The Renal Dopamine Receptors

Pedro A. Jose, John R. Raymond, Michael D. Bates, Anita Aperia, Robin A. Felder, and Robert M. Carey

P.A. Jose, Georgetown University Children's Medical Center, Washington, D.C.
J.R. Raymond, M.D. Bates, Duke University School of Medicine, Durham, NC
J.R. Raymond, Section of Nephrology, Durham VA Medical Center, Durham, NC
A. Aperia, Department of Pediatrics, St. Goran’s Children's Hospital, Karolinska Institute, Stockholm, Sweden
R.A. Felder, University of Virginia Medical Center, Charlottesville, VA
R.M. Carey, University of Virginia School of Medicine, Charlottesville, VA

ABSTRACT
Dopamine is an endogenous catecholamine that modulates many functions including behavior, movement, nerve conduction, hormone synthesis and release, blood pressure, and ion fluxes. Dopamine receptors in the brain have been classically divided into D1 and D2 subtypes, based on pharmacological data. However, molecular biology techniques have identified many more dopamine receptor subtypes. Several of the receptors cloned from the brain correspond to the classically described D1 and D2 receptors. Several D1 receptor subtypes have been cloned (D1A, D1B, and D5) and are each coupled to the stimulation of adenylyl cyclase. The D2 receptor has two isoforms, a shorter form, composed of 415 amino acids, is termed the D2short receptor. The long form, called the D2long receptor, is composed of 444 amino acids; both are coupled to the inhibition of adenylyl cyclase. The D3 and D4 receptors are closely related to, but clearly distinct from, the D2 receptor. They have not yet been linked to adenylyl cyclase activity. Outside of the central nervous system, the peripheral dopamine receptors have been classified into the DA1 and DA2 subtypes, on the basis of synaptic localization. The pharmacological properties of DA1 receptors roughly approximate those of D1 and D2 receptors, whereas those of DA2 receptors approximate those of D2 receptors. A renal dopamine receptor with some pharmacological features of the D2 receptor but not linked to adenylyl cyclase has been described in the renal cortex and inner medulla. In the inner medulla, this D2-like receptor, termed DA2k, is linked to stimulation of prostaglandin E2 production, apparently due to stimulation of phospholipase A2. Of the cloned dopamine receptors, only the mRNA of the D3 receptor has been reported in the kidney. The DA1 receptor in the kidney is associated with renal vasodilation and an increase in electrolyte excretion. The DA1-related vasodilation and inhibition of electrolyte transport is mediated by cAMP. The role of renal DA2 receptors remains to be clarified. Although DA1 and DA2 receptors may act in concert to decrease transport in the renal proximal convoluted tubule, the overall function of DA2 receptors may be actually the opposite of those noted for DA1 receptors. Dopamine has been postulated to act as an intrarenal natriuretic hormone. Moreover, an aberrant renal dopaminergic system may play a role in the pathogenesis of some forms of hypertension. A decreased renal production of dopamine and/or a defective transduction of the dopamine signal is present in some animal models of experimental hypertension as well as in some forms of human essential hypertension.

Key Words: Receptor subtypes, sodium transport, Na+/H+ antiport, Na+/K+ ATPase activity, hypertension

Dopamine is an endogenous catecholamine that serves not only as a precursor for norepinephrine and epinephrine but also as a neurotransmitter. Dopamine is involved in a wide variety of physiological processes both in the central nervous system and
Dopamine receptors

Dopamine receptors belong to a large family of related proteins, some aspects of the signal transduction pathways are well established. For example, in the case of the D1A (7-10), D1B (24), and D2 (19) receptors, interaction of the hormone with the receptor induces an interaction with a G protein (guanine nucleotide-binding protein), called Gs, because of its ability to stimulate the enzyme adenylyl cyclase. The G protein is a heterotrimeric protein composed of distinct α, β, and γ subunits. After binding of dopamine to its receptor, the α subunit dissociates from the β-γ heterodimer, allowing interaction of the G protein with the effector enzyme. In this case, the enzyme is adenylyl cyclase, which converts ATP into the second messenger cAMP. The cAMP thus produced then binds to and activates protein kinase A.

Dopamine interaction with the D2 receptor causes an opposite effect on cellular cAMP. The pathway involved is similar to that used for the D1 receptor. The D2 receptor interacts with a G protein called Gi. The α subunit of Gi dissociates from the β-γ heterodimer, causing an inhibition of adenylyl cyclase. However, there appear to be other pathways involved in dopaminergic signal transduction. For example, D3 receptors can activate K+ channels in the brain (25) and pituitary (26).

A dopamine D1 receptor capable of activating the enzyme phospholipase C, leading to the generation of inositol phosphates and diacylglycerol, which was first described in the kidney (27), has also been shown to be present in brain striatum (28,29) and the retina (30). However, it is not clear whether this D1 receptor is the same D1 receptor as that which stimulates adenylyl cyclase. Additionally, the relationships of these receptors to peripheral dopamine receptors, termed DA1 and DA2 receptors, to renal dopamine receptors, and to the D1-like receptors expressed in cultured cell lines, such as the OK (31,32) and LLC-PK1 cell (33), are not clear.

Other questions in this area are equally complex. For instance, two new receptor subtypes, termed D3 and D4 receptors, have recently been cloned (17,18). Although their pharmacological characteristics resemble the dopamine D2 receptor, they have not yet been linked to inhibition of adenylyl cyclase activity and their signal transduction pathways are as yet undefined. Finally, the coupling of various dopamine receptors to cellular kinases is not completely delineated. For example, the role of a cellular phosphoprotein, termed DARPP-32 (dopamine-related phosphoprotein), in the signal transduction processes of dopamine receptors, remains to be elucidated (34,35).

MOLECULAR STRUCTURE

Molecular cloning studies have revealed that dopamine receptors belong to a large family of related proteins. The thick lines around the ovals depict pertussis toxin-sensitive G protein subunits. D1 and D5 receptors are subunits of Gs, and D2 receptors inhibit AC, leading to increased levels of cellular cAMP. D1 receptors (same or different as the D1 receptors that stimulate adenylyl cyclase) also activate PIP2 hydrolysis by stimulating phospholipase C. D2 receptors inhibit AC, activate K+ channels, hyperpolarize membranes, and activate phospholipase A3—all through pertussis toxin-sensitive G proteins. D2 receptors can also activate PLC in some cells, leading to calcium mobilization and phospholipid turnover.

Abbreviations: AC, adenylyl cyclase; PKA, cAMP-dependent protein kinase; protein kinase A; PKC, Ca2+ and phospholipid-dependent protein kinase; protein kinase C; PIP2, phosphatidylinositol 4,5 bisphosphate; IP3, inositol 1,4,5 triphosphate; DAG, diacylglycerol; PLC, phospholipase C; PLA2, phospholipase A2.
receptor proteins, which include adrenergic and muscarinic acetylcholine receptors, visual opsins, and receptors for serotonin, tachykinins, and a variety of peptide hormones (36–38). Other members of this family include a slime mold chemotactic factor receptor, a yeast mating factor receptor, and the mas-myc oncogene. It has become clear that these receptors are ubiquitous and numerous. In fact, nearly 100 members of this superfamily of receptors that couple to G proteins have been cloned from various species over the last 3 yr (38). Figure 2 depicts the putative arrangement of the human D2 and D1A dopamine receptors within the plasma membrane. This arrangement is based on analysis of hydropathicity and by analogy with rhodopsin and β-adrenergic receptors. These receptors have seven stretches of 20 to 26 hydrophobic amino acids, which form potential transmembrane domains. The seven transmembrane domains are probably configured as amphipathic α helices with charged residues facing inward, forming a dopamine-binding pocket, and uncharged residues facing the membrane lipids. There are potential sites for N-linked glycosylation on the extracellular domains. The intracellular loops and the carboxy termini contain several sites for potential phosphorylation by various kinases such as protein kinase C, protein kinase A, and the β-adrenergic receptor kinase.

By analogy with rhodopsin and β-adrenergic receptors, some highly conserved residues may also play important roles. For example, two highly conserved cysteines in the second and third extracellular loop may form a disulfide bond that stabilizes the binding pocket (39–41). By analogy with the β-adrenergic receptor, an aspartate residue in the third transmembrane domain (42,43) and two serines in the fifth

---

Figure 2. (a) Human D2 dopamine receptor. The putative transmembrane topology of the human D2 receptor is depicted. There are three putative extracellular glycosylation sites. The cytoplasmic carboxyl terminus may be anchored by a membrane-bound palmitate. The third intracellular loop contains an alternative splicing sequence. This receptor, with its large third intracellular loop and short carboxyl terminus, is prototypical of receptors that inhibit adenylyl cyclase. (b) Human D1 dopamine receptor. There are two putative extracellular glycosylation sites. The cytoplasmic carboxyl terminus may be anchored by a membrane-bound palmitate. This receptor, with its short third intracellular loop and long carboxyl terminus, is prototypical of receptors that stimulate adenylyl cyclase.
transmembrane domain (44) may be specific residues that interact with the amine and hydroxyl groups of dopamine. Finally, a highly conserved cysteine in the carboxyl terminus may participate in a fatty acylation with palmitate, which may serve as a membrane anchor. This residue has been shown to be fatty acylated by palmitate for the β-adrenergic receptor (45) and rhodopsin (46). Mutations of this cysteine caused alterations in the β-adrenergic receptor-G protein coupling, thereby leading O'Dowd and his colleagues (45) to speculate that the palmitate may serve as a membrane anchor, thus creating a fourth intracellular loop, which may have a tertiary structure important for G protein interaction. These structural features are conserved among the six cloned dopamine receptors. Five of the cloned receptors correspond to the classically described D1 (D1A, D1B, and D5) and D2 (D2short, D2long) receptors (6–19). Other receptors, termed the D3 and D4 receptors, are apparently closely related to, but clearly distinct from, the D2 receptor (17,18).

The genetic organization of these receptors may also provide insight into aspects of the molecular functions of dopamine. The D1A, D1B, and D2 receptors have no introns. Like many of the G protein-coupled receptors, these receptors are encoded by a single exon. These receptors are highly homologous to one another. Thus, the D1A receptor is approximately 50% identical at the amino acid level to both D1B and D2 receptors, whereas the D1B and D2 receptors are 83% identical to one another. The D3, D5, and D4 receptors are encoded by a mosaic of exons (11,17,18). The D2 receptor has six introns, two of which participate in an alternative splicing scheme, which leads to the expression of two isoforms of the D2 receptor. The shorter form (less abundant), composed of 415 amino acids, is termed the D2short. The long form, which is called the D2long receptor, is composed of 444 amino acids, including a sequence of 29 extra amino acids in the putative third intracellular loop (Figure 2a) and seems to be the more abundant form, at least in rat tissues. The significance of the presence of the two forms of the D2 receptors is unclear. Giros and colleagues could not demonstrate any differences in the affinities of a small series of compounds between the shorter of the D2short and the longer alternatively spliced D2long receptors (13). This is not surprising, because the 29-amino-acid insert occurs in the third intracellular loop, and not in or near the transmembrane regions, which are thought to form the ligand-binding pocket. Because the cytoplasmic regions comprising the second and third intracellular loop and carboxy terminus are thought to be critical in coupling to G proteins, the alternative splicing may affect signal transduction of the two isoforms of the D2 receptor (36–38). Another D2 receptor different from the D2short and D2long receptors has been described by Todd et al. (47). The D3 receptor has also been shown to have alternative splice variants (48). Although few mutagenesis studies of dopamine receptors have been reported, Neve et al. (49) recently demonstrated that aspartate 80 of the D2short receptor was critical in conferring pH and sodium sensitivity to ligand binding. This residue may also play an important role in modulating adenyl cyclase activity.

**SIGNAL TRANSDUCTION**

Several intracellular regions close to the membrane have been implicated for G protein coupling of these receptors. These regions comprise the carboxyl end of the second intracellular loop, the amino and carboxyl ends of the third intracellular loop, and the amino portion of the carboxyl terminus (36–38). The relative importance of these regions is an area of active investigation, but the third loop regions seem to be most important in conferring specificity of coupling to G proteins. Molecular cloning studies allow some general speculations about the relationship of the amino acid sequence and the potential G protein and second messenger couplings of dopamine receptors. For example, the D1A, D1B, and D2 receptors have relatively small third intracellular loops and a very long carboxyl terminus. These features are often associated with receptors that couple to Gi and that stimulate adenyl cyclase. The D2 receptors, with their long third intracellular loops and a short carboxyl termini are prototypical of receptors that couple to the inhibitory Gi.

Evidence from renal tissues and other sources suggest strongly that one or several D1 receptors couple to the stimulation of cAMP and to the activation of phospholipase C (20,21,25,27–30,50,51). The question is whether these couplings are from the same receptor, or are two distinct molecular entities. One potential scheme is that two distinct D1 receptors couple individually to single specific pathways. This could occur through the same or different cells. Alternatively, a single pluripotent receptor could couple through the same or different G proteins to both second messenger pathways. Obviously, these couplings could be affected by the available cellular signaling machinery. A third possibility would be an indirect stimulation of one second messenger pathway by another.

Thus far, the cloned D1A and D1B receptors have been shown to couple only to the stimulation of adenyl cyclase and not to phosphatidylinositol hydrolysis in several host cell lines (7,24). This evidence suggests that other closely related D1 receptor subtypes may exist that couple to other effector enzymes such as phospholipase C. This does not rule out the possibility that this receptor can couple to phosho-
lipase C in other host cell lines but does strongly suggest that a receptor with similar pharmacological properties may exist that couples primarily to phosphatidylinositol hydrolysis.

The pluripotent nature of the cloned D₃ receptor has been much more clearly defined. This receptor appears to uniformly couple to the inhibition of adenyl cyclase through pertussis toxin-sensitive G proteins (7,16,52). However, it also couples efficiently to multiple signal transduction pathways in at least three different host cell lines (52). For example, in pituitary GH₄C₁ cells, the cloned D₃ receptor inhibits adenyl cyclase, activates phosphatidylinositol hydrolysis through phospholipase C, and increases intracellular Ca²⁺ levels. By contrast, the cloned D₂₉ short receptor expressed at similar levels in mouse Ltk⁻ fibroblasts inhibits adenyl cyclase, activates phosphatidylinositol hydrolysis through phospholipase C, and increases intracellular Ca²⁺ (by mobilizing intracellular Ca²⁺ stores and increasing Ca²⁺ entry). D₂₉ long receptors transfected in Chinese hamster ovary cells mediate the potentiation of arachidonic acid release by a mechanism involving protein kinase C but independent of the D₃ receptor’s inhibition of adenyl cyclase (53). These studies provide compelling evidence that coupling mechanisms of dopamine receptors depend not only on the intrinsic properties of the receptor macromolecules themselves, but also on their cellular milieu. Clearly, further molecular cloning and expression studies are needed before the exact number and nature of brain and renal dopamine receptors are determined.

**CLASSIFICATION OF RENAL Dopamine RECEPTORS**

Outside of the central nervous system, Goldberg et al. divided the peripheral dopamine receptors into two groups, the DA₁ and DA₂ subtypes, based on synaptic localization (54). The pharmacological properties of DA₁ receptors roughly approximate those of D₁ₐ, D₁₉, and D₅ receptors, whereas those of DA₂ receptors approximate those of D₂ receptors (20,21,50,55,56). A renal dopamine receptor with some pharmacological features of the D₂ receptor but unlinked to adenyl cyclase has been described in the renal cortex and inner medulla. In the inner medulla, this D₂-like receptor, termed DA₂₉, has been shown to be linked to stimulation of prostaglandin E₂ production apparently due to stimulation of phospholipase A₂ (57). Of the cloned dopamine receptors, only the mRNA of the D₃ receptor has been reported in the kidney (13). Preliminary studies have reported the cloning of novel dopamine receptors from rat renal proximal tubules (58) and OK cells (59). Whether the peripheral dopamine receptors are distinct from the cloned brain dopamine receptors remains to be determined. In the following discussion, the renal dopamine receptor will be divided into the DA₁ receptor, on the basis of a greater affinity to D₁ agonists (e.g., fenoldopam, SKF38393) and antagonists (e.g., SCH 23390, SCH 23982) and linkage to adenyl cyclase stimulation, and the DA₂ receptor, on the basis of a greater affinity to D₂ agonists (e.g., quinpirole [LY171555], bromocriptine) and antagonists (e.g., YM-09151, domperidone, spiroperidol). It must be realized however, that the “D₁” drugs have similar affinities to the D₄ₐ, D₄₉, and D₅ receptors, and all are linked to stimulation of adenylate cyclase activity (19), except that dopamine and agonists seem to have a higher affinity to the D₅ and D₄₉ receptors than to the D₄ₐ receptor. Moreover, the “D₂” drugs have similar affinities to the D₂, D₅, and D₄ receptors with some exceptions. For example, the rank order potency for clozapine affinities is D₅ > D₂ > D₃; spiroperidol is D₃ > D₅ = D₂; domperidone is D₃ > D₂; quinpirole is D₅ > D₄ > D₂. For dopamine, the rank order affinity is D₃ > D₅ > D₂ > D₄ > D₁.

**LOCALIZATION OF RENAL Dopamine RECEPTORS**

The prototype DA₁ receptor is found in the renal arterial tree and is associated with vasodilation (54). The DA₂ receptor is associated with inhibition of norepinephrine release (54). Like the D₁ receptors, the DA₁ receptor is located postsynaptically; the DA₂ receptor, like the D₃ receptor, may be located at both presynaptic and postsynaptic sites (54). By using radioligand binding in isolated nephron segments, autoradiography, and linkage to adenyl cyclase, and on the basis of the classification scheme of Goldberg and coworkers (54), dopamine receptor subtypes have been localized to specific segments of the renal vasculature and the nephron (Table 1). DA₁ receptors have been identified in the renal vasculature from the muscular layers of the main renal artery to the afferent arteriole. DA₂ receptors are also present in these vessels (adventitial and endothelial cell layers). Specific glomerular DA₂ radioligand-binding sites have also been reported in homogenates but not in autoradiography studies. Whether this apparent discrepancy is related to methodological differences remains to be determined. DA₁ receptors, which are not present in glomeruli, may become apparent in mesangial cells after several days of culture. DA₁ and DA₂ receptors are present in the proximal tubule. DA₁ receptors are also present in the cortical collecting duct and medullary thick ascending limb. Specific DA₁ radioligand-binding sites are also present in other segments of the nephron, albeit in much smaller quantities. A novel DA₂-like receptor called the DA₂₉ has also been described in inner medullary collecting duct cells.
<table>
<thead>
<tr>
<th>Location</th>
<th>Dopamine Receptor Subtype as Determined by (Ref. No.)</th>
<th>Adenylyl Cyclase (Ref. No.)</th>
<th>Linkage to Activation of (Ref. No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Radioligand Binding</td>
<td>Autoradiography</td>
<td>Phospholipase C</td>
</tr>
<tr>
<td>Renal Artery</td>
<td>DA&lt;sub&gt;2&lt;/sub&gt; ([H]dopamine) (146)</td>
<td>DA&lt;sub&gt;2&lt;/sub&gt; ([H]dopamine) (147)</td>
<td>Inhibition (148)</td>
</tr>
<tr>
<td></td>
<td>DA&lt;sub&gt;2&lt;/sub&gt; ([H]haloperidol) (149)</td>
<td>DA&lt;sub&gt;2&lt;/sub&gt; ([H]dopamine) (147)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Stimulation (148, 152)</td>
</tr>
<tr>
<td>Renal Arterioles</td>
<td></td>
<td></td>
<td>Stimulation (153)</td>
</tr>
<tr>
<td>Glomeruli</td>
<td>DA&lt;sub&gt;2&lt;/sub&gt; ([H]haloperidol) (154)</td>
<td>—&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Inhibition (154)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Proximal Convoluted Tubule</td>
<td>DA&lt;sub&gt;1&lt;/sub&gt; ([H]dopamine) (70, 129, 156)</td>
<td></td>
<td>Stimulation (70, 129, 156)</td>
</tr>
<tr>
<td>Proximal Straight Tubule</td>
<td>DA&lt;sub&gt;1&lt;/sub&gt; ([H]dopamine) (20)</td>
<td>DA&lt;sub&gt;1&lt;/sub&gt; ([H]dopamine) (157)</td>
<td>Stimulation (51)</td>
</tr>
<tr>
<td></td>
<td>DA&lt;sub&gt;1&lt;/sub&gt; ([H]dopamine) (20)</td>
<td>DA&lt;sub&gt;1&lt;/sub&gt; ([H]dopamine) (147, 151)</td>
<td>Stimulation (27, 51, 158)</td>
</tr>
<tr>
<td>Proximal Tubules</td>
<td>DA&lt;sub&gt;1&lt;/sub&gt; ([H]dopamine) (51)</td>
<td>DA&lt;sub&gt;1&lt;/sub&gt; ([H]dopamine) (157)</td>
<td>Stimulation (51)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DA&lt;sub&gt;1&lt;/sub&gt; ([H]dopamine) (147, 151)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DA&lt;sub&gt;1&lt;/sub&gt; ([H]dopamine) (150)</td>
<td></td>
</tr>
<tr>
<td>Medullary Thick Ascending</td>
<td>DA&lt;sub&gt;2&lt;/sub&gt; ([H]dopamine) (51)</td>
<td>Unlinked (51)</td>
<td>Unlinked (51)</td>
</tr>
<tr>
<td>Limb</td>
<td>DA&lt;sub&gt;2&lt;/sub&gt; ([H]dopamine) (51)</td>
<td>Unlinked (51)</td>
<td>Unlinked (51)</td>
</tr>
<tr>
<td>Cortical Collecting Duct</td>
<td>DA&lt;sub&gt;1&lt;/sub&gt; ([H]dopamine) (108, 159)</td>
<td>Stimulation (108)</td>
<td>Stimulation (109)</td>
</tr>
<tr>
<td>Inner Medullary Collecting</td>
<td>DA&lt;sub&gt;1&lt;/sub&gt; ([H]dopamine) (159)</td>
<td></td>
<td>Stimulation (108)</td>
</tr>
<tr>
<td>Duct</td>
<td></td>
<td></td>
<td>Stimulation (109)</td>
</tr>
<tr>
<td></td>
<td>DA&lt;sub&gt;2&lt;/sub&gt; ([H]dopamine) (57)</td>
<td>—&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Stimulation (57)</td>
</tr>
</tbody>
</table>

<sup>a</sup>DA<sub>2</sub> vascular receptors were not detected (151).
<sup>b</sup> [H]dopamine binding was not found in autoradiographic studies (57, 147).
<sup>c</sup>DA<sub>2</sub> stimulated adenyl cyclase was noted in mesangial cells after several days in culture (155).
<sup>d</sup>DA<sub>2</sub> receptors in medullary membranes may be linked to inhibition of adenylate cyclase (160).
FUNCTION OF DOPAMINE RECEPTORS IN NORMAL RENAL PHYSIOLOGY

Most of the dopamine produced in the kidney is synthesized in renal proximal tubular cells (60-66) and possibly in the inner medulla (57). When dopamine production is increased by the in vivo or in vitro administration of levodopa (L-dopa), norepinephrine does not increase (67). Carbodopa decreases renal dopamine but not norepinephrine production. These studies suggest that most of renal dopamine production is extraneuronal. Nonetheless, renal dopaminergic and adrenergic nerves may contribute a minor component of total renal dopamine (68-74). Biochemical and morphological studies indicate that there are dopamine-containing neurons within the kidney. Adrenergic but not dopaminergic nerves contain dopamine-β-hydroxylase (69). There is no compelling evidence that the relationship of these neurons to renal dopamine receptors is functionally significant (75). However, under certain conditions in the dog, intrarenal dopaminergic nerve terminals may modulate GFR as well as distal water reabsorption (68). When dopamine-β-hydroxylase activity is inhibited, intrarenal dopamine increases (70). The increase in intrarenal dopamine is associated with a decrease in DA1 receptor density and the ability of the DA1 agonist to stimulate adenylate cyclase activity (70). When renal nerves are stimulated, renal venous outflow (index of nerve activity) of norepinephrine and dopamine is increased (71). Dopamine released by this maneuver may function to counteract the vasoconstrictor effects of norepinephrine during renal nerve stimulation. Such a function has also been suggested for dopamine released at the lower limits of autoregulation (72). There are additional interactions between renal nerves and dopamine. Neural but not tubular dopamine regulates GFR (76). Indeed, the intrarenal infusion of a DA1 agonist increased GFR to a greater extent in denervated than in innervated kidneys (77).

Because dopamine is synthesized and stored within the kidneys in close proximity to dopamine receptors, it is possible that dopamine released intrarenally acts as a paracrine substance and alters renal function (78-81). Because exogenous dopamine increases sodium excretion, it is possible that dopamine produced by the kidney (mainly from proximal tubules) can serve as an intrarenal natriuretic hormone (80). The natriuresis associated with acute volume expansion with isotonic saline or increased dietary sodium is associated with increased urinary excretion of dopamine (82,83). This natriuresis is abrogated when dopamine synthesis is blocked (84-86). Dopamine antagonists with selectivity to D1a, D1b, and D2 receptors (which we will call simply D1 antagonists), but not those with selectivity to the D2, D3, and D4 receptors, attenuate the natriuresis induced by exogenous dopamine (87) or by sodium loading (79,81,88-91). Criticisms of the above studies include that the drugs were administered systemically and that the experiments were performed in anesthetized animals. These drawbacks were addressed by the studies of Siragy et al. in the conscious unanesthetized dog (78). These investigators found that the intrarenal arterial infusion of the D1 antagonist SCH 23390 induced a dose-dependent decrease in urine flow rate and sodium excretion without any changes in RPF, GFR, systemic arterial pressure, plasma aldosterone concentration, or PRA. The specificity of SCH 23390 as a D1 antagonist was demonstrated by the reversal of these effects with the coadministration of the D1 agonist, fenoldopam. Thus, these studies support the hypothesis that intrarenal dopamine acts as an intrarenal natriuretic hormone by activation of renal DA1 receptors. Furthermore, it seems that endogenous dopamine exerts its natriuretic effect only under conditions of moderate sodium balance and does not play a role at the extremes of loading or depletion (78,79). The role of renal DA2 receptors on sodium excretion is controversial (92,93). Some DA2 agonists (e.g., bromocriptine, quinpirole) have been reported to increase RBF and superficial nephron GFR (92,94,95) without affecting urinary sodium excretion. By contrast, in the isolated perfused kidney, haloperidol (a dopamine antagonist with affinity to both D1 and D2 receptors [as well as D3 and D4 receptors]) increased sodium excretion (96). Siragy et al., using a protocol similar to that employed for SCH 23390 described above, infused the D2 antagonist, YM 09151, into the renal artery of conscious dogs (97). In picomolar concentrations, YM 09151 engendered a dose-related increase in RPF, GFR filtration fraction, and a natriuresis and diuresis. The diuresis and natriuresis could be completely explained by the changes in renal hemodynamics. These changes were not accompanied by any changes in plasma aldosterone concentration, PRA, or systemic arterial pressure and were blocked completely by coadministration of quinpirole, a DA2 agonist. Because renal presynaptic DA2 receptors are not thought to have a physiological role in modulating peripheral sympathetic function (93), action on postsynaptic vascular or glomerular DA2 receptors was proposed. This impression was strengthened by recent studies in the conscious experimental animal model in which LY 171555 administered intrarenally produced antidiuresis, antinatriuresis, and a decrease in renal hemodynamic function (98). The authors speculated that DA2 antagonists may release vasodilator substances such as prostanoids or other endothelium-relaxing factors or antagonize endothelium-derived vasoconstrictor factors (97). More recently, a novel DA2 receptor termed DA2k has been described in inner medullary cells (57). These receptors are apparently linked to activation of phospholipase A2, leading to the for-
mation of prostaglandin E2. The role of this receptor in renal function remains to be determined.

Potentially important interactions between the intrarenal dopaminergic and renin-angiotensin systems have also been described. Blockade of angiotensin II production with angiotensin-converting enzyme inhibitors in anesthetized rats led to the enhancement of the natriuretic response to the DA1 agonist, fenoldopam (99). Thus, these renal paracrine systems may oppose each other in the control of renal sodium excretion.

Although the discussion so far has been devoted to the effects of dopamine on sodium excretion, dopamine can also increase calcium and phosphate excretion (100–102). Some preliminary studies suggest that intrarenal dopamine may also be a physiological regulator of phosphate excretion (103).

MECHANISM OF THE NATRIURETIC ACTION OF DOPAMINE

The natriuretic effect of dopamine is due to both hemodynamic and tubular factors (20,22,23,54). When intrarenal dopamine subserves a paracrine function at renal DA1 receptors, the natriuretic action is probably due entirely to its tubular effects. The inhibitory effect of dopamine on sodium transport is most likely due mainly to its ability to stimulate adenyl cyclase activity (20). The action of dopamine (via DA1 receptors) to increase cAMP levels and protein kinase A activity results in an inhibition of Na+/H+ exchange activity at the brush border membranes in proximal tubular cells (104–106). The effect of dopamine on the Na+/H+ exchanger is unrelated to its stimulatory effect on phospholipase C activity (107). In the medullary thick ascending limb of Henle, dopamine via DA1 receptors also increases cAMP production and inhibits Na+/K+ ATPase activity (34). Aperia and coworkers have suggested that the stimulation of a cAMP-dependent protein kinase by dopamine via DA1 receptors leads to the phosphorylation of a protein, DARPP-32 (dopamine-related phosphoprotein) (22,34,35). An increased amount of phospho-DARPP-32 inhibits protein phosphatase activity, leading to a putative increase in the state of phosphorylation of Na+/K+ ATPase; presumably, phosphorylated Na+/K+ ATPase is inactive (35). Similar mechanisms may apply in the cortical collecting duct, because DA1 agonists stimulate adenyl cyclase activity (108) and inhibit Na+/K+ ATPase activity (109,110). In this nephron segment, arachidonic acid metabolites released by the stimulation of phospholipase A2 may also be involved (109,110). This is of interest because dopamine apparently does not stimulate phospholipase A2 activity in brush border membranes (111) but does stimulate this enzyme in inner medullary cells via a novel DA2-like receptor (DA2k) (57). In the proximal tubule, dopamine also inhibits Na+/K+ ATPase activity (112). Although clearly cAMP is involved, apparently both DA1 and DA2 receptors are needed to inhibit Na+/K+ ATPase (113). A similar mechanism also occurs in isolated neurons (114). Protein kinase C (via phospholipase C) is also involved (115), but, like the arachidonic acid pathway, the mechanisms of interaction among adenyl cyclase, phospholipase C, and phospholipase A2 remain to be resolved. In the face of the apparent necessity of both DA1 and DA2 receptor agonists to inhibit Na+/K+ ATPase activity in the proximal convoluted tubule, the reports of a natriuretic effect of the D2 blockers YM-09151 and haloperidol need to be reconciled (96,97). Whether these discrepancies can be explained by actions on unique DA2 receptors waiting to be cloned or by compartmentalized actions on different nephron segments remains to be seen. What is clear, however, is that the inhibitory effect of dopamine on tubular transport is best explained by its ability to increase cAMP production with inhibition of Na+/H+ exchange and Na+/K+ ATPase activities. The role of other second messengers on the modulation of Na+/K+ ATPase activity and prostanooids remains to be determined.

FUNCTION OF DOPAMINE RECEPTORS IN HYPERTENSION

Because some forms of hypertension are dependent or aggravated by sodium loading and because dopamine is important in aiding the organism to eliminate "excess" sodium, it has been postulated that an aberrant dopaminergic system may play a role in the pathogenesis of some forms of hypertension. Some forms of human hypertension are associated with a decreased ability to increase renal dopamine production in response to a sodium load (116–121). A decreased ability to increase renal dopamine production in response to a salt load has also been described in subset normotensive subjects with a positive family history of hypertension (122). In other studies, no differences in urinary dopamine excretion were noted between normotensive subjects with or without a history of hypertension (123). In these normotensive subjects without a family history of hypertension, urinary sodium and dopamine were positively correlated, whereas no such correlation was noted in the normotensive subjects with a positive family history of hypertension (103). Thus, in human hypertension, decreases in renal dopamine production as well as post-first messenger defects are possible etiological causes.

An animal model of salt-sensitive hypertension, the Dahl salt-sensitive rat, has a decreased ability to generate renal dopamine (124,125). By contrast, the spontaneously hypertensive rat (SHR), whose hypertension can be aggravated by a sodium loading (126),
has no defect in the ability to generate dopamine (125,127,128). In the young SHR, sodium retention occurs in the face of increased renal dopamine production (125,127,128). One possible mechanism for this apparent inability of dopamine to enhance renal sodium excretion is a down-regulation of dopamine receptors in the face of increased renal dopamine concentrations. Indeed, increasing renal dopamine by inhibiting dopamine β-hydroxylase activity resulted in a down-regulation of the density of DA1 receptors. The ability of DA1 agonists to stimulate adenyl cyclase activity in these proximal convoluted tubules was also decreased (70). The ability of dopamine to down-regulate DA1 receptors has also been demonstrated in kidney cell lines (32). In the SHR, however, DA1 receptor density (measured by radioligand binding with [125]I SCH 23390) is not different from that in normotensive Wistar-Kyoto rat (WKY) controls. Indeed, DA1 receptor density in proximal convoluted tubules is similar in WKY rats and SHR even at 3 wk of age, when renal dopamine levels are not different from each other (125). However, the ability of dopamine and other DA1 agonists to stimulate adenyl cyclase activity in SHR is decreased as early as 3 wk of age (129). This decreased ability of DA1 agonists to stimulate adenyl cyclase activity in the proximal convoluted tubule of the SHR is not due to defective adenyl cyclase or G proteins but rather due to a defect in the coupling between the DA1 receptor and the G protein/adenyl enzyme complex (129). Although, photoaffinity labeling studies with [125]I MAB did not show differences in protein-labeled bands, some evidence was offered in favor of a receptor defect. First, agonist-, but not antagonist-binding affinities were decreased in SHR but not in the WKY rats. With the DA1 antagonist, [125]I SCH 23982, low- and high-affinity-binding sites were noted in the proximal tubule of the WKY rats, whereas only a low-affinity-binding site was noted in the SHR; these characteristics were noted in both membrane-bound (130) and solubilized receptors (131). There was specificity of the apparent DA1 receptor defect because the ability of parathyroid hormone to stimulate adenyl cyclase in the proximal tubule of the SHR was not impaired (129). The defect seems to be localized to the proximal convoluted tubule because dopamine or DA1 agonists stimulated adenyl cyclase activity in cortical collecting ducts and striatum to a similar degree in SHR and WKY rat (132). The possibility of a genetic defect was also suggested because the defect was noted as early as 3 wk of age. Moreover, the defect cosegregated with the hypertensive phenotype (133). Of interest is the preliminary report of Yoshihara et al. showing that i.v. administered dopamine increased urinary cAMP to a lesser degree in SHR than in WKY rats (134).

A consequence of a defective dopamine receptor coupling mechanism in the proximal convoluted tubule in the SHR is a decreased ability of DA1 agonists to inhibit Na+/H+ exchange activity in renal brush border membrane vesicles (135,136) and proximal tubules (137). In the SHR, this decreased inhibitory effect of the DA1 agonist on Na+/H+ exchange activity was noted as early as 3 wk of age (136). A decreased ability of dopamine to inhibit Na+/K+ ATPase activity in renal proximal convoluted tubule has also been reported in the Dahl salt-sensitive rat (138). A decreased ability of dopamine and DA1 agonists to stimulate adenyl cyclase in the proximal convoluted tubule of Dahl salt-sensitive rats has also been reported (139). This defect was noted before the onset of hypertension.

A physiological correlate of the decreased ability of dopamine and DA1 agonists to stimulate adenyl cyclase activity and the decreased ability to inhibit Na+/H+ exchange activity in the proximal tubule of SHR has also been reported (140). The selective DA1 agonist SKF 38393 administered into the renal artery increased fractional sodium excretion in WKY rats but not in SHR (140). A decreased natriuretic effect of a non-catechol DA1/DA2 agonist, pramipexole, and another dibenzazepine DA1 agonist, fenoldopam, administered i.v. has also been reported (141). Because the ability of DA1 agonists to increase RBF in SHR (142) is not impaired, the "DA1 defect" is probably of a tubular nature. A defect in renal tubular DA1 receptor function has also been proposed to cause a diminished natriuresis to volume expansion in the SHR (143). In aggregate, these studies clearly demonstrate a close link between DA1 receptors, impaired signal transduction and solute transport, and hypertension.

Whether or not a defect in dopamine receptor coupling exists in the renal proximal tubule of hypertensive humans awaits further investigation. Studies of the renal response to dopamine or fenoldopam, a DA1 agonist, in normal and hypertensive subjects would be of major critical importance. Although Bughi et al. (144) as well as other investigators (145) have shown substantial natriuretic responses to fenoldopam or dopamine in some patients with essential hypertension, these studies were not designed specifically to address whether or not a DA1 receptor coupling defect may exist in salt-sensitive hypertension.

CONCLUSION

Dopamine produced by renal tubules acts as a paracrine substance regulating tubular electrolyte and water transport. Dopamine DA1 receptors are associated with renal vasodilation and inhibition of sodium transport via cAMP. DA1 receptors are also linked to stimulation of phospholipase C and possibly phospholipase A2, but the role of these effector enzymes on tubular transport remains to be defined. DA2 and receptors may also regulate renal hemody-
dynamics and renal tubular transport. Both an increase and a decrease in RBF and sodium excretion have been linked to DA2 receptors; the effector mechanism needs to be established. The role on renal function of a novel DA2 receptor, DA2α, also remains to be determined. DA1 and DA2 receptors may also interact to inhibit sodium transport at least in the proximal convoluted tubule. The DA2 receptors may be linked to inhibition of adenyl cyclase, whereas the DA2α may be linked to stimulation of phospholipase A2. Several dopamine receptor subtypes (D1 through D5) have been linked from the brain. It remains to be seen whether the "renal" dopamine receptors are similar to or different from the "brain" dopamine receptors. The study of these receptors is important because renal dopamine receptors may play a role in salt-sensitive hypertension.

REFERENCES

25. Lacey MG, Mereur NB, North RA: Dopamine acts on D3 receptors to increase potassium conductance in neurons of the rat substantia nigra zona compacta. J Physiol 1987;398:397–416.
28. Mahan LC, Burch RM, Monsma FJ Jr, Sibley


60. Hagedo J, Richet G: Proximal tubule dopamine histofluorescence in renal slices incubated


111. Sheik-Hamad D, Jo OD, Yanagawa N: Dopamine (DA) antagonizes the effect of angiotensin II (ATII) on sodium (Na) transport by rabbit renal brush border membrane (BBM). Kidney Int 1990;37:359A.


129. Kinosita S, Sidhu A, Felder RA: Defective dopamine-1 receptor adenylate cyclase coupling in the proximal convoluted tubule from the spontaneously hypertensive rat. J Clin In-
Dopamine Receptors

vest 1989;84:1849-1856.


