Lack of Renal Dopamine D$_5$ Receptors Promotes Hypertension

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ABSTRACT
Disruption of the dopamine D$_5$ receptor gene in mice increases BP and causes salt sensitivity. To determine the role of renal versus extrarenal D$_5$ receptors in BP regulation, we performed cross-renal transplantation experiments. BP was similar between wild-type mice and wild-type mice transplanted with wild-type kidneys, indicating that the transplantation procedure did not affect BP. BP was lower among D$_5^{-/-}$ mice transplanted with wild-type kidneys than D$_5^{-/-}$ kidneys, demonstrating that the renal D$_5$ receptors are important in BP control. BP was higher in wild-type mice transplanted with D$_5^{-/-}$ kidneys than wild-type kidneys but not significantly different from syngenic transplanted D$_5^{-/-}$ mice, indicating the importance of the kidney in the development of hypertension. On a high-salt diet, all mice with D$_5^{-/-}$ kidneys excreted less sodium than mice with wild-type kidneys. Transplantation of a wild-type kidney into a D$_5^{-/-}$ mouse decreased the renal expression of AT$_1$ receptors and Nox-2. Conversely, transplantation of a D$_5^{-/-}$ kidney into a wild-type mouse increased the expression of both, suggesting that both renal and extrarenal factors are important in the regulation of AT$_1$ receptor and Nox-2 expression. These results highlight the role of renal D$_5$ receptors in BP homeostasis and the pathogenesis of hypertension.


Dopamine is an important regulator of systemic BP.$^{1-3}$ In the kidney it regulates fluid and electrolyte balance by its actions on hemodynamics and epithelial transport and by regulation of hormones and humoral agents.$^{1-2,4-6}$ Dopamine also controls BP by actions on neuronal cardiovascular centers and the heart, as well as arterial and venous vessels,$^{1-4}$ and modulates fluid and sodium intake via “appetite” centers in the brain and via gastrointestinal transport.$^{7,8}$

Dopamine is produced locally in the kidney, independent of innervation, and its actions are exerted through five subtypes of receptors: the D1-like receptors comprised of the D$_1$ (D$_1$R) and D$_5$ (D$_5$R) receptor subtypes and the D2-like receptors comprised of the D$_2$, D$_3$, and D$_4$ receptor subtypes.$^{1-3}$ Renal dopamine receptors are important in the regulation of NaCl transport in almost all segments of the nephron$^{1-3}$ and are responsible for
more than 50% of incremental sodium excretion when NaCl intake is increased.9–11

The D5R has a higher affinity for dopamine than the D1R and is constitutively active.12,13 In the kidney, D5R is expressed in proximal and distal tubules and tunic media of arterioles14,15 and together with the D1 receptor may mediate the diuretic and natriuretic effects of D1-like receptors. However, the role of renal D5R in the regulation of BP is not completely understood because of the lack of drugs that selectively stimulate or antagonize this receptor.1–3

We reported that disruption of the D5R in mice resulted in elevated systolic, diastolic, and mean BPs, as well as heart weights. The increased BP in these mice, measured under anesthesia, appears to be, in part, related to increased sympathetic tone primarily attributable to the central nervous system. However, further studies suggested that the kidney may play a significant role in the hypertension of D5R−/− mice. A high salt diet increases BP further in D5R−/− mice, indicating that renal D5Rs are important in the control of BP via regulation of sodium transport.17 The renal expression of angiotensin type 1 receptor (AT1R) is increased in D5R−/− mice relative to D5R+/+ littermates,18,19 and chronic intraperitoneal administration of the AT1R antagonist losartan normalizes BP in pentobarbital-anesthetized D5R−/− mice but minimally affects BP in D5R+/+ littermates.19 Renal and brain reactive oxygen species and oxidative stress are increased in D5R−/− mice.17

To determine the role of renal D5R in the regulation of BP, we performed cross-transplantation studies in D5R−/− and wild-type mice in which one kidney of a D5R−/− mouse was transplanted into a bilaterally nephrectomized wild-type mouse or one kidney of a wild-type mouse was transplanted into a bilaterally nephrectomized D5R−/− mouse. Syngenic transplants (wild-type kidney to wild-type mouse and D5R−/− kidney to D5R−/− mice) were also performed. We studied the effects of renal cross-transplantation on BP on normal and high salt diet and determined the renal expression of D5R and AT1R and NADPH oxidase isoform 2 (Nox-2) and nitrotyrosine.

RESULTS

BP in Unmanipulated D5R−/− Mice and Wild-type Littermates
Systolic and diastolic BPs measured under anesthesia were significantly higher in unmanipulated D5R−/− mice than in unmanipulated D5R wild-type littermates (systolic: 124 ± 2 versus 97 ± 3 mmHg; diastolic: 93 ± 4 versus 70 ± 3 mmHg). These results are consistent with our previous studies in anesthetized D5R−/− mice.16,17,19

BP in Transplanted Mice
Four groups of mice were generated from the cross-transplantation procedure between genetically matched wild-type (D5R+/+) and D5R−/− mice. The mice were genotyped for the presence of the wild-type D5R or the D5R knockout (D5R−/−) gene that is truncated in the second extracellular loop, resulting in the absence of D5R function.16 Wild-type mice transplanted with wild-type kidneys expressed the wild-type D5R in both renal and nonrenal tissues (WT-WT). Wild-type mice transplanted with a kidney from a D5R−/− mouse expressed wild-type D5R only in nonrenal tissues (KO-WT). D5R−/− mice transplanted with a wild-type kidney expressed the wild-type D5R only in the kidney (WT-KO). D5R−/− mice transplanted with a kidney from D5R−/− mouse did not express the wild-type D5R in any tissue (KO-KO).

Cross-transplantation of a kidney of a wild-type mouse into a bilaterally nephrectomized wild-type mouse did not affect either systolic or diastolic BP (Figure 1) or heart rate (Supplemental Table 1). Both systolic and diastolic BPs were similar to those in unmanipulated mice (systolic: 98 ± 2 versus 97 ± 2; diastolic: 69 ± 4 versus 70 ± 3). This indicates that the transplantation and its consequences (e.g. renal denervation) do not affect BP.

Cross-transplantation of a kidney of a wild-type mouse into a D5R−/− mouse did not affect heart rate (Supplemental Table 1) but decreased BP when compared with D5R−/− mice transplanted with a syngenic kidney (systolic: 105 ± 5 versus 128 ± 2 mmHg; diastolic: 73 ± 5 versus 94 ± 5 mmHg) (Figure 1) or unmanipulated D5R−/− mice. These values were slightly higher but not significantly different from D5R+/+ mice transplanted with a syngenic kidney (Figure 1). This shows that D5Rs in nonrenal tissues do not play a major role in the regulation of BP and that renal mechanisms are the main determining factors of chronic BP levels.

Cross-transplantation of a kidney of a D5R−/− mouse into a wild-type mouse increased BP (systolic: 117 ± 3 versus 98 ± 2 mmHg; diastolic: 85 ± 4 versus 69 ± 4 mmHg) so that systolic BP in the transplanted mice was no longer different from that in syngenic transplanted D5R−/− (Figure 2) or unmanipulated D5R−/− mice. Heart rate was not affected (Supplemental Table 1). This indicates that renal D5Rs are important in the regula-
Absence of D5Rs increases BP. Innervation is not involved in the mechanisms by which the transplanted with D5 mouse also did not affect BP (systolic: 128 ± 2 versus 124 ± 2 mmHg; diastolic: 94 ± 5 versus 93 ± 4 mmHg) (Figure 1) or heart rate when compared with those in unmanipulated D5−/− mice (Supplemental Table 1). This indicates that renal innervation is not involved in the mechanisms by which the absence of D5Rs increases BP.

There was no cardiac hypertrophy in the wild-type mice transplanted with D5−/− kidneys, as judged by heart weights. Both groups of wild-type mice transplanted with wild-type or D5−/− kidneys had the same heart weight (WT-D5: 140 ± 7.5 mg; D5-D5: 140 ± 6.7 mg), whereas both groups of D5−/− mice had higher heart weights (WT-D5: 161 ± 4.8 mg; D5-D5: 162 ± 16.2 mg). However, mice were transplanted for only 2 weeks. This period may not be long enough for the mice to develop significant hypertrophy.17

The functional and anatomical viability of the transplant was assessed. Serum creatinines were similar in all groups (Table 1). Histologic study of renal sections showed no gross abnormalities in any of the transplanted kidneys and no evidence of ischemic injury (Supplemental Figure 1).

Effect of Salt Loading on BP and Sodium Excretion in Transplanted Mice
Salt loading did not induce any significant change in absolute BP levels in any of the groups. We have reported that BP in D5−/− mice increases after dietary salt loading.17 However, neither systolic nor diastolic BP increased on high salt diet in either syngenic transplanted D5−/− or wild-type mice transplanted a D5−/− kidney (Figure 2, top panel, and Table 1). In contrast to the apparent absence of an effect of the high salt diet on absolute BP levels, the directional change, i.e., an increase in systolic BP with high salt diet, was significantly different in mice transplanted with D5−/− kidneys, either syngenic or nonsyngenic, than in wild-type mice transplanted with syngenic kidneys (Figure 2, bottom panel). A plot of the relationship between BP and sodium excretion was shifted to the right in mice with D5−/− kidneys, indicating that in these mice higher BPs are necessary to excrete comparatively less sodium (Figure 3).

Renal Expression of D1 and AT1 Receptors, Nox-2 and Nitro-tyrosine
The renal expression of D1Rs was similar in all groups (WT-WT: 100 ± 24; WT-KO: 108 ± 28; KO-WT: 68 ± 20; KO-KO: 96 ± 12 expressed as percentages of WT-WT and corrected for protein loading). The expression of AT1Rs was highest in D5−/− mice with syngenic transplanted kidneys and lowest in wild-type mice with syngenic transplanted kidneys. As mentioned previously we have already reported that renal expression of AT1Rs is increased in D5−/− mice.18,19 Surprisingly nonsyngenic cross-transplanted D5−/− kidneys expressed approximately 50% fewer AT1Rs than kidneys that were syngenic transplanted. Conversely nonsyngenic cross-transplanted wild-type kidneys expressed twice as many AT1Rs relative to

Table 1. Systolic and diastolic blood pressures, serum, and urinary sodium and creatinine concentrations in cross renal-transplanted mice on high NaCl diet (6%) for 1 week

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<td>Systolic pressure (mmHg)</td>
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<td>273 ± 34</td>
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<td>Serum creatinine (mg/dl)</td>
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<td>0.40 ± 0.12</td>
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<td>Urinary creatinine (mg/ml)</td>
<td>0.09 ± 0.03</td>
<td>0.07 ± 0.02</td>
<td>0.19 ± 0.03</td>
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Wild-type mice transplanted with wild-type kidneys (WT-WT, n = 5); D5−/− mice transplanted with wild-type kidneys (WT-KO, n = 6), wild-type mice transplanted with D5−/− kidneys (KO-WT, n = 9), and D5−/− mice transplanted with D5−/− kidneys (KO-KO, n = 4) were used. *P < 0.05 versus WT-WT and WT-KO. One-way ANOVA and Student-Newman-Keul’s test.

bP < 0.01 versus WT-WT and WT-KO.
those that were syngenic transplanted (Figure 4). The renal expression of Nox-2 showed a pattern similar to that of AT1Rs. It was lowest in syngenic transplanted wild-type mice and highest in syngenic transplanted D5/H11002/H11002 mice. This is in agreement with our previous report showing that renal and brain Nox-2 expression is increased in D5/H11002/H11002 mice. However, Nox-2 expression in the kidneys of D5/H11002/H11002 mice transplanted into wild-type mice was lower than in syngenic transplanted D5/H11002/H11002 mice, and its expression in kidneys of wild-type mice transplanted into D5/H11002/H11002 mice was higher than in syngenic transplanted wild-type mice (Figure 5).

The presence of nitro-tyrosine on proteins, a marker for peroxynitrite formation in vivo, was also determined. Nitro-tyrosine expression was lower in syngenic or congenic transplanted wild-type kidneys than in D5/H11002/H11002 kidneys transplanted into either D5/H11002/H11002 or wild-type mice (Figure 6).

**DISCUSSION**

Our cross-transplantation studies demonstrate an important role for renal D5Rs in the regulation of BP. This is supported by the finding of increased systolic and diastolic BPs when a kidney lacking D5Rs is transplanted into a wild-type mouse and a decrease in BP when a kidney from a wild-type mouse is transplanted into a mouse lacking D5Rs. The rodent kidney expresses D5Rs in proximal and distal convoluted tubules, cortical...
collecting ducts, medullary ascending limbs of Henle, and arteries, but not in the glomeruli, juxtaglomerular cells, or macula densa. The thick ascending limb of Henle and the cortical collecting duct preferentially express the D1R over the D3R. Stimulation of D1-like receptors induces diuresis and natriuresis in all of the mammalian species studied, including rats and mice. Similarly to the D1R, the D3R also increases cAMP production, which mediates, in part, the inhibition of renal sodium transport by decreasing the activities of NHE3, Na+/H+ exchange, and Na+/HCO3− exchange. Thus, wild-type mice transplanted with kidneys lacking the D3R may have increased renal sodium reabsorption because of a lack of the inhibitory effects of the constitutively active D3R on tubular sodium transport. In fact, on a high salt diet, wild-type mice transplanted with a kidney lacking D3Rs or syngenic transplanted D5−/− mice excrete comparatively less sodium than syngenic transplanted wild-type mice. Our data also show that renal D1R cannot compensate for the lack of D3Rs, suggesting that renal D1R and D3R functions are not redundant.

The D3R may also affect renal tubular sodium reabsorption by interacting with AT1R. Previous studies have shown that inhibition of renal angiotensin II production or blockade of AT1R increases the inhibitory effect of the D1-like receptor agonist fenoldopam on sodium transport. We have shown that the high BP of D5−/− mice is associated with increased renal AT1R protein and is normalized by AT1R blockade. This indicates that in basal conditions the constitutively active D3Rs can decrease AT1R expression. Furthermore, activation of the D3R decreases the AT1R protein level by increasing AT1R degradation via an ubiquitin/proteasome pathway. However, renal AT1Rs are lower in D5−/− kidneys transplanted into wild-type mice than in syngenic transplanted D5−/− mice. Conversely, wild-type kidneys transplanted into D5−/− mice express more AT1Rs than syngenic transplanted D5−/− mice. These suggest that extrarenal factors other than D3Rs are also involved in the regulation of the renal expression of AT1R.

We have reported that the generation of ROS is increased in D5−/− mice. The expression of NADPH oxidase activity and proteins (Nox-2 and p47phox) in the brain and kidney of D5−/− mice is increased, as well as plasma thiobarbituric acid reactive substances, an index of systemic oxidative stress. In the transplanted kidneys the pattern of expression of nitrotyrosine was somewhat different from that of Nox-2. This may indicate the presence of other sources of oxidative stress in the kidneys of D5−/− mice. Oxidative stress and angiotensin II signaling regulate each other by multiple mechanisms, and oxidative stress induces upregulation of AT1R in several tissues. Thus, it is possible that increased systemic oxidative stress in D5−/− mice may increase AT1R expression in the transplanted wild-type kidney. Similarly, increased renal AT1Rs in D5−/− mice may be, in part, caused by increased systemic oxidative stress; thus, transplanting a D5−/− kidney into a wild-type mouse that does not have increased systemic oxidative stress would result in a decrease in the expression of renal AT1Rs.

The diastolic BP was lower and the systolic BP tended to be lower in wild-type mice transplanted with D5−/− kidneys than in syngenic transplanted D5−/− mice. The decreased renal AT1R expression in nonsyngenic transplanted kidneys may be responsible for this effect. Conversely, D5−/− mice transplanted with wild-type kidneys tended to have higher BP levels than syngenic transplanted wild-type mice and showed increased renal AT1R, which may be responsible for the slightly elevated BP. However, the BP levels cannot be completely explained by the changes in the expression of Nox-2 and AT1R, indicating that other AT1R actions are just as important, i.e., regulation of sodium transport. Indeed, the pressure-natriuresis plot is shifted to the right in mice with kidneys that lack D3Rs. Regardless of the possible mechanisms by which the absence of the D3R increases BP, our studies show the pre-eminence of the kidney in the long-term regulation of BP. The important role of the kidney in the long-term regulation of BP using cross-transplantation experiments was reported by Crowley et al. They showed that the renal expression is more important than the extrarenal expression of the AT1R in the regulation of BP.

We have reported that D3Rs in nonrenal tissues participate in the short-term regulation of BP. However, these studies indicate that D3Rs in nonrenal tissues do not seem to have a prominent role in the long-term regulation of BP. In this study BP was measured under isoflurane anesthesia. In mice, isoflurane produces fewer systemic hemodynamic effects than pentobarbital anesthetics but may induce a reduction in centrally generated sympathetic activity. If this were the case the contribution of D3Rs other than in the kidney may be underestimated. However, this should similarly affect D3−/− mice syngenic or congenic transplanted and wild-type mice transplanted with wild-type or D5−/− kidneys, making unlikely a significant underestimation of the effect of nonrenal D3Rs on BP regulation. In the cardiovascular system D3Rs are expressed in smooth muscle of the tunica media of pial, pulmonary, coronary, and mesenteric artery branches, and in vivo administration or in vitro application of D1-like receptor agonists induces vasodilation in the cerebral, coronary, and mesenteric vascular beds, reduces vascular resistance, and causes hypotension. Dopamine and its analogs, acting via D1-like receptors, are coronary vasodilators in animals and humans. The vasodilation of coronary arteries, mediated by D3Rs, is attributed, in part, to activation of hyperpolarizing vasorelaxant potassium channels via cAMP/protein kinase G. It is possible that the lack of the D3R-induced vasodilation is compensated by other vasodilatory systems that may include increased D3R function, although in other organs like brain and kidney, D3R function has not been shown to compensate for the lack of D3R. In our studies, D3R expression is not altered by the absence of the D3R in renal and nonrenal areas.

We have reported that D5−/− mice have a greater reduction in mean arterial pressure after acute adrenalectomy or acute...
α-adrenergic blockade compared with D$_5^{+/+}$ mice, suggesting that increased sympathetic activity ascribed to central nervous system mechanisms may be involved in the hypertension of D$_5^{-/-}$ mice, although plasma and urinary catecholamines were normal in these mice. Our cross-transplantation studies demonstrate that increased sympathetic activity does not play a major role in the chronic regulation of BP, such as the BP increase in D$_5^{-/-}$ mice because wild-type mice with D$_5^{-/-}$ kidneys that should have normal sympathetic activity have BP levels that are indistinguishable from syngenic transplanted D$_5^{-/-}$ animals, and D$_5^{-/-}$ mice with wild-type kidneys that should have increased sympathetic activity have BP levels similar to syngenic transplanted wild-type mice.

Renal sympathetic innervation may be a major contributor to the increase in BP brought about by high salt intake because renal nerves can modulate sodium handling. In salt-sensitive hypertension, increased salt intake results in increased renal sympathetic nerve activity via actions in the central nervous system leading to increased renal sodium retention and increased BP. Sympathetic innervation is impaired by the surgical procedure in the transplanted kidney. This may explain why salt sensitivity is lost in syngenic transplanted D$_5^{-/-}$ mice and in wild-type mice transplanted with a D$_5^{-/-}$ kidney that would be expected to be salt-sensitive and indicate the need for renal nerves to impart salt sensitivity. Nevertheless other renal mechanisms may be involved in the salt sensitivity of D$_5^{-/-}$ mice because although not significantly, both groups of mice with D$_5^{-/-}$ kidneys have a tendency to higher BP values on high salt diet.

The locus of DRD5, 4p15.1 to 16.1, and its pseudogenes, 1q21.1 and 2p11.1-p11.2, have been linked to human essential hypertension. Moreover, humans have single nucleotide polymorphisms in the DRD5 gene with diminished D$_1$R function and abnormal coupling to adenylyl cyclase. Our results indicate that diminished renal D$_1$R function may increase the susceptibility to hypertension. Genetic testing for polymorphisms associated with decreased function may be developed and applied for personalized treatment of hypertension.

**CONCISE METHODS**

**Mice**

Wild-type and D$_5^{-/-}$ mice were bred at the National Institute of Health. The generation of the mouse model is described in the Supplemental Methods. We studied male knockout mice and their wild-type littermates that were at least 8 weeks old. The mice were genotyped using a PCR-based protocol. Mouse genomic DNA was isolated from tail biopsies and renal tissue using standard methods.

**Mouse Kidney Transplantation**

The mice were uninephrectomized 1 week before the transplantation procedure. The remaining native kidney was removed 1 week later. All of the experiments were started 1 week after the last surgery. Creatine clearances in all of the groups of mice were similar to those of unmanipulated and uninephrectomized control mice. The detailed transplantation procedure is described in the Supplemental Methods.

**BP Measurements on Normal and High Salt Diet**

The mice were allowed to fully recover and acclimatize for a week, during which they were fed a normal salt diet (0.75% NaCl). At the end of the week, BP levels under isoflurane anesthesia were measured by cannulation of the femoral artery (PE-50 with tip heat stretched to 180 μm). The catheter was advanced to the aorta and then connected to a BP detection equipment (Cardiomax II). After a day of full recovery from the femoral arterial cannulation, the normal rodent chow feed was replaced with a high salt diet (6.0% NaCl) for 1 week. The mice were then anesthetized, and the BP was taken as described above through the cannulation of the other femoral artery. A urine sample was collected from the bladder by paracentesis, and blood was collected for measurement of serum and urinary electrolytes and creatinine. The mice were then sacrificed by an overdose of pentobarbital (100 mg/kg body wt), after which the kidney and other organs were collected, flash frozen in isopentane over dry ice, and stored at −80°C until assayed. BP was also measured in a group of unmanipulated D$_5^{-/-}$ mice and D$_5$ wild-type littermates, as described above.

**Immunoblotting**

Mouse kidney homogenates were subjected to immunoblotting, as previously reported. The primary antibodies used were rabbit polyclonal directed against the D$_1$R that was generated, affinity purified, and characterized in our laboratory, rabbit polyclonal against AT$_1$R (Santa Cruz Biotechnology, Santa Cruz, California), mouse monoclonal anti-Nox-2 (a kind gift of Dr. M. T. Quinn, Department of Veterinary Molecular Biology, Montana State University, Bozeman, Montana), and rabbit polyclonal nitro-tyrosine (Abcam, Cambridge, Massachusetts). The densitometry values were corrected for protein loading (amounts of protein on the loading gel before and after membrane transfers were quantified) and expressed as the mean densities of the syngenic transplanted wild-type mice.

**Histopathological Evaluation of the Kidney**

Fixed kidney tissues were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. All of the tissues were examined by a pathologist without knowledge of the experimental groups.

**Statistical Analyses**

The data are the means ± SEM, except as indicated. Comparison between two groups was performed using a t test. Statistical differences among the four groups were performed using one-way ANOVA followed by post hoc analysis using the Student-Newman-Keul’s multiple comparison test. Comparisons between normal and high salt diet were performed using two-way repeated-measure ANOVA followed by Student-Newman-Keul’s multiple comparison test. Comparisons of the change in systolic BP from normal to high salt were done using a Kuskal-Wallis test followed by a Dunn multiple comparison test. P < 0.05 was considered statistically significant.
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DISCLOSURES

None.

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