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

M. PRAVENEC¹, V. LANDA¹, V. ZÍDEK¹, P. MLEJNEK¹, J. ŠILHAVÝ¹, S. A. MIR², S. M. VAINGANKAR², J. WANG³, T. W. KURTZ³

¹Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic, ²University of California, San Diego, USA, ³University of California, San Francisco, USA

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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 expectations, 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.

Key words
Spontaneously hypertensive rat • Transgenic • Dopamine beta hydroxylase • Catecholamines • Blood pressure • Left ventricular mass

Corresponding author
M. Pravenec, Institute of Physiology of the Czech Academy of Sciences, Vídeňská 1083, 14220 Prague 4, Czech Republic. Fax: (420)241062488. E-mail: pravenec@biomed.cas.cz

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 manufacturers’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.

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 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).

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).

Table 1. Levels of catecholamines in plasma, adrenals and brainstem in SHR versus SHR-Dbh transgenic rats of lines 611 and 625.

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

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

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.
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.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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References


