Functional characterization of basolateral and luminal dopamine receptors in rabbit CCD

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DOPAMINE (DA) CAUSES NATRIURESIS in various mammals, such as dogs, rats, rabbits, and humans (11, 22, 24). An increase in glomerular filtration rate (GFR) is a definite factor in natriuresis. In addition, DA causes vasodilatation of the renal artery, and this is the major mechanism for increasing GFR. As an endogenous catecholamine, DA also has been shown to inhibit angiotensin II-induced mesangial cell contraction (8). A physiological role of DA in glomerular contractility is supported by the presence of dopaminergic nerve endings close to the vascular pole of the glomeruli and juxtaglomerular cells (9).

With regard to electrolyte metabolism, DA directly suppresses tubular sodium reabsorption with a resultant increase in fractional excretion of sodium by inhibiting the activity of Na+/K+-ATPase in several nephron segments and also by suppressing the apical Na+/H+ exchange in the proximal tubule (6). The first two subsegments of the proximal tubule, i.e., S1 and S2, produce DA from l-dopa by the action of aromatic l-amino-acid decarboxylase. Proximal convoluted tubule also secretes the DA to basolateral and luminal sides (7). Thus the proximal convoluted tubule-derived DA can act as a paracrine factor at adjacent and more distal segments (37). In the medullary thick ascending limb of the loop of Henle, dopamine- and cAMP-regulated phosphoprotein (DARPP-32) has been identified in the kidney of rat, mouse, and rabbit (23). This is another line of evidence that DA, as an endogenous hormone, may regulate the distal nephron functions. Indeed, in the collecting ducts, DA has been reported to inhibit sodium reabsorption (15, 17).

The dopamine receptor family is divided into two major groups with pharmacological and molecular character, the D1-like and D2-like dopamine receptors, respectively (34, 35). The D1-like receptors (D1 and D5) couple to the G protein Gs and activate adenylyl cyclase. The D2-like receptors (D2, D3, and D4) are prototypical G protein-coupled receptors that inhibit adenylyl cyclase (Gi) and activate K+ channels (18, 36). All subtypes of the dopamine receptors are expressed in the kidney (14, 25, 31). In the rat cortical collecting duct (CCD), the D1 receptor has been detected by in situ hybridization or immunohistochemistry (27). Also, Satoh, et al. (32) have shown that DA decreases Na+-K+-ATPase activity in the CCD and medullary thick ascending limb of the rat. On the other hand, physiological studies suggested the presence of the D2-like receptor in rat (39) and rabbit CCD (26, 40). Thus there still is controversy concerning the dominant receptor subtype in this segment. In addition, the polarity of the dopamine receptor localization, i.e., basolateral or luminal side of the tubule, remains unexplored.

In the present study, rabbit CCD was perfused in vitro, and the response of transepithelial voltage (Vt) and Na+ transport (JNa+) to basolateral and luminal DA was examined to characterize the subtypes and localization of the DA receptors.

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METHODS

In vitro microperfusion. Single CCDs were dissected from kidneys of anesthetized (intravenous pentobarbital sodium, 1 mg/kg) female Japanese White rabbits, weighing 1.5–2.5 kg and perfused in a Lucite bath chamber on the stage of an inverted microscope at 37°C using the methods described previously (2, 5). To facilitate luminal perfusate exchange during each experiment, a polyethylene tube (PE-10; Clay-Adams, Parsippany, NJ) was placed inside pipette B, which was connected to the tubular lumen for perfusion. The luminal perfusate flow rate was adjusted by hydrostatic pressure. Luminal perfusate exchange was performed manually by injecting perfusate into the polyethylene tube, washing out the preexisting medium in pipette B.

The composition of bath medium and isotonic luminal perfusate was as follows (in mM): 105 NaCl, 25 NaHCO3, 10 sodium acetate, 2.3 Na2HPO4, 10 NaH2PO4, 5 KCl, 1.8 CaCl2, 1.0 MgSO4, 8.3 glucose, and 5 alanine (osmolality 300 mosmol/kgH2O).

Before use, all solutions were bubbled to equilibration at 37°C with a 95% O2-5% CO2 gas mixture to achieve a pH of ~7.40 and a PCO2 of ~40 Torr. Bath medium was continuously exchanged during the experiment at a flow rate of 25 ml/h by using a syringe pump (model STC-521, Terumo, Tokyo, Japan). To exchange bath medium, 2 ml of new bath medium, which was warmed to 37°C, were rapidly infused three times into the bath chamber through another set of syringe and polyethylene tubing. This maneuver was completed within 30 s. Luminal perfusates were also shielded in plastic syringes until use. The pH of the solutions was checked again each time just before use by a pH meter (model MEZ-7.40, Nihon Kohden, Tokyo, Japan) by using standard techniques and recorded on a chart (model R202, Rikadenki, Tokyo, Japan).

Experiments were started after perfusion of tubules for 60–90 min at 37°C to obtain a stable Vt and to eliminate residual actions of endogenous arginine vasopressin (AVP; equilibration period) (4). To detect cell damage and perfusate leak, luminal perfusates contained 0.2 mg/ml FD&C green dye. Appearance of the tubular cells and dye leakage were continuously monitored under the microscope. Tubules with dye leakage or an increasing number of cells stained with the dye were discarded.

Measurement of Vt. The voltage difference between two calomel cell electrodes connected by Ringer-agarose bridges to the bath medium and perfusate in calomel cell electrodes connected by Ringer-agarose bridges (pipette B, which was connected to the tubular lumen for perfusion. The luminal perfusate flow rate was adjusted by hydrostatic pressure. Luminal perfusate exchange was performed manually by injecting perfusate into the polyethylene tube, washing out the preexisting medium in pipette B.

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When Vt (in mV) was exclusively measured, a high perfusion rate (~30 nI/min) was chosen, and the perfused fluid was collected in a large-volume (~10-µl) pipette to avoid changes in perfusion rate and perfusion pressure that might affect Vt during exchange or collection of luminal perfusate. After the equilibration period, stability of the Vt was confirmed by exchanging the luminal perfusate for that containing vehicle alone. Because Vt was significantly different from tubule to tubule (~5 to ~30 mV), when appropriately adequate for comparison, the degree of depolarization (%Vt change) was calculated from the Vt at maximal depolarization (Vt dep, max) and the corresponding basal Vt (Vt, basal) as follows

\[
\%V_t\text{ change} = \frac{V_t\text{ dep, max} - V_t\text{, basal}}{V_t\text{, basal}} \times 100\%
\]

Agonists and antagonists. DA agonists and antagonists exert various binding specificities to the D1- and D2-like receptors (1, 19, 20, 25, 43). In the present study, we used SKF-81297, a D1 and D5 agonist, as a D1-like receptor agonist and SCH-23390, a D1 and D5 antagonist, as a D1-like receptor antagonist. With reference to the D2-like receptor, we chose bromocriptine, a D2-like receptor agonist (D2, D3, and, partially, a D4 agonist) and domperidone (D2, D3, and D4 antagonist) as a D2-like receptor antagonist, respectively (33, 34, 35, 38). For further

Table 1. Pharmacological profile of dopamine receptors

<table>
<thead>
<tr>
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<th>D1-Like</th>
<th>D2-Like</th>
<th>D3-Like</th>
<th>D4-Like</th>
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<tr>
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<tr>
<td>Dopamine</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
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<tr>
<td>Bromocriptine</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
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<tr>
<td>SKF81297</td>
<td>+</td>
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<td>+</td>
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<td><strong>Antagonists</strong></td>
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<tr>
<td>SCH-23390</td>
<td>+++</td>
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<tr>
<td>Domperidone</td>
<td>−</td>
<td>−</td>
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<td>RB1-257</td>
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</tbody>
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++, +++, +++++, inhibition constant (Kᵢ) <1.0 nM; ++, 1.0 nM < Kᵢ <5 nM; +, 5 nM < Kᵢ <50 nM; ++, 50 nM < Kᵢ <500 nM; +++, 500 nM < Kᵢ <1 µM; −, Kᵢ > 1 µM.

Fig. 1. Original trace of change in transepithelial voltage (Vt) induced by basolateral dopamine (DA).

Fig. 2. Dose-dependent change in Vt (%Vt change) in response to basolateral DA (n = 8). P value vs. basal Vt is shown.
confirmation of D₂-like receptor subtype, a specific D₄ receptor antagonist, RBI-257, was also used (19). Their pharmacological profiles are shown in Table 1. All these reagents were purchased from Sigma (St. Louis, MO).

Effect of DA on Na⁺ transport. In this series of experiments, ²²NaCl (DuPont, Wilmington, DE) was added to the luminal perfusate as a tracer. Lumen-to-bath Na⁺ efflux, \( J_{Na} \) (peq min⁻¹ mm tubule⁻¹), was calculated from the disappearance rate of ²²Na⁺ from the luminal perfusate according to the following equations

\[
V_o = V_p / t
\]

\[
J_{Na} = (K_i - K_o) \times V_o / L
\]

where \( V_o \) is the collection rate (in nl/min); \( V_p \) and \( t \) are the volume of the constriction pipette and collection time, respectively; \( K_i \) and \( K_o \) are the concentrations of Na⁺ in the perfusate and collected fluid, respectively; and \( L \) is the length of the tubule, which was measured directly at the end of each experiment with the eyepiece reticle. The radioactivity of ²²Na⁺ was counted by using a liquid scintillation counter (Auto-Gamma 5650, Packard Japan, Tokyo, Japan).

After the equilibration period mentioned above, two or three collections were made (basal period). Then, in 10–15 min, when \( V_t \) depolarization was stabilized after addition of DA, three collections were made (experimental period).

To detect the reverse leakage of the bath medium into the tubular lumen, ²²Na⁺ activity of the first one or two basal collections was counted immediately. If leakage was suggested by an unusually low count rate, compared with those in the previous time control experiments (30), the experiment was discontinued, although leakage was not observed in the present series of experiments.

Statistics. Data are presented as means ± SE. Unless specified otherwise, differences among groups were assessed...
by analysis of variance (Scheffe's post hoc test for multiple comparisons), using Statview 5.0J software. A difference with a \( P \) value of \( < 0.05 \) was considered significant.

**RESULTS**

**Effect of basolateral DA on \( V_t \).** Basolateral DA (1 nM-10 \( \mu \)M) induced dose-dependent depolarization of \( V_t \) (26.3 \( \pm \) 2.8, 44.3 \( \pm \) 5.3, and 75.1 \( \pm \) 6.9\%, respectively) (Figs. 1 and 2). To evaluate the receptor subtype, basolateral DA was applied after the pretreatment with 10 \( \mu \)M basolateral SCH-23390, an antagonist of both the D\(_1\) and D\(_5\) receptors, or 10 \( \mu \)M basolateral domperidone, a D\(_2\)-like receptor antagonist that antagonizes all D\(_2\), D\(_3\), and D\(_4\) receptors (Table 1). In the presence of SCH-23390, which by itself did not change \( V_t \), the depolarization induced by DA was not significantly altered (28.7 \( \pm \) 8.2\% at 100 nM, 49.3 \( \pm \) 9.3\% at 10 \( \mu \)M) (Figs. 3A and 4A). On the other hand, the DA-induced depolarization was significantly suppressed in the presence of domperidone, which by itself did not alter the \( V_t \) (1.2 \( \pm \) 0.5\% at 100 nM, 20.8 \( \pm \) 4.2\% at 10 \( \mu \)M, \( n = 5 \)) (Figs. 3B and 4B).

For further confirmation, agonist studies were performed. Basolateral SKF-81297 (10 \( \mu \)M), which agonizes both the D\(_1\) and D\(_5\) receptors (Table 1), induced no significant \( V_t \) change (3.5 \( \pm \) 3.8\% at 100 nM and 5.6 \( \pm \) 5.8\% at 10 \( \mu \)M) (Figs. 5A and 6), whereas 100 nM and 10 \( \mu \)M basolateral bromocriptine, a D\(_2\)-like receptor agonist (mainly of D\(_2\) and D\(_3\) and, partially, of D\(_4\)) (Table 1), caused a dose-dependent depolarization (21.7 \( \pm \) 5.8\% at 100 nM and 33.5 \( \pm \) 5.1\% at 10 \( \mu \)M, \( n = 6 \)) (Figs. 5B and 6). These results demonstrated that basolateral DA causes depolarization of the \( V_t \) not via a D\(_1\)-like receptor but via a D\(_2\)-like receptor.

As recent papers suggested the existence of a D\(_4\) receptor in CCD (39, 40), we performed further evaluation for the subtype of the basolateral D\(_2\)-like receptor. DA was applied in the presence of basolateral RBI-257, a specific D\(_4\) receptor antagonist (19). Basolateral RBI-257 (10 \( \mu \)M) did not change the \( V_t \) by itself, whereas it abolished the action of 100 nM and 10 \( \mu \)M basolateral DA. After washing out of RBI-257, significant depolarization was observed with a 10 \( \mu \)M basolateral DA rechallenge (100 nM DA with RBI-257, 2.2 \( \pm \) 0.4\%; 10 \( \mu \)M DA with RBI-257, 5.2 \( \pm \) 0.5\%; 10 \( \mu \)M basolateral DA rechallenge, 73.2 \( \pm \) 5.1\%, respectively; \( n = 5 \)) (Fig. 7).

**Effect of luminal DA on \( V_t \).** Next, we investigated whether luminal DA exerts an effect on \( V_t \). Because urinary concentration of DA is 10–100 times higher than that at the plasma level (41), we chose higher concentrations for luminal study. Although 1 \( \mu \)M luminal DA did not cause a significant depolarization (0.2 \( \pm \) 2.5\%; \( n = 5 \)), 10 and 100 \( \mu \)M luminal DA depolarized \( V_t \) significantly (14.6 \( \pm \) 6.5\% at 10 \( \mu \)M, 18.2 \( \pm \) 6.8\% at 100 \( \mu \)M, respectively, \( n = 8 \)) (Figs. 8 and 9). Pretreatment with 10 \( \mu \)M luminal SCH-23390 did not change \( V_t \) by itself (4.1 \( \pm \) 2.2\%; \( n = 4 \)); however, it completely blocked the luminal DA-induced depolarization (5.6 \( \pm \) 3.7\%; \( n = 4 \)) (Fig. 10A). In contrast, 10 \( \mu \)M luminal domperidone, which also did not affect \( V_t \) by itself (0.2 \( \pm \) 1.2\%; \( n = 4 \)), failed to suppress the luminal

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**Fig. 6.** Effect of basolateral SKF-81297 (D\(_1\)-like agonist) or bromocriptine (D\(_2\)-like agonist) on \( V_t \). *\( P < 0.05 \) and **\( P < 0.005 \) vs. basal \( V_t \) (\( n = 6 \)).

**Fig. 7.** Effect of basolateral RBI-257 (D\(_4\)-specific antagonist) on basolateral DA-induced depolarization. RBI-257 blocked basolateral DA-induced depolarization completely. *\( P < 0.001 \) vs. basal \( V_t \) (\( n = 5 \)).
DA-induced depolarization (37.5 ± 4.9%; n = 4) (Fig. 10B). In good agreement with these antagonist studies, 10 μM luminal SKF-81297 mimicked the effect of luminal DA, whereas 10 μM luminal bromocriptine had no significant effect (37.7 ± 6.3% with luminal SKF-81297, 28.3 ± 7.5% with luminal bromocriptine; n = 6) (Figs. 11 and 12).

Thus, like basolateral DA, luminal DA also induced depolarization of the $V_t$. However, the receptor subtype responsible for this action was not a D2-like receptor but a D1-like receptor. Besides the difference in receptor subtype, it was noted that the depolarization induced by luminal DA required 10,000 times higher concentrations (>10 μM) than those for basolateral DA (>1 nM). Also, the depolarization itself was much smaller than that induced by basolateral DA. Namely, at 10 μM, basolateral and luminal DA depolarized $V_t$ by 75 and 15%, respectively (Fig. 2 vs. Fig. 9).

Effect of the DA function on Na$^+$ transport. It is known that the $V_t$ in CCD primarily represents lumen-to-basolateral Na$^+$ flux (16). To explore the mechanism of basolateral DA-induced depolarization, we performed an Na$^+$ flux study. In the presence of 100 nM and 10 μM basolateral DA, $J_{Na}$ was significantly decreased (basal, 51.4 ± 4.2; 100 nM, 44.9 ± 3.2; 10 μM, 32.0 ± 2.9 peq·min$^{-1}$·mm tubule$^{-1}$, respectively) (Fig. 13A). Pretreatment with 10 μM basolateral domperidone reversed the DA-induced suppression of $J_{Na}$ (basal, 49.2 ± 3.8; domperidone alone, 48.5 ± 1.6; 10 μM DA with domperidone, 46.6 ± 3.5 peq·min$^{-1}$·mm tubule$^{-1}$, respectively) (Fig. 13B).

In contrast, however, 100 μM luminal DA caused no significant change in $J_{Na}$ despite significant depolarization of $V_t$ at 100 μM (data not shown).

DISCUSSION

In rat CCD, the presence of a D1-like receptor has been reported (29, 42), whereas some previous studies demonstrated D2-like receptor activity in the same segment of the rabbit and the rat (26, 39). Also, in vivo studies suggested that D2-like receptors are responsible for natriuresis (12, 17). Differences between animal species may be one of the reasons for the discrepancy. In addition, polarized localization of different DA receptors in the apical and basolateral side of the epithelium may exist, like AVP or PGE2 (2, 3, 5, 13), yielding conflicting results in the previous studies. Indeed, apical DA receptors have been reported in the proximal tubules (10) as well as in the collecting duct (28, 40).

To characterize receptor subtypes of DA in the rabbit CCD, we thus applied DA not only from the basolateral side but also from the luminal side. It was considered that the DA receptor resides on both sides of the CCD epithelium. Namely, DA depolarized the $V_t$ not only when applied from the basolateral side but also from the luminal side. Interestingly, however, the depolarization was mediated by distinct subtypes of the DA receptor. From the basolateral side, DA appeared to induce depolarization via a D2-like receptor. The D2-like receptor includes D2, D3, and D4 receptor subtypes (34, 35). Our basolateral agonist study showed that the D2-like receptor was dominant, and the basolateral antagonist study also confirmed this result. Moreover, the D4 subtype seemed to be the major basolateral DA receptor (Fig. 7). However, concerning D2-like receptor subtypes, these results seemed slightly discrepant because bromocriptine agonizes the D2-like receptor (mainly via D2 and D3 receptors), and the affinity of the D2 and D3 receptors is nearly 10–100 times higher than that of the D4 recep-
tor (Table 1). However, DA itself also indicated a much lower affinity to the D₄ receptor than to the D₂ and D₃ receptors. Indeed, the affinity of DA to the D₄ receptor has been shown to be only 10 times higher than that of bromocriptine (33, 38). In our results, RBI-251 showed complete inhibition of the effects of basolateral DA. Interestingly, RBI-251 is a D₄-specific antagonist. If the D₂ or D₃ receptor existed mainly in the basolateral compartment.

Fig. 10. Effect of luminal DA on Vᵢ in the presence of luminal SCH-23390 (D₁-like antagonist) or domperidone (D₂-like antagonist). A: pretreatment with luminal SCH-23390. SCH-23390 significantly suppressed the 10 μM luminal DA-induced depolarization (n = 4). B: pretreatment with luminal domperidone. Domperidone did not prevent the 10 μM luminal DA-induced depolarization (n = 4).

Fig. 11. Original trace of the Vᵢ change induced by luminal bromocriptine (D₂-like agonist) or SKF-81297 (D₁-like agonist). SKF-81297 but not bromocriptine mimicked the luminal DA-induced depolarization.
CCD, DA might cause the significant depolarization in the presence of RBI-251. Thus we concluded that basolateral DA caused depolarization via a D₂-like receptor, especially to the D₄ subtype.

In contrast, the D₁-like receptor is the major apical DA receptor. Because there is no adequate antagonist or agonist to distinguish D₁ and D₅ receptors available at present (25, 33, 38), in this study we could not specify the subtype of the D₁-like receptor on the apical side.

In rat CCD, only D₃ and D₄ receptor subtypes have been demonstrated by in situ hybridization studies (28, 40). The D₃ receptor resides exclusively on the apical side (28), whereas D₄ receptor immunostaining was found on both sides of the epithelia (40). These discrepancies between rat and rabbit argue for the species difference in DA receptor subtype in the CCD.

With respect to the role of basolateral and luminal DA receptors in Na⁺ transport, the present study demonstrated that basolateral DA receptors are primarily responsible for the inhibition of Na⁺ transport. Although luminal DA caused modest depolarization at concentrations >10 μM (Fig. 9), it failed to cause a significant suppression of Na⁺ transport (data not shown). Thus the mechanism of depolarization induced by luminal DA remains unknown. However, a similar Na⁺-independent change in V₉₅ has been noted in rabbit CCD when AVP is applied from the luminal side (5). Luminal AVP has been shown to suppress H⁺ secretion in this segment (21). Therefore, luminal DA may depolarize the V₉₅ by modulating electrogenic transport other than that of Na⁺. Further studies are needed to evaluate the regulation between basolateral and luminal DA.

![Graph showing the effect of luminal bromocriptine (D₂-like agonist) or SKF-81297 (D₁-like agonist) on V₉₅. Luminal SKF-81297 but not bromocriptine caused depolarization. P value vs. basal V₉₅ is shown (n = 6).](image)

![Graph showing the effect of basolateral DA on Na⁺ efflux (J₉₅). A: changes in V₉₅ and J₉₅ induced by basolateral DA. Basolateral DA suppressed J₉₅. *P < 0.01 vs. basal. ** P < 0.001 vs. basal. †P < 0.01 vs. 100 nM DA (n = 6). B: effect of basolateral DA in the presence of basolateral domperidone (D₂-like antagonist) (n = 6). In the presence of basolateral 10 μM domperidone, the effect of 10 μM basolateral DA was abolished. Thin lines in J₉₅ represent individual experiments.](image)
DOPAMINE RECEPTORS IN RABBIT CCD

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