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


Abstract—Dopamine receptors are important in systemic blood pressure regulation. D1 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 (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 D1 receptor increases blood pressure, possibly via increased angiotensin II type 1 receptor expression. (Hypertension. 2006;47:1-8.)

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 environment 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 vasopressins. In addition, dopamine can control blood pressure by acting on neuronal cardiovascular centers, heart, and arterial and venous vessels.3,4 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 pathophysiologic mechanisms.10–13 The cardiovascular consequences of disruption of the D1 receptor have not been reported. D1 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 D1 receptor gene (11p15.5) have been linked to hypertension18,19 and polymorphisms of the D1 receptor gene are associated with hypertension.20 Therefore, we tested the hypothesis that the D1−/− mouse has a cardiovascular phenotype.

Methods

Generation of D1 Dopamine–Receptor Mutant Mice

The original F2 hybrid strain (129/Sv × C57BL/6) carrying a mutant form of the D1 dopamine receptor was initially generated and backcrossed to C57BL/6 mice. Heterozygous (D1+/−) mice were mated to obtain D1−/− and D1+/+ littermates, and the D1−/− 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.

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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 day 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 phenotolamine (5 mg/kg per minute), angiotensin II type 1 receptor (AT1) antagonist losartan (3 mg/kg over 30 s), endothelin A receptor antagonist BQ610 (100 μg/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-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 mg/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. Na+/K+-ATPase activity in renal cortex or medulla was measured as the ouabainsensitive dephosphorylation of (tris)-p-nitrophenyl phosphate by K+-p-nitrophenyl phosphatase. The kidneys were homogenized with 0.1 mol/L HClO4 and centrifuged at 6000 g for 20 minutes at 4°C. The supernatant and urine catechol concentrations 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).
TABLE 1. The Effect of Saline Loading on Renal Function in D4+/+ (n=6) and D4−/− (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>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.

Beckman). After sacrifice, kidneys, brains, and hearts were homogenized, as reported previously.25,26 In additional studies, mouse kidney homogenates were centrifuged at 42,000g to obtain membrane fractions.

Semiquantitative Immunoblotting
Rabbit polyclonal antibodies recognizing the AT1 receptor and actin were purchased from Santa Cruz (SC-1173) and Sigma (AS0606), respectively. The specificity of the AT1 antibody has been reported.27–29 Semiquantitative immunoblotting was used to compare AT1 protein expression, as described previously.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+/+ 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 D4−/− 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 D4−/− mice (SBP, 128±2; DBP, 98±1; MAP, 108±1; n=27) than in D4+/+ 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+/+ mice (female: 93±1, n=10; male: 90±1, n=8) and D4−/− mice (female: 113±4, n=10 male: 110±2, n=18). Body weights were the same in D4+/+ and D4−/− mice (28±1 g in both groups). However, heart weights (% body weight) were greater in D4−/− than in D4+/+ mice (50.5±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−/− mice were also higher than in D4+/+ littermates (Figure 1B). Conscious blood pressures were also higher in the tenth-generation D4−/− than in D4+/+ 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−/− and D4+/+ 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−/− and D4+/+ mice. Saline loading increased dopamine excretion in both mouse strains without affecting the excretion of other catecholines (Table 2).

Table 2. The Effect of Saline Loading on Urinary Catechols (pg/min) in D4+/+ (n=5) and D4−/− (n=17) Mice

<table>
<thead>
<tr>
<th>Collection Periods</th>
<th>L-DOPA (pg/min)</th>
<th>DOPAC (pg/min)</th>
<th>Dopamine (pg/min)</th>
<th>Epinephrine (pg/min)</th>
<th>Norepinephrine (pg/min)</th>
</tr>
</thead>
<tbody>
<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 Al/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−/− (3855 ± 2097 ng Al/mL 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 hypertensive effect of losartan persisted for >45 minutes in D4+/+ mice (Figure 3a). In contrast, the V1 vasopressin receptor antagonist (Figure 3b) and ETB receptor antagonist (Figure 3c) increased blood pressure in both D4−/− and D4+/+ mice (Figure 3d). Bolus injections of angiotensin II (0.1, 0.3, 1, 3, and 10 ng/kg per mouse) dose-dependently increased blood pressure in both D4−/− mice [Yc = 16.45 + (5.8 × dose), n = 5] and D4+/+ mice [Yc = 13.89 + (3.37 × dose), n = 5; P < 0.05]. Although, a higher MAP was found in D4−/− mice than in D4+/+ mice.

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. (b) V1 vasopressin receptor antagonist, [1-β-mercaptopropionic 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 became evident at 8 minutes (P < 0.05, t test). (c) ETB receptor antagonist, BO Q788, 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 BO Q788 infusion (P < 0.05, ANVR, Newman-Keuls test). Differences between D4−/− and D4+/+ mice became evident at 8 minutes (P < 0.05, t test). (d) ETA antagonist BO 610 or the α-adrenergic antagonist phentolamine was infused intravenously (D4−/−, n = 6; D4+/+, n = 4). There were no effects of BO 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 dose-dependently (0.1, 0.3, 1, 3, and 10 ng/kg per mouse) increased the blood pressure in both D4−/− [Yc = 16.45 + (5.8 × 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.
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 C57BL/6 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.③

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 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>0.6±1.0</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. BWs 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.

AT₁ Receptor Protein Expression
To determine a mechanism for the involvement of the AT₁ receptor in the hypertension of D₄⁻/⁻ mice, we measured AT₁ receptor protein expression in the ration-fed tenth-generation D₄⁻/⁻ mice and C57/BL6 mice. AT₁ receptor expression (45 kDa) in whole-kidney homogenates of D₄⁻/⁻ mice was increased (330±53%, normalized by the band density of D₄⁺/⁺ set to 100%, n=5; *P<0.05) compared with D₄⁺/⁺ mice (100±12%, n=5; Figure 5A). AT₁ receptor expression was also increased in whole-brain homogenates of D₄⁻/⁻ mice (272±69%; *P<0.05) compared with D₄⁺/⁺ mice (100±32%; Figure 5B). In contrast, there were no differences in AT₁ expression in whole heart homogenates between the 2 mouse strains (Figure 5C).

In additional experiments, tenth-generation D₄⁻/⁻ and D₄⁺/⁺ mice littermates (mixed gender, 4 to 5 months old) were studied to achieve a >99% C57/BL6 genetic background.③ 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%; % of D₄⁺/⁺; *P<0.05; Figure 6), in agreement with studies using renal homogenates.

Discussion
The present studies show that the complete lack of D₄ 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.③ The prolonged hypotensive effect of losartan in the D₄⁻/⁻ mice is also

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.
Evidence has accumulated that the increase in blood pressure caused by central AT1 activation is mediated with\textsuperscript{37–40} or without\textsuperscript{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 \(\text{D}4_{-/-}\) and \(\text{D}4_{+/-}\) mice. This observation may be specific to the \(\text{D}4_{-/-}\) mice, because the \(\alpha\)-adrenergic blockade decreased blood pressure to a greater extent in \(\text{D}4_{+/-}\) and \(\text{D}4_{+/-}\) mice than in their wild-type counterparts.\textsuperscript{11,13} There were also no differences in renal or urinary catechol excretion between \(\text{D}4_{+/-}\) and \(\text{D}4_{-/-}\) 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.\textsuperscript{13} However, in the current study, V1 vasopressin receptor blockade did not decrease but actually produced a slight increase in blood pressure in \(\text{D}4_{+/-}\) mice, suggesting a different mechanism to be involved.

Endothelin receptors may mediate pressor and depressor effects.\textsuperscript{37–40} We have reported that disruption of the D2 dopamine receptor in mice increased blood pressure possibly via the vasoconstrictor ETB\textsubscript{2} (presumably in vascular smooth muscle or in brain centers).\textsuperscript{11} The D4 receptor, like the D2 receptor, is not expressed in endothelial cells.\textsuperscript{50} Thus, neither D3 nor D2 receptors can activate the vasodilatory ETB\textsubscript{1} receptors in endothelial cells. The modest increase in blood pressure by blockade of ETB receptors might be mediated via vasoconstrictor ETB\textsubscript{2} in the \(\text{D}4_{+/-}\) mice.

Dopamine has been shown to act as a potent intrarenal natriuretic hormone in humans and rodents.\textsuperscript{3,4} D4 receptors have been shown to antagonize vasopressin- and aldosterone-dependent sodium reabsorption in the cortical collecting duct.\textsuperscript{51,52} In the present study, we did not find significant differences in renal Na\textsuperscript{+}/K\textsuperscript{+}-ATPase activity and urinary dopamine excretion between \(\text{D}4_{+/-}\) and \(\text{D}4_{+/-}\) mice. The \(\text{D}4_{+/-}\) mice did not have an impaired ability to excrete an acute saline load. There are also no differences in sodium excretion between conscious \(\text{D}4_{+/-}\) mice and \(\text{D}4_{+/-}\) littermates in spite of an increase in AT1 receptors in the kidney of \(\text{D}4_{+/-}\) mice. Possibly, the high blood pressure may have elicited a pressure natriuresis through other mechanism that obfuscated any deficits in the renal handling of sodium in \(\text{D}4_{+/-}\) mice.\textsuperscript{53,54}

In summary, we have found that disruption of the D3 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 D3 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

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**Figure 6.** Immunoblots of AT1 receptors of renal membranes from tenth-generation \(\text{D}4_{+/-}\) and \(\text{D}4_{-/-}\) littermates. Top,munoblot of AT1 receptors in membranes from whole kidneys of \(\text{D}4_{+/-}\) (left, \(n=6\)) and \(\text{D}4_{-/-}\) (right, \(n=4\)) littermates. Bottom, bar graphs; AT1 receptor expression corrected by \(\alpha\)-actin was increased in \(\text{D}4_{-/-}\) mice (relative to \(\text{D}4_{+/-}\) littermates; \(n=8\)). *P<0.05 vs \(\text{D}4_{+/-}\), \(t\) test.

similar to the effect in \(\text{D}3_{+/-}\) mice.\textsuperscript{12} The duration of the hypertensive effect was longest in the \(\text{D}3_{+/-}\) mice, intermediate in the \(\text{D}3_{+/-}\) mice, and shortest in the \(\text{D}3_{+/-}\) mice. There were no differences in plasma or renal renin concentrations between \(\text{D}3_{+/-}\) and \(\text{D}3_{+/-}\) mice. This contrasts with the elevation of renal renin concentration in the hypertensive \(\text{D}4_{+/-}\) mice.\textsuperscript{12}

The mechanism underlying the AT1-dependent high blood pressure in the \(\text{D}3_{+/-}\) mice is not readily apparent. Aberrant interactions between D3 and AT1 receptors were found in spontaneously hypertensive rats.\textsuperscript{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 \(\text{D}4_{+/-}\) mice is, in part, central in origin. This may also explain why the acute hypertensive effect of angiotensin II was reduced in \(\text{D}4_{+/-}\) receptor–deficient mice compared with wild-type mice. However, AT1 protein expression in whole brain and renal homogenates and membranes of \(\text{D}4_{+/-}\) mice were increased relative to \(\text{D}4_{+/-}\) mice. The finding that the increase in AT1 receptor protein in the kidneys of \(\text{D}4_{+/-}\) mice was not associated with sodium retention may indicate a counteracting effect of another receptor stimulated by angiotensin II outside the central nervous system, possibly the AT2 receptor.\textsuperscript{36} The absence of a difference in AT1 receptor expression in the heart of \(\text{D}4_{+/-}\) and \(\text{D}4_{+/-}\) mice also suggests tissue-specific D4 regulation of the AT1 receptor. These remain speculative at this time.
receptor interacts with the AT$_1$ receptor in the central regulation of blood pressure. AT$_1$ 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 D$_3$ receptor gene locus is linked to and D$_3$ receptor variants are associated with hypertension, the relevance of the D$_3$ receptor in the pathogenesis of human essential hypertension needs to be evaluated.

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References


