with the current guidelines for the care and use of experimental animals of the National Cardiovascular Center.

Results

Confirmation of the Differential Expression of *Comt*

The expression levels of *Comt* mRNA in the kidneys and adrenal glands from DS and LEW rats were determined by Northern blotting (Fig. 1A). The expression levels of *Comt* mRNA in DS kidneys were 2-fold lower than those in LEW kidneys (p=0.0002) (Fig. 1B). COMT protein levels were also lower in DS kidneys than in LEW kidneys (p=0.0116) (Fig. 2). *Comt* mRNA in DS rats appeared to be longer than that in LEW rats. RT-PCR analysis indicated that expression levels of *Comt* mRNA in livers, adrenal glands and brains were significantly lower in DS than in LEW rats (data not shown).

Sequencing and 3′-End Analyses of *Comt*

Sequence analysis of *Comt* in DS and LEW rats identified 10 polymorphisms (Fig. 3). Of these, 7 polymorphisms were

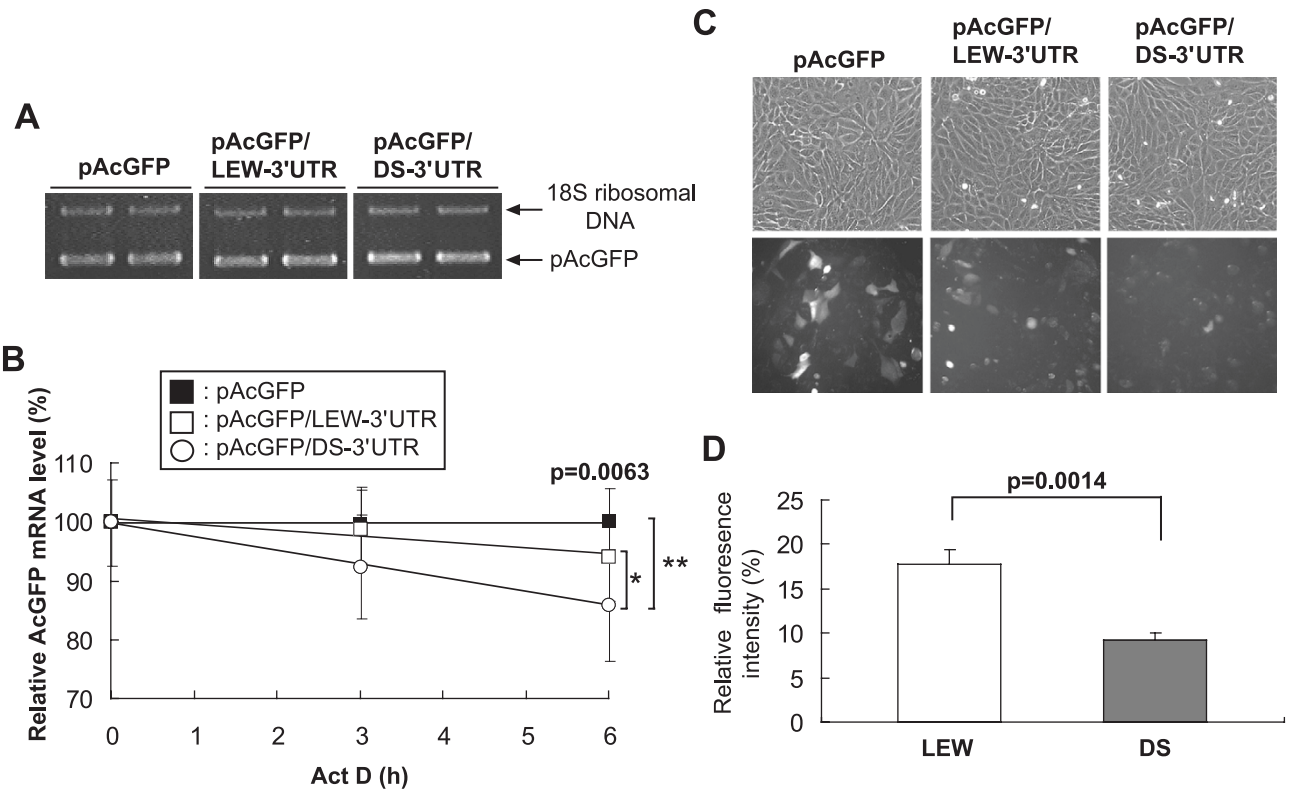

Fig. 5. *Inhibitory effects of DS Comt 3′-UTR on transcription and translation. A: The DNA level of the transgene was analyzed by PCR using 18S ribosomal DNA as a control. B: The AcGFP/luciferase mRNA ratio was determined by RT-PCR. The ratio at time 0 h in each transfectant was defined as 100%. Data are the mean±SD. At 6 h after treatment with actinomycin D (Act D), the mRNA levels were significantly different among the transfectants ($p=0.0063$). The mRNA level of AcGFP with DS-type 3′-UTR was significantly decreased compared to those of AcGFP (**$p<0.01$, $n=6$) and LEW-type 3′-UTR (*$p<0.05$, $n=6$). C: At 24 h after transfection, the cells were washed with 1× PBS and AcGFP expression was observed by fluorescence microscopy (×200). D: The fluorescence intensity/luciferase activity ratio in the transfectants was measured. The ratio in cells that had been transfected with the pAcGFP control was defined as 100%. Data are the mean±SD.*

Table 2. Association between the Genotype of the *Comt* Gene and Blood Pressure Values

	Comt genotype			*p*-value
	DD	DL	LL	
Number	28	57	22	
D-SBP (mmHg)	132.08±12.33	127.40±12.50	138.18±11.45	0.0025
D-DBP (mmHg)	89.24±9.60	86.75±10.11	92.15±8.56	0.0809
N-SBP (mmHg)	143.07±12.44	138.94±12.86	150.28±11.92	0.0021
N-DBP (mmHg)	98.70±9.81	96.92±11.49	102.01±8.79	0.1614
D-PP (mmHg)	42.84±4.73	40.65±4.80	46.03±4.47	<0.0001
N-PP (mmHg)	44.37±5.60	42.03±5.23	48.27±6.65	0.0001

Data represent mean±SD. Association analyses with blood pressure (BP) and pulse pressure (PP) values were performed for the genotype of the *Comt* gene of 107 F2 rats. D-SBP, daytime systolic BP; D-DBP, daytime diastolic BP; N-SBP, nighttime systolic BP; N-DBP, nighttime diastolic BP; D-PP, daytime PP; N-PP, nighttime PP.

identified in the region corresponding to the 3′-UTR in exon 4 (Fig. 3B). No polymorphisms were detected in the promoter region sequenced (up to −1 kb). Intriguingly, a 212-bp palindromic fragment was inserted near the 3′-end of exon 4 in DS rats. The entire sequence of this insertion is shown in Fig. 4A. The computational prediction of the secondary structure indi-

Table 3. The Genes Localized to the Chromosome (Ch)11 Showing Significant Difference in the Expression Levels between DS and LEW Kidneys

Gene title	Gene symbol	GenBank ID	Alignments	Experiment 1			Experiment 2			Experiment 3		
				LEW	DS	log ratio	LEW	DS	log ratio	LEW	DS	log ratio
A disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 1	*Adamts1*	NM_024400	Chr11:25433792–25443150 (−)//95.74//q11	134.7	801.2	2.6	237.5	960.6	2.0	198.5	848.3	2.1
Catechol-*O*-methyl-transferase	*Comt*	NM_012531	Chr11:84617363–84622303 (+)//84.53//q23	2,854.3	490.3	−2.5	5,764.7	468.6	−3.6	4,353.2	387.8	−3.5

Microarray analyses in the Dahl salt-sensitive (DS) and Lewis (LEW) kidneys identified 2 differentially expressed genes on the Ch11. *Comt* in the Ch11 QTL region is the only gene that is differentially expressed between DS and LEW kidneys.

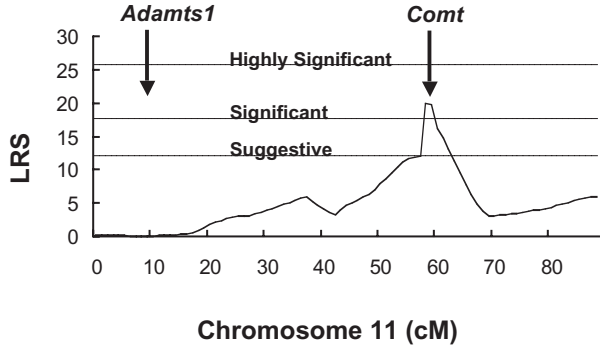

Fig. 6. *Interval mapping analyses of pulse pressure QTL on chromosome 11. Interval mapping analyses of pulse pressure values were performed on chromosome 11. Likelihood ratio statistics (LRS) plots for PP are shown.*

cated the existence of a stable and tight stem-loop structure (Fig. 4B). The 3′RACE revealed that exon 4 was the end of *Comt* mRNA in DS and LEW kidneys, and the insertion appears to be the reason for the longer mRNA in DS rats detected by Northern blotting.

Effect of 3′-UTR on the Differential Expression of *Comt* in DS and LEW Rats

To examine whether the insertion in exon 4 is responsible for the reduced expression of *Comt* mRNA in DS kidneys, we constructed reporter vectors (AcGFP expression vectors) with either DS or LEW 3′-UTR, and then transfected MDCK cells with these reporter constructs. At 24 h after transfection, the DNA level of the transgene was not significantly different among the transfectants (Fig. 5A). Next, to clarify whether the mRNA decay rate is enhanced in DS-type transfectants, we examined the rate of AcGFP mRNA decay after the inhibition of RNA polymerase II-dependent transcription by actinomycin D (Act D) (*18*). The AcGFP-DS-type 3′-UTR mRNA exhibited a faster decay than both the control AcGFP and AcGFP-LEW-type 3′-UTR mRNA (Fig. 5B). Moreover, the cells were observed by fluorescence microscopy after transfection (Fig. 5C), and the fluorescence intensity of AcGFP and luciferase activity in these transfectants were then measured. The relative fluorescence intensity of AcGFP per luciferase activity in the DS-type transfectants was significantly decreased relative to that in LEW-type transfectants ($p=0.0014$) (Fig. 5D). Similar results were obtained by Western blotting using AcGFP-specific antibody (Clontech) (data not shown). These findings indicate that DS-type *Comt*-3′-UTR decreases the stability of its mRNA, leading to a reduction of the protein level.

QTL Analyses for BP and PP

We previously performed analyses to determine the QTL region that influenced BP using an F2 cohort from DS and LEW rats (*1*). In the present study, we newly determined the genotype of *Comt* in 107 F2 rats by PCR based on insertion of a 212-bp sequence. Marker regression analysis indicated that the Ch11 *Comt* region was not significantly associated with any of the 4 BP values (below a suggestive LRS=12.2). However, the *Comt* genotype was nominally associated with systolic blood pressure (SBP) values, but not with diastolic blood pressure (DBP) values (Table 2). Intriguingly, QTL analysis indicated that the *Comt* locus corresponds to a significant locus for PP (Fig. 6). PP values of F2 rats with the DS genotype were lower than those of F2 rats with the LEW genotype (Table 2).

Identification of Differentially Expressed Genes

We performed microarray analyses using RNA samples from DS and LEW kidneys. Within the Ch11 QTL region for PP, *Comt* was the only gene that was differentially expressed between DS and LEW kidneys (Table 3).

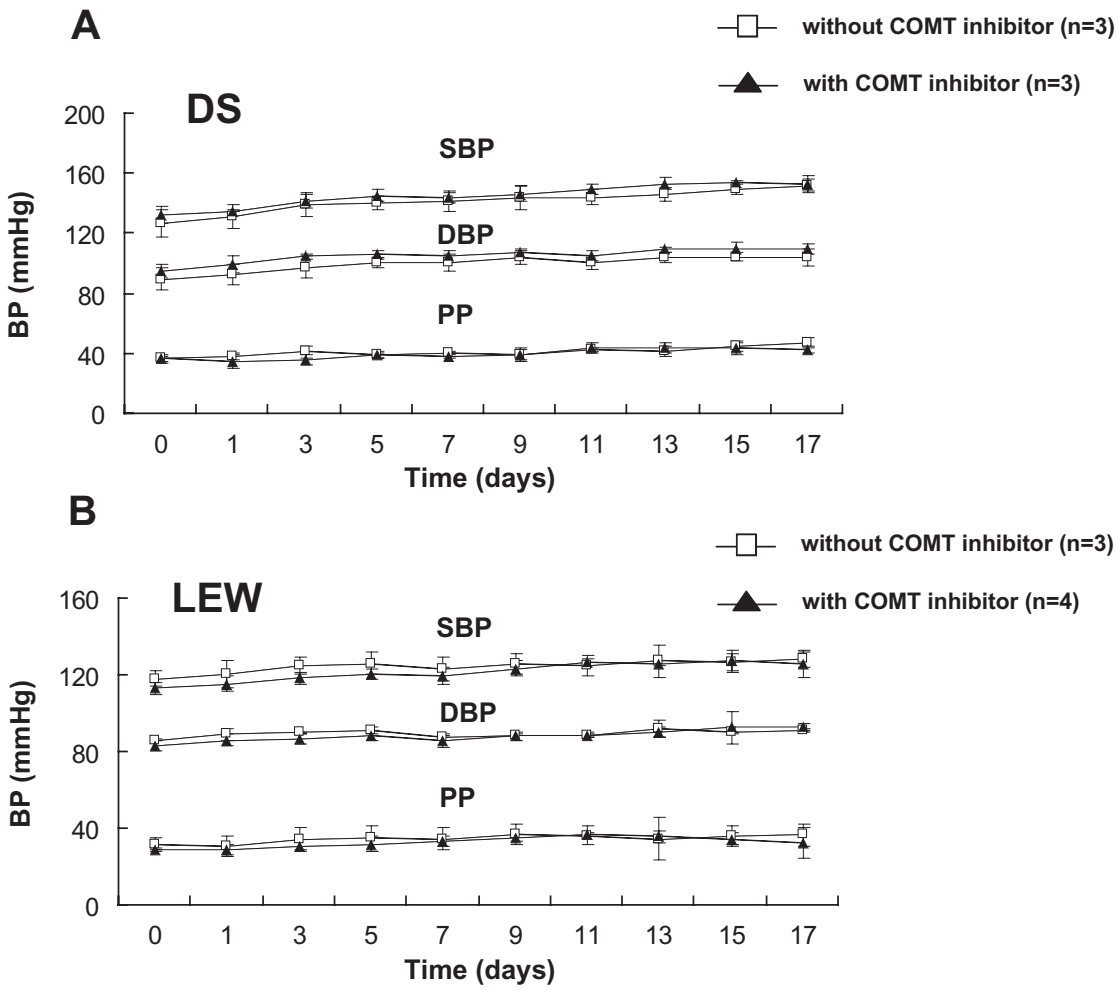

Fig. 7. *Effect of COMT inhibitor on blood pressure (BP) levels in DS and LEW rats. Comparisons of BP values as determined by radiotelemetry between the control (□) and the COMT inhibitor–treated groups (▲) in the DS (A) and LEW rats (B). The dates of administration of drug or vehicle are shown. Day 0 denotes baseline. Each point represents a 24-h mean±SD. SBP, systolic BP; DBP, diastolic BP; PP, pulse pressure.*

Effects of COMT Inhibitors on BP Levels

Figure 7A and B show the effects of oral administration of the COMT inhibitor, 3,5-dinitrocatechol, on SBP, DBP and PP throughout the study period in the DS and LEW rats, respectively. No significant differences in BP values over time were observed between the COMT inhibitor–treated group and the control group in either DS (by MANOVA; $p=0.37$, 0.21 and 0.15 for SBP, DBP and PP, respectively) or LEW rats (by MANOVA; $p=0.38$, 0.26 and 0.56 for SBP, DBP and PP, respectively).

Discussion

Recently, mice with disrupted *Comt* have been reported to be resistant to salt-induced hypertension (*10*). Previously, we noted that the expression levels of *Comt* mRNA in DS kidneys were lower than those in LEW kidneys (*4*). Thus, the present study was undertaken to investigate the hypothesis that the reduced expression of *Comt* mRNA in DS kidneys is a genetic compensatory mechanism to prevent further aggravation of salt-sensitive hypertension in DS rats.

Although QTL analysis indicated that the *Comt* locus was a suggestive QTL for PP and *Comt* was the only one gene differentially expressed between DS and LEW rats in this chromosomal region, COMT inhibitors had no significant effects on BP in either DS or LEW rats. The dose of 3,5-dinitrocatechol used in our study was 30 mg/kg, which was higher than that in the earlier study where its inhibitory effect on COMT activity as well as serum formation of 3-*O*-methyldopa was demonstrated in rats at an oral dose of 3 mg/kg (*17*). It is likely that inhibition of COMT in the whole body by oral administration of the COMT inhibitor does not mimic the tissue-specific differential expression of COMT in DS rats.

However, these pharmacological experiments strongly suggest that *Comt* is not the gene contributing to BP regulation in DS rats.

We were not able to identify other intriguing candidate genes in this Ch11 region based on microarray analyses, which mainly focused on protein-coding genes. Similar situations have been observed in the genetic analyses of spontaneously hypertensive rats, another prevalently used genetic model for hypertension (*19*). The concept of a gene is now being re-evaluated (*20*). Large transcriptional surveys have indicated that more than half of transcripts are non-coding (*21*, *22*). The genome is now considered to be full of overlapping transcripts (*20*). Moreover, protein-coding sequences are now considered to have no clear beginning or end (*23*, *24*). To advance our understanding of polygenic diseases, including hypertension, we need to understand the total genomic function and the RNA world.

In conclusion, the reduced expression of *Comt* mRNA in DS kidneys appears to be genetically determined by the polymorphism in 3′-UTR through mRNA stability. However, this reduced expression of COMT in DS rats does not seem to contribute to salt-sensitive hypertension or salt resistance. The true gene contributing to pulse pressure in this *Comt* locus remains to be determined. Further studies on non-coding RNA will be required to clarify all of the genes contributing to salt-sensitive hypertension in DS rats.

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