Essential hypertension is a major risk factor for the development of cardiovascular disease. It is a heterogeneous disease in which both genetics and environmental influence blood pressure. Dopamine affects cardiovascular regulatory mechanisms by its actions on renal hemodynamics and ion and water transport and by its regulation of hormones and humoral agents, such as aldosterone, catecholamines, endothelin, prolactin, proopiomelanocortin, renin, and vasopressin. In addition, dopamine can control blood pressure by acting on neuronal cardiovascular centers, heart, and arterial and venous vessels.

Dopamine exerts its actions by occupation of the D₁-like (D₁ and D₅) and D₂-like (D₂, D₃, and D₄) family of cell surface G protein–coupled receptors. We have reported that disruption of the D₁, D₂, D₃, and D₄ receptors leads to hypertension in mice, via specific pathophysiological mechanisms. The cardiovascular consequences of disruption of the D₄ receptor have not been reported. D₄ receptors are expressed in the heart, renal collecting ducts, juxtaglomerular cells, and in brain nuclei known to affect blood pressure, but their role in cardiovascular regulation is unknown. Loci near the D₄ receptor gene (11p15.5) have been linked to hypertension, and polymorphisms of the D₄ receptor gene are associated with hypertension. Therefore, we tested the hypothesis that the D₄ receptor deficiency increases blood pressure, possibly via increased angiotensin II type 1 receptor expression.

**Key Words:** dopamine receptors, angiotensin II mice, hypertension, angiotensin II, endothelin, vasopressin

**Methods**

**Generation of D₄ Dopamine–Receptor Mutant Mice**

The original F₁ hybrid strain (129/Sv × C57BL/6) carrying a mutant form of the D₄ dopamine receptor was initially generated and backcrossed to C57BL/6 mice. Heterozygous (D₄+/−) mice were mated to obtain D₄−/− and D₄+/+ littermates, and the D₄−/− mice were backcrossed with C57BL/6 mice to obtain sixth- and tenth-generation mice in the Department of Physiology and Pharmacology, Oregon Health and Science University. We used sixth-generation mice for acute studies and tenth-generation mice for chronic and immunoblotting studies. All of the animals were genotyped and treated in accordance with National Institutes of Health guidelines for ethical treatment and handling of animals in research.

**Revised Form**

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Blood Pressure Measurement

**Blood Pressures Under Anesthesia**

Mice were anesthetized with pentobarbital (50 mg/kg IV) and tracheotomized (PE100). Catheters (PE50 heat-stretched to 180-μm tip) were inserted into the femoral vessels for fluid administration and blood pressure monitoring. Blood pressures were recorded (CardioMaxII, Columbus Instrument) after 1 hour of equilibration.

**Blood Pressures Without Anesthesia**

Conscious blood pressures were measured in 2 sets of mice. In the first set of studies, blood pressures were measured via a femoral artery catheter, coated with 5% heparin complex, threaded upward and out of a 5-mm incision at the nape of the neck. The catheter was flushed immediately (1/2 mg plasmin and 1000 U heparin/mL of sterile saline) and every 2 days thereafter. One to 3 days after catheter placement, blood pressures were measured in freely moving, unanesthetized mice. In the second set of studies, TA-PAC20 transmitters (DSI) were implanted into 1 carotid artery, and blood pressures were measured by telemetry 1 week after the surgery.

**Acute Saline Loading Study**

After a 60-minute stabilization period after the catheter insertion and a baseline 60-minute period for blood pressure measurement, a normal saline load equivalent to 5% body weight was infused intravenously for 30 minutes. Urine was collected during saline loading via suprapubic cystostomy for another 30 minutes; 3 more urine collection periods of 60 minutes each were obtained after loading. Blood (50 μL) was obtained from the femoral artery before the load and at the end of the last urine collection. The kidneys were homogenized with 0.1 M HClO₄ and centrifuged at 6000g for 20 minutes at 4°C. The supernatant and urine catechol concentrations were determined by high-performance liquid chromatography and electrochemical detection.

**Acute Drug Infusion Studies**

In additional experiments, several antagonists of receptors known to influence blood pressure were infused via a central venous catheter, and blood pressure and heart rate were monitored. The drugs were α-adrenergic antagonist phenolamine (5 ng/kg per minute), angiotensin II type 1 receptor (AT₁) antagonist losartan (3 mg/kg per minute and blood pressure and heart rate were monitored. After a 60-minute stabilization period after the catheter insertion and a baseline 60-minute period for blood pressure measurement, a normal saline load equivalent to 5% body weight was infused intravenously for 30 minutes. Urine was collected during saline loading via suprapubic cystostomy for another 30 minutes; 3 more urine collection periods of 60 minutes each were obtained after loading. Blood (50 μL) was obtained from the femoral artery before the load and at the end of the last urine collection. The kidneys were homogenized with 0.1 M HClO₄ and centrifuged at 6000g for 20 minutes at 4°C. The supernatant and urine catechol concentrations were determined by high-performance liquid chromatography and electrochemical detection.

**Measurement of Renin, Na⁺/K⁺-ATPase, Activity, and Catechols**

Plasma and renal renin concentrations were assessed by radioimmunoassay measuring the generation of angiotensin I. Plasma and renal renin concentrations were assessed by radioimmunoassay measuring the generation of angiotensin I. Na⁺/K⁺-ATPase activity in renal cortex or medulla was measured as the ouabain-sensitive dephosphorylation of (tris)-p-nitrophenyl phosphate by K⁺/Na⁺-ATPase: n=18, 8- to 12-months old; A) and tenth generation (4- to 6-months old; 7 pairs; B). Data are mean±SE. *P<0.05 vs D4<sup>+/+</sup> mice, t test.

**Chronic Sodium Balance Study With Ration Feeding in Metabolic Cages**

The mice were maintained in metabolic cages to allow quantitative urine collections and ration feeding, modified from a rat-diet protocol. The baseline sodium diet (5 g/25 g body weight per day, TD.90228, Harlan Teklad) consisted of a gelled mixture of distilled water (10 mL/5 g of mouse chow), agar (0.04 g/10 mL of water), and 0.4% NaCl added before gelation. The sodium replete diet was the same, except for the addition of 0.8% NaCl. All of the animals received the same amount of gelled food, determined by weighing the gelled mixture. On day 3, the mice were ration-fed 0.4% NaCl gelled food. On day zero, the ration was changed to 0.8% NaCl for 1 week. Seven days later, the mice were euthanized after blood pressures were recorded with anesthesia. Serum and urinary samples were analyzed for Na, K, Cl, Cr, and creatinine concentrations (Creatinine Analyzer).

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**Figure 1.** BPs of D4<sup>−/−</sup> and D4<sup>+/+</sup> mice measured under pentobarbital anesthesia. BPs were obtained in mice of both genders from the sixth generation (D4<sup>−/−</sup>: n=18; D4<sup>+/+</sup>: n=18, 8- to 12-months old; A) and tenth generation (4- to 6-months old; 7 pairs; B). Data are mean±SE. *P<0.05 vs D4<sup>+/+</sup> mice, t test.

**Figure 2.** BPs of conscious D4<sup>−/−</sup> and D4<sup>+/+</sup> mice (tenth generation, 4- to 5-months old). (A) BPs of mice measured via a chronic femoral artery catheter (3 pairs). Blood pressures were measured by telemetry (3 pairs). Data are mean±SE. *P<0.05 vs D4<sup>+/+</sup> mice, t test, with Bonferroni correction (B).
Beckman). After sacrifice, kidneys, brains, and hearts were homogenized, as reported previously.\textsuperscript{25,26} In additional studies, mouse kidney homogenates were centrifuged at 42 000g to obtain membrane fractions.

**Semiquantitative Immunoblotting**

Rabbit polyclonal antibodies recognizing the AT\textsubscript{1} receptor and actin were purchased from Santa Cruz (SC-1173) and Sigma (A5060), respectively. The specificity of the AT\textsubscript{1} antibody has been reported.\textsuperscript{25–29} Semiquantitative immunoblotting was used to compare AT\textsubscript{1} protein expression, as described previously.\textsuperscript{25,26,28,29} The bands were scanned and quantified by the NIH Image J program. The densitometry values were corrected by actin and shown as percentage of mean density of D\textsubscript{4\textsuperscript{+/+}} mice.

**Statistical Analysis**

Data expressed as mean±SE were analyzed by repeated-measures ANOVA for comparisons within groups and 1-way factorial ANOVA for comparisons among groups. Student t test was used for 2-group comparison, with Bonferroni correction as indicated. \(P<0.05\) was considered significant.

**Results**

**Blood Pressure and Other Physiological Data in D\textsubscript{4\textsuperscript{+/+}} Mice**

Figure 1A shows that the systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressures (mm Hg) measured under anesthesia in sixth-generation mice were higher in D\textsubscript{4\textsuperscript{+/+}} mice (SBP, 128±2; DBP, 98±1; MAP, 108±1; \(n=27\)) than in D\textsubscript{4\textsuperscript{+/+}} littermates (SBP, 104±1; DBP, 79±1; MAP, 87±1; \(n=18\); 8- to 12-months old, mixed gender). No differences in MAP between genders were found in D\textsubscript{4\textsuperscript{+/+}} mice (male: 93±1, \(n=10\); male: 90±1, \(n=8\)) and D\textsubscript{4\textsuperscript{+/-}} mice (female: 113±4, \(n=10\); male: 110±2, \(n=18\)). Body weights were the same in D\textsubscript{4\textsuperscript{+/+}} and D\textsubscript{4\textsuperscript{+/-}} mice (28±1 g in both groups). However, heart weights (% body weight) were greater in D\textsubscript{4\textsuperscript{+/+}} than in D\textsubscript{4\textsuperscript{+/-}} mice (0.50±0.02 versus 0.43±0.01; \(P<0.05\)), whereas kidney weights (1.15±0.08 versus 1.23±0.06) were similar. The heart rates (447±6 versus 430±8 bpm) were not different between the 2 mouse strains.

In pentobarbital-anesthetized tenth-generation (4- to 6-months old, mixed gender), the blood pressures of D\textsubscript{4\textsuperscript{+/-}} mice were also higher than in D\textsubscript{4\textsuperscript{+/+}} littermates (Figure 1B). Conscious blood pressures were also higher in the tenth-generation D\textsubscript{4\textsuperscript{+/-}} than in D\textsubscript{4\textsuperscript{+/+}} mice (4- to 6-months old, mixed gender) measured via the femoral artery (Figure 2A) or by telemetry (Figure 2B and 2C). SBPs measured by telemetry were lower than those measured via the femoral catheter, presumably because there were no distractions in the mice studied by telemetry.

**Arterial Blood Pressure, Renal Function, and Catechol Excretions in Response to an Acute Sodium Load**

SBP, DBP, and MAP were not affected by an acute sodium load in D\textsubscript{4\textsuperscript{+/-}} and D\textsubscript{4\textsuperscript{+/+}} mice (data not shown). Glomerular filtration rate was not different between the 2 mouse strains and was not affected by saline loading (Table 1). Urine flow and sodium excretion, which were increased by saline loading, were also similar in the 2 mouse strains. Urinary catechol excretions were similar in D\textsubscript{4\textsuperscript{+/-}} and D\textsubscript{4\textsuperscript{+/+}} mice. Saline loading increased dopamine excretion in both mouse strains without affecting the excretion of other catechols (Table 2).

**Table 1: The Effect of Saline Loading on Renal Function in D\textsubscript{4\textsuperscript{+/+}} (n=6) and D\textsubscript{4\textsuperscript{+/-}} (n=18) Mice**

<table>
<thead>
<tr>
<th>Collection Periods</th>
<th>GFR (nL/g kidney weight per minute)</th>
<th>V (μL/min)</th>
<th>UNaV (nEq/min)</th>
<th>FENa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D\textsubscript{4\textsuperscript{+/+}}</td>
<td>D\textsubscript{4\textsuperscript{+/-}}</td>
<td>D\textsubscript{4\textsuperscript{+/+}}</td>
<td>D\textsubscript{4\textsuperscript{+/-}}</td>
<td>D\textsubscript{4\textsuperscript{+/+}}</td>
</tr>
<tr>
<td>Baseline</td>
<td>1225±256</td>
<td>1598±123</td>
<td>1.21±0.09*</td>
<td>1.11±0.07*</td>
</tr>
<tr>
<td>Loading</td>
<td>1390±107</td>
<td>1823±136</td>
<td>2.94±0.27</td>
<td>4.28±0.84</td>
</tr>
<tr>
<td>Postload 1</td>
<td>1240±77</td>
<td>1493±203</td>
<td>1.72±0.33*</td>
<td>3.36±0.72</td>
</tr>
<tr>
<td>Postload 2</td>
<td>1146±167</td>
<td>1304±80*</td>
<td>1.32±0.06*</td>
<td>2.53±0.52</td>
</tr>
<tr>
<td>Postload 3</td>
<td>1260±133</td>
<td>1247±102*</td>
<td>1.19±0.10*</td>
<td>1.32±0.21*</td>
</tr>
</tbody>
</table>

Data are mean±SE. Each period lasted 60 minutes. MAPs were not affected by time or saline infusion. GFR, glomerular filtration rate; V, urine flow; UNaV, sodium excretion; FENa, fractional sodium excretion.

\*\(P<0.05\) vs loading period or postload 1, ANVR, Newman Keuls test.

\#\(P<0.05\) vs baseline, ANVR, Newman Keuls test.

**Table 2: The Effect of Saline Loading on Urinary Catechols (pg/min) in D\textsubscript{4\textsuperscript{+/+}} (n=5) and D\textsubscript{4\textsuperscript{+/-}} (n=17) Mice**

<table>
<thead>
<tr>
<th>Collection Periods</th>
<th>L-DOPA</th>
<th>DOPAC</th>
<th>Dopamine</th>
<th>Epinephrine</th>
<th>Norepinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>D\textsubscript{4\textsuperscript{+/+}}</td>
<td>D\textsubscript{4\textsuperscript{+/-}}</td>
<td>D\textsubscript{4\textsuperscript{+/+}}</td>
<td>D\textsubscript{4\textsuperscript{+/-}}</td>
<td>D\textsubscript{4\textsuperscript{+/+}}</td>
<td>D\textsubscript{4\textsuperscript{+/-}}</td>
</tr>
<tr>
<td>Baseline</td>
<td>10.6±2.3</td>
<td>15.2±2.4</td>
<td>105±26</td>
<td>82±9</td>
<td>498±87*</td>
</tr>
<tr>
<td>Loading</td>
<td>15.1±4.0</td>
<td>18.0±3.9</td>
<td>231±61</td>
<td>149±31</td>
<td>884±107</td>
</tr>
<tr>
<td>Postload 1</td>
<td>14.0±4.5</td>
<td>20.5±5.5</td>
<td>183±67</td>
<td>143±37</td>
<td>608±112*</td>
</tr>
<tr>
<td>Postload 2</td>
<td>12.5±3.7</td>
<td>31.3±14.0</td>
<td>131±51</td>
<td>144±44</td>
<td>492±37*</td>
</tr>
<tr>
<td>Postload 3</td>
<td>9.0±2.3</td>
<td>33.7±9.7</td>
<td>140±59</td>
<td>151±43</td>
<td>518±86*</td>
</tr>
</tbody>
</table>

Data are mean±SE. Each period lasted 60 minutes. DOPA indicates dihydroxyphenylalanine; DOPAC, dihydroxyphenylacetic acid.

\*\(P<0.05\) vs loading.

\#\(P<0.05\) vs baseline, ANVR, Newman Keuls test.
Renal and Plasma Renin Concentrations in D4$^{+/+}$ Mice
Renal renin concentrations (µg A/g kidney per hour) were similar in D4$^{-/-}$ mice (57.5±7.2, n=18) and D4$^{+/+}$ mice (70.2±18.3, n=5). Plasma renin concentrations were also not significantly different between D4$^{-/-}$ (3835±2097 ng A/g/mL/hour, n=18) and D4$^{+/+}$ mice (3540±2881, n=5) mice.

Na$^{+}$/K$^{+}$-ATPase Activity
Renal cortical and medullary Na$^{+}$/K$^{+}$-ATPase activities were not significantly changed in D4$^{-/-}$ mice (cortex = 34.2±0.5 nmol Pi/mg protein per minute, medulla = 34.2±0.5, n=17) compared with D4$^{+/+}$ mice (cortex = 33.4±0.2 nmol Pi/mg protein per minute, medulla = 31.5±2.6, n=5). The D4 receptor agonist, SKF81297 (1 μmol/L), decreased Na$^{+}$/K$^{+}$-ATPase activity to a similar extent in cortex (D4$^{-/-}$ = 25.98±2.91%, D4$^{+/+}$ = 21.60±1.41%) and medulla (D4$^{-/-}$ = 23.17±3.34%, D4$^{+/+}$ = 20.02±1.37%; P>0.05, ANOVA) in the 2 mouse strains.

Role of Blood Pressure–Regulating Systems in the Hypertension of D4$^{-/-}$ Mice
Bolus intravenous injection of losartan decreased MAP promptly and to a similar degree initially in the 2 strains. However, the effect dissipated quickly with recovery toward baseline 10 minutes after the injection in D4$^{-/-}$ mice, whereas the hypotensive effect of losartan persisted for 45 minutes in D4$^{+/+}$ mice (Figure 3a). In contrast, the V1 vasopressin receptor antagonist [1-(β-mercapto-β-2-(O-methyl)-tyrosine) arginine vasopressin, was given intravenously (D4$^{-/-}$, n=6; D4$^{+/+}$, n=6). A slight decrease in blood pressure was noted in D4$^{-/-}$ mice 8 minutes after the start of BQ 788 infusion (P<0.05, ANOVA, Newman Keuls test). Differences between D4$^{-/-}$ and D4$^{+/+}$ mice became evident at 8 minutes (*P<0.05, t test). (c) ETB receptor antagonist, BQ 788, was infused intravenously (D4$^{-/-}$, n=6; D4$^{+/+}$, n=6). A slight decrease in blood pressure was noted in D4$^{-/-}$ but not in D4$^{+/+}$ mice immediately after the injection (P<0.05, ANVR, Newman Keuls test). However, a significantly longer duration of BP suppression was observed in D4$^{-/-}$ mice compared with D4$^{+/+}$ mice beginning 8 minutes after losartan administration and lasting as long as 45 minutes. *P<0.05 vs D4$^{+/+}$, t test. (d) V1 vasopressin receptor antagonist, [1-(β-mercapto-β-2-(O-methyl)-tyrosine) arginine vasopressin, was given intravenously (D4$^{-/-}$, n=6; D4$^{+/+}$, n=4). There were no effects of BQ 610 on blood pressure, but a significant decrease in blood pressure was noted 5 minutes after the infusion of phenolamine in D4$^{-/-}$ mice and 7 minutes in D4$^{+/+}$ mice (P>0.05, ANVR, Newman Keuls test).

Figure 3. Effect of antagonists to α-adrenergic, AT1, endothelin (A and B), and V1 vasopressin receptors on MAP pressure in anesthetized D4$^{+/+}$ and D4$^{-/-}$ mice. (a) AT1 receptor antagonist, losartan, was given as an intravenous bolus injection (D4$^{-/-}$, n=6; D4$^{+/+}$, n=5). A decrease in blood pressure was noted in both D4$^{-/-}$ and D4$^{+/+}$ mice immediately after the injection (P<0.05, ANVR, Newman Keuls test). However, a significantly longer duration of BP suppression was observed in D4$^{-/-}$ mice compared with D4$^{+/+}$ mice beginning 8 minutes after losartan administration and lasting as long as 45 minutes. *P<0.05 vs D4$^{+/+}$, t test. (b) V1 vasopressin receptor antagonist, [1-(β-mercapto-β-2-(O-methyl)-tyrosine) arginine vasopressin, was given intravenously (D4$^{-/-}$, n=6; D4$^{+/+}$, n=4). A slight decrease in blood pressure was noted in D4$^{-/-}$ mice 2 minutes after the administration. In contrast, a slight increase in blood pressure was noted in D4$^{+/+}$ mice 10 minutes after the administration of the V1 vasopressin antagonist (P<0.05, ANVR, Newman Keuls test). Differences between D4$^{-/-}$ and D4$^{+/+}$ mice became evident at 8 minutes (*P<0.05, t test). (c) ETB receptor antagonist, BQ 788, was infused intravenously (D4$^{-/-}$, n=6; D4$^{+/+}$, n=6). A slight decrease in blood pressure was noted in D4$^{-/-}$ but not in D4$^{+/+}$ mice immediately after the injection of BQ 788 (P<0.05, ANVR, Newman Keuls test). Differences between D4$^{-/-}$ and D4$^{+/+}$ mice became evident at 9 minutes (*P<0.05, t test). (d) ETA antagonist BQ 610 or the α-adrenergic antagonist phentolamine was infused intravenously (D4$^{-/-}$, n=6; D4$^{+/+}$, n=4). There were no effects of BQ 610 on blood pressure, but a significant decrease in blood pressure was noted 5 minutes after the infusion of phenolamine in D4$^{-/-}$ mice and 7 minutes in D4$^{+/+}$ mice (P>0.05, ANVR, Newman Keuls test).

Figure 4. Effect of angiotensin II on MAP in anesthetized D4$^{+/+}$ and D4$^{-/-}$ mice. D4$^{-/-}$ mice immediately after the injection (D4$^{-/-}$, n=6; D4$^{+/+}$, n=5). A decrease in blood pressure was noted in both D4$^{-/-}$ and D4$^{+/+}$ mice immediately after the injection (P<0.05, ANVR, Newman Keuls test). However, a significantly longer duration of BP suppression was observed in D4$^{-/-}$ mice compared with D4$^{+/+}$ mice beginning 8 minutes after losartan administration and lasting as long as 45 minutes. *P<0.05 vs D4$^{+/+}$, t test. (b) V1 vasopressin receptor antagonist, [1-(β-mercapto-β-2-(O-methyl)-tyrosine) arginine vasopressin, was given intravenously (D4$^{-/-}$, n=6; D4$^{+/+}$, n=4). A slight decrease in blood pressure was noted in D4$^{-/-}$ mice 2 minutes after the administration. In contrast, a slight increase in blood pressure was noted in D4$^{+/+}$ mice 10 minutes after the administration of the V1 vasopressin antagonist (P<0.05, ANVR, Newman Keuls test). Differences between D4$^{-/-}$ and D4$^{+/+}$ mice became evident at 8 minutes (*P<0.05, t test). (c) ETB receptor antagonist, BQ 788, was infused intravenously (D4$^{-/-}$, n=6; D4$^{+/+}$, n=6). A slight decrease in blood pressure was noted in D4$^{-/-}$ but not in D4$^{+/+}$ mice immediately after the injection of BQ 788 (P<0.05, ANVR, Newman Keuls test). Differences between D4$^{-/-}$ and D4$^{+/+}$ mice became evident at 9 minutes (*P<0.05, t test). (d) ETA antagonist BQ 610 or the α-adrenergic antagonist phentolamine was infused intravenously (D4$^{-/-}$, n=6; D4$^{+/+}$, n=4). There were no effects of BQ 610 on blood pressure, but a significant decrease in blood pressure was noted 5 minutes after the infusion of phenolamine in D4$^{-/-}$ mice and 7 minutes in D4$^{+/+}$ mice (P>0.05, ANVR, Newman Keuls test).
TABLE 3. Body Weight, Blood Pressure, Creatinine Clearance, and Sodium and Water Excretion in Ration-Fed Mice

<table>
<thead>
<tr>
<th>Variable</th>
<th>D_{4.5}/+ (n=5)</th>
<th>D_{4.5}/- (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g) day 3</td>
<td>26.8±0.5</td>
<td>20.7±0.7*</td>
</tr>
<tr>
<td>Day 0</td>
<td>27.6±0.7</td>
<td>21.4±0.6*</td>
</tr>
<tr>
<td>Day 10</td>
<td>27.8±0.5</td>
<td>22.3±0.4*</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>96±3</td>
<td>119±1*</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>81±5</td>
<td>90±2*</td>
</tr>
<tr>
<td>Serum Na (mM)</td>
<td>150±2</td>
<td>151±2</td>
</tr>
<tr>
<td>Creatinine clearance (mL/g BW per day)</td>
<td>6.0±1.1</td>
<td>4.8±0.7</td>
</tr>
<tr>
<td>Water excretion (mL/g BW per day)</td>
<td>0.10±0.02</td>
<td>0.08±0.02</td>
</tr>
<tr>
<td>Na excretion (mmol/g BW per day)</td>
<td>0.017±0.002</td>
<td>0.014±0.002</td>
</tr>
</tbody>
</table>

Values are mean±SE. BW indicates body weight; BP, blood pressure. Mice: female, 6 months old. BPs were measured under pentobarbital anesthesia. Salt intake: 0.8% NaCl for 10 days, 5 g mouse chow plus 10 mL water/25g of BW per day.

*P<0.05 vs D_{4.5}/+, t test.

mice at any dose, the MAP tended to increase to a greater extent in D_{4.5}/+ than in D_{4.5}/- mice, reaching significance at 10 ng (ΔMAP, mm Hg; D_{4.5}/+: 67±4, n=5; D_{4.5}/-: 44±4, n=4; P<0.05, t test; Figure 4).

Chronic Sodium Balance Study

Age- and gender-matched D_{4.5}/+ mice and D_{4.5}/- littermates (tenth-generation) and C57/BL6 mice (Taconic, Germantown, NY) were housed in the animal facility under the same conditions for >1 month before use. Backcrossing to the sixth generation or to more than the tenth generation results in a C57/BL6 genetic background >98% or >99% congenic, respectively. In agreement with data shown in Figure 1A and 1B, SBP and DBP under pentobarbital anesthesia were higher in D_{4.5}/- than in D_{4.5}/+ mice (female, 6 months old). D_{4.5}/- mice weighed less than D_{4.5}/+ mice possibly because of the ration feeding (sodium intake=0.8% NaCl) in metabolic cages. Sodium and water excretions (normalized by body weight) tended to be lower in D_{4.5}/- than in D_{4.5}/+ mice, but the differences did not reach statistical significance. Creatinine clearance and serum sodium concentration were similar in D_{4.5}/- and D_{4.5}/+ mice (Table 3).

AT_{1} Receptor Protein Expression

To determine a mechanism for the involvement of the AT_{1} receptor in the hypertension of D_{4.5}/- mice, we measured AT_{1} receptor protein expression in the ration-fed tenth-generation D_{4.5}/- and C57/BL6 mice. AT_{1} receptor expression (45 kDa) in whole kidney homogenates of D_{4.5}/- mice was increased (330±53%, normalized by the band density of D_{4.5}/+ set to 100%, n=5; P<0.05) compared with D_{4.5}/+ mice (100±12%, n=5; Figure 5A). AT_{1} receptor expression was also increased in whole-brain homogenates of D_{4.5}/- mice (272±69%; P<0.05) compared with D_{4.5}/+ mice (100±32%; Figure 5B). In contrast, there were no differences in AT_{1} expression in whole heart homogenates between the 2 mouse strains (Figure 5C).

In additional experiments, tenth-generation D_{4.5}/- and D_{4.5}/+ littermates (mixed gender, 4 to 5 months old) were studied to achieve a >99% C57/BL6 genetic background. There were no differences in body weight between D_{4.5}/- mice (31±2 g, n=10) and D_{4.5}/+ littermates (27±2 g, n=8), whereas blood pressures were higher in D_{4.5}/- mice than in D_{4.5}/+ littermates (Figure 1B). AT_{1} receptor expression was measured in kidney membrane fractions; receptors in the cytosol would not be responsive to angiotensin II stimulation. AT_{1} receptor expression in kidney membrane fractions was increased in D_{4.5}/- mice relative to D_{4.5}/+ littermates (D_{4.5}/+: 100±14%; D_{4.5}/- mice: 289±28; % of D_{4.5}/+, P<0.05; Figure 6), in agreement with studies using renal homogenates.

Discussion

The present studies show that the complete lack of D_{4} dopamine receptors resulted in increased blood pressure. Furthermore, the increased blood pressure in the mutant mice was associated with a prolonged depressor response caused by the AT_{1} receptor antagonist losartan. The transient effect of losartan in the D_{4.5}/- mice was related to the dose and similar to the hypotensive effect in D_{4.5}/+ mice. The prolonged hypotensive effect of losartan in the D_{4.5}/+ mice is also

Figure 5. Immunoblots of AT_{1} receptors from kidney, brain, and heart homogenates in ration-fed D_{4.5}/- mice (left, n=5; tenth generation) and age- and gender-matched C57/BL6 mice (Taconic, Germantown, NY; right, n=5). (A) Immunoblots of AT_{1} receptors in homogenates from whole kidneys. Each lane was loaded with a sample from a different mouse. The AT_{1} receptor band densities were corrected by actin expression. AT_{1} receptor expression was increased in D_{4.5}/- mice relative to D_{4.5}/+ mice (n=5; P<0.05 vs D_{4.5}/+, t test). (B) Immunoblots of AT_{1} receptors in homogenates from whole brain. AT_{1} receptor expression was increased in D_{4.5}/- mice relative to D_{4.5}/+ mice (P<0.05 vs D_{4.5}/+, t test). (C) Immunoblots of AT_{1} receptors in homogenates from whole heart. There were no differences between D_{4.5}/- and D_{4.5}/+ mice.
Evidence has accumulated that the increase in blood pressure caused by central AT\(_1\) activation is mediated with\(^{37-40}\) or without\(^{41-44}\) activation of the sympathetic nervous system. In the present study, \(\alpha\)-adrenergic blockade with phentolamine decreased blood pressure to a similar extent in D\(_{4}^{-/-}\) and D\(_{3}^{-/-}\) mice. This observation may be specific to the D\(_{4}^{-/-}\) mice, because the \(\alpha\)-adrenergic blockade decreased blood pressure to a greater extent in D\(_{3}^{-/-}\) and D\(_{5}^{-/-}\) mice than in their wild-type counterparts.\(^{11,13}\) There were also no differences in renal or urinary catechol excretion between D\(_{4}^{-/-}\) and D\(_{3}^{-/-}\) mice. The pressor effect mediated by AT\(_1\) receptors expressed in the central nervous system has also been shown to be mediated, in part, by activation of central V\(_1\) receptors.\(^{13}\) However, in the current study, V\(_1\) vasopressin receptor blockade did not decrease but actually produced a slight increase in blood pressure in D\(_{4}^{-/-}\) mice, suggesting a different mechanism to be involved.

Endothelin receptors may mediate pressor and depressor effects.\(^{37-49}\) We have reported that disruption of the D\(_{2}\) dopamine receptor in mice increased blood pressure possibly via the vasoconstrictor ETB\(_2\) (presumably in vascular smooth muscle or in brain centers).\(^{11}\) The D\(_{2}\) receptor, like the D\(_{1}\) receptor, is not expressed in endothelial cells.\(^{50}\) Thus, neither D\(_{2}\) nor D\(_{3}\) receptors can activate the vasodilatory ETB\(_1\) receptors in endothelial cells. The modest increase in blood pressure by blockade of ETB receptors might be mediated via vasoconstrictor ETB\(_2\) in the D\(_{4}^{-/-}\) mice.

Dopamine has been shown to act as a potent intrarenal natriuretic hormone in humans and rodents.\(^{3,4}\) D\(_{4}\) receptors have been shown to antagonize vasopressin- and aldosterone-dependent sodium reabsorption in the cortical collecting duct.\(^{51,52}\) In the present study, we did not find significant differences in renal Na\(^+/\)K\(^{-}\)-ATPase activity and urinary dopamine excretion between D\(_{4}^{-/-}\) and D\(_{3}^{-/-}\) mice. The D\(_{4}^{-/-}\) mice did not have an impaired ability to excrete an acute saline load. There are also no differences in sodium excretion between conscious D\(_{4}^{-/-}\) mice and D\(_{3}^{-/-}\) littermates in spite of an increase in AT\(_{1}\) receptors in the kidney of D\(_{4}^{-/-}\) mice. Possibly, the high blood pressure may have elicited a pressure natriuresis through other mechanisms that obfuscated any deficits in the renal handling of sodium in D\(_{3}^{-/-}\) mice.\(^{53,54}\)

In summary, we have found that disruption of the D\(_{2}\) receptor in mice causes hypertension that is associated with increased expression of AT\(_{1}\) receptors in the brain and kidney but not in the heart. The mechanism by which D\(_{2}\) receptors regulate AT\(_{1}\) receptor expression remains to be determined.

**Perspectives**

This study demonstrates that disruption of the D\(_{4}\) dopamine receptor results in increased blood pressure that may be related to activation of AT\(_{1}\) receptors in the brain. However, determination of AT\(_{1}\) receptor expression and function in specific brain nuclei are needed to understand how the D\(_{4}\)
receptor interacts with the AT1 receptor in the central regulation of blood pressure. AT1 receptors are also increased in the kidneys of D4−/− mice, but these mice do not have an impaired ability to excrete an acute sodium load. It is possible that D4−/− mice may not be able to excrete a chronic sodium load, but that remains to be determined. Because the D4 receptor gene locus is linked to and D4 receptor variants are associated with hypertension,20 the relevance of the D4 receptor in the pathogenesis of human essential hypertension needs to be evaluated.

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References


Angiotensin-II Type 1 Receptor–Mediated Hypertension in D₄ Dopamine Receptor–Deficient Mice


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In the article by Bek et al in the February 2006 issue of *Hypertension* (Bek MJ, Wang X, Asico LD, Jones JE, Zheng S, Li X, Eisner GM, Grandy DK, Carey RM, Soares-da-Silva P, Jose PA. Angiotensin-II type 1 receptor–mediated hypertension in D₄ dopamine receptor–deficient mice. *Hypertension* 2006;47:288–292) the symbols in the legend for Figure 2B were incorrect. The correct Figure 2 appears below. The authors regret the error.

![Figure 2](image_url)

**Figure 2.** BPs of conscious D₄⁻/⁻ and D₄⁺/⁺ mice (tenth generation, 4- to 5-months old). (A) BPs of mice measured via a chronic femoral artery catheter (3 pairs). The bihourly SBP (B) and nighttime SBP and MAP (C) of mice measured by telemetry (3 pairs). Data are mean±SE. *P<0.05 vs D₄⁻/⁻ mice, t test, with Bonferroni correction (B).