Functional genomics of the dopaminergic system in hypertension

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—Abnormalities in dopamine production and receptor function have been described in human essential hypertension and rodent models of genetic hypertension. Under normal conditions, D1-like receptors (D1 and D5) inhibit sodium transport in the kidney and intestine. However, in the Dahl salt-sensitive and spontaneously hypertensive rats (SHRs) and in humans with essential hypertension, the D1-like receptor-mediated inhibition of epithelial sodium transport is impaired because of an uncoupling of the D1-like receptor from its G protein/effecter complex. The uncoupling is receptor specific, organ selective, nephron-segment specific, precedes the onset of hypertension, and cosegregates with the hypertensive phenotype. The defective transduction of the renal dopaminergic signal is caused by activating variants of G protein-coupled receptor kinase type 4 (GRK4: R65L, A142V, A486V). The GRK4 locus is linked to and GRK4 gene variants are associated with human essential hypertension, especially in salt-sensitive hypertensive subjects. Indeed, the presence of three or more GRK4 variants impairs the natriuretic response to dopaminergic stimulation in humans. In genetically hypertensive rats, renal inhibition of GRK4 expression ameliorates the hypertension. In mice, overexpression of GRK4 variants causes hypertension either with or without salt sensitivity according to the variant. GRK4 gene variants, by preventing the natriuretic function of the dopaminergic system and by allowing the antinatriuretic factors (e.g., angiotensin II type 1 receptor) to predominate, may be responsible for salt sensitivity. Subclasses of hypertension may occur because of additional perturbations caused by variants of other genes, the quantitative interaction of which may vary depending upon the genetic background.

THE LONG-TERM REGULATION OF blood pressure rests on renal and nonrenal mechanisms (22, 34, 41, 82, 85, 138, 143, 172). The sympathetic nervous (48, 91, 133, 198, 240) and the renin-angiotensin (48, 62, 64, 81, 82, 122, 132, 143, 171, 205, 221) systems have been shown to be important in the pathogenesis of essential hypertension, including that associated with obesity (43). However, there are several counter-regulatory pathways (39, 76, 135, 142, 169, 174, 184, 205, 219) (e.g., dopamine pathway), aberrations of which are involved in the pathogenesis of essential hypertension (3, 4, 8–18, 20, 21, 23–26, 29–33, 35, 37, 42, 44–46, 52–60, 67–70, 73–75, 77, 79, 80, 84, 86, 93–95, 97–103, 106–111, 114, 116, 118, 119, 124, 126–128, 130, 131, 136, 140, 141, 144–146, 148–156, 159–161, 163–166, 176–179, 182, 183, 186, 189, 192, 193, 195, 197, 203, 207, 209–216, 218, 224, 226–239), including that associated with obesity (16, 37, 201). Dopamine can regulate blood pressure by renal and nonrenal mechanisms (e.g., intestines and central nervous system) (93, 130, 131, 207) that also involve the renin-angiotensin system (12, 29, 46, 209, 211, 212, 226, 235, 236).

Because the kidney is important in the long-term regulation of blood pressure, many studies have focused on abnormal renal handling of sodium chloride in the pathogenesis of essential hypertension (82, 143). Indeed, human essential hypertension is associated with increased sodium transport in the renal proximal tubule and medullary thick ascending limb (13, 50, 158). The increase in proximal tubule sodium reabsorption in hypertension could be the result of increased transport, per se, or impairment of factors that decrease sodium transport.

**Renal Dopamine and Autocrine/Paracrine Function**

During the past decade dopamine has been recognized as an important modulator of blood pressure, sodium balance, and adrenal (1, 93, 223), intestinal (130, 131, 207), and renal function (3, 4, 8–18, 20, 21, 23–26, 29–33, 35, 37, 42, 44–46, 52–60, 67–70, 73–75, 77, 79, 80, 84, 86, 93–95, 97–103,
autocrine dopaminergic regulation of sodium excretion is mediated by tubular but not by hemodynamic mechanisms (106, 193). Therefore, systemically administered dopaminergic drugs may not mimic the autocrine/paracrine function of dopamine.

**Dopamine Receptor Subtypes**

Dopamine exerts its actions via two families of cell surface receptors that belong to the rhodopsin-like family of G-protein-coupled receptors (GPCRs) (26, 72, 101, 108–110, 190). D<sub>1</sub>-like receptors are composed of the D<sub>1</sub> and D<sub>5</sub> subtypes, whose rat homologs are D<sub>1A</sub> and D<sub>1B</sub>, and couple to the stimulatory G proteins G<sub>A</sub><sup>ol</sup> and G<sub>A</sub><sup>olf</sup> and stimulate adenyl cyclases. Both D<sub>1</sub> and D<sub>5</sub> receptors can also couple to G<sub>q/11</sub> but not to G<sub>i14</sub> (92). Under certain circumstances, for example, in the presence of pertussis toxin, the D<sub>1</sub> but not the D<sub>5</sub> receptor can couple to any of the three isoforms of G<sub>i</sub> (G<sub>i1</sub>, G<sub>i2</sub>, and G<sub>i3</sub>) (4, 203). The D<sub>1</sub>, but not the D<sub>5</sub> receptor, couples to G protein γ<sub>7</sub> (208). In contrast, the D<sub>5</sub> but not the D<sub>1</sub> receptor can couple to G<sub>i</sub> (191) and to G<sub>12/13</sub> (134, 238). The D<sub>2</sub>-like receptors are composed of the D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> subtypes, which couple to the inhibitory G proteins G<sub>i</sub> and G<sub>α</sub>, and inhibit adenyl cyclase and calcium channel activities and modulate potassium channel activity (72, 110, 190).

**Renal Dopamine Receptors**

All the dopamine receptor subtypes are expressed in the kidney (Fig. 2). The D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub> receptors are located in the adventitia and the adventitia-media of renal arteries and prejunctional nerves (6, 173). D<sub>1</sub>, D<sub>4</sub>, and D<sub>5</sub> receptors are expressed in the tunica media (6, 7, 148, 150). The expression of dopamine receptors in the endothelial cell layer in renal arterioles has not been described but the D<sub>3</sub> receptor is expressed in the endothelium of rat mesenteric arteries (237). D<sub>2</sub> and D<sub>4</sub> but not D<sub>1</sub> and D<sub>5</sub> receptors are present in glomeruli (7, 148, 150, 173). The proximal tubule expresses D<sub>1</sub>, D<sub>5</sub>, D<sub>3</sub>, and D<sub>4</sub> receptors (6, 7, 148, 150, 197, 238). The medullary but not the cortical thick ascending limb of Henle expresses D<sub>1</sub>, D<sub>3</sub>, and D<sub>5</sub> receptors (6, 7, 148, 150, 197, 238). The collecting ducts (cortical and medullary) express D<sub>1</sub>, D<sub>3</sub>, D<sub>4</sub>, and D<sub>5</sub> receptors (6, 7, 148, 150, 197, 238). The macula densa and juxtaglomerular cell express D<sub>1</sub> and D<sub>3</sub> receptors (148, 180, 226). There may be species differences, because the D<sub>1</sub> receptor is not expressed in human juxtaglomerular cells (159).

Dopamine inhibits sodium transport at multiple sites along the nephron and acts on multiple transporters [NHE1, NHE3, Na<sup>+</sup>/P<sub>i</sub>, Na<sup>+</sup>/HCO<sub>3</sub> -, Cl<sup>-</sup>/HCO<sub>3</sub> - (SLC26A6), and Na<sup>+</sup>/K<sup>+</sup>-ATPase] (3, 8, 9, 11, 14–16, 20, 21, 24, 32, 33, 44, 45, 52–57, 67, 70, 73–75, 77, 94, 95, 97–103, 111, 118, 127, 128, 136, 144–146, 153, 161, 163, 166, 176, 218, 224) (Fig. 3). The inhibitory effect of dopamine on Na<sup>+</sup>/K<sup>+</sup>-ATPase is influenced by intracellular sodium and calcium; dopamine inhibits Na<sup>+</sup>/K<sup>+</sup>-ATPase activity when intracellular sodium concentrations (24, 53) are high (>20 mM) and when intracellular
calcium concentrations are low (≤150 nM) (32). Because dopamine can inhibit sodium transport in several nephron segments, even a small inhibitory effect in each nephron segment in which it acts can lead to a marked increase in sodium excretion.

There are remarkable parallels in the abnormal dopamine metabolism and signaling via D1-like receptors in rodent hypertensive models and in humans with essential hypertension (26, 37, 50, 101, 107, 110, 116, 124, 158). In both, there is a well-documented failure of the normal inhibition by D1-like receptors of NHE3, Na/HCO3, Cl/HCO3, and Na–K–ATPase (but not Na–H or NHE1) activities in the renal proximal tubule and thick ascending limb of Henle in genetic hypertension (3, 30, 44, 67, 94, 98–100, 102, 118, 128, 131, 144, 160, 161, 224). The impairment of D1-like receptor inhibition of sodium transport in thick ascending limb of Henle in hypertension (144) is in agreement with the hypothesis that increased sodium transport in this nephron segment is causal of sodium sensitivity (13, 50, 158).

Renal Dopamine Receptor Defect in Hypertension

Decreased renal synthesis of dopamine may be involved in the pathogenesis of hypertension in some human subjects (116). Some salt-sensitive subjects with or without hypertension do not increase renal dopamine production in response to an NaCl or protein load (35, 42, 68, 69, 124, 195). However, a decreased renal production of dopamine does not explain the impaired function of endogenous dopamine in many cases of essential hypertension. Urinary dopamine and dopamine metabolites are actually increased in young patients with essential hypertension (116, 177, 178). The urinary excretion of dopamine is not decreased in the SHR or in the Dahl salt-sensitive rat, compared with their normotensive controls (79, 164, 170). Increasing renal dopamine production in the SHR does not enhance the ability of D1-like agonists to inhibit renal cortical NHE3 activity or sodium excretion to the degree seen in WKY rats (107).

To determine whether renal D1-like receptors play a role in the pathogenesis of human essential hypertension, the effect of fenoldopam on cAMP accumulation was studied in human renal proximal tubular cells (59, 179). A D1-like receptor agonist, fenoldopam, increased cAMP accumulation in renal proximal tubule cells from normotensive subjects. This effect was markedly impaired in cells from hypertensive subjects, even though there were no differences in basal cAMP levels. An impaired ability of D1-like agonists to stimulate cAMP production and other second messengers has been reported in the renal proximal tubule and thick ascending limb of Henle of SHRs and Dahl salt-sensitive rats. The impaired ability of D1-like agonists to increase the production of second messengers and inhibit sodium transport is not due to abnormalities in G proteins, second messenger producing enzymes (adenyl cyclase, phospholipase C), sodium transporters, or the sodium pump, per se (3, 26, 30, 44, 58, 59, 94, 98–100, 102, 107, 114, 118, 128, 144, 154, 155, 160, 161, 179, 186, 192, 203, 224, 229, 230, 233, 234).

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In Dahl salt-sensitive and SHRs and humans with essential hypertension, the D1-like receptor-mediated inhibition of epithelial sodium transport is impaired because of an uncoupling of the D1-like receptor from its G protein/effector complex (3, 26, 30, 44, 58, 59, 94, 98–100, 102, 107, 114, 118, 128, 144, 154, 155, 160, 161, 179, 186, 192, 203, 224, 229, 230, 233, 234). The uncoupling of the renal D1-like receptor from its effector complex is receptor specific because the effects of cholecystokinin (119), parathyroid hormone (114, 137, 157, 179), and β-adrenergic receptor agonist (137) in the kidney are preserved when the uncoupling of the D1-like receptor is already evident. Additionally, the uncoupling is organ selective because it is present in the kidney and small intestines (131) but not in the brain striatum (58). It is nephron-segment specific because it is observed in the renal proximal tubule and thick ascending limb of Henle (3, 26, 30, 44, 58, 59, 94, 98–100, 102, 107, 114, 118, 128, 144, 154, 155, 160, 161, 179, 186, 192, 203, 224, 229, 230, 233, 234) but not the cortical collecting duct (153). Because the systemic and distal tubular responses to dopamine are preserved in human essential hypertension (149), exogenously given D1-like receptor agonists can decrease blood pressure and induce a natriuresis in hypertensive subjects (139). This may not be true in rodent models of genetic hypertension (29, 46) and in some patients with essential hypertension (25). D1-like receptors are important in the regulation of basal blood pressure because blood pressure is increased when dopamine receptors are chronically blocked (saline-loaded Wistar rats and normotensive humans).

**Genetic Evidence for the Renal D1-Like Receptor Defect in Hypertension**

The uncoupling of the D1-like receptor from its effectors precedes the onset of hypertension. The decreased ability of D1-like receptor agonists to increase adenyl cyclase activity and inhibit NHE3 activity in renal proximal tubules is evident as early as 3–4 wk of age, before the establishment of hypertension in SHRs (58, 67, 94, 114, 128). The impaired D1-like
receptor is seen in normotensive Dahl salt-sensitive rats while on a low-salt diet and prior to the establishment of hypertension induced by a high salt intake (155).

The SHR is both hypertensive and hyperactive (2, 88, 89) (Fig. 4). Disturbances in central nervous system function, including that of dopamine, have been related to hyperactivity in the SHR (175). Hendley and coworkers (88, 89) separated the hypertensive from the hyperactive phenotype in two inbred rat strains derived from the SHR: the hyperactive normotensive rat (WK-HA) and the hypertensive normoactive rat (WK-HT). In microdissected renal proximal tubule basal adenylyl cyclase activity and response to a nonhydrolyzable analogue of GTP (GppNHp) are similar in WK-HA and WK-HT. However, in these tubules, the D1-like agonist, fenoldopam, stimulates adenylyl cyclase activity in WK-HA but not in WK-HT (154). In contrast, renal calmodulin kinase activity is increased in WK-HA relative to WK-HT (121). To determine whether the impaired D1-like receptor regulation of NHE in proximal tubules is related to hypertension, studies were performed in the F2 generation from female Wistar-Kyoto (WKY) and male SHR crosses (3). A D1-like agonist, SKF-81297, inhibits NHE activity in brush-border membranes of normotensive F2 rats but not in hypertensive F2 rats. Furthermore, a D1-like agonist, SKF-38393, when infused into the renal artery, dose-dependently increases sodium excretion in normotensive F2 rats without altering renal blood flow but is inactive in hypertensive F2 rats (3). The cosegregation of the uncoupling of the D1-like receptor from its G protein/effecter complex with the hypertensive phenotype is suggestive of a genetic defect (3, 154).

Genetics of the Renal Dopaminergic Defect in Hypertension

Additional support for a role of the D1-like receptors in hypertension comes from gene knockout studies. Disruption of either the D1 (3) or the D5 receptor gene (93) in mice increases blood pressure to a level as high as, or higher than, the increases associated with the mutation of other candidate genes of hypertension. Mice heterozygous or homozygous for the D1 receptor null mutation have increased systolic, diastolic, and mean arterial pressure. The renal proximal tubules of these mice do not increase adenylyl cyclase activity in response to D1-like agonist stimulation but do so to parathyroid hormone (3). Because these studies were performed in D1 −/− mice on a C57BL/6 and B129 background, the cardiovascular phenotype of the D1 −/− mice needs to be restudied in a homogenous genetic background. In contrast, D5 −/− mice are hypertensive whether on a mixed C57BL/6 and B129 background or on a C57BL/6 background (>F6) (228). Additional evidence for the importance of D1-like receptors in the regulation of blood pressure comes from studies using D1-like receptor antagonist; salt-loaded Wistar rats given the dopamine antagonist, metoclopramide (188), and humans given the long-acting D1 receptor antagonist, ecopipam (83), have increased blood pressure.

Fig. 4. Cosegregation of renal D1-like receptor dysfunction with the hypertensive but not with the hyperactive phenotype in the SHR. The SHR is a model for both hyperactivity and hypertension (88, 89, 175). D1-like receptors have an impaired ability to stimulate adenylyl cyclase activity in hypertensive but normoactive (WK-HT) rats, like the SHR (154). Renal D1 receptor function is normal in hyperactive but normotensive (WK-HA) rats, similar to that observed in the WKY rat. In contrast, CaMK-dependent phosphorylation is greater in WK-HA than in WK-HT. In addition, serine phosphorylation of a 36-kDa and a 24-kDa protein is 5-fold and 3-fold greater in WK-HA than in WK-HT. The increased CaMK activity in the renal cortical membrane may serve to inhibit GRK activity in WK-HA and prevent the development of hypertension (121). To determine further whether impaired D1 receptor function is related to hypertension, F2 generation of rats were produced by crossbreeding F1 rats bred from WKY female and SHR male rats using the stock at the University of California San Diego (3). In parental WKY rats, and in F2 female pentobarbital-anesthetized rats with normal systolic blood pressures (<140 mmHg), D1 receptor agonist inhibits NHE3 activity in renal proximal tubule brush-border membranes but not in parental SHRs or F2 rats with elevated systolic blood pressures (>140 mmHg). There is a significant correlation between blood pressure (systolic or mean) with the D1-like receptor percent inhibition of NHE3 activity (3). In anesthetized WKY rats (31, 60) and F2 rats with normal blood pressure, the intrarenal infusion of a D1-like receptor antagonist (SKF-38393) produced a diuresis and a natriuresis but not in SHRs (31, 60) or F2 rats with hypertension (4). *Significantly different vs. others (ANOVA, Bonferroni). #Significantly different vs. others except SHR (ANOVA, Bonferroni).
There are polymorphisms in the coding region of the human D3 receptor (40), but these have not been associated with essential hypertension. A renal D3 receptor defect may be present in genetic hypertension, because D3 receptor expression is decreased in the renal cortex of SHRs (235). A polymorphism in the noncoding region of the D1 receptor gene has been associated with human essential hypertension (185), but renal D1 receptor expression is not altered in either genetically hypertensive rodents or humans with essential hypertension (58, 99, 100, 114, 155, 179, 186, 192, 224, 233). No polymorphisms or mutations in the coding region of the D1 receptor gene have been associated with hypertension, suggesting that the D1-like receptor affinity and function may be secondary to an aberrant posttranslational modification.

**Role of G Protein-Coupled Receptor Kinase 4 (GRK4)**

The uncoupling of the D1-like receptor from its G protein/effector complex in hypertension is similar to that seen in the desensitization process. An initial step in desensitization following receptor occupation is the phosphorylation or some other action of GRKs on the GPCR. The action of GRKs on GPCRs, including D1-like receptors, leads to their binding with arrestins and other adaptor proteins, an uncoupling of the receptor from its G protein complex, and a decrease in function (27, 61, 66, 105, 113, 115, 120, 162, 167, 168, 187, 199, 206, 216, 233). The phosphorylated D1 receptor and arrestin complex undergo internalization, via clathrin-coated pits, into an endosome where the GPCR is dephosphorylated, although dephosphorylation of the D1 receptor may occur at the cell surface membrane (66). A role for protein phosphatase 2A has been suggested in the case of D1 receptors (54, 230). The dephosphorylated GPCR is recycled back to the plasma membrane; otherwise, it is degraded by lysosomes or proteasomes.

There is evidence for a constitutive desensitization of the renal D1 receptor but not the D2 receptor in hypertension (211, 236; and Gildea J, Jose PA, and Felder RA, unpublished studies). The D1 receptor is hyper-serine-phosphorylated and not properly targeted to the cell surface membrane of the renal tubule cell, especially the proximal tubule (179, 233). Although an impaired protein phosphatase 2A function may play a role in the hyperphosphorylation of the renal D1 receptor in the SHR (230), the activity of the enzyme is actually increased in renal proximal tubule cells from hypertensive subjects (Yu P, Felder RA, and Jose PA, unpublished observations). However, the hyper-serine-phosphorylation of the D1 receptor in the renal proximal tubule, in the absence of ligand occupation, is caused by increased GRK activity (59). Indeed, decreasing GRK expression or activity in renal proximal tubule cells from hypertensive subjects normalizes the ability of D1-like agonists to increase cAMP accumulation (59) (Fig. 5).

Fig. 5. GRK regulation of D1 receptor function. The role of GRKs in the homologous desensitization of D1 receptors was evaluated using heparin, an inhibitor of GRK activity (168). Renal proximal tubule cells (105 cells/24-well plate) from hypertensive and normotensive human subjects were plated and allowed to grow to 80% confluence (59, 179, 216). Heparin 10−7 mol/l (porcine intestinal mucosa, sodium salt, molecular mass = 3–5 kDa) entry into the cells was facilitated by coincubating the cells with lipofectin (5 g/ml) overnight (168, 216). The cells were washed twice with dextrose phosphate-buffered saline. After the second wash, the cells were incubated at 37°C at varying times and concentrations with the D1 agonist, fenoldopam, and cAMP accumulation was measured as reported (59, 179, 216). Basal cAMP activities were 1.057 ± 0.15 for normotensive subjects (n = 3) and 1.028 ± 0.8 fmol/mg protein h−1·10−3 min−1 for hypertensive subjects (n = 3). Fenoldopam stimulated cAMP to a greater extent in cells from normotensive than those from hypertensive subjects (*P < 0.05 vs. Student’s t-test). Heparin normalized the stimulatory effect of fenoldopam in cells from hypertensive subjects. Data are for vehicle-treated cells from normotensive subjects (■), heparin-treated cells from normotensive subjects (●), vehicle-treated cells from hypertensive subjects (□), heparin-treated cells from hypertensive subjects (●).

GRK activity and GRK2 expression are increased in the lymphocytes of patients with essential hypertension and SHRs (78). However, in SHRs, the increases in GRK activity and GRK2 expression follow rather than precede the hypertensive process (78). Overexpression of GRK2, GRK3, and GRK5 in human embryonic kidney cells desensitizes the D1 receptor; GRK5 has the greatest effect, reducing the maximal activation of the D1 receptor (199). GRK5 can also be increased in hypertension, but like GRK2, its activity also follows the increase in blood pressure (104). Although dopamine hypersensitivity occurs with disruption of the GRK6 gene in mice (65), GRK6 does not regulate the renal D1 receptor (225).

GRK activity is increased in renal proximal tubule cells from hypertensive humans, and inhibition of GRK activity normalizes the ability of D1-like receptors to increase cAMP production (Fig. 5). In renal proximal tubules, GRK4 is more important than other GRKs (e.g., GRK2) in the desensitization of D1 receptors (216). In humans with essential hypertension, the constitutive desensitization of the D1 receptor occurs as a result of a constitutively activated GRK4 gene variants (R65L, A142V, A486V) (59) (Table 1). GRK4 activity is also increased in kidneys of SHRs; chronic renal interstitial infusion of GRK4 antisense oligonucleotides attenuates the increase in blood pressure that occurs with age in SHRs (181). The D1 receptor functional defect noted in renal proximal tubules and medullary thick ascending limb of Henle in hypertension is replicated by expression of GRK4γ gene variants in cell lines (CHO cells) and is rectified by the prevention of GRK4γ expression. In mice, overexpression of GRK4γ 142V impairs the natriuretic
Fig. 6. Regulation of D1 receptor trafficking by G protein-coupled receptor kinase (GRK) in renal proximal tubules. The D1 receptor with signaling molecules (e.g., G protein subunits, adenylyl cyclase, PKA, protein phosphatase 2A) resides in caveolin (with GRK2) and non-caveolin-related (with GRK4) microdomains (234). Caveolin-2 in rat renal proximal tubules enhances D1 receptor-mediated cAMP production; caveolin also inhibits GRK activity. Occupation of the D1 receptor results in its dissociation from its G protein subunits and production of GPCR-regulated cytoplasmic second messengers. There is also recruitment of cytosolic receptors to the surface membrane (23, 201). The occupied D1 receptor, also becomes a substrate for GRKs, which initiate receptor desensitization with or without internalization (105, 199, 216). The desensitization of the D1 receptor occurs in minutes to hours compared with seconds to minutes time frame for α₂-adrenergic receptors (113). GRK2, to a lesser extent, and GRK4, to a greater extent, initiate endogenously expressed human D1 receptor desensitization in renal proximal tubules (216) [GRKs 2, 3, and 5 desensitize D1 receptors heterologously expressed in HEK-293 cells (199)]. G protein β- and γ-subunits mediate GRK2 membrane anchoring, while palmitoylation subserves this function for GRK4 (162, 167). The carboxy terminus of the D1 receptor is phosphorylated first, followed by phosphorylation of the 3rd cytoplasmic loop (66, 105, 120, 199), allowing the binding of arrestin 3 (β-arrestin 2) (common to class A GPCRs) (147) to the 3rd cytoplasmic loop (113). Arrestin attracts clathrin and other components of the endocytotic machinery, resulting in the internalization of GPCRs to early or sorting endosomes (115, 187). GRK2 is associated with both caveolin and clathrin; GRK4 is associated with clathrin but not caveolins (234). The interaction of the D1 receptor with proteins in the sorting endosome sends the receptor to the late endosome for degradation by lysosomes or proteasomes or the rapid recycling endosome (class A receptor), where it is dephosphorylated (protein phosphatase 2A) and recycled back to the surface membrane (54, 230); dephosphorylation can also occur in surface membranes (66). The sodium/hydrogen exchanger regulatory cofactor (NHERF) has been shown to influence not only sodium/hydrogen exchanger function but also agonist-mediated endocytosis and recycling for several GPCRs but not dopamine receptors (90). The sorting protein for the D1 receptor remains to be determined. However, synexin 1 may be the sorting protein for D5 receptor degradation. Degradation can occur via proteasomes or lysosomes. A dopamine receptor interacting protein (DRIP 78) (19) and neurofilament M (112) retard D1 receptor cell surface expression. Variants of GRK4 (65L, 142V, and 486V) constitutively phosphorylate, internalize, and desensitize the D1 receptor (59).

Table 1. Characteristics in mutant mice and implications for humans

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<tr>
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<th>Implication in Human Essential Hypertension</th>
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action of D1 receptors and produces hypertension (59). GRK4\(^{\gamma}\) 142V transgenic mice have high blood pressure that is independent of sodium intake (59). In contrast, GRK4\(^{\gamma}\) 486V transgenic mice become hypertensive, after an increase in sodium intake (212, 213). The hypertensive phenotype is independent of transgene copy number and renal mRNA expression.

The GRK4 locus, 4p16.3, is linked to hypertension (5, 28). We have reported that the GRK4 486V is associated with salt-sensitive hypertensive Italians, recapitulating the mouse transgenic study (18). The latter study was recently corroborated by Speirs et al. (196) in 168 unrelated Caucasians with essential hypertension. In the Japanese, the presence of all three GRK4 variants, 65L, 142V, and 486V, predicts the salt-sensitive hypertensive phenotype with a 94% accuracy (182). The ability to excrete a sodium load in these hypertensive subjects is inversely related to the number of GRK4 alleles with a high degree of correlation ($r^2 = 0.99$), indicating a gene dose effect. However, the presence of three GRK4 variants impairs the natriuretic effect of a dopaminergic drug, even in normotensive subjects. Thus salt sensitivity, per se, may be imparted by GRK4 gene variants.

Six polymorphisms of GRK4 have been reported (GRK4 R65L, GRK4 A142V, GRK4 V2471, GRK4 A253T, GRK4 A486V, and GRK4 G562D). The frequency of these polymorphisms varies according to ethnicity. A Japanese population was found to carry only the wild-type GRK4 V2471, GRK4 A253T, and GRK4 G562D (Sanada H, Scott M, Williams SM, Ritchie MD, Yatabe J, Morikdorika S, Hashimoto S, Watanabe T, Sasaki M, Jose PA, and Felder RA, unpublished data). GRK4 65L and 142V are more frequent among Ghanaians and African Americans than other ethnic groups studied (Chinese, Hispanics, Japanese, and Caucasians), whereas GRK4 486V is more frequent in Chinese and Japanese subjects (196, 220, 222; and Lohmueller KE, Wong LC, Liang M, Felder RA, Jose PA, and Williams SM, unpublished data). Thirteen polymorphisms of eight genes in hypertensive Ghanaians [angiotensinogen (AGT), angiotensin I converting enzyme (ACE), angiotensin II receptor type 1 (AT1R), GRK4, nitric oxide synthases 1 and 3 (NOS1 and NOS3), and carbamyl phosphate synthase 1 (CPS1), which affects NO production, and CYP2C8, the putative endothelium-derived hyperpolarizing factor synthase] were reported recently (222). The best combination that was predictive of hypertension, not classified according to salt sensitivity, was ACE and GRK4, with an estimated prediction success of 70% (222). Among Japanese, the best combination that was predictive of hypertension, not classified according to salt sensitivity, was GRK4, ACE, and CYP11B2, with an estimated prediction success of 63% (Sanada H, Yatabe J, Moridikawa S, Hashimoto S, Watanabe T, Sasaki M, Moore JH, Ritchie MD, Williams SM, Pezzullo JC, Eisner GM, Jose PA, and Felder RA, unpublished data). We also found that the single best genetic model for low-renin hypertension in Japanese included only GRK4 A142V and CYP11B2, with an estimated prediction success of 84% (182; and Sanada H, Yatabe J, Moridikawa S, Hashimoto S, Watanabe T, Sasaki M, Moore JH, Ritchie MD, Williams SM, Pezzullo JC, Eisner GM, Jose PA, and Field RA, unpublished data). These results show that underlying genetic models of salt-sensitive, low-renin, and possibly other subclasses of hypertension are different.

### Summary

Glazier et al. (71) recently proposed criteria to assign genes that underlie complex diseases. We believe that GRK4 gene, as a cause of hypertension, meets these criteria. Thus GRK4 gene locus is linked to and GRK4 variants are associated with hypertension. GRK4 variants impair D1 receptor function in renal proximal tubules. The D1 receptor dysfunction in renal proximal tubules present in human essential hypertension could be related to a defect in the inhibitory function of D1 receptors on renal proximal sodium transport (Fig. 6). Expression of GRK4 variants in cell lines replicates the D1 receptor defect noted in renal proximal tubules. Inhibition of GRK4 function or expression normalizes D1 receptor function in cell lines expressing GRK4 gene variants and renal proximal tubule cells from humans with essential hypertension. Overexpression of GRK4\(^{\gamma}\) 142V variant in mice produces hypertension and impairs the natriuretic but not the acute vasodepressor effect of D1 receptors. GRK4\(^{\gamma}\) 486V imparts sodium sensitivity to mice that are otherwise normotensive. Moreover, selective renal inhibition of GRK4 gene expression attenuates the increase in blood pressure in SHRs. It is possible that GRK4 inhibitors may lead to a novel treatment for salt sensitivity that would reduce the morbidity and mortality associated with this condition, even in normotensive subjects (47, 87, 96, 202, 217).

### NOTE ADDED IN PROOF

Both rBAT (in rat) and LAT-2 (in OK cell) are involved in the renal proximal tubule apical uptake of L-DOPA; LAT-2 is involved in basolateral uptake in both rat kidney and OK cells (Gomes P and Soares-da-Silva P. Na+-independent transporters, LAT-2 and b0, +, exchange L-DOPA with neutral and basic amino acids in two clonal renal cell lines. J. Membr Biol 186: 63–80, 2002; Quinones H, Collazo R, and Moo OW. The dopamine precursor L-dihydroxyphenylalanine is transported by the amino acid transporters rBAT and LAT2 in renal cortex. Am J Physiol Renal Physiol 287: F74–F80, 2004; doi:10.1152/ajprenal00237.2003).

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### REFERENCES


75. Hendley ED, Ohlsson WG, and Mustey RE. Interstrain aggression in hypertensive and/or hyperactive rats: SHR, WKY, WKHA, WKHT. *Physiol Behav* 51: 1041–1046, 1992.


