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Effects of Transgenic Expression of Dopamine Beta Hydroxylase (*Dbh*) Gene on Blood Pressure in Spontaneously Hypertensive Rats

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Summary

The spontaneously hypertensive rat (SHR) is the most widely used animal model of essential hypertension and left ventricular hypertrophy. Catecholamines play an important role in the pathogenesis of both essential hypertension in humans and in the SHR. Recently, we obtained evidence that the SHR harbors a variant in the gene for dopamine beta hydroxylase (*Dbh*) that is associated with reduced adrenal expression of *Dbh* mRNA and reduced DBH enzymatic activity which correlated negatively with blood pressure. In the current study, we used a transgenic experiment to test the hypothesis that reduced *Dbh* expression predisposes the SHR to hypertension and that augmentation of *Dbh* expression would reduce blood pressure. We derived 2 new transgenic SHR-*Dbh* lines expressing *Dbh* cDNA under control of the Brown Norway (BN) wild type promoter. We found modestly increased adrenal expression of *Dbh* in transgenic rats versus SHR non-transgenic controls that was associated with reduced adrenal levels of dopamine and increased plasma levels of norepinephrine and epinephrine. The observed changes in catecholamine metabolism were associated with increased blood pressure and left ventricular mass in both transgenic lines. We did not observe any consistent changes in brainstem levels of catecholamines or of mRNA levels of *Dbh* in the transgenic strains. Contrary to our initial expections, these findings are consistent with the possibility that genetically determined decreases in adrenal expression and activity of DBH do not represent primary determinants of increased blood pressure in the SHR model.

Keywords

Spontaneously hypertensive rat; Transgenic; Dopamine beta hydroxylase; Catecholamines; Blood pressure; Left ventricular mass

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Conflict of Interest

There is no conflict of interest.

Introduction

Increased sympathoadrenal activity may play an important role in the pathogenesis of high blood pressure in humans with essential hypertension (DeQuattro and Feng 2002, Esler 2000) and in spontaneously hypertensive rats (SHR) (Cabassi *et al.* 1998), the most widely used animal model of essential hypertension and left ventricular hypertrophy. Increased sympathoadrenal activity was observed in young SHR (Nagatsu *et al.* 1974, Grobecker *et al.* 1975) as well as in normotensive humans with family history of hypertension which suggests that it might represent a primary genetic determinant of risk for hypertension (Esler 2000). Recently, we used integration of transcriptional, biochemical and linkage analyses in the adrenal tissue of the BXH/HXB recombinant inbred (RI) strains, derived from the SHR and Brown Norway (BN) progenitors, and obtained evidence that genetically determined expression of the dopamine beta hydroxylase (*Dbh*) gene in the RI strains is regulated in *cis* and correlates with DBH enzymatic activity (Jirout *et al.* 2010). DBH catalyzes the oxidative hydroxylation of dopamine to form norepinephrine. Norepinephrine is the principal transmitter in the sympathetic nervous system which maintains heart rate and blood pressure. The SHR *Dbh* allele was associated with a lower expression and activity of DBH which was accompanied by an increase in dopamine, a substrate for DBH. The functional significance of the *Dbh* variant of SHR origin is suggested by the fact that a quantitative trait locus (QTL) for adrenal dopamine levels was detected in the vicinity of *cis*-regulated expression QTL (eQTL) for *Dbh*. These results suggested "…that lower *Dbh* in the young SHR presents a 'bottleneck' in catecholamine biosynthesis, leading to dopamine accumulation and catecholamine depletion in adrenergic cells, which then contributes to the pathogenesis of hypertension" (Jirout *et al.* 2010). It has been suggested that reduced DBH activity in the brain leads to higher dopamine and lower norepinephrine levels which might represent a possible mechanism connecting reduced central DBH activity with increased blood pressure (Howes *et al.* 1984, Cornish *et al.* 1997, van den Buuse 1997, Takami *et al.* 1993). However, in our previous studies, we did not determine *Dbh* expression levels or catecholamine levels in the brainstem (Jirout *et al.* 2010). The aim of the current study was to perform a transgenic rescue experiment to test the hypothesis that genetically determined reduced expression of *Dbh* gene and DBH activity represents a primary defect predisposing the SHR to increased blood pressure. We anticipated that transgenic augmentation of *Dbh* expression would reduce blood pressure in the SHR strain.

Methods

Animals

Transgenic SHR lines SHR/Ola-Tg(*Dbh*-*Dbh*)611 and SHR/Ola-Tg(*Dbh*-*Dbh*)625 (hereafter referred to as the transgenic SHR-*Dbh* lines 611 and 625) were derived by microinjecting fertilized eggs with a mix of the Sleeping Beauty (SB) construct containing BN *Dbh* cDNA (identical to the SHR cDNA) under control of the BN *Dbh* promoter and mRNA of the SB100X transposase (Ivics *et al.* 2014). Transgenic rats were detected using PCR with the following primers: *Dbh*-F 5′-ATC TGG AAT CCG CAT CTT TG-3′ and *Dbh*-R 5′-GGT GGA CAG TCA CAG CAT TC-3′. Positive transgenic rats showed 170 bp fragment of cDNA together with 540 bp fragment of endogenous genomic DNA. Blood pressures, heart

rates and left ventricular mass were determined in 3-month-old males from transgenic SHR-*Dbh* lines 611 and 625 (N=8 per group) and age-matched SHR controls (N=8). The rats were housed in an air-conditioned animal facility and allowed free access to standard diet and water. All experiments were performed in agreement with the Animal Protection Law of the Czech Republic and were approved by the Ethics Committee of the Institute of Physiology of the Czech Academy of Sciences.

Blood pressure measurement

Arterial blood pressures were measured continuously by radiotelemetry (Data Sciences International, St. Paul, USA) in paired experiments in conscious, unrestrained male rats. All rats were allowed to recover for at least 7 days after surgical implantation of radiotelemetry transducers before the start of blood pressure recordings. Pulsatile pressures were recorded in 5-s bursts every 10 min throughout the day and night, and 24-h averages for systolic and diastolic arterial blood pressure were calculated for each rat. The results from each rat in the same group were then averaged to obtain the group means.

Biochemical analyses

Adrenal glands and brainstem were harvested, immediately frozen and stored at −80°C. Tissues for biochemical phenotyping were homogenized in 1.9 ml of 10 mM MES buffer (pH=6.0), using a Tissuemizer (Tekmar, Cincinnati, OH, USA). Frozen tissues were placed into pre-cooled (4°C) buffer, homogenized, spun at 13 000 g for 1 min to clear debris, the supernatants divided into aliquots and placed immediately on dry ice to freeze. Aliquots were stored at −80°C. Catecholamines (dopamine, epinephrine and norepinephrine) in plasma as well as in adrenal and brainstem extracts were measured by an enzyme immunoassay using commercially available 3-CAT ELISA kit (BA E-6600; LDN, Germany) following manufactures's instructions. A standard curve was drawn from known standards and absolute amounts of the catecholamines in samples were estimated from the curve. Catecholamine values were normalized to milligram weight tissue.

Gene expression determined by real time PCR

Total RNA was extracted from tissues using Trizol reagent (Invitrogen), and cDNA was prepared and analyzed by real-time PCR testing using QuantiTect SYBR Green reagents (Qiagen, Inc.) on an Opticon continuous fluorescence detector (MJ Research). The following primers were used to amplify both endogenous and transgenic *Dbh* genes: *Dbh*-F 5′-ACC TCG TCA TGC TCT GGA CT-3′ and *Dbh*-R 5′-GAG CAG GGA TAG GCT GTT TG-3′. Gene expression levels were normalized relative to the expression of peptidylprolyl isomerase A (*Ppia*) (cyclophilin) gene which served as the internal control using the following primers: *Ppia*-F 5′-AGC ATA CAG GTC CTG GCA T-3′ and *Ppia*-R 5′-TCA CCT TCC CAA AGA CCA C-3′. Results were determined in triplicate. The expression results are reported as fold increases in relation to the mean expression level in the SHR strain that was arbitrarily defined as 1.

Statistical analysis

The data are expressed as means ± SEM. Strain differences were analyzed by one-way ANOVA with adjustments for multiple comparisons by Holm-Sidak testing. Normality of distribution was tested by Shapiro-Wilk method. The 24-h mean values of systolic and diastolic blood pressures were analyzed by repeated measures ANOVA with grouping effect of treatment and repeated measurements in time. Statistical significance was defined as $P<0.05$.

Effects of Dbh transgenic expression on catecholamine metabolism

In comparison to the SHR controls, both SHR-*Dbh* transgenic lines showed a similar pattern of changes in catecholamine concentrations, except for norepinephrine levels in the brainstem which were reduced in line 611 but not significantly affected in line 625 (Table 1). Both transgenic lines versus SHR controls showed increased plasma levels of epinephrine and norepinephrine while levels of plasma dopamine were not significantly different. Both lines showed significantly reduced dopamine levels in the adrenals while levels of adrenal epinephrine and norepinephrine were not significantly different when compared to SHR controls (Table 1).

Results

Derivation of SHR-Dbh transgenic lines and expression of endogenous and transgenic Dbh genes

Altogether we obtained 7 SHR-*Dbh* transgene positive founders from 57 rats born after transfer of microinjected fertilized eggs into foster mothers which is approximately 12 % effectiveness. Two randomly selected male founders (lines 611 and 625) were crossed with SHR females and heterozygotes intercrossed to obtain homozygous SHR-*Dbh* transgenic rats. Figure 1 shows combined expression of *Dbh* transgene and *Dbh* endogenous gene in SHR-*Dbh* transgenic lines versus nontransgenic SHR controls that express only the *Dbh* endogenous gene. The adrenal expression of *Dbh* in both SHR-*Dbh* transgenic lines was significantly increased when compared to the expression of endogenous *Dbh* gene in nontransgenic SHR controls. On the other hand, no significant strain differences were observed in *Dbh* expression in the brainstem (Fig. 1).

Effects of transgenic expression of Dbh on blood pressure and left ventricular mass

Figure 2 shows results for systolic blood pressures and left ventricular mass. Transgenic expression of *Dbh* was associated with significantly increased systolic blood pressures and higher left ventricular mass in both SHR-*Dbh* transgenic lines when compared to SHR controls. No significant differences were observed in diastolic blood pressure and heart rates when both transgenic lines were compared to SHR controls (data not shown).

Discussion

Results of our previous studies in the BXH/HXB recombinant inbred (RI) strains strongly suggested that genetically reduced (*cis*-regulated) adrenal expression of *Dbh* and DBH activity might represent a primary defect predisposing the SHR to increased blood pressure

(Jirout *et al.* 2010). The responsible mechanisms remained unclear although it has been proposed that reduced DBH activity might somehow be linked to increased blood pressure through higher dopamine and lower norepinephrine levels in the brain. To test the possible causal role of reduced adrenal DBH activity in the pathogenesis of hypertension in the SHR, we transgenically expressed *Dbh* cDNA under control of the BN strain *Dbh* promoter in SHR. We expected that if reduced adrenal expression of *Dbh* was contributing to hypertension in the SHR, transgenic augmentation of adrenal *Dbh* expression might attenuate hypertension in the SHR. However, compared to non-transgenic SHR, the SHR-*Dbh* transgenic lines showed greater blood pressures with greater expression levels of total *Dbh* mRNA in the adrenals. The SHR-*Dbh* transgenic lines also showed greater levels of norepinephrine and epinephrine in plasma and significantly lower levels of dopamine in adrenal tissue compared to those in the non-transgenic SHR. There were no consistent differences in brainstem catecholamine levels or in brainstem *Dbh* mRNA levels between the strains. In the transgenic SHR, the increases in circulating levels of norepinephrine and epinephrine were also associated with increases in left ventricular mass.

Although we previously found an association between increased blood pressure and reduced adrenal expression and activity levels of DBH, other investigators have reported that decreased blood pressure is associated with genetic deficiency of DBH and/or downregulation of DBH activity. For example, genetic deficiency of DBH is associated with hypotension in both humans and knockout mice (Robertson *et al.* 1991, Swoap *et al.* 2004). In addition, inhibition of DBH activity reduced hypertension in the SHR (Ohlstein *et al.* 1987). Furthermore, it has been demonstrated that inhibition of cerebral DBH using intracerebroventricular injections of anti-DBH antibody conjugated to the ribosomal toxin saporin leads to reduced blood pressure and heart rate in male Sprague-Dawley rats (Chang *et al.* 2013). These reports strongly suggest that DBH deficiency, both peripheral and central, may promote reduced blood pressure. In addition, it has been reported that in humans, the C-970T or C-2073T variants in the promoter region of DBH are associated with higher plasma DBH levels and increased blood pressure (Chen *et al.* 2010, Chen *et al.* 2011). Consistent with these reports, the results of the current study suggest that genetically mediated increases in expression and activity of adrenal DBH, not decreases in DBH expression and activity, may be contributing to the pathogenesis of increased blood pressure in some circumstances. The results of the previous linkage studies in recombinant inbred strains (Jirout *et al.* 2010) showing an association between reduced adrenal DBH activity and increased blood pressure may have been related to reverse causation effects, and or to blood pressure effects of genetic variants linked to the cis-acting expression QTL that appeared to account for variation in DBH activity and blood pressure.

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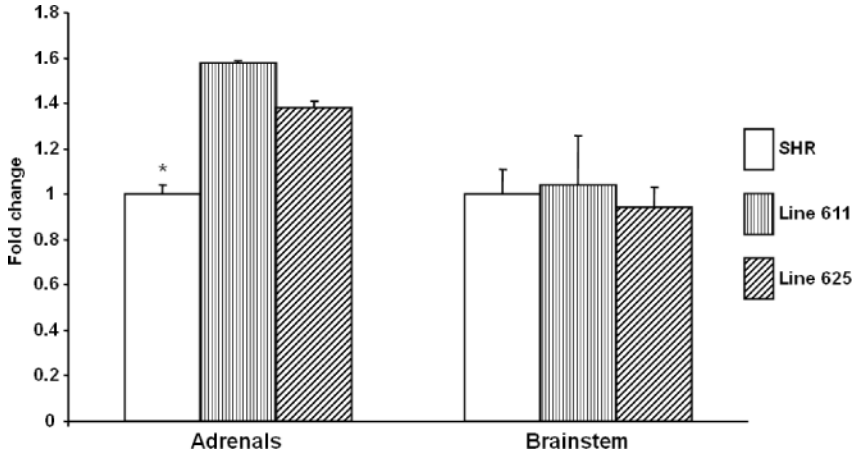

Fig. 1.

Expression of *Dbh* (endogenous + transgenic) gene in the adrenals and brainstems from SHR-*Dbh* transgenic lines 611 and 625 versus SHR controls. SHR control expression values have been arbitrarily set at 1. *denotes P<0.05.

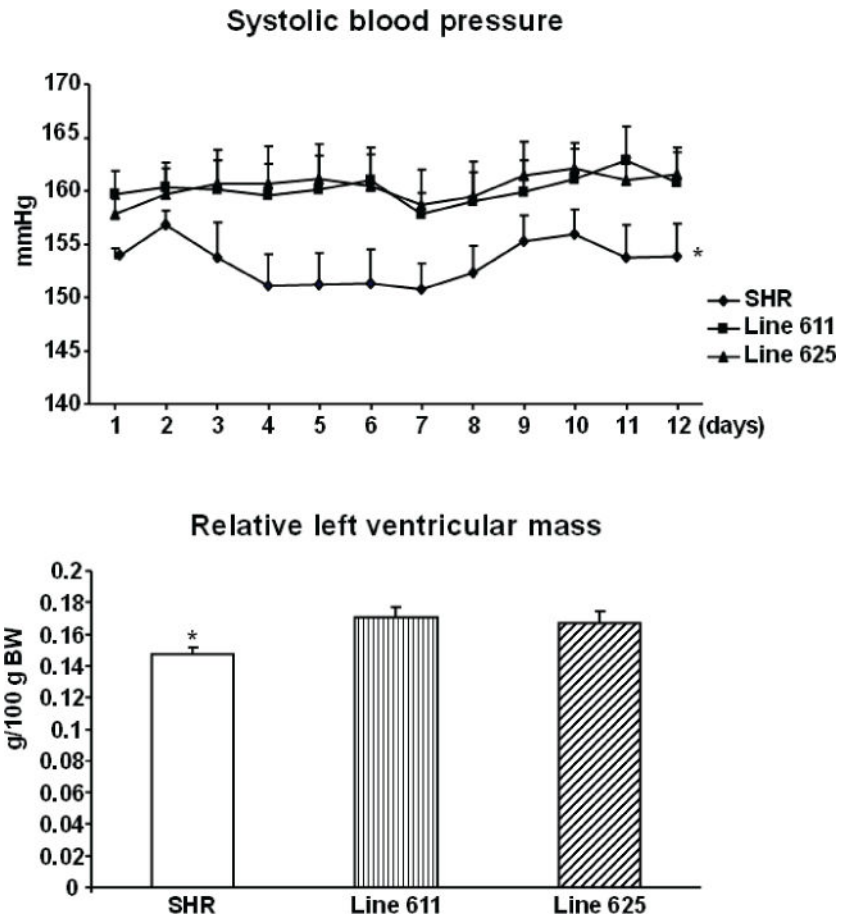

Fig. 2.

Systolic blood pressure measured by telemetry and relative left ventricular mass were significantly increased in SHR-*Dbh* transgenic lines 611 and 625 when compared to nontransgenic SHR controls. *denotes $P<0.05$ determined by repeated measures of ANOVA for blood pressure and by one-way ANOVA for relative left ventricular mass.

Table 1

Levels of catecholamines in plasma, adrenals and brainstem in SHR versus SHR-*Dbh* transgenic rats of lines 611 and 625.

Trait	SHR	SHR-*Dbh* Line 611	SHR-*Dbh* Line 625
Plasma			
Epinephrine (ng/ml)	10.8±4.6	78.7±17.5 *	76.4±25.2 *
Norepinephrine (ng/ml)	28.0±6.6	111.8±33.3 *	45.5±5.6 *
Dopamine (ng/ml)	27.7±3.6	32.3±3.0	33.9±3.5
Adrenals			
Epinephrine (ng/μg protein)	2829±361	2459±94	2352±166
Norepinephrine (ng/μg protein)	431±48	404±27	365±26
Dopamine (ng/μg protein)	50.6±16.6	2.8±1.7 *	7.2±4.3 *
Brainstem			
Epinephrine (pg/μg protein)	137±27	149±33	165±35
Norepinephrine (pg/μg protein)	3079±149	2182±129 *	2848±171
Dopamine (pg/μg protein)	582±150	710±214	794±187

* denotes P<0.05 compared to SHR group; N=8 per each group.