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Research Article

Since dopamine produced by the kidney is an intrarenal regulator of sodium transport, an abnormality of the dopaminergic system may be important in the pathogenesis of hypertension. In the spontaneously hypertensive rat (SHR), in spite of normal renal production of dopamine and receptor density, there is defective transduction of the D1 receptor signal in renal proximal tubules, resulting in decreased inhibition of sodium transport (Na+/H+ exchanger [NHE] and Na+/K+ATPase activity) by dopamine. To determine if impaired D1 receptor regulation of NHE in proximal tubules is related to hypertension, studies were performed in a F2 generation from female Wistar Kyoto (WKY) and male SHR crosses. A D1 agonist, SKF 81297, inhibited (37.6 +/- 4.7%) NHE activity in brush border membranes of normotensive F2s (systolic blood pressure < 140 mm Hg, n = 7) but not in hypertensive F2s (n = 21). Furthermore, a D1 agonist, SKF 38393, when infused into the renal artery, dose dependently increased sodium excretion in normotensive F2s (n = 3) without altering renal blood flow but was inactive in hypertensive F2s (n = 21). Since the major D1 receptor gene expressed in renal proximal tubules is the D1A subtype, we determined the importance of this gene in the control of blood pressure in mice lacking functional D1A receptors. Systolic blood pressure was greater in homozygous (n = […]

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Role of the D_{1A} Dopamine Receptor in the Pathogenesis of Genetic Hypertension

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Abstract

Since dopamine produced by the kidney is an intrarenal regulator of sodium transport, an abnormality of the dopaminergic system may be important in the pathogenesis of hypertension. In the spontaneously hypertensive rat (SHR), in spite of normal renal production of dopamine and receptor density, there is defective transduction of the D_1 receptor signal in renal proximal tubules, resulting in decreased inhibition of sodium transport (Na^+/H^+ exchanger [NHE] and Na^+/K^+ATPase activity) by dopamine. To determine if impaired D_1 receptor regulation of NHE in proximal tubules is related to hypertension, studies were performed in a F2 generation from female Wistar Kyoto (WKY) and male SHR crosses. A D_1 agonist, SKF 81297, inhibited ($37.6 \pm 4.7\%$) NHE activity in brush border membranes of normotensive F2s (systolic blood pressure < 140 mm Hg, $n = 7$) but not in hypertensive F2s ($n = 21$). Furthermore, a D_1 agonist, SKF 38393, when infused into the renal artery, dose dependently increased sodium excretion in normotensive F2s ($n = 3$) without altering renal blood flow but was inactive in hypertensive F2s ($n = 21$). Since the major D_1 receptor gene expressed in renal proximal tubules is the D_{1A} subtype, we determined the importance of this gene in the control of blood pressure in mice lacking functional D_{1A} receptors. Systolic blood pressure was greater in homozygous ($n = 6$) and heterozygous ($n = 5$) mice compared to normal sex matched litter mate controls ($n = 12$); moreover, the mice lacking one or both D_{1A} alleles developed diastolic hypertension. The cosegregation with hypertension of an impaired D_1 receptor regulation of renal sodium transport and the development of elevated systolic and diastolic pressure in mice lacking one or both D_{1A} alleles suggest a causal relationship of the D_{1A} receptor gene with hypertension. (*J. Clin. Invest.* 1996. 97:2283–2288.) Key words: hypertension • dopamine • dopamine receptor • sodium transport • genetics

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Introduction

Essential hypertension is a heterogeneous disease thought to be due to genetic and environmental factors that interact to increase blood pressure. The blood pressure difference between a hypertensive individual and a normotensive control has been attributed to the influence/interaction of 2–6 genetic loci (1). Several candidate genes have been suggested for roles in the pathogenesis of hypertension (2–6). Some forms of hypertension are dependent upon or aggravated by sodium loading. Recently, homozygous mice carrying a mutant proANP allele were found to have hypertension which was aggravated by sodium loading (6). Because dopamine produced by renal tubules is an important intrarenal regulator of sodium excretion, a dysfunction of the renal dopaminergic system has been proposed as a pathogenetic factor in genetic hypertension (7). Dopamine can regulate blood pressure by actions in the central and peripheral nervous system as well as in target endocrine (e.g., adrenal) glands and transporting organs (7). Dopamine produced by renal proximal tubules can serve as an intrarenal natriuretic factor by direct tubular action, independent of hemodynamic mechanisms (7); this paracrine effect is most prominent during sodium loading (8–11). In conscious, as well as in anesthetized animals, up to 60% of sodium excretion during an acute volume load is mediated by D_1 receptors (7); this is due both to an increase in renal dopamine production and to an increased sensitivity of sodium transporters to dopaminergic inhibition (7, 12). In the spontaneously hypertensive rat (SHR),[1] the ability of exogenous and endogenous renal dopamine to engender a natriuresis is impaired (13–15). Since renal dopamine levels in genetic models of hypertension are not lower than those found in their normotensive controls (16, 17), the impaired intrarenal paracrine effect of dopamine in these animal models of hypertension appears to be receptor or postreceptor-mediated. In the SHR, the diminished ability of dopamine and D_1 agonists to inhibit Na^+/H^+ exchange and Na^+/K^+ATPase activity in the kidney is due, at least in part, to their impaired ability to stimulate production of second messenger effector enzymes (adenylyl cyclase, phospholipase C) (14, 18–22). This phenomenon is not due to differences in D_1 receptor density, D_1 antagonist affinity, G-proteins, effector enzyme activity per se, renal brush border Na^+/H^+ exchanger or Na^+/K^+ATPase activity (18, 19). The decreased ability of dopamine and D_1 agonists to stimulate adenylyl cyclase activity

1. *Abbreviations used in this paper:* BBMV, brush border membrane vesicles; GFR, glomerular filtration rate; NHE, sodium/hydrogen exchanger; SHR, spontaneously hypertensive rat; WKY, Wistar-Kyoto.

in the renal proximal tubules of SHR occurs as early as 3 wk of age and is receptor, organ, and nephron segment specific (18, 23, 24). Thus, dopamine and D_1 agonists stimulate adenylyl cyclase activity in the striatum and the cortical collecting duct to a similar extent in the SHR and in its normotensive control, the Wistar-Kyoto rat (WKY) (18, 23, 24); parathyroid hormone stimulates adenylyl cyclase activity in renal proximal tubules to a similar extent in SHR and WKY (18). Impaired transduction of the renal D_1 receptor has also been reported in another model of genetic hypertension, the Dahl salt sensitive rat (14, 22).

Many physiologic and biochemical differences between hypertensive and normotensive strains have been explained by genetic drift and random fixation of alleles in loci that may not be involved in blood pressure regulation (3). Because fortuitously fixed characteristics in the SHR, such as hyperactivity and hyper-reactivity to stress, detract from the suitability of the SHR as a model of genetic hypertension, the hyperactive/hyper-reactive trait was bred out of the SHR. One strain, the WK-HA, exhibits the hyperactive phenotype without hypertension; the other strain, the WK-HT, exhibits hypertension without the accompanying hyperactivity. The D_1 receptor/adenylyl cyclase coupling was defective in the renal proximal convoluted tubule of the WK-HT at 3 wk of age but was normal in the WK-HA strain; in the WK-HT this coupling defect was present in the proximal tubule but not in the cortical collecting duct, another site of dopamine action (25). Studies were designed to determine if the decreased ability of D_1 agonists to inhibit Na^+–H^+ exchange activity in renal brush border membranes in vitro or to induce a natriuresis in vivo is associated with hypertension in a F 2 population of rats generated from WKY and SHR crosses. Two D_1-like receptor genes have been cloned from mammals (26–28). Although both receptor genes are expressed in the kidney, the D_{1A} subtype is more abundant than the D_{1B} subtype in renal proximal tubules. We, therefore, studied the effects of deletion of D_{1A} receptors in mice generated by homologous recombination (29).

Methods

Reagents. D_1 agonists (SKF 81297 and SKF 38393), and the D_1 antagonist, SCH 23390 were purchased from Research Biochemicals International (Natick, MA), dopamine, 3-isobutyl-1-methyl xanthine, and parathyroid hormone from (Sigma Chemical Co., St. Louis, MO), and ^{125}I-SCH 23982 from Dupont NEN (Boston, MA).

Cosegregation studies in rats

F2 generation of rats produced by cross breeding F1 rats bred from WKY female and SHR male crosses using the stock at the University of California at San Diego (UCSD). The rats were maintained on standard Purina rat chow at UCSD and shipped to Georgetown University at 10–14 wk of age. The rats were continued on Purina rat chow and studied between 31–41 wk of age.

Preparation of brush border membranes. At 4–6 mo of age, female F2 rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally). The femoral artery was cannulated and blood pressure recorded. The kidneys were removed and the cortex separated from the medulla. Brush border membrane vesicles (BBMV) were prepared by $MnCl_2$ and differential centrifugation and purity of BBMV determined as described previously (30). Under these conditions, cAMP is not generated and the inhibitory effect of D_1 agonists on NHE activity is linked to G-proteins (30); the inhibitory effect is not due to dissipation of the proton gradient (31). In these studies, equilibrium values at 1–2 h were similar in drug or vehi-

cle treated BBMV. Gamma glutamyl transpeptidase and alkaline phosphatase, brush border membrane marker enzymes, were enriched about eightfold. Na^+/K^+ ATPase, a basolateral membrane marker enzyme was depleted such that it was barely detectable in BBMV preparations in either drug or vehicle treated tissues.

Na/H exchange activity (NHE). NHE activity was assayed by measuring the 100 μM 5-(N-methyl-N-isobutyl)-amiloride sensitive uptake of $^{22}Na^+$ at room temperature by a Millipore filtration technique with 0.65 μm nitrocellulose filters (30, 31). BBMV were incubated with drugs or vehicle for 20 min at room temperature. $^{22}Na^+$ uptake was measured by mixing 20 μl of the membrane vesicle suspension (150–350 μg protein) and 30 μl of uptake medium (final concentration in mM: 142 KCl, 14.7 KOH, 10MES, 9 Hepes, and 1 NaCl, containing 0.1–0.2 μCi of 22Na$^+$; 1 Ci = 37 GBq, 7.5 final pH). The studies were performed in the presence of an outwardly directed pH gradient (pH_{in} = 5.5, pH_{out} = 7.5) and an inwardly directed Na^+ gradient ($[Na^+]_{out}$, = 1 mM, $[Na^+]_{in}$ = 0 mM). Since amiloride sensitive $^{22}Na^+$ uptake at 3 s is due mainly to the exchanger, comparisons were made at this time period (30, 32).

In vivo studies. The rats were anesthetized with pentobarbital (50 mg/kg body weight, intraperitoneally), placed on a heated table to maintain rectal temperature between 36–37°C, and tracheotomized (PE-240). Anesthesia was maintained by the infusion of pentobarbital at 0.8 mg/100 g body weight/h (33). Catheters (PE-50) were placed into the external jugular and femoral veins and femoral artery. Systemic arterial pressure was monitored electronically using Cardiomax II (Columbus Instruments, Columbus, OH). Laparotomy was performed and both the right and left ureters catheterized (PE-10). The right renal artery was exposed and the right suprarenal artery (which originates from the right renal artery) was catheterized (PE-10 heat stretched to 180 μm). A Transonic probe to measure renal blood flow was also placed around the renal artery (32). The abdomen was closed with surgical clips. The duration of these surgical procedures was about 60 min. Fluid losses during surgery were replaced with 5% albumin at 1% body weight over 30 min. For the determination of glomerular filtration rate (GFR), the animals received an intravenous infusion of normal saline containing ^{14}C-inulin (0.01 mCi/ 10 ml infusate; New England Nuclear, Boston, MA) at a rate of 5 ml/ 100 grams body weight for 30 min, followed by a rate of 1 ml/100 g body weight/h until the end of the experiment. After an equilibration period of 120 min, 40 min urine collections for clearance determinations were begun, and eight collections were obtained.

Control group. In the control group, normal saline, the vehicle, was infused alone into the right suprarenal artery at a rate of 20 μl/h for eight collection periods. The rates for both intravenous and intrarenal arterial infusions were the same in all the groups.

SKF 38393 group. After two control periods, SKF 38393, a D_1 agonist, was infused via the supra renal artery for 80 min at a dose of 12 ng/ 300 grams body weight/min (13). Two 40-min collections were then obtained. The infusion was then changed back to the vehicle alone and 1 urine collection period was obtained. Then, the dose of SKF 38393 was increased to 120 ng/300 grams body weight/min and two more urine collection periods were obtained. Subsequently, the infusion was changed to the vehicle alone and one last urine collection period was obtained.

Blood samples were obtained prior to the first collection period, before the fifth collection period and at the end of the experiment. Radioactivity and sodium concentrations were assayed in the blood and urine samples.

At the conclusion of the experiment, the position of the intrarenal arterial catheter was verified with lissamine green infusion. The rats were then sacrificed by an overdose of pentobarbital (100 mg/kg body wt). The kidneys were removed, blotted dry and weighed for calculation of corrected GFR.

Studies in mice

Generation of mutant mice. The targeting construct contained 7.0 kb of 129/Sv-derived D_{1A} dopamine receptor genomic sequence in
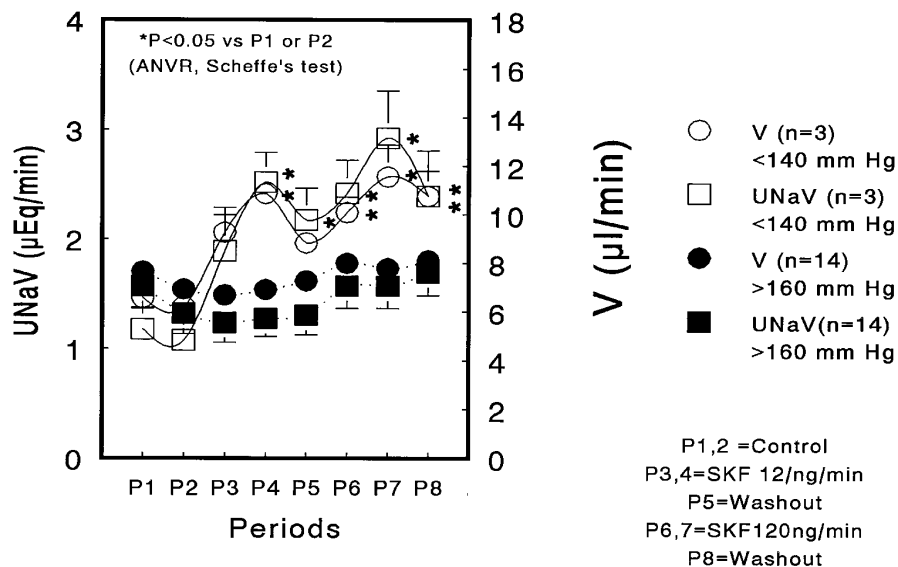
Figure 1. Apparent cosegregation of the decreased efficacy of the D_1 dopamine agonist, SKF 81297 (5×10^{-7} M), to inhibit Na^+/H^+ exchange (NHE) activity in BBMV from F2 rats; SKF 81297 inhibited NHE only in rats with systolic blood pressures < 140 mm Hg. Offspring (F1) of male SHR and female WKY rats (University of California, San Diego) were mated to produce F2 offspring. Data are mean±SE. *$P < 0.05$ vs. other groups, analysis of variance for repeated measures, Scheffe's test.

pPNT (29). The gene was targeted in the J1 line of ES cells (a gift from R. Jaenisch, Whitehead Institute). Homologous recombination events were identified by Southern blotting. Blastocyst injections were then performed. Mice heterozygous for the D_{1A} receptor deletion were generated by mating male chimeras with C57BL/6 females and mice homozygous for the D_{1A} receptor deletion were generated by heterozygous mouse intermatings (29). Only male mice were studied and their normal sex matched litter mates served as controls. For the radioligand binding and cAMP studied, the mice were anesthetized with pentobarbital (0.5 mg/10 grams body weight, IP) or Avertin (1.25 mg/10 grams body weight). The femoral artery was cannulated (PE 50 stretched to 180 μm) and blood pressure monitored electronically by Cardiomax II as above. In other mice, after anesthesia, the kidneys were obtained for radioligand binding and cAMP assay.

Radioligand binding. The cortices were separated from the medullae. The cortex was then homogenized in ice-cold buffer containing in mM: 140 KCl, 10 Hepes, 7 Tris-HCl, 3 Tris-Base, 10 mannose, 2.5 glutamine, and 1 μg/ml leupeptin, in a glass/Teflon® homogenizer. The homogenate was polytroned on ice at a setting of seven for 15 s, 3×, separated by a pause of 30 s. The homogenate was then centrifuged at 2000 g for 10 min and then the supernatant was centrifuged at 35,000 g for 20 min. The pellet was resuspended in KCl buffer (150 mM KCl, 25 M MES) and pelleted at 35,000 g for 20 min. The pellet was resuspended in sucrose buffer containing (in mM): 250 sucrose, 0.1 PMSF, 0.5 DTT, and Tris-Cl, pH 7.6 and flash frozen until the radioligand binding assay was performed. Prior to radioligand binding the pellet was thawed on ice and washed in Dulbecco's PBS (pH 7.4) containing glucose (1 grams/liter). Radioligand binding was performed using ^{125}I-SCH 23982, a D_1 antagonist; non-specific binding was defined by 1 μM SCH 23390 as described previously (18, 20, 22–25).

Cyclic AMP accumulation. The renal cortex was minced using a straight edged razor. The minceate was then sieved through a polypropylene mesh (210 μm) to obtain renal proximal tubules (21, 24) and washed with DME buffer containing 1 μg/ml leupeptin. The suspension was then centrifuged at 500 g for 5 min at 4°C. The pellet was then resuspended in DMEM with 1 μg/ml leupeptin and equilibrated for 15 min at 37°C. Then, IBMX (5×10^{-4} M, final concentration) was added and immediately afterward (1 μl each) vehicle (DDH$_2$O), dopamine (5×10^{-5} M), or parathyroid hormone (10^{-7} M) was added and allowed to incubate for 15 min. EDTA was then added immediately to final concentration of 4 mM. Aliquots were removed for protein analysis and the remaining sample was boiled for 3 min. The tubes were then centrifuged at high speed in a microcentrifuge for 3 min and the resulting supernatant was frozen until cAMP was determined by radioimmunoassay (18, 20, 22–25).

Statistical analyses. Within group comparisons were made using ANOVA for repeated measures and among group comparisons were made using ANOVA. Scheffe's test was used for post hoc test in both instances. When the left and the right kidney were being compared, paired t test was used. A $P < 0.05$ was considered significant.

Results

Studies in rats. Na^+–H^+ exchanger activity: In female F2 rats with systolic blood pressures < 140 mm Hg, the D_1 agonist, SKF 81297 (5×10^{-7} M), inhibited Na^+–H^+ exchange activity; the inhibition was clearly attenuated in those with systolic blood pressure between 140–159 mm Hg and in those with systolic blood pressure > 159 mm Hg (Fig. 1). Indeed, a significant inverse correlation existed between systolic or mean arterial pressure and percent inhibition of Na^+–H^+ exchange activity by the D_1 agonist, SKF 81297 (5×10^{-7} M) (linear regression, $r = 0.54$, $P = 0.04$, polynomial 2 regression, $r = 0.45$, $P < 0.01$). In the parental strains, basal NHE activity was similar in WKY and SHR (1.51±0.07 vs. 1.59±0.07 nmol/mg protein/min, $n = 4$, respectively); however, SKF 81297 (5×10^{-7} M) decreased NHE activity to a greater extent in WKY than SHR (0.67±0.07 vs. 1.10±0.05, nmol/mg protein/min, $n = 4$, respectively) ($P < 0.01$ ANOVA, Scheffe's test). The percent inhibition of NHE activity by SKF 81297 (5×10^{-7} M) was also greater ($P < 0.01$ t test) in the WKY than in the SHR (56±3% vs. 30±5%, $n = 4$/group, respectively). Even at a higher concentration of the agonist (5×10^{-5} M), there was still a differential inhibition ($P < 0.01$ t test) between WKY and SHR (100±0 vs. 88±1%, $n = 2$/group, respectively).

To determine if the differences noted in vitro can also be observed in vivo, we used a D_1 dopamine agonist, SKF 38393, in doses previously found to produce a natriuresis without increasing renal blood flow or GFR in WKY(13, data not shown). The infusion of the agonist into the right renal artery of anesthetized rats in Period 3 (P3) after two control periods P1 and P2), increased sodium excretion and urine flow in a dose-related manner in normotensive but not hypertensive male F2s (Fig. 2). No changes were observed in the contralateral uninfused kidney (data not shown).
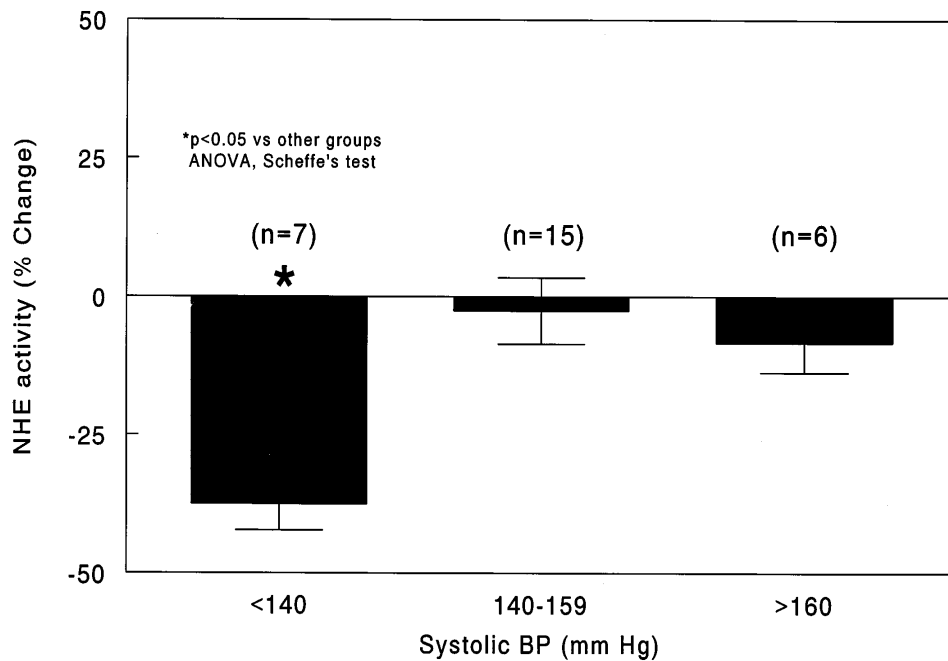
Figure 2. Effect of the intrarenal arterial infusion of the D_1 dopamine agonist, SKF 38393, on urine flow (V) and sodium excretion (UNaV) in male F2s. SKF 38393 was chosen as the agonist in these studies since it does not increase renal blood flow or GFR. Vehicle (control period, P1 and P2, P4, P8) or agonist (12 ng/min, P3, P4, 120 ng/min P6, P7) was infused into the right renal artery via the right suprarenal artery at a constant rate of 1.2 ml/h as described (36). Each period P lasted 40 min. The diuretic and natriuretic effect of SKF 38393 was evident in the normotensive F2s (systolic blood pressure < 140 mm Hg, $n = 3$) but not in the hypertensive F2s (systolic blood pressure > 160 mm Hg, $n = 14$). GFR and renal blood flow did not change during these experiments. SKF 38393 was also ineffective in engendering a diuresis or a natriuresis in rats with systolic blood pressures between 140 and 159 mm Hg ($n = 7$). Data are mean±SE. *$P < 0.05$ vs. P1 or P2, analysis of variance for repeated measures (ANVR), Scheffe's test.

Studies in mice. Radioligand binding studies revealed negligible specific binding in the kidney of mice homozygous for the mutant allele (Table I). D_1 dopamine receptor density in heterozygous mice was intermediate between the normal and the homozygous mice. Basal cAMP accumulation in renal proximal tubules was similar among the three groups. Dopamine stimulated cAMP production in renal proximal tubules in normal mice, did so inconsistently in the heterozygous and not at all in the D_{1A} receptor deficient homozygous mice. This effect was specific, since parathyroid hormone stimulated cAMP accumulation to a similar extent among the three groups of mice. Blood urea nitrogen, serum electrolytes and calcium were similar among the groups (data not shown). Of interest is the fact that systolic blood pressure (on normal chow) was greater in homozygous and heterozygous mice compared to normal sex matched litter mate controls; more-

over, the mice lacking one or both D_{1A} alleles developed diastolic hypertension (Fig. 3). The mice lacking both D_{1A} alleles were smaller than the wild type or the heterozygous mice. The growth retardation of the homozygous mice has been documented previously (29). However, feeding the mice with moistened food allowed growth to $\sim 70\%$ of their littermate controls as reported previously (Table I and reference 29). Fertility was not impaired in the mutant mice.

Discussion

These studies show that the decreased renal tubular response to D_1 agonists cosegregates with hypertension in a F2 generation of rats produced by cross breeding F1 rats bred from WKY female and SHR male crosses. In the experimental set up of these studies, any inhibition of the Na^+/H^+ exchanger by

Table I. Studies in Renal Tubules in Mice Lacking a Functional D_{1A} Receptor

Variables	Normal mice	Heterozygous mice	Homozygous mice
B_{max} (pmol/mg protein)	2.1±0.4 ($n = 4$)*	0.57±0.18 ($n = 4$)*	0 ($n = 4$)
K_d (nM)	40±18 ($n = 4$)*	29±10 ($n = 4$)	0 ($n = 4$)
Basal cAMP accumulation (fmol/mg protein/min)	29.1±8.9	27.7±9.6	22.6±5.6
Effect of dopamine (5×10^{-5} M) cAMP (fmol/mg protein/min)	46.2±7.8*‡§	52.6±11.7	23.0±5.8
Effect of parathyroid hormone (10^{-7} M) cAMP (fmol/mg protein/min)	923±257§	870±252§	858±177§
Weight (grams)	24.5±1.0 ($n = 12$)*	23.4±1.0 ($n = 5$)*	17.3±1.4 ($n = 6$)
Age (d)	99±2 ($n = 12$)	88±9 ($n = 5$)	80±9 ($n = 6$)

*$P < 0.05$ vs. homozygous (ANOVA, Scheffe's test). *‡$P < 0.05$ normal vs. null mutants (t test). §$P < 0.05$ vs. basal (t-pair). $n = 5$ unless indicated.
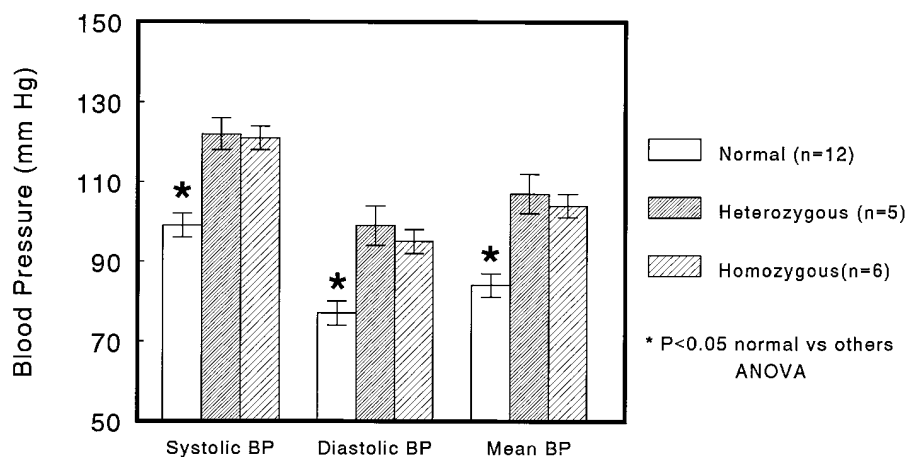
Figure 3. Arterial blood pressure in mice completely lacking a functional D_{1A} dopamine receptor (homozygous) compared with normal and heterozygous mice. The mice ($\sim$ 3 mo of age, see Table I for details) were anesthetized with either Avertin (10 mg/ml, 0.3 ml/mouse in two divided within 15 min as needed) or pentobarbital (5 mg/kg body weight). Arterial blood pressure was recorded from the femoral artery. Systolic, diastolic, and mean arterial pressure were greater in mice homozygous and heterozygous for the mutant D_{1A} allele. Data are mean$\pm$SE. *$P < 0.05$ vs. other groups, analysis of variance (ANOVA), Scheffe's test.

dopamine is independent of second messengers but probably mediated by G-proteins (30). We have shown that the Na^+/H^+ exchanger in the renal brush border membranes of both SHR and WKY is inhibited to a similar extent by non-hydrolyzable guanine nucleotide analogues. These data indicate that the decreased ability of a D_1 agonist to inhibit the exchanger in the SHR or in the hypertensive F2s is not due to a defect of G-protein-end effector action but rather on more proximal sites, presumably on the receptor itself (34). The in vitro studies are corroborated by the in vivo studies, since the diuretic and natriuretic effects of the intrarenal arterial infusion of the D_1 agonist, SKF38393 are evident in normotensive but not in hypertensive male F2 rats.

Decreased renal production of dopamine has been reported in human essential hypertension, especially those with salt-sensitive hypertension; those patients have been reported to have an exaggerated natriuretic response to exogenous dopamine. There are also studies suggesting that the natriuretic effect of endogenous dopamine is impaired in human hypertensive subjects (35). A preliminary report suggests that the ability of a D_1 agonist, fenoldopam, to decrease renal proximal sodium transport is much less in salt sensitive hypertensive humans than in salt resistant hypertensive or control normotensive subjects (36).

The dopamine receptors cloned from brain fall into two groups. The two D_1-like receptors (D_{1A} and D_{1B} in rats; D_{1B} is also known as D_5 in humans) are linked to stimulation of adenylyl cyclase, while the three D_2-like receptors (D_2, D_3, D_4) are linked to inhibition of adenylyl cyclase (1, 26–28). Additional signal transduction pathways have been described (1, 26–28). All the dopamine receptors cloned from the brain are also present in tissues outside the central nervous system, including the kidney (1). The D_2 and D_3 receptor genes are expressed in glomeruli, renal microvessels, proximal tubules, and outer and inner medullary tubules (references in 7). The D_{1A} and D_{1B} receptor genes are expressed in renal microvessels ($D_{1B} > D_{1A}$) (37) and proximal tubules ($D_{1A} > D_{1B}$) where most of the renal D_1-like receptors are located (38, 39). D_1-like receptors are also present in the medullary thick ascending limb and cortical collecting duct. Since the D_{1A} receptor gene is expressed predominantly over the D_{1B} receptor in renal proximal tubules where the defective coupling between the D_1-like receptor and its signal transduction pathways occurs (14, 18–22), we examined the renal D_1 receptor and measured the blood pressure in mutant mice lacking functional D_{1A} receptors (29). These stud-

ies suggest that deletion of even one D_{1A} allele leads to the development of hypertension. Since blood pressure is influenced by strain, Smithies suggested that the comparison between heterozygous offspring provides a test of the effects of targeted modifications on the phenotype of interest completely free from any genetic heterogeneity introduced by the use of two strains (40). In rats, the hypertensive gene(s) has been shown to be transmitted as a partial autosomal dominant trait (41). However, the apparent similar blood pressure between the homozygous and heterozygous mice may have been related to the smaller size of the D_{1A} receptor null mutants (Table I) (29). Although there is substantial evidence that the hypertension is related to abnormal dopaminergic regulation of renal sodium transport, the possibility remains that it may be secondary to changes known to be present in the brain of D_{1A} receptor mutants (29, 42).

In summary, there is abundant evidence that a defect in the ability of dopamine and D_1 agonists to inhibit sodium transport in vivo and in vitro may result in some forms of hypertension in rats and in humans. This defect apparently cosegregates with hypertension in rats. Furthermore, functional ablation of the D_{1A} receptor gene produces diastolic hypertension in homozygous and heterozygous mice. The molecular mechanism of the D_1 receptor defect remains to be defined, since a mutation in the coding region of this receptor gene has not been described (34). Nevertheless, our results indicate that a nonfunctional D_{1A} dopamine receptor contributes to genetic hypertension.

Acknowledgments

References

1. Yen, T.T., P. Yu, H. Roeder, and P.W. Willard. 1974. A genetic study of hypertension in Okamoto-Aoki spontaneously hypertensive rats. *Heredity.* 33: 309–316.

2. Hilbert, P., L. Lindpaintner, J.S. Beckman, T. Serikawa, C. Soubrier, C. Dubay, P. Cartwright, B. De Gouyon, C. Julier, S. Takahasi, M. Vincent, D. Ganten, M. Georges, and G.M. Lathrop. 1991. Chromosomal mapping of two genetic loci associated with blood-pressure regulation in hereditary hypertensive rats. *Nature (Lond.).* 353:521–529.

3. Kurtz, T.W. 1993. Genetics of essential hypertension. *Am. J. Med.* 94: 77–84.

4. Deng, Y., and J.P. Rapp. 1992. Cosegregation of blood pressure with angiotensin converting enzyme and atrial natriuretic peptide receptor genes using Dahl salt sensitive rats. *Nature Genet.* 1:267–272.

5. Jacob, H.J., K. Lindpaintner, S.E. Lincoln, K. Kusumi, R.K. Bunker, Y.P. Mao, G. Ganten, V.J. Dzau, and E.S. Lander. 1991. Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. *Cell.* 67:213–224.

6. John, S.W.M., J.H. Krege, P.M. Oliver, J.R. Hagaman, J.B. Hodgin, S.C. Pang, T.G. Flynn, and O. Smithies. 1995. Genetic decreases in atrial natriuretic peptide and salt-sensitive hypertension. *Science (Wash. DC).* 267:679–681.

7. Jose, P.A., J.R. Raymond. M.D. Bates, A. Aperia, R.A. Felder, and R.M. Carey. 1992. The renal dopamine receptors. *J. Am. Soc. Nephrol.* 2:1265–1278.

8. Pelayo J.C., R.D. Fildes, G.M. Eisner, and P.A. Jose. 1983. Effects of dopamine blockade on renal sodium excretion. *Am. J. Physiol.* 245:F247–F253.

9. Siragy H.M., R.A. Felder, N.L. Howell, R.L. Chevalier, M.J. Peach, and R.M. Carey. 1989. Evidence that intrarenal dopamine acts as a paracrine substance at the renal tubule. *Am. J. Physiol.* 257:F469–F477.

10. Hegde, S.S., A.L. Jadhav, and M.F. Lokhandwala. 1989. Role of kidney dopamine in the natriuretic response to volume expansion in rats. *Hypertension.* 13:828–834.

11. Hansell, P., and A. Fasching. 1991. The effect of dopamine receptor blockade on natriuresis is dependent on the degree of hypervolemia. *Kidney Int.* 39:253–258.

12. Bertorello, A., T. Hökfelt, M. Goldstein, and A. Aperia. 1988. Proximal tubule Na^+-K^+ATPase activity is inhibited during high-salt diet: evidence for DA-mediated effect. *Am. J. Physiol.* 254:F795–F801.

13. Felder, R.A., M.G. Seikaly, P. Cody, G.M. Eisner, and P.A. Jose. 1990. Attenuated renal response to dopaminergic drugs in spontaneously hypertensive rats. *Hypertension.* 15: 560–569.

14. Nishi, A., A-C. Eklöf, A.M. Bertorello, and A. Aperia. 1993. Dopamine regulation of renal Na^+, K^+-ATPase activity is lacking in Dahl salt-sensitive rats. *Hypertension.* 21:767–771.

15. Chen, C.J., S.J. Vyas, J. Eichberg, and M.F. Lokhandwala. 1992. Diminished phospholipase C activation by dopamine in spontaneously hypertensive rats. *Hypertension.* 19:102–108.

16. Grossman, E., A. Hoffman, M. Tamrat, I. Armando, H.R. Keiser, and D.S. Goldstein. 1991. Endogenous dopa and dopamine responses to dietary salt loading in salt-sensitive rats. J. Hypertens. 9:259–263.

17. Racz, K., O. Kuchel, N.T. Buu, and S. Tenneson. 1986. Peripheral dopamine synthesis and metabolism in spontaneously hypertensive rats. *Circ. Res.* 58:889–897.

18. Kinoshita, S., A. Sidhu, and R.A. Felder. 1989. Defective dopamine-1 receptor adenylate cyclase coupling in the proximal convoluted tubule from the spontaneously hypertensive rat. *J. Clin. Invest.* 84: 1849–1856.

19. Chen, C.J., R.E. Beach, and M.F. Lokhandwala. 1993. Dopamine fails to inhibit renal tubular sodium pump in spontaneously hypertensive rats. *Hypertension.* 21:364–372.

20. Horiuchi, A., F. Albrecht, G.M. Eisner, P.A. Jose, and R.A. Felder. 1992. Renal dopamine receptors and pre- and post-cAMP mediated sodium transport defect in the spontaneously hypertensive rat. *Am. J. Physiol.* 263: F1105–F1111.

21. Gesek, F.A., and A.C. Schoolwerth. 1991. Hormone responses of proximal Na^+-H^+ exchanger in spontaneously hypertensive rats. *Am. J. Physiol.* 261: F526–F536.

22. Ohbu, K., F.J. Kaskel, S. Kinoshita, and R.A. Felder. 1995. Dopamine-1 receptors in the proximal convoluted tubule of the Dahl rat: defective coupling to adenylate cyclase. *Am. J. Physiol.* 268 :R231-R235.

23. Ohbu, K., and R.A. Felder. 1993. Nephron segment specificity of dopamine receptor/adenylyl cyclase defect in spontaneous hypertension. *Am. J. Physiol.* 264:F274–F279.

24. Felder, R.A., S. Kinoshita, K. Ohbu, M.M. Mouradian, D.R. Sibley, F.J. Monsma, Jr., L.M. Canessa, and P.A. Jose. 1993. Renal dopamine-1 receptor mRNA and organ specificity of the dopamine-1 receptor/adenylyl cyclase coupling defect in the spontaneously hypertensive rat. *Am. J. Physiol.* 264:R726-R732.

25. Ohbu, K., E.D. Hendley, I. Yamaguchi, and R.A. Felder. 1993. Renal dopamine-1 receptors in hypertensive inbred rat strains with and without hyperactivity. *Hypertension.* 21:485–490.

26. Gingrich, J.A., and M.G. Caron. 1993. Recent advances in the molecular biology of dopamine receptors. *Annu. Rev. Neurosci.* 16:299–321.

27. Civelli, O., J.R. Bunzow, and D.K. Grandy. 1993. Molecular diversity of the dopamine receptors. *Annu. Rev. Pharmacol. Toxicol.* 33:281–307.

28. Sibley, D.R., and F.J. Monsma, Jr. 1992. Molecular biology of dopamine receptors. *Trends Pharmacol. Sci.* 13:61–69.

29. Drago, J., C.R. Gerfen, J.E. Lachowicz, H. Steiner, T.R. Hollon, P.E. Love, G.T. Ooi, A. Grinberg, E.J. Lee, S.P. Huang, P.F. Bartlett, P.A. Jose, D.R. Sibley, and H. Westphal. 1994. Altered striatal function in a mutant mouse lacking D_{1A} dopamine receptors. *Proc. Natl. Acad. Sci. USA.* 91:12564–12568.

30. Felder, C.C., F.E. Albrecht, T. Campbell, G.M. Eisner, and P.A. Jose. 1993. Cyclic AMP independent-G protein linked inhibition of Na^+/H^+ exchange activity in renal cortical brush border membranes by dopamine-1 agonists. *Am. J. Physiol.* 264:F1032–F1037.

31. Felder, C.C., T. Campbell, F. Albrecht, and P.A. Jose. 1990. Dopamine inhibits Na^+-H^+ exchanger activity in renal BBMV by stimulation of adenylate cyclase. *Am. J. Physiol.* 259:F297–F303.

32. Kinsella, J.L., and P.S. Aronson. 1980. Properties of the Na^+-H^+ exchanger in renal microvillus membrane vesicles. *Am. J. Physiol.* 238:F461–F469.

33. Eisner, G.M., I. Yamaguchi I, R.A. Felder, L.D. Asico, and P.A. Jose. 1994. *Am. J. Physiol.* 267:F965–F970.

34. Albrecht, F.E., J.E. Robillard, G.M. Eisner, and P.A. Jose. 1995. Receptor and G-protein regulation of the renal apical sodium/hydrogen exchanger (NHE) in genetic hypertension. *Pediatr. Res.* 37: 358A.

35. Clark, B.A., R.M. Rosa, F.H. Epstein, J.B. Young, and L. Landsberg. 1992. Altered dopaminergic responses in hypertension. *Hypertension.* 19:589–594.

36. Carey, R.M., D.P. O'Connell, and R.A. Felder RA. 1994. Renal dopaminergic mechanisms in salt sensitive hypertension. 5th Int Conf Peripheral Dopamine, Kyoto, Japan. 36.

37. Yao, L., X. Ruan, W.J. Arendshorst, and P.A. Jose. 1995. Dopamine receptor subtype (D_{1A} and D_{1B}) expression in rat renal microvessels. *Pediatr. Res.* 37: 374A.

38. Burgess, L.H., F.J. Monsma, Jr., Y. Shen, P.A. Jose, and D.R. Sibley. 1993. Further characterization of D_{1A} and D_{1B} dopamine receptors in rat kidney. *Soc. Neurosci. Abstr.* Wash., DC. 75.

39. Nash, S.R., N. Godinot, and M.G. Caron. 1993. Cloning and characterization of the opossum kidney cell D1 dopamine receptor: expression of identical D1A and D1B dopamine receptor mRNAs in opossum kidney and brain. *Mol. Pharmacol.* 44:918–925.

40. Smithies O. 1995. Unraveling the genetics of complex cardiovascular diseases with the help of the mouse. *In* The Mouse as a Model for Cardiovascular and Pulmonary Research, Bethesda, MD. 40.

41. G. Schlager, and C-S. Chao. 1991. The role of dominance and epistasis in the genetic control of blood pressure in rodent models of hypertension. *Clin. Exp. Hypertens.* A13:947–953.

42. Lin, M.T., and J.J. Yang. 1994. Stimulation of the nigrostriatal dopamine system produces hypertension and tachycardia in rats. *Am. J. Physiol.* 266: H2489–H2496.