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


Abstract—Dopamine receptors are important in systemic blood pressure regulation. D4 receptors are expressed in the kidney and brain, but their role in cardiovascular regulation is unknown. In pentobarbital-anesthetized mice, systolic and diastolic blood pressures were elevated in sixth-generation D4 receptor–deficient (D4−/−) mice and in tenth-generation D4−/− mice compared with D4 wild-type (D4+/+) littermates. The conscious blood pressures measured via a chronic arterial (femoral) catheter or telemetry (carotid) were also higher in D4−/− mice than in D4 littermates. Basal renal and plasma renin concentrations were similar in the 2 mouse strains. The protein expression of angiotensin II type 1 receptor was increased in homogenates of kidney (330 ± 53%, n = 5) and brain (272 ± 69%, n = 5) of D4−/− mice relative to D4+/+ mice (kidney: 100 ± 12%, n = 5; brain: 100 ± 32%, n = 5). The expression of the receptor in renal membrane was also increased in D4−/− mice (289 ± 28%, n = 8) relative to D4+/+ mice (100 ± 14%, n = 10). In contrast, the expression in the heart was similar in the 2 strains. Bolus intravenous injection of angiotensin II type 1 receptor antagonist losartan initially decreased mean arterial pressures to a similar degree in D4−/− and D4+/+ littermates. However, the hypotensive effect of losartan dissipated after 10 minutes in D4−/− mice, whereas the effect persisted for >45 minutes in D4+/+ mice. We conclude that the absence of the D4 receptor increases blood pressure, possibly via increased angiotensin II type 1 receptor expression. (Hypertension. 2006;47:288-295.)

Key Words: dopamine receptors, angiotensin II mice hypertension angiotensin II endothelin vasopressins

Essential hypertension is a major risk factor for the development of cardiovascular disease.1 It is a heterogeneous disease in which both genetics and environmental influence blood pressure.2 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.3-9 Dopamine exerts its actions by occupation of the D1-like (D1 and D2) or D2-like (D3, D4, and D5) 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 pathophysiologic mechanisms.10-13 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.14-17 Loci near the D4 receptor gene (11p15.5) have been linked to hypertension,18,19 and polymorphisms of the D4 receptor gene are associated with hypertension.20 Therefore, we tested the hypothesis that the D4−/− mouse has a cardiovascular phenotype.

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.21 We used sixth-generation mice for acute studies and tenth-generation mice for chronic and immunoblotting studies. All of the animals were genotyped21 and treated in accordance with National Institutes of Health guidelines for ethical treatment and handling of animals in research.
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 obtained for determination of renin concentration.

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 phentolamine (5 ng/kg per minute), angiotensin II type 1 receptor (AT1) antagonist losartan (3 mg/kg per minute for 10 minutes), endothelin B receptor (ETB) antagonist BQ788 (6.6 µg/kg per minute for 15 minutes), and V1 vasopressin receptor (V1) antagonist [1-(β-mercapto-β, β-cyclopentamethylenepropionic acid)-2-(O-methyl)-tyrosine] arginine vasopressin (10 µg/kg over 30 s). The rationale and dosages of these drugs have been validated. The dose of losartan had been shown previously to have an effect of limited duration in wild-type mice. We also studied the acute effect on blood pressure of varying doses of bolus intravenous injections of angiotensin II (0.1, 0.3, 1, 3, and 10 ng/kg per mouse). Mice were euthanized with pentobarbital (100 mg/kg) at the end of the experiments.

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 urinary catecholamines were determined by high-performance liquid chromatography and electrochemical detection.

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 (E4A Electrolyte system, Beckman) and creatinine concentrations (Creatinine Analyzer2, Beckman) and creatinine concentrations (Creatinine Analyzer2, Beckman).
Beckman). After sacrifice, kidneys, brains, and hearts were homogenized, as reported previously.\textsuperscript{27,29} Semiquantitative immunoblotting was used to compare AT1 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 D4\textsuperscript{−/−} mice.

**Statistical Analysis**

Data expressed as mean±SE were analyzed by repeated-measures 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 D4\textsuperscript{−/−} Mice**

Figure 1A shows that the systolic (SBP), diastolic (DBP), and mean arterial (MAP) blood pressures (mm Hg) measured under anesthesia in six-generation mice were higher in D4\textsuperscript{−/−} mice (SBP, 128±2; DBP, 98±1; MAP, 108±1; n=27) than in D4\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 D4\textsuperscript{−/−} mice (female: 93±1, n=10; male: 90±1, n=8) and D4\textsuperscript{+/+} mice (female: 113±4, n=10 male: 110±2, n=18). Body weights were the same in D4\textsuperscript{+/+} and D4\textsuperscript{−/−} mice (28±1 g in both groups). However, heart weights (% body weight) were greater in D4\textsuperscript{−/−} than in D4\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 D4\textsuperscript{−/−} mice were also higher than in D4\textsuperscript{+/+} littermates (Figure 1B). Conscious blood pressures were also higher in the tenth-generation D4\textsuperscript{−/−} than in D4\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 D4\textsuperscript{−/−} and D4\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 D4\textsuperscript{−/−} and D4\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 D4\textsuperscript{+/+} (n=6) and D4\textsuperscript{−/−} (n=18) Mice

<table>
<thead>
<tr>
<th>Collection Periods</th>
<th>GFR (nL/min kidney weight per minute)</th>
<th>V (µL/min)</th>
<th>UNaV (nEq/min)</th>
<th>FENa (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D4\textsuperscript{+/+}</td>
<td>D4\textsuperscript{−/−}</td>
<td>D4\textsuperscript{+/+}</td>
<td>D4\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.

**TABLE 2. The Effect of Saline Loading on Urinary Catechols (pg/min) in D4\textsuperscript{+/+} (n=5) and D4\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></td>
<td>D4\textsuperscript{+/+}</td>
<td>D4\textsuperscript{−/−}</td>
<td>D4\textsuperscript{+/+}</td>
<td>D4\textsuperscript{−/−}</td>
<td>D4\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>606±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.
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 increase in blood pressure was noted in D4−/− but not in D4+/−, 8 minutes after the start of BQ 788 infusion (*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). 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 ETA vasopressin antagonist (*P<0.05, ANVR, Newman Keuls test). Differences between D4+/− and D4−/− mice were not significant on the effects of BQ 610 on blood pressure, but a significant decrease in blood pressure was noted 5 minutes after the infusion of phentolamine in D4−/− mice and 7 minutes in D4+/− mice (P>0.05, ANVR, Newman Keuls test).

Renal and Plasma Renin Concentrations in D4−/− Mice
Renal renin concentrations (µg A/µg 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/µL per hour, n=18) and D4+/− (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±2.0 nmol Pi/mg protein per minute, medulla =31.5±2.6, n=5). The D1 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 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-mercaptopropyl)-β-cyclopentamethylenepropionic acid]-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 were not significant for the effects of BQ 610 on blood pressure, but a significant decrease in blood pressure was noted 5 minutes after the infusion of phentolamine 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-mercaptopropyl)-β-cyclopentamethylenepropionic acid]-2-(O-methyl)-tyrosine] arginine vasopressin, was given intravenously (D4−/−, n=6; D4+/−, n=4). There were no differences of ETA 610 on blood pressure, but a significant decrease in blood pressure was noted 5 minutes after the infusion of phentolamine 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. Angiotensin II (0.1, 0.3, 1, 3, and 10 ng/kg per mouse) increased the blood pressure in both D4+/− [Yc=16.45+(5.6×dose), n=5] and D4−/− mice [Yc=13.89+(3.37×dose), n=4; P<0.05]. Although, a higher MAP was found in D4−/− mice than in D4+/− mice.

Angiotensin-II, ng/kg
mice at any dose, the MAP tended to increase to a greater extent in D4+/− than in D4−/− mice, reaching significance at 10 ng (ΔMAP, mm Hg, D4+/−: 67±4, n=5; D4−/−: 44±4, n=4; \(P<0.05\); t test; Figure 4).

**Chronic Sodium Balance Study**

Age- and gender-matched D4−/− mice and D4+/− 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 resulted in a >99% C57/BL6 genetic background. In additional experiments, tenth-generation D4−/− and D4+/− mice were studied to achieve a >99% C57/BL6 genetic background. There were no differences in body weight between D4−/− mice (31±2 g, n=10) and D4+/− littermates (27±2 g, n=8), whereas blood pressures were higher in D4−/− mice than in D4+/− littermates (Figure 1B). AT1 receptor expression was measured in kidney membrane fractions; receptors in the cytosol would not be responsive to angiotensin II stimulation. AT1 receptor expression in kidney membrane fractions was increased in D4−/− mice relative to D4+/− littermates (D4−/− mice: 100±14%; D4+/− mice: 289±28%; \(P<0.05\); Figure 6), in agreement with studies using renal homogenates.

**Discussion**

The present studies show that the complete lack of D1 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 AT1 receptor antagonist losartan. The transient effect of losartan in the D4−/− mice was related to the dose and similar to the hypotensive effect in D4+/− mice. The prolonged hypotensive effect of losartan in the D4−/− mice is also

**TABLE 3. Body Weight, Blood Pressure, Creatinine Clearance, and Sodium and Water Excretion in Ration-Fed Mice**

<table>
<thead>
<tr>
<th>Variable</th>
<th>D4−/− (n=5)</th>
<th>D4+/− (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 10</td>
<td>27.6±0.7</td>
<td>21.4±0.6*</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>27.8±0.5</td>
<td>22.3±0.4*</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>96±3</td>
<td>119±1*</td>
</tr>
<tr>
<td>Serum Na (mM)</td>
<td>81±5</td>
<td>90±2*</td>
</tr>
<tr>
<td>Creatinine clearance (mL/g BW per day)</td>
<td>0.10±0.02</td>
<td>0.08±0.02</td>
</tr>
<tr>
<td>Water excretion (mL/g BW per day)</td>
<td>1.017±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 D4+/−, t test.

Figure 5. Immunoblots of AT1 receptors from kidney, brain, and heart homogenates in ration-fed D4−/− mice (left, n=5, tenth generation) and age- and gender-matched C57/BL6 mice (Taconic, Germantown, NY; right, n=5). (A) Immunoblots of AT1 receptors in homogenates from whole kidneys. Each lane was loaded with a sample from a different mouse. The AT1 receptor band densities were corrected by actin expression. AT1 receptor expression was increased in D4−/− mice relative to D4+/− mice (n=5; \(P<0.05\); vs D4+/−, t test). (B) Immunoblots of AT1 receptors in homogenates from whole brain. AT1 receptor expression was increased in D4−/− mice relative to D4+/− mice (\(P<0.05\) vs D4+/−, t test). (C) Immunoblots of AT1 receptors in homogenates from whole heart. There were no differences between D4−/− and D4+/− mice.
Blood pressure became significant at later rather than earlier time points, suggesting tissue-specific D4 regulation of the AT1 receptor. D4 receptors are similar to the effect in D3 receptor–deficient mice.12 The duration of the hypertensive effect was longest in the D4+/− mice, intermediate in the D4+/+ mice, and shortest in the D4−/− mice. There were no differences in plasma or renal renin concentrations between D4+/− and D4+/+ mice. This contrasts with the elevation of renal renin concentration in the hypertensive D4−/− mice.12

The mechanism underlying the AT1-dependent high blood pressure in the D4−/− mice is not readily apparent. Aberrant interactions between D4 and AT1 receptors were found in spontaneously hypertensive rats.28 It is possible that the D4 receptor similarly regulates the AT1 receptor. D4 receptors are expressed in brain areas that contribute to the regulation of blood pressure, for example, nucleus tractus solitarius.32–35 Because the differential effect of AT1 receptor blockade on blood pressure became significant at later rather than earlier time points, we presumed that the AT1 receptor-mediated hypertension in D4−/− mice is, in part, central in origin. This may also explain why the acute hypertensive effect of angiotensin II was reduced in D4−/− mice. This observation may be specific to the D4−/− mice, because the α-adrenergic blockade decreased blood pressure to a greater extent in D4−/− and D4+/+ mice than in their wild-type counterparts.11,13 There were also no differences in renal or urinary catechol excretion between D4+/+ and D4−/− mice. The pressor effect mediated by AT1 receptors expressed in the central nervous system has also been shown to be mediated, in part, by activation of central V1 receptors.38,45,46 The increased blood pressure caused by disruption of the D4 dopamine receptor is characterized by activation of central V1 vasopressin receptors.13 However, in the current study, V1 vasopressin receptor blockade did not decrease but actually produced a slight increase in blood pressure in D4−/− mice, suggesting a different mechanism to be involved.

Evidence has accumulated that the increase in blood pressure caused by central AT1 activation is mediated with37–40 or without41–44 activation of the sympathetic nervous system. In the present study, α-adrenergic blockade with phentolamine decreased blood pressure to a similar extent in D4−/− and D4+/+ mice. This observation may be specific to the D4−/− mice, because the α-adrenergic blockade decreased blood pressure to a greater extent in D4−/− and D4+/+ mice than in their wild-type counterparts.11,13 There were also no differences in renal or urinary catechol excretion between D4+/+ and D4−/− 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 D4−/− mice.53,54

In summary, we have found that disruption of the D4 dopamine receptor in mice causes hypertension that is associated with increased expression of AT1 receptors in the brain and kidney but not in the heart. The mechanism by which D4 receptors regulate AT1 receptor expression remains to be determined.

**Perspectives**

This study demonstrates that disruption of the D4 dopamine receptor results in increased blood pressure that may be related to activation of AT1 receptors in the brain. However, determination of AT1 receptor expression and function in specific brain nuclei are needed to understand how the D4 receptors mediate pressor and depressor effects.37–49 We have reported that disruption of the D2 dopamine receptor in mice increased blood pressure possibly via the vasoconstrictor ETB2 (presumably in vascular smooth muscle or in brain centers).11 The D2 receptor, like the D4 receptor, is not expressed in endothelial cells.50 Thus, neither D4 nor D2 receptors can activate the vasodilatory ETB2 receptors in endothelial cells. The modest increase in blood pressure by blockade of ETB receptors might be mediated via vasoconstrictor ETB2 in the D4−/− mice.

Dopamine has been shown to act as a potent intrarenal natriuretic hormone in humans and rodents.3,4 D4 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 D4+/− and D4+/+ mice. The D4−/− mice did not have an impaired ability to excrete an acute saline load. There are also no differences in sodium excretion between conscious D4−/− mice and D4+/+ littermates in spite of an increase in AT1 receptors in the kidney of D4−/− mice. Possibly, the high blood pressure may have elicited a pressure natriuresis through other mechanisms that obscured any deficits in the renal handling of sodium in D4−/− mice.53,54
receptor interacts with the AT1 receptor in the central regu-
lation 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 D3
receptor gene locus is linked to and D3 receptor variants are
associated with hypertension,20 the relevance of the D3
receptor in the pathogenesis of human essential hypertension
needs to be evaluated.

Acknowledgments

This work was supported in part by grants from the National
Institutes of Health: HL23081, HL68696, DK39308, DK52612
(P.A.J.), HL074940 (P.A.J. and R.A.F.), DA12062 (D.K.G.);
National Kidney Foundation and Else Kröner-Fresenius-Stiftung
(M.B.); and POCTI/53747/FCT/2000 from Foundation for Science
and Technology, Portugal (P.S.). We also thank Dr Marcelo
Rubinstein for generating the D4−/− mice and Katherine Suchland
for excellent technical assistance with the mice.

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Hypertension. 2006;47:288-295; originally published online December 27, 2005; doi: 10.1161/01.HYP.0000198427.96225.36

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/47/2/288

An erratum has been published regarding this article. Please see the attached page for:
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Data Supplement (unedited) at:
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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 D_{4} 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)

**Figure 2.** BPs of conscious D_{4}^{+/+} and D_{4}^{-/-} 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 D_{4}^{+/+} mice, t test, with Bonferroni correction (B).