Role of the D_{1A} Dopamine Receptor in the Pathogenesis of Genetic Hypertension


Georgetown University Medical Center, Departments of *Pediatrics, †Physiology and Biophysics, and **Medicine, Washington, DC 20007; ‡Monash University, Neuroscience Unit, Wellington Road, Department of Anatomy, Clayton 3168, Australia; §University of Virginia Health Sciences Center, Department of Pathology, Charlottesville, Virginia 22908; ¶‡Department of Pharmacology, University of California, San Diego, La Jolla, California 92161; ¶§University of Iowa Medical Center, Department of Pediatrics, Iowa City, Iowa 52242; ¶¶National Institute of Neurological Disorders and Stroke, Experimental Therapeutics Branch, Bethesda, Maryland 20892; and **National Institute of Child Health and Human Development, Laboratory of Mammalian Genes and Development, Bethesda, Maryland 20892

Abstract

Since dopamine produced by the kidney is an intrarenal regulator of sodium transport, an abnormality of the dopaminergic system may be important in the pathogenesis of hypertension. In the spontaneously hypertensive rat (SHR), in spite of normal renal production of dopamine and receptor density, there is defective transduction of the D_{1} receptor signal in renal proximal tubules, resulting in decreased inhibition of sodium transport (Na^+/H^+ exchanger [NHE] and Na^+/K^+ ATPase activity) by dopamine. To determine if impaired D_{1} receptor regulation of NHE in proximal tubules is related to hypertension, studies were performed in a F2 generation from female Wistar Kyoto (WKY) and male SHR crosses. A D_{1} agonist, SKF 81297, inhibited (37.6±4.7%) NHE activity in brush border membranes of normotensive F2s (systolic blood pressure < 140 mm Hg, n = 7) but not in hypertensive F2s (n = 21). Furthermore, a D_{1} agonist, SKF 38393, when infused into the renal artery, dose dependently increased sodium excretion in normotensive F2s (n = 3) without altering renal blood flow but was inactive in hypertensive F2s (n = 21). Since the major D_{1} receptor gene expressed in renal proximal tubules is the D_{1A} subtype, we determined the importance of this gene in the control of blood pressure in mice lacking functional D_{1A} receptors. Systolic blood pressure was greater in homozygous (n = 6) and heterozygous (n = 5) mice compared to normal sex matched litter mate controls (n = 12); moreover, the mice lacking one or both D_{1A} alleles developed diastolic hypertension. The cosegregation with hypertension of an impaired D_{1} receptor regulation of renal sodium transport and the development of elevated systolic and diastolic pressure in mice lacking one or both D_{1A} alleles suggest a causal relationship of the D_{1A} receptor gene with hypertension. (J. Clin. Invest. 1996. 97:2283–2288.) Key words: hypertension • dopamine • dopamine receptor • sodium transport • genetics

Introduction

Essential hypertension is a heterogeneous disease thought to be due to genetic and environmental factors that interact to increase blood pressure. The blood pressure difference between a hypertensive individual and a normotensive control has been attributed to the influence/interaction of 2–6 genetic loci (1). Several candidate genes have been suggested for roles in the pathogenesis of hypertension (2–6). Some forms of hypertension are dependent upon or aggravated by sodium loading. Recently, homozygous mice carrying a mutant proANP allele were found to have hypertension which was aggravated by sodium loading (6). Because dopamine produced by renal tubules is an important intrarenal regulator of sodium excretion, a dysfunction of the renal dopaminergic system has been proposed as a pathogenetic factor in genetic hypertension (7). Dopamine can regulate blood pressure by actions in the central and peripheral nervous system as well as in target endocrine (e.g., adrenal) glands and transporting organs (7). Dopamine produced by renal proximal tubules can serve as an intrarenal natriuretic factor by direct tubular action, independent of hemodynamic mechanisms (7); this paracrine effect is most prominent during sodium loading (8–11). In conscious, as well as in anesthetized animals, up to 60% of sodium excretion during an acute volume load is mediated by D_{1} receptors (7); this is due both to an increase in renal dopamine production and to an increased sensitivity of sodium transporters to dopaminergic inhibition (7, 12). In the spontaneously hypertensive rat (SHR), the ability of exogenous and endogenous renal dopamine to engender a natriuresis is impaired (13–15). Since renal dopamine levels in genetic models of hypertension are not lower than those found in their normotensive controls (16, 17), the impaired intrarenal paracrine effect of dopamine in these animal models of hypertension appears to be receptor or postreceptor-mediated. In the SHR, the diminished ability of dopamine and D_{1} agonists to inhibit Na^+/H^+ exchange and Na^+/K^+ ATPase activity in the kidney is due, at least in part, to their impaired ability to stimulate production of second messenger effector enzymes (adenyl cyclase, phospholipase C) (14, 18–22). This phenomenon is not due to differences in D_{1} receptor density, D_{1} antagonist affinity, G-proteins, effector enzyme activity per se, renal brush border Na^+/H^+ exchanger or Na^+/K^+ ATPase activity (18, 19). The decreased ability of dopamine and D_{1} agonists to stimulate adenyl cyclase activity

1. Abbreviations used in this paper: BBMV, brush border membrane vesicles; GFR, glomerular filtration rate; NHE, sodium/hydrogen exchanger; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto.
in the renal proximal tubules of SHR occurs as early as 3 wk of age and is receptor, organ, and nephron segment specific (18, 23, 24). Thus, dopamine and D₁ agonists stimulate adenyllyl cyclase activity in the stratum and the cortical collecting duct to a similar extent in the SHR and in its normotensive control, the Wistar-Kyoto rat (WKY) (18, 23, 24); parathyroid hormone stimulates adenyllyl cyclase activity in renal proximal tubules to a similar extent in SHR and WKY (18). Impaired transduction of the renal D₁ receptor has also been reported in another model of genetic hypertension, the Dahl salt sensitive rat (14, 22).

Many physiologic and biochemical differences between hypertensive and normotensive strains have been explained by genetic drift and random fixation of alleles in loci that may not be involved in blood pressure regulation (3). Because fortuitously fixed characteristics in the SHR, such as hyperactivity and hyper-reactivity to stress, detract from the suitability of the SHR as a model of genetic hypertension, the hypertensive/hyper-reactive trait was bred out of the SHR. One strain, the WK-HA, exhibits the hyperactive phenotype without hypertension; the other strain, the WK-HT, exhibits hypertension without the accompanying hyperactivity. The D₁ receptor/adenyllyl cyclase coupling was defective in the renal proximal convoluted tubule of the WK-HT at 3 wk of age but was normal in the WK-HA strain; in the WK-HT this coupling defect was present in the proximal tubule but not in the cortical collecting duct, another site of dopamine action (25). Studies were designed to determine if the decreased ability of D₁ agonists to inhibit Na⁺/H⁺ exchange activity in renal brush border membranes in vitro or to induce a natriuresis in vivo is associated with hypertension in a F₂ population of rats generated from WKY and SHR crosses. Two D₁-like receptor genes have been cloned from mammals (26–28). Although both receptor genes are expressed in the kidney, the D₁A subtype is more abundant than the D₁B subtype in renal proximal tubules. We, therefore, studied the effects of deletion of D₁A receptors in mice generated by homologous recombination (29).

Methods

Reagents. D₁ agonists (SKF 81297 and SKF 38393), and the D₁ antagonist, SCH 23390 were purchased from Research Biochemicals International (Natick, MA), dopamine, 3-isobutyl-1-methyl xanthine, and parathyroid hormone from (Sigma Chemical Co., St. Louis, MO), and 125I-SCH 23982 from Dupont NEN (Boston, MA).

Co-segregation studies in rats

F₂ generation of rats produced by cross breeding F₁ rats bred from WKY female and SHR male crosses using the stock at the University of California at San Diego (UCSD). The rats were maintained on standard Purina rat chow at UCSD and shipped to Georgetown University at 10–14 wk of age. The rats were continued on Purina rat chow and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F₂ rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and studied between 31–41 wk of age.
pPNT (29). The gene was targeted in the J1 line of ES cells (a gift from R. Jaenisch, Whitehead Institute). Homologous recombination events were identified by Southern blotting. Blastocyst injections were then performed. Mice heterozygous for the D1A receptor deletion were generated by mating male chimeras with C57BL/6 females and mice homozygous for the D1A receptor deletion were generated by heterozygous mouse intermarriages (29). Only male mice were studied and their normal sex matched litter mates served as controls. For the radioligand binding and cAMP studies, the mice were anesthetized with pentobarbital (0.5 mg/10 grams body weight, IP) or Avertin (1.25 mg/10 grams body weight). The femoral artery was cannulated (PE 50 stretched to 180 μm) and blood pressure monitored electronically by Cardiomax II as above. In other mice, after anesthesia, the kidneys were obtained for radioligand binding and cAMP assay.

Radioligand binding. The cortices were separated from the medullae. The cortex was then homogenized in ice-cold buffer containing in mM: 140 KCl, 10 Hepes, 7 Tris-HCl, 3 Tris-Base, 10 mannose, 2.5 glutamine, and 1 μg/ml leupeptin, in a glass/Teflon® homogenizer. The homogenate was polytronized on ice at a setting of seven for 15 s, separated by a chase of 30 s. The homogenate was then centrifuged 1000 g for 10 min and then the supernatant was centrifuged at 35,000 g for 20 min. The pellet was reisolated in KCl buffer (150 mM KCl, 25 M MES) and pelleted at 35,000 g for 20 min. The pellet was resuspended in sucrose buffer containing (in mM): 250 sucrose, 0.1 PMSEF, 0.5 DTT, and Tris-Cl, pH 7.6 and flash frozen until the radioligand binding assay was performed. Prior to radioligand binding the pellet was thawed on ice and washed in Dulbecco’s PBS (pH 7.4) containing glucose (1 grams/liter). Radioligand binding was performed using 3H-SCH 23982, a D1A antagonist; non-specific binding was defined by 1 μM SCH 23390 as described previously (18, 20, 22–25).

Cyclic AMP accumulation. The renal cortex was minced using a straight edge razor. The minceate was then sieved through a polypropylene mesh (210 μm) to obtain renal proximal tubules (21, 24) and washed with DME buffer containing 1 μg/ml leupeptin. The suspension was then centrifuged at 500 g for 5 min at 4°C. The pellet was then resuspended in DMEM with 1 μg/ml leupeptin and equilibrated for 15 min at 37°C. Then, IBMX (5 × 10^{-4} M, final concentration) was added and immediately afterward (1 μl each) vehicle (DDEH/O), dopamine (5 × 10^{-4} M), or parathyroid hormone (10^{-7} M) was added and allowed to incubate for 15 min. EDTA was then added immediately to final concentration of 4 mM. Aliquots were removed for protein analysis and the remaining sample was boiled for 3 min. The tubes were then centrifuged at high speed in a microcentrifuge for 3 min and the resulting supernatant was frozen until cAMP was determined by radioimmunoassay (18, 20, 22–25).

Figure 1. Apparent cosegregation of the decreased efficacy of the D1 dopamine agonist, SKF 81297 (5 × 10^{-7} M), to inhibit Na⁺/H⁺ exchange (NHE) activity in BBMV from F2 rats; SKF 81297 inhibited NHE only in rats with systolic blood pressures <140 mm Hg. Offspring (F1) of male SHR and female WKY rats (University of California, San Diego) were mated to produce F2 offspring. Data are mean±SE. *P < 0.05 vs. other groups, analysis of variance for repeated measures, Scheffe’s test.

Statistical analyses. Within group comparisons were made using ANOVA for repeated measures and among group comparisons were made using ANOVA. Scheffe’s test was used for post hoc test in both instances. When the left and the right kidney were being compared, paired t test was used. A P < 0.05 was considered significant.

Results

Studies in rats. Na⁺—H⁺ exchanger activity: In female F2 rats with systolic blood pressures < 140 mm Hg, the D1 agonist, SKF 81297 (5 × 10^{-7} M), inhibited Na⁺—H⁺ exchange activity; the inhibition was clearly attenuated in those with systolic blood pressure between 140–159 mm Hg and in those with systolic blood pressure > 159 mm Hg (Fig. 1). Indeed, a significant inverse correlation existed between systolic or mean arterial pressure and percent inhibition of Na⁺—H⁺ exchange activity by the D1 agonist, SKF 81297 (5 × 10^{-7} M) (linear regression, r = 0.54, P = 0.04, polynomial 2 regression, r = 0.45, P < 0.01). In the parental strains, basal NHE activity was similar in WKY and SHR (1.51 ± 0.07 nmol/mg protein/min, n = 4, respectively); however, SKF 81297 (5 × 10^{-7} M) decreased NHE activity to a greater extent in WKY than SHR (0.67 ± 0.07 vs. 1.10 ± 0.05, nmol/mg protein/min, n = 4, respectively) (P < 0.01 ANOVA, Scheffe’s test). The percent inhibition of NHE activity by SKF 81297 (5 × 10^{-7} M) was also greater (P < 0.01 t test) in the WKY than in the SHR (56 ± 3% vs. 30 ± 5%, n = 4/group, respectively). Even at a higher concentration of the agonist (5 × 10^{-5} M), there was still a differential inhibition (P < 0.01 t test) between WKY and SHR (100 ± 0 vs. 88 ± 1%, n = 2/group, respectively).

To determine if the differences noted in vitro can also be observed in vivo, we used a D1 dopamine agonist, SKF 38393, in doses previously found to produce a natriuresis without increasing renal blood flow or GFR in WKY(13, data not shown). The infusion of the agonist into the right renal artery of anesthetized rats in Period 3 (P3) after two control periods (P1 and P2), increased sodium excretion and urine flow in a dose-related manner in normotensive but not hypertensive male F2s (Fig. 2). No changes were observed in the contralateral uninfused kidney (data not shown).
Studies in mice. Radioligand binding studies revealed negligible specific binding in the kidney of mice homozygous for the mutant allele (Table I). D1 dopamine receptor density in heterozygous mice was intermediate between the normal and the homozygous mice. Basal cAMP accumulation in renal proximal tubules was similar among the three groups. Dopamine stimulated cAMP production in renal proximal tubules in normal mice, did so inconsistently in the heterozygous and not at all in the D1 receptor deficient homozygous mice. This effect was specific, since parathyroid hormone stimulated cAMP accumulation to a similar extent among the three groups of mice. Blood urea nitrogen, serum electrolytes and calcium were similar among the groups (data not shown). Of interest is the fact that systolic blood pressure (on normal chow) was greater in homozygous and heterozygous mice compared to normal sex matched litter mate controls; moreover, the mice lacking one or both D1 alleles developed diastolic hypertension (Fig. 3). The mice lacking both D1 alleles were smaller than the wild type or the heterozygous mice. The growth retardation of the homozygous mice has been documented previously (29). However, feeding the mice with moistened food allowed growth to ~70% of their littermate controls as reported previously (Table I and reference 29). Fertility was not impaired in the mutant mice.

### Discussion

These studies show that the decreased renal tubular response to D1 agonists cosegregates with hypertension in a F2 generation of rats produced by cross breeding F1 rats bred from WKY female and SHR male crosses. In the experimental set up of these studies, any inhibition of the Na+/H+ exchanger by...

### Table I. Studies in Renal Tubules in Mice Lacking a Functional D1A Receptor

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal mice</th>
<th>Heterozygous mice</th>
<th>Homozygous mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>B&lt;sub&gt;max&lt;/sub&gt; (pmol/mg protein)</td>
<td>2.1±0.4 (n = 4)*</td>
<td>0.57±0.18 (n = 4)*</td>
<td>0 (n = 4)</td>
</tr>
<tr>
<td>K&lt;sub&gt;d&lt;/sub&gt; (nM)</td>
<td>40±18 (n = 4)*</td>
<td>29±10 (n = 4)</td>
<td>0 (n = 4)</td>
</tr>
<tr>
<td>Basal cAMP accumulation (fmol/mg protein/min)</td>
<td>29.1±8.9</td>
<td>27.7±9.6</td>
<td>22.6±5.6</td>
</tr>
<tr>
<td>Effect of dopamine (5 × 10&lt;sup&gt;-5&lt;/sup&gt; M)</td>
<td>46.2±7.8&lt;sup&gt;±11&lt;/sup&gt;</td>
<td>52.6±11.7</td>
<td>23.0±5.8</td>
</tr>
<tr>
<td>cAMP (fmol/mg protein/min)</td>
<td>923±257&lt;sup&gt;§&lt;/sup&gt;</td>
<td>870±252&lt;sup&gt;§&lt;/sup&gt;</td>
<td>858±177&lt;sup&gt;§&lt;/sup&gt;</td>
</tr>
<tr>
<td>Effect of parathyroid hormone (10&lt;sup&gt;-7&lt;/sup&gt; M)</td>
<td>24.5±1.0 (n = 12)*</td>
<td>23.4±1.0 (n = 5)*</td>
<td>17.3±1.4 (n = 6)</td>
</tr>
<tr>
<td>cAMP (fmol/mg protein/min)</td>
<td>99±2 (n = 12)</td>
<td>88±9 (n = 5)</td>
<td>80±9 (n = 6)</td>
</tr>
</tbody>
</table>

* P < 0.05 vs. homozygous (ANOVA, Scheffe’s test). ** P < 0.05 normal vs. null mutants (t test). § P < 0.05 vs. basal (t-pair). n = 5 unless indicated.
dopamine is independent of second messengers but probably mediated by G-proteins (30). We have shown that the Na\(^+\)/H\(^+\) exchanger in the renal brush border membranes of both SHR and WKY is inhibited to a similar extent by non-hydrolyzable guanine nucleotide analogues. These data indicate that the decreased ability of a D\(_1\) agonist to inhibit the exchanger in the SHR or in the hypertensive F2s is not due to a defect of G-protein-end effector action but rather on more proximal sites, presumably on the receptor itself (34). The in vitro studies are corroborated by the in vivo studies, since the diuretic and natriuretic response to exogenous dopamine is impaired in human hypertensive subjects (35). A preliminary report suggests that the natriuretic response to exogenous dopamine is impaired in human essential hypertension, especially those with salt-sensitive hypertension; those patients have been reported in human essential hypertension in Okamoto-Aoki spontaneously hypertensive rats. Furthermore, functional ablation of the D\(_1\) receptor gene produces diastolic hypertension in homozygous and heterozygous mice. The molecular mechanism of the D\(_1\) receptor defect remains to be defined, since a mutation in the coding region of this receptor gene has not been described (34). Nevertheless, our results indicate that a nonfunctional D\(_1\) dopamine receptor contributes to genetic hypertension.

**Acknowledgments**

This project was supported in part by National Institutes of Health grants HL-23081, DK-49361, DK447-56, DK-39308, and DK-42185.

**References**


**Figure 3.** Arterial blood pressure in mice completely lacking a functional D\(_{1A}\) dopamine receptor (homozygous) compared with normal and heterozygous mice. The mice (~ 3 mo of age, see Table I for details) were anesthetized with either Avertin (10 mg/ml, 0.3 ml/mouse in two divided within 15 min as needed) or pentobarbital (5 mg/kg body weight). Arterial blood pressure was recorded from the femoral artery. Systolic, diastolic, and mean arterial pressure were greater in mice homozygous and heterozygous for the mutant D\(_{1A}\) allele. Data are mean±SE. \( \ast P < 0.05 \) vs. other groups, analysis of variance (ANOVA), Scheffe’s test.

**Figure 3.**

![Blood Pressure Graph](https://example.com/blood-pressure-graph.png)

- Normal (n=12)
- Heterozygous (n=5)
- Homozygous (n=6)

\[ P < 0.05 \] normal vs others

ANOVA

**ANOVA**

**Heterozygous**

**Homzygous**

**Normal**

**Systolic BP**

**Diastolic BP**

**Mean BP**

**Table I.**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean BP (mm Hg)</th>
<th>Systolic BP (mm Hg)</th>
<th>Diastolic BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>12</td>
<td>110</td>
<td>130</td>
<td>90</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>5</td>
<td>115</td>
<td>135</td>
<td>95</td>
</tr>
<tr>
<td>Homozygous</td>
<td>6</td>
<td>120</td>
<td>140</td>
<td>100</td>
</tr>
</tbody>
</table>

*P < 0.05 normal vs others ANOVA*


