Adrenergic and Endothelin B Receptor–Dependent Hypertension in Dopamine Receptor Type-2 Knockout Mice

Xiao Xi Li, Martin Bek, Laureano D. Asico, Zhiwei Yang, David K. Grandy, David S. Goldstein, Marcelo Rubinstein, Gilbert M. Eisner, Pedro A. Jose

Abstract—Polymorphism of the dopamine receptor type-2 (D_2) gene is associated with essential hypertension. To assess whether D_2 receptors participate in regulation of blood pressure (BP), we studied mice in which the D_2 receptor was disrupted. In anesthetized mice, systolic and diastolic BPs (in millimeters of mercury) were higher in D_2 homozygous and heterozygous mutant mice than in $D_2+/+$ littermates. BP after α-adrenergic blockade decreased to a greater extent in $D_2-/-$ mice than in $D_2+/+$ mice. Epinephrine excretion was greater in $D_2-/-$ mice than in $D_2+/+$ mice, and acute adrenalectomy decreased BP to a similar level in $D_2-/-$ and $D_2+/+$ mice. An endothelin B (ET[B]) receptor blocker for both ET(B1) and ET(B2) receptors decreased, whereas a selective ET(B1) blocker increased, BP in $D_2-/-$ mice but not $D_2+/+$ mice. ET(B) receptor expression was greater in $D_2-/-$ mice than in $D_2+/+$ mice. In contrast, blockade of ET(A) and V_1 vasopressin receptors had no effect on BP in either $D_2-/-$ or $D_2+/+$ mice. The hypotensive effect of an AT_1 antagonist was also similar in $D_2-/-$ and $D_2+/+$ mice. Basal Na^+,K^+-ATPase activities in renal cortex and medulla were higher in $D_2+/+$ mice than in $D_2-/-$ mice. Urine flow and sodium excretion were higher in $D_2-/-$ mice than in $D_2+/+$ mice before and after acute saline loading. Thus, complete loss of the D_2 receptor results in hypertension that is not due to impairment of sodium excretion. Instead, enhanced vascular reactivity in the D_2 mutant mice may be caused by increased sympathetic and ET(B) receptor activities. **(*Hypertension*. 2001;38:303-308.)**

Key Words: dopamine ■ receptors, dopamine ■ receptors, endothelin ■ Na^+,K^+ transporting ATPase ■ kidney

Dopamine can regulate cardiovascular function by its actions on central cardiovascular centers, the pituitary and adrenal glands, kidney, cardiac and vascular smooth muscle, and the sympathetic nervous system.[1,2] Effects of dopamine are mediated by dopamine receptor type-1 and type-2 (D_1- and D_2)–like receptors that belong to the G protein–coupled receptor family.[1–3] Both D_1- and D_2-like receptors have been shown to regulate arterial blood pressure (BP).[1,2] Genetic hypertension in rodents and humans has been shown to be associated with decreased activity of D_1-like receptors in the kidney and central nervous system.[1,2] Decreased D_2-like dopaminergic activity in the central nervous system has been reported in essential hypertension.[1,4] Several studies in animal models of genetic hypertension also support the notion of altered central D_2-like dopaminergic activity in hypertension.[5,6] However, 3 D_2-like dopamine receptors, D_2, D_3, and D_4, exist, and which of the 3 cloned D_2-like receptors participate in the D_2-like regulation of BP is unclear.[1–3] Disruption of D_3 receptors in mice produces hypertension mediated, at least in part, by activation of the renin-angiotensin system.[7] The D_2 receptor could be involved in D_2-like–mediated hypertension because it is the major D_2-like dopamine receptor.[3,8–11] Moreover, BP decreased when a segment of chromosome 8 that contained the D_2 receptor gene was transferred from a normotensive Brown Norway rat to a spontaneously hypertensive rat background.[12]

Several variants of the human D_2 dopamine receptor have been reported.[13] Abnormalities of D_2 receptor genes could play a role in the pathogenesis of essential hypertension, because the association of a D_2 dopamine receptor polymorphism with obesity and hypertension has been reported.[14] To determine whether D_2 receptors play a role in the regulation of BP, we measured arterial pressure in congenic B6 mice mutants for the D_2 receptor.[8,9] Because D_2-like receptors have been shown to interact with vasopressor systems,[1,6,15,16] interactions between D_2 receptors and other vasopressor systems were also studied.

Methods

D_2 Receptor–Deficient Mice

The original F_2 hybrid strain (129/SvXC57BL/6J, Oregon Health Sciences University, Portland) that contained the mutated D_2 recep-

Received October 23, 2000; first decision December 7, 2000; revision accepted March 1, 2001.

From the Departments of Pediatrics (X.X.L., M.B., L.D.A., Z.Y., P.A.J.), Physiology and Biophysics (Z.Y., P.A.J.), and Medicine (G.M.E.), Georgetown University Medical Center, Washington, DC; the Departments of Physiology and Pharmacology (D.K.G.), Oregon Health Sciences University, The Vollum Institute, Portland, Ore; the National Institute of Neurological Disorders and Stroke (D.S.G.), Bethesda, Md; and Instituto de Investigaciones en Ingeniería Genética y Biología Molecular (M.R.), Universidad de Buenos Aires, Argentina.

Correspondence to Pedro A. Jose, MD, PhD, Professor of Pediatrics and Physiology and Biophysics, Georgetown University Medical Center, 3800 Reservoir Rd NW, Washington, DC 20007. E-mail josep@gunet.georgetown.edu

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tor allele was backcrossed to wild-type C57BL/6J for 5 generations and genotyped.[8,9] These mice acquired normal basic motor skills without tremor, ataxia, or abnormal stance or posture but had decreased initiation of movement.[8] All studies were approved by the Georgetown University Animal Care and Use Committee.

BP and Renal Function Studies

Mice were anesthetized with pentobarbital 50 mg/kg IP, placed on a heated board to maintain body temperature at 37°C, and tracheotomized.[7] Mice were euthanized (pentobarbital 100 mg/kg) at the conclusion of the study.

Effect of Agonists and Antagonists on BP

Because preliminary studies indicated that arterial pressures were higher in $D_2-/-$ than $D_2+/+$ mice, we determined the mechanism of increase in BP by intravenous infusion of antagonists of pressor agents. After a 60-minute stabilization period, drugs were infused in random order: [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)-2-(O-methyl)-tyrosine] arginine vasopressin (V_1 vasopressin antagonist; Peninsula Laboratories, Inc) 10 μg/kg IV over 30 seconds[17]; BQ-610 (endothelin receptor antagonist, ET[A]; Peninsula) 100 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 10 minutes[18]; BQ-788 (endothelin B ET[B]1/ET[B]2 antagonist; Peninsula) 6.6 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 15 minutes[19]; RES-701-1 (ET[B1] antagonist; American Peptide) 100 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 1 hour[20]; phentolamine (α-adrenergic antagonist; Research Biochemicals International) 5 $ng \cdot kg^{-1} \cdot min^{-1}$ for 30 minutes[21]; and losartan (AT_1 antagonist) 3 mg/kg IV over 30 seconds.[7] BP was allowed to stabilize at preinfusion values for 30 to 60 minutes before new drug administration. These antagonists block the vasopressor effects of their respective agonists: 40 μL of various concentrations of vasopressin, phenylephrine, endothelin-1 (ET-1), and angiotensin II, respectively, given over 30 seconds. Effects on BP of bolus injections of ET(B1) agonist sarafotoxin Sc6 0.01 to 1.0 nmol/kg (American Peptide)[20] and ET-1 0.1 to 1.3 pmol/kg were also studied.

Adrenalectomy

Effect of adrenalectomy on BP was also studied in some mice. After a midline abdominal incision, adrenal gland was separated from kidney, ligated, crushed with forceps, and excised. BP readings were obtained after a 20-minute stabilization period.

Immunoblotting Studies

Because blockade of ET(B) receptors normalized BP in $D_2-/-$ mice (see Results), we immunoblotted for ET(B) receptors (Maine Biotechnology Services, Inc) of liver; ET-1 has been reported to induce constriction of hepatic circulation through both ET(A) and ET(B) receptors.[22] Renal D_2 receptors were also immunoblotted with purified rat striatum as positive control (Chemicon).[7] Negative controls included rabbit IgG instead of primary antibody, secondary antibody alone, and D_2 antibody preadsorbed with immunizing peptide. D_2-specific bands (49 and 98 kDa) and ET(B)-specific bands (49 kDa) were visualized by use of enhanced chemiluminescence Western blotting detection kit (Amersham Biotech).

Determination of Na^+,K^+-ATPase Activity

Na^+,K^+-ATPase activity is as described previously.[23]

Determination of Catecholamines

Kidneys were homogenized with 0.1 mol/L $HClO_4$ and centrifuged at 6000*g* for 20 minutes at 4°C. Supernatant, urine, and plasma were flash-frozen and stored at −70°C until assay.[24]

Determination of Plasma Endothelin-Immunoreactive Levels

Fifty microliters of plasma was diluted with 1% TCA and centrifuged at 14 000 rpm for 20 minutes at 4°C. Supernatants were loaded into separation columns preequilibrated with 100% acetonitrile and washed with 1% TCA. Peptides were eluted (60% acetonitrile in 1% TCA), lyophilized, and quantified by ELISA (Peninsula).

TABLE 1. Characteristics of D_2 Mutant Mice

Variable	$D_2+/+$ Mice (n=13)	$D_2+/-$ Mice (n=11)	$D_2-/-$ Mice (n=25)
Age, mo	6–12	6–12	6–12
Body weight, g	25±1	31±2*	25±1
Heart weight, % body weight	0.45±0.01	0.42±0.01	0.44±0.02
Kidney weight, % body weight	1.21±0.08	1.22±0.10	1.20±0.05
Heart rate, bpm	418±8	439±13	452±8†
Blood pressure, mm Hg			
Systolic	104±2‡	129±4	128±2
Diastolic	77±1‡	98±3	97±2
Mean	85±1‡	108±3	107±2

Data are mean±SE.

*$P<0.05$ vs $D_2+/+$ or $D_2-/-$ mice; †$P<0.05$ vs $D_2+/+$ mice; ‡$P<0.05$ vs mutant mice by ANOVA, Newman-Keuls test.

Statistical Analyses

Data (mean±SE) were analyzed by 1-way or repeated ANOVA or *t* test as indicated.

Results

General Characteristics

$D_2+/-$ mice were heavier than either $D_2+/+$ or $D_2-/-$ mice (Table 1). Heart and kidney weights as a percentage of body weight were similar among the groups. Heart rate was significantly elevated in $D_2-/-$ mice versus $D_2+/+$ mice. Furthermore, systolic, diastolic, and mean arterial pressures were also higher in mice heterozygous and homozygous for mutated D_2 receptor allele compared with wild-type mice. D_2 receptors (47 and 98 kDa) were detected in rat striatal tissues (positive control) but not in renal cortical or medullary tissue from rats or from $D_2-/-$ or $D_2+/+$ mice (data not shown).

Effect of Saline Loading

Basal urine flow rate and sodium excretion were greater in $D_2-/-$ mice than in $D_2+/+$ mice (Figure 1). Saline loading increased urine flow and absolute (Figure 1) and fractional sodium (data not shown) excretion in all the mice. However, the increase was greater in $D_2-/-$ mice than in $D_2+/+$ mice.

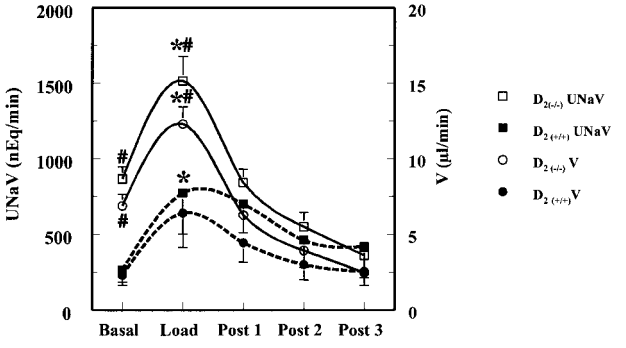

Figure 1. Urine flow (V) and sodium excretion (UNaV) in $D_2+/+$ and $D_2-/-$ mice. Natriuresis and diuresis before (Basal) and during (Load) but not after (Post 1, 2, and 3) saline loading were greater in $D_2-/-$ mice than in $D_2+/+$ mice. #$P<0.05$, $D_2-/-$ vs $D_2+/+$ mice, *t* test; *$P<0.05$ vs basal, ANOVA for repeated measures, Newman-Keuls test; n=6 to 9 per group. Each period lasted 60 minutes.

Glomerular filtration rate was similar in $D_2-/-$ and $D_2+/+$ mice and was not affected by saline loading. Arterial pressures were not affected by the acute saline load (data not shown).

Na^+,K^+-ATPase Activity

In agreement with reports that D_2-like agonists stimulate Na^+,K^+-ATPase activity in renal tubules,[2] Na^+,K^+-ATPase activity was lower ($P<0.05$, *t* test) in both cortical and medullary regions in $D_2-/-$ mice (cortex, 22.97 ± 3.26; medulla, 22.09 ± 2.07 nmol of inorganic phosphate per milligram of protein per minute, n=5) than in $D_2+/+$ mice (cortex, 33.45 ± 2.01; medulla, 31.52 ± 2.62 nmol of inorganic phosphate per milligram of protein per minute, n=7).

Effect of Receptor Ligands on BPs

Effects of AT_1 and V_1 Receptor Antagonists

AT_1 receptor antagonist decreased BP in $D_2+/+$ and $D_2-/-$ mice, which indicated that AT_1 receptors maintain a tonic control of BP in anesthetized wild-type and mutant mice. In the first 5 minutes after AT_1 antagonist administration, however, decrease in BP was greater in $D_2+/+$ than $D_2-/-$ mice (Figure 2A). V_1 vasopressin antagonist [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid)-2-(O-methyl)-tyrosine] arginine vasopressin had minimal effects on BP (Figure 2A).

Effect of α-Adrenergic Antagonist

Phentolamine, an α-adrenergic antagonist, markedly decreased BP in D_2 mutant and wild-type mice. The magnitude of the decrease in BP was greater in $D_2-/-$ than $D_2+/+$ mice (Figure 2B), ultimately resulting in similar BPs at the nadir of the phentolamine effect. Moreover, acute adrenalectomy decreased BP in $D_2-/-$ (mean BP in mm Hg before adrenalectomy, 101 ± 5; after adrenalectomy, 52 ± 14; n=3), $D_2+/-$ (mean BP before adrenalectomy, 101 ± 2; after adrenalectomy, 61 ± 4; n=4) and $D_2+/+$ (mean BP before adrenalectomy, 82 ± 2; after adrenalectomy, 47 ± 8; n=2) mice, such that BPs were no longer different among groups.

Effects of ET(A) and ET(B) Antagonists

ET(A) antagonist BQ-610 had minimal effects on BP in $D_2+/+$ and $D_2-/-$ mice (Figure 2A). In contrast, ET(B1)/ET(B2) antagonist BQ-788 decreased BP in $D_2-/-$ but not $D_2+/+$ mice, which indicated that increased activity of ET(B) receptors contributes to elevation of BP in D_2 mutant mice (Figure 3A). Because ET(B) receptor subtypes have differential vascular effects (ET[B1] decreases and ET[B2] increases BP),[20] additional studies were performed with ET(B1) antagonist RES-701-1 (Figure 3A). RES-701-1 increased BP to a greater extent in $D_2-/-$ than $D_2+/+$ mice.

Effects of ET-1 and ET(B) Agonist

Because ET(B1) and ET(B2) effects were increased in D_2 mutant mice, we determined the effect of ET-1 on BP. ET-1 0.1 to 1.3 nmol/kg tended to increase BP to a greater extent in $D_2+/+$ than $D_2-/-$ mice (maximum increase, $23\pm5\%$ and $17\pm5\%$, respectively; n=3 to 6 per group), but significant differences were not found. ET(B) agonist sarafotoxin S6c also tended to increase BP to a greater extent in $D_2+/+$ than $D_2-/-$ mice (maximum increase, $33\pm10\%$ and $30\pm4\%$, respectively) but reached significance only at 0.3 nmol/kg ($D_2+/+$, $21\pm5\%$ versus $D_2-/-$, $10\pm1\%$; $P<0.05$ by *t* test; n=3 to 6 per group).

Endothelin Receptor Protein and Endothelin-Like Immunoreactive Levels

Immunoreactive ET(B) receptors were 3-fold greater in $D_2-/-$ than $D_2+/+$ mice (Figure 3B). Plasma immunoreactive endothelin levels were not different between $D_2+/+$ (1.20 ± 0.42 ng/mL) and $D_2-/-$ (0.75 ± 0.24 ng/mL) mice, although a trend occurred toward lower values in $D_2-/-$ mice. These values are 5 to 100 times greater than that previously reported in mice,[25] because the antibody used cross-reacts with ET-1, ET-2, and big endothelin (Peninsula).

Catechol Levels

Renal catechol levels were similar in $D_2+/+$ and $D_2-/-$ mice (data not shown). Urinary catechols were also similar except for urinary epinephrine. Epinephrine excretion rates were generally higher before (baseline, urine period 1), during (urine period 2), and after saline loading (urine periods 3 through 5) in $D_2-/-$ versus $D_2+/+$ mice (Table 2). Urinary dopamine and norepinephrine tended to increase with saline

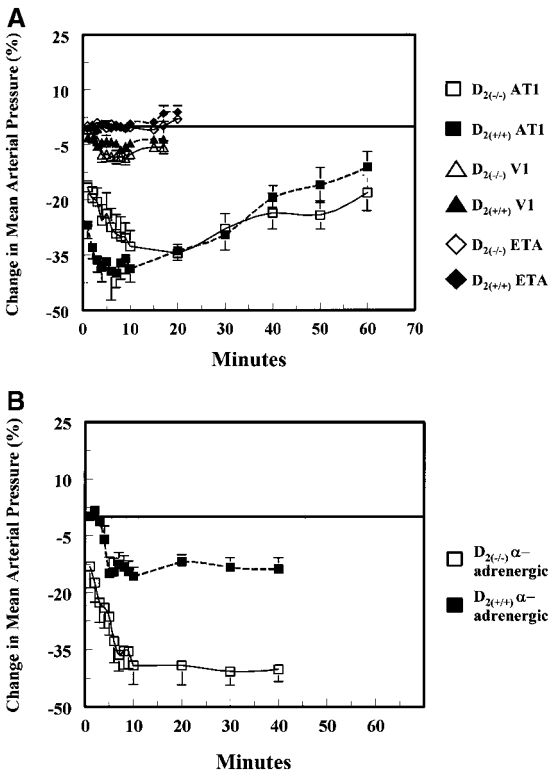

Figure 2. A, Effect of the AT_1 antagonist losartan, V_1 vasopressin antagonist, and ET(A) antagonist BQ 610 on mean arterial pressure in $D_2+/+$ and $D_2-/-$ mice. AT_1 antagonist significantly decreased BP in both mouse strains ($P<0.05$ by ANOVA for repeated measures, Newman-Keuls test; n=6 to 9 mice per group). Decrease in BP in first 5 minutes was modestly greater in $D_2+/+$ mice than in $D_2-/-$ mice ($P<0.05$ by *t* test). B, Effect of α-adrenergic antagonist phentolamine on mean arterial pressure in $D_2+/+$ and $D_2-/-$ mice. α-adrenergic antagonist decreased BP ($P<0.05$ by ANOVA for repeated measures, Newman-Keuls test) after 5 minutes to a greater extent in $D_2-/-$ (n=9) than $D_2+/+$ (n=7) mice ($P<0.05$ by *t* test).

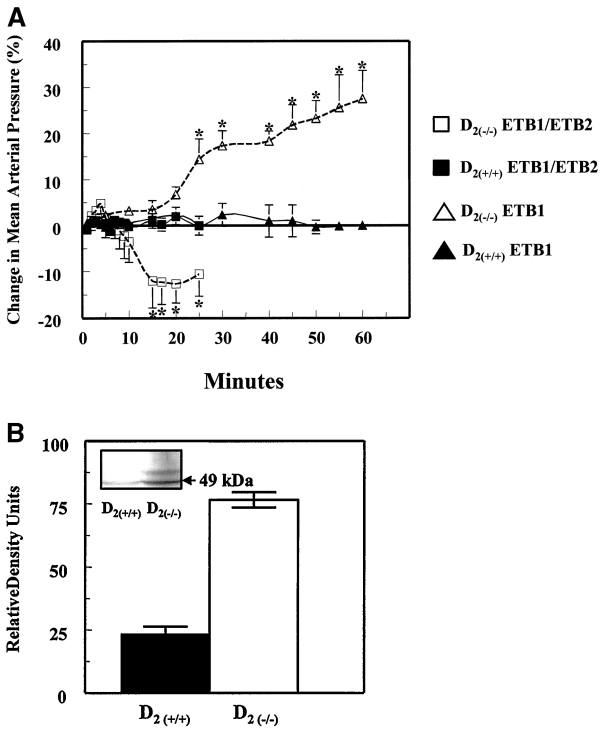

Figure 3. A, Effect of an ET(B1) and ET(B2) antagonist (BQ 788) and a selective ET(B1) antagonist (RES-701-1) on mean arterial pressure in $D_2+/+$ and $D_2-/-$ mice. ET(B1)/ET(B2) antagonist decreased BP in $D_2-/-$ but not $D_2+/+$ mice. In contrast, ET(B1) antagonist increased BP in $D_2-/-$ but not $D_2+/+$ mice (*$P<0.05$ vs baseline by ANOVA for repeated measures, Newman-Keuls test; *$P<0.05$ vs $D_2+/+$ by *t* test; n=6 to 8 mice per group). B, Immunoreactive ET(B) receptors in $D_2+/+$ and $D_2-/-$ mice. ET(B) receptor expression in liver was greater in $D_2-/-$ mice than in $D_2+/+$ mice (*$P<0.05$ by *t* test, n=5 per group). Inset is a representative immunoblot.

loading in $D_2+/+$ mice and reached statistical significance in $D_2-/-$ mice; the percentage increases in urinary dopamine (85%) and urinary norepinephrine (108%) with saline loading (compared with baseline) were similar in $D_2+/+$ and $D_2-/-$ mice. These changes were associated with increased urine flow but not with glomerular filtration rate (data not shown).

Discussion

Our data suggest that disruption of the D_2 dopamine receptor, a member of the family of D_2-like receptors, increases systolic and diastolic BPs in $D_2+/-$ mice and $D_2-/-$ mice. No gender effect occurred. Presence of hypertension in $D_2+/-$ mice may be taken to be an indication that few spare D_2 receptors regulate this phenotype, similar to what has been reported for locomotor and pituitary lactotroph function.[7,8] The hypertensive phenotype was unlikely to be caused by genetic heterogeneity, because mice have been inbred to the fifth generation.[7]

Both α_2-adrenergic and D_2 dopamine receptors are involved in prejunctional inhibition of catecholamine release.[3,15,16] α_{2A}-Adrenergic receptor has been shown to inhibit sympathetic outflow, and disruption of this receptor in mice increased BP.[26] Stimulation of prejunctional D_2-like receptors also inhibited sympathetic outflow.[3,15,16] In contrast, stimulation of postsynaptic D_2-like receptors in the nervous system and arterial vessels increased vascular resistance or BP.[6,27] The pressor effect of intravenously administered D_2-like drugs was transient, whereas the peripheral vasodilator effect, presumably caused by actions at prejunctional D_2-like receptors, was persistent.[16] The decrease in BP after α-adrenergic blockade in $D_2-/-$ mice suggests that sympathetic activity may have increased as a result of withdrawal of D_2 receptor actions at prejunctional receptors. Thus, acute adrenalectomy decreased BP such that BPs were no longer different among groups. Moreover, urinary epinephrine levels were elevated in $D_2-/-$ compared with $D_2+/+$ mice; D_2 receptors in the adrenal medulla inhibit epinephrine release.[27] Similar renal and urinary norepinephrine levels in $D_2+/+$ and $D_2-/-$ mice may be explained by observations of catecholamine metabolism in striatum of $D_2-/-$ mice. Monoamine levels in striatum were similar in $D_2-/-$ and $D_2+/+$ mice, although dopamine metabolites were increased.[9,10]

An unexpected finding in these studies was the ability of BQ-788, an ET(B1)/ET(B2) antagonist, to decrease and normalize BP, whereas ET(B1) antagonist RES-701-1 increased BP in $D_2-/-$ mice without affecting BP in $D_2+/+$ mice. ET(A) antagonist BQ-610 had no effect on BP in either $D_2-/-$ or D_2 +/+ mice. The endothelins (ET-1, ET-2, and ET-3), which are generally vasoconstrictors, exert their actions by means of ET(A) and ET(B) receptors.[18–20,25,28] However, endothelins can also mediate vasodilation.[19,20,25,28] On the basis of pharmacological evidence, 2 types of ET(B) receptors have been postulated: ET(B1), which is a relaxant,

TABLE 2. Catechol Excretion in $D_2+/+$ and $D_2-/-$ Mice

Urine Catechols, pg/min	$D_2+/+$ Mice (n=7)					$D_2-/-$ Mice (n=9)				
	U1	U2	U3	U4	U5	U1	U2	U3	U4	U5
DHPG	148±23	299±156	166±56	201±100	220±139	173±19	213±21	11.8±14	87±14*	71±12*
DOPA	65±41	181±112	163±118	99±49	228±193	75±66	37±23	22±12	25±14	14±6
DOPAC	35±6	56±18	52±17	39±14	36±12	48±7	61±8	36±7	37±18	24±9
Dopamine	437±79	810±264	513±139	516±197	631±408	353±55†	656±118	498±50	400±74	386±67†
Epinephrine	9±3	24±7	31±13	11±5	7±3	63±23	156±49‡	125±36‡	73±19‡	113±50
Norepinephrine	131±43	273±77	164±56	128±39	146±87	191±20†	401±80	237±28	210±36	210±31

U indicates urine periods 1–5: U1, baseline; U2, saline loading; and U3 through U5, after saline loading. DHPG indicates dihydroxyphenyl glycol; DOPA, dihydroxyphenylalanine; and DOPAC, dihydroxyphenylacetic acid.

*$P<0.05$ vs U1 or U2 in D−/− mice by ANVR, Newman-Keuls test; †$P<0.05$ vs U2 in D−/− mice by ANVR, Newman-Keuls test; ‡$P<0.05$ vs $D_2+/+$ mice, *t* test.

and ET(B2) which is a constrictor.[19,20,25,28] In ET(B) knockout mice, both vasodilatory and vasoconstrictor effects of ET(B) receptors were eliminated, which suggests that ET(B1) and ET(B2) receptors are the same receptor.[29] The difference in their actions may be related to the sites at which these receptors are expressed. For example, ET(B) (ET[B1]) receptors expressed in endothelial cells are vasodilatory because of their linkage to nitric oxide and prostaglandins[25,29]; the ability of prejunctional ET(B) (ET[B1]) receptors to inhibit catecholamine release may also contribute to decreasing vascular resistance.[30] Elimination of the ET(B1)-mediated stimulation of endothelial prostacyclin production has been suggested to be the cause of hypertension in ET(B)-deficient mice.[25] If an ET(B1)-mediated increase in endothelial or prostacyclin production does not occur, however, then the vasoconstrictor effect of ET(B2) would become unopposed,[25,28,29] which would result in hypertension. The anticipated inhibitory effect of ET(B1) on catecholamine release[30] would partially offset the absence of the usual inhibitory effect of D_2 receptors on catecholamine release in this model. This occurrence may explain the modest changes in urinary catechol levels in $D_2-/-$ mice.

Interestingly, ET(B) expression was 3 times greater in $D_2-/-$ than $D_2+/+$ mice. Absence of D_2 receptors conceivably could have led to increased vasodilatory ET(B1) receptors and vasoconstrictor ET(B2) receptors. Because D_2 receptors are expressed at the junction of adventitia and tunica media,[31] disruption of D_2 receptors should affect expression of ET(B1) and ET(B2) receptors in the tunica media–adventitia but not ET(B1) in the tunica intima because no dopamine receptors are expressed in this blood vessel layer.[1,31] Minimal differential effect of ET-1 and the ET(B1) agonist sarafotoxin S6c occurred on BP in $D_2+/+$ versus $D_2-/-$ mice, presumably because ET(B1) expression in tunica media was not altered in $D_2-/-$ mice. The predominant effect in D_2 mutant mice was an increase in ET(B2) action, however, because the D_2 mutant mice were hypertensive. Hence, the BP-lowering effect of ET(B1)/ET(B2) antagonist BQ-788 in D_2 mutant mice and the greater increase in BP in D_2 mutant versus $D_2+/+$ mice after the ET(B1) antagonist RES-101-1. Although renin levels were not measured in these studies, the greater hypotensive response to AT_1 blockade in D_2 wild-type versus $D_2-/-$ mice is suggestive of decreased activity of the renin-angiotensin system in $D_2-/-$ mice. ET(B) receptors have been reported to inhibit renin gene expression in mouse juxtaglomerular cells.[32]

The site of interaction between D_2 dopamine receptors and ET(B) receptors was not determined in the present studies. Dopamine, dopamine receptors, endothelin, and ET(B) receptors have been found in brain and spinal regions known to control cardiovascular function.[33] Depletion of dopamine production in the striatum has been reported to decrease ET receptors.[33] Decreased clearance of dopamine in $D_2-/-$ mice[9,10] may have led to upregulation of vasoconstricting ET(B2) receptors in the tunica media of resistance vessels. Another explanation may be that the absence of inhibitory effect of D_2 receptors on ET(B1) receptors in adrenal medulla of $D_2-/-$ mice could have led to the increase in circulating epinephrine.[30,34]

We have reported that disruption of the D_3 receptor in mice leads to development of renin-dependent hypertension and decreased ability to excrete sodium load.[7] In the present studies, $D_2-/-$ mice not only had a greater basal urine flow rate and sodium excretion than $D_2+/+$ mice but also responded to saline loading with greater natriuresis and diuresis than the $D_2+/+$ mice. Increased sodium excretion in the $D-/-$ mice was associated with lower Na^+,K^+-ATPase activity, the cause of which remains to be determined. However, ET(B) receptors have been reported to decrease chloride transport at the thick ascending limb of Henle.[35] Pressure natriuresis also may have contributed to increased sodium excretion in the $D_2-/-$ mice.

In summary, on the basis of our examination of D_2 receptor–deficient mice, we conclude that D_2 dopamine receptors expressed on sympathetic neurons normally act to inhibit sympathetic outflow from the nervous system.[15,16] When $\geq$50% of the D_2 receptor population is depleted, concomitant increases occur in sympathetic outflow and expression of ET(B) receptors in several tissues, including liver and, presumably, vascular smooth muscle cells as well. The absence of inhibitory tone on sympathetic outflow mediated by the D_2 dopamine receptor coupled with increased ET(B) activity may predispose the animal to hypertension.

Acknowledgments

The present work was supported in part by grants from the National Institutes of Health HL-58536, HL-23081, DK-39308, and DK-52612. We also thank Courtney Holmes for performing the catechol assays.

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