Ouabain-insensitive acidification by dopamine in renal OK cells: primary control of the Na\(^+\)/H\(^+\) exchanger

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Gomes, Pedro, M. A. Vieira-Coelho, and P. Soares-da-Silva. Ouabain-insensitive acidification by dopamine in renal OK cells: primary control of the Na\(^+\)/H\(^+\) exchanger. Am J Physiol Regulatory Integrative Comp Physiol 281: R10–R18, 2001.—The present study was aimed at evaluating the role of D\(_1\)- and D\(_2\)-like receptors and investigating whether inhibition of Na\(^+\) transepithelial flux by dopamine is primarily dependent on inhibition of the apical Na\(^+\)/H\(^+\) exchanger, inhibition of the basolateral Na\(^+\)-K\(^+\)-ATPase, or both. The data presented here show that opossum kidney cells are endowed with D\(_1\)- and D\(_2\)-like receptors, the activation of the former, but not the latter, accompanied by stimulation of adenylyl cyclase (EC\(_{50}\) = 220 ± 2 nM), marked intracellular acidification (IC\(_{50}\) = 58 ± 2 nM), and attenuation of amphotericin B-induced decreases in short-circuit current (28.6 ± 4.5% reduction) without affecting intracellular pH recovery after CO\(_2\) removal. These results agree with the view that dopamine, through the activation of D\(_1\)- but not D\(_2\)-like receptors, inhibits both the Na\(^+\)/H\(^+\) exchanger (0.001933 ± 0.000121 vs. 0.000887 ± 0.000073 pH unit/s) and Na\(^+\)-K\(^+\)-ATPase without interfering with the Na\(^+\)-independent HCO\(_3\)\(^-\) transporter. It is concluded that dopamine, through the action of D\(_1\)-like receptors, inhibits both the Na\(^+\)/H\(^+\) exchanger and Na\(^+\)-K\(^+\)-ATPase, but its marked acidifying effects result from inhibition of the Na\(^+\)/H\(^+\) exchanger only, without interfering with the Na\(^+\)-independent HCO\(_3\)\(^-\) transporter and Na\(^+\)-K\(^+\)-ATPase.

REGULATION OF Na\(^+\) TRANSPORT across the proximal tubules can be influenced by dopamine (28), an intrarenal natriuretic hormone (4, 21, 36, 37), that was demonstrated to result in inhibition of the Na\(^+\)-K\(^+\)-ATPase (1), the Na\(^+\)/H\(^+\) exchanger (15), and the Na\(^+\)-HCO\(_3\)\(^-\) cotransporter (31). The magnitude of the importance of dopamine on renal function is clearly evidenced by the findings that a defect in renal dopamine receptor function and/or dopamine production may play a role in the pathogenesis of hypertension (26, 27). However, despite the amount of information available on the inhibitory effects of dopamine on mechanisms responsible for the regulation of Na\(^+\) transepithelial flux, changes in intracellular pH (pH\(_i\)) during activation of dopamine receptors in renal cells, to our knowledge, have not been systematically reported in the literature (18). Furthermore, it is still a matter of debate whether inhibition of Na\(^+\) transepithelial flux by dopamine is primarily dependent on inhibition of the apical Na\(^+\)/H\(^+\) exchanger, inhibition of the basolateral Na\(^+\)-K\(^+\)-ATPase, or both. Another controversial issue concerns the effect of D\(_2\)-like receptors on Na\(^+\)/H\(^+\) exchange and Na\(^+\)-K\(^+\)-ATPase activities. In renal proximal tubular cells, where both D\(_1\)- and D\(_2\)-like receptors are expressed, D\(_2\) agonists have been reported to have no effect (7, 14), to act in concert with D\(_1\) agonists to inhibit Na\(^+\)-K\(^+\)-ATPase activity (5), or to stimulate the Na\(^+\) pump (24, 25, 40). Similarly, stimulation of D\(_2\)-like receptors was found to have no effect on (14) or to inhibit the Na\(^+\)/H\(^+\) exchanger (16).

The present study was aimed at evaluating the role of D\(_1\)- and D\(_2\)-like receptors and investigating whether inhibition of Na\(^+\) transepithelial flux by dopamine is primarily dependent on inhibition of the apical Na\(^+\)/H\(^+\) exchanger, inhibition of the basolateral Na\(^+\)-K\(^+\)-ATPase, or both. For this purpose, we used opossum kidney (OK) cells, which are known to express several transport systems characteristic of proximal tubular cells, namely the Na\(^+\)/H\(^+\) exchanger, which is involved in extruding H\(^+\) from the cell after an acid load (3, 30); the Na\(^+\)-independent HCO\(_3\)\(^-\) transport system (34); and the Na\(^+\)-K\(^+\)-ATPase, which is responsible for maintaining the driving force for vectorial Na\(^+\) transport from the apical to the basolateral membrane (35). The findings reported here show that dopamine, through the action of D\(_1\)-like receptors, inhibits both the Na\(^+\)/H\(^+\) exchanger and Na\(^+\)-K\(^+\)-ATPase, but its marked acidifying effects result from inhibition of the Na\(^+\)/H\(^+\) exchanger only, without interfering with the Na\(^+\)-independent HCO\(_3\)\(^-\) transporter and Na\(^+\)-K\(^+\)-ATPase.

MATERIALS AND METHODS

Cell culture. OK cells, an established cell line derived from the kidney of a female American opossum that retains several properties of proximal tubular epithelial cells in culture (29) were obtained from the American Type Culture Collection (ATCC 1840 CRL, Rockville, MD) and maintained in a humidified atmosphere of 5% CO\(_2\)-95% air at 37°C. OK cells were grown in minimum essential medium (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (Sigma), 100
U/ml penicillin G, 0.25 μg/ml amphotericin B, 100 μg/ml streptomycin (Sigma), and 25 mM HEPES (Sigma). For subcellular, the cells were dissociated with 0.05% trypsin-EDTA (Sigma), split 1:5, and subcultured in Petri dishes with a 21-cm² growth area (Costar, Badhoevedorp, The Netherlands). The cell medium was changed every 2 days, and the cells reached confluence after 3–5 days of initial seeding. For 24 h before each experiment, the cell medium was free of fetal bovine serum. Experiments were generally performed 2–3 days after cells reached confluence and 6–7 days after initial seeding, and each square centimeter contained about 100 μg of cell protein.

Radioisotopic binding studies. OK cells were homogenized in 10 mM Tris·HCl, pH 7.4, containing 250 mM sucrose, 1 mM phenylmethylsulfonyl fluoride, 1 mM EDTA, and 5 μg/ml each of leupeptin and pepstatin with a Potter-Elvehjem Teflon homogenizer and centrifuged (20,000 g, 20 min, 4°C). Pellets were resuspended to a concentration of 2 mg/ml in 10 mM Tris·HCl, pH 7.4, with 5 mM MgCl₂ and 250 mM sucrose and stored aliquoted at −80°C. Membranes were thawed and homogenized at room temperature, 3°C for 20 min, 4°C, and resuspended in binding buffer (50 mM Tris·HCl, pH 7.4, with 120 mM NaCl, 5 mM KCl, 2 mM CaCl₂, and 1 mM MgCl₂). Saturation experiments were performed in quadruplicate in 96-well enzyme-immunnoassay (EIA)/RIA plates (Costar) in a final volume of 0.2 ml binding buffer containing 0.05–1.6 nM [³H]-Sch-23390 or 0.015–10 nM [³H]-YM-09151–2 and 100–200 μg membrane protein. Nonspecific binding was determined in the presence of 10 μM of unlabeled Sch-23390 or 10 μM of unlabeled YM-09151–2 (19, 23). After 30 min incubation at 30°C in a shaking water bath, assays were terminated by vacuum filtration through glass-fiber filter mats with the Brandel 96-cell Harvester (Brandel, Gaithersburg, MD). Filters were washed three times with 200 μl of cold 50 mM Tris·HCl, pH 7.4, dried, and impregnated with MultiLex A (Wallac, Turku, Finland), and radioactivity measured in a Microbeta counter (Wallac) with 20% efficiency.

cAMP measurement. cAMP was determined with an EIA kit (Assay Designs, Ann Arbor, MI), as previously described (8). OK cells were preincubated for 15 min at 37°C in Hanks’ medium (medium composition in mM: 137 NaCl, 5 KCl, 0.8 MgSO₄, 0.33 Na₂HPO₄, 0.44 KH₂PO₄, 0.25 CaCl₂, 1.0 MgCl₂, 0.15 Tris·HCl, and 1.0 sodium butyrate, pH 7.4), containing 100 μM IBMX, a phosphodiesterase inhibitor, in the presence or absence of antagonists. Cells were then incubated for 15 min with dopaminergic or specific dopaminergic agonists. At the end of the experiment, the reaction was stopped by the addition of 0.1 M HCl. Aliquots were taken for the measurement of total cAMP content.

pH measurements. In pH measurement experiments, OK cells were grown in 10 mm-width collagen-coated glass coverslips. pH was measured as previously described (39). At days 6–8 after seeding, the glass coverslips were incubated at 37°C for 40 min with 5 μM of the acetoxymethyl ester of sodium-binding benzofuran isophthalate (SBFI-AM). Coverslips were then washed twice with prewarmed dye-free modified Krebs buffer (see above) before initiation of the fluorescence recordings. Cells were mounted diagonally in a 1 × 1-cm acrylic fluorometric cuvette and were placed in the sample compartment of a Fluoromax-2 spectrofluorometer (Jobin Yvon-SPEX, Edison, NJ). The cuvette volume of 3.0 ml was constantly stirred and perfused at 5.0 ml/min with modified Krebs buffer prewarmed to 37°C. Under these conditions, the cuvette medium was replaced within 150 s. After 5 min, fluorescence was measured every 5 s alternating between 440- and 490-nm excitation (1 nm slit size) at 525-nm emission (3 nm slit size). The ratio of intracellular BCECF fluorescence at 490 and 440 nm was converted to pH, values by comparison with values from an intracellular calibration curve using the nigericin (10 μM) and high-K⁺ method (38). Na⁺/H⁺ antiporter activity. Na⁺/H⁺ exchanger activity was assayed as the initial rate of pH₁ recovery after an acid load imposed by 10 mM NH₄Cl followed by removal of sodium from the Krebs-modified buffer solution in the absence of CO₂/HCO₃⁻ (20, 22). These experiments, NaCl was replaced by an equimolar concentration of tetramethylammonium chloride. Test compounds were added to the extracellular fluid during the acidification and Na⁺-dependent pH₁ recovery periods.

Measurement of [Na⁺]. At days 6–8 after seeding, the glass coverslips were incubated at 37°C for 2 h with 5 μM of the membrane-permeable acetoxyethyl ester of sodium-binding benzofuran isophthalate (SBFI-AM). Coverslips were then washed twice with prewarmed dye-free modified Krebs buffer (see above) before initiation of the fluorescence recordings. Cells were mounted diagonally in a 1 × 1-cm acrylic fluorometric cuvette and were placed in the sample compartment of a Fluoromax-2 spectrofluorometer. The cuvette volume of 3.0 ml was constantly stirred and was perfused at 5.0 ml/min with modified Krebs buffer prewarmed to 37°C. After 5 min, fluorescence was measured every 5 s alternating between 340 and 390 nm excitation at 510 nm emission. The ratio of intracellular SBFI fluorescence at 340 and 390 nm was an index of [Na⁺], levels.

Na⁺-independent HCO₃⁻ transport system activity. Na⁺-independent HCO₃⁻ transport system activity was assayed as the initial rate of pH₁ recovery after an alkaline load (CO₂/HCO₃⁻ removal) in the presence of Na⁺, as previously described (34). The Krebs-HCO₃⁻ buffer had the following composition (in mM): 115 NaCl, 25 NaHCO₃, 5.4 KCl, 2.5 CaCl₂, 1.2 MgSO₄, 0.3 NaH₂PO₄, 0.3 KH₂PO₄, 10 HEPES, 5 glucose, pH 7.4 (adjusted with Tris base). When an HCO₃⁻-free medium was used, NaHCO₃ was replaced by an equimolar concentration of sodium gluconate.

Na⁺-K⁺-ATPase activity in OK cells. Cell monolayers were continuously monitored for changes in short-circuit current (Isc, μA/cm²) after the addition of amphotericin B to the apical-side reservoir to increase the sodium delivered to Na⁺-K⁺-ATPase at the saturating level. Under short-circuit conditions, the resulting current is due to the transport of sodium across the basolateral membrane mediated by Na⁺-K⁺-ATPase, as indicated by complete prevention by ouabain (100 μM) and removal of sodium from the medium bathing the apical side of the monolayer. OK cells grown on polycarbonate filters (Snapwell, Costar) were mounted in Ussing chambers (window area 1.0 cm²) equipped with water-jacketed gas lifts bathed on both sides with 10 ml of Krebs-Hensleit solution, gassed with 95% O₂ and 5% CO₂, and maintained at 37°C. The Krebs-Hensleit solution contained (in mM): 118 NaCl, 4.7 KCl, 25 NaHCO₃, 1.2 KH₂PO₄, 2.5 CaCl₂, and 1.2 MgSO₄; pH was adjusted to 7.4 after gassing with 5% CO₂ and 95% O₂. Monolayers were continuously voltage clamped to zero potential differences by application of external current with compensation for fluid resistance by means of an automatic voltage-current clamp (DVC 1000, World Precision Instruments, Sarasota, FL). Transepithelial resistance (Ω·cm²) was determined by altering the membrane potential stepwise (±3 mV) and applying the Ohmic relationship. The voltage-current clamp unit was connected to a...
personal computer via a BIOPAC MP1000 data-acquisition system (BIOPAC Systems, Goleta, CA). Data analysis was performed using AcqKnowledge 2.0 software (BIOPAC Systems).

Protein assay. The protein content of monolayers of OK cells was determined by the method of Bradford (6) with human serum albumin as a standard.

Data analysis. Arithmetic means are given with SE or geometric means with 95% confidence values. Statistical analysis was done with a one-way ANOVA followed by Newman-Keuls test for multiple comparisons. A P value < 0.05 was assumed to denote a significant difference.

Drugs. Amphotericin B, DIDS, IBMX, ouabain, and trypan blue were purchased from Sigma. (±)-SKF-83566 hydrochloride, S-(−)-sulpiride, (±)-SKF-38393 hydrochloride, and quinerolane hydrochloride were obtained from Research Biochemicals International (Natick, MA). BCECF-AM, SBFI-AM, and nigericin were obtained from Molecular Probes (Eugene, OR). [3H]Sch-23390 ([N-methyl-[3H]R(+)-7-chloro-2,3,4,5-tetrahydro-3-methyl-1-phenyl-1H-3-benzazepine-8-ol, specific activity 81.4 Ci/mmol) was purchased from New England Nuclear (Boston, MA). [3H]YM-09151–02 (specific activity 85.5 Ci/mmol) was also purchased from New England Nuclear.

RESULTS

Radioligand binding studies. Though OK cells have been demonstrated to express both D1- and D2-like dopamine receptors (8), the first series of experiments was aimed to examine the presence of specific D1 and D2 binding sites in these cells. Specific binding of [3H]Sch-23390, a D1-like receptor antagonist, and [3H]YM-09151–2, a D2-like receptor antagonist, to OK cell membranes revealed the presence of both D1- and D2-like receptors in this cell line (Table 1).

cAMP measurements. In the next series of experiments, by measuring cAMP production, we examined the extent to which the effects of dopamine in OK cells were linked to adenyl cyclase. Dopamine effects on cellular cAMP production were determined in the presence of 100 μM IBMX. IBMX was used to facilitate detection of small changes in cAMP formation that, in the absence of IBMX, might escape detection. As shown in Fig. 1A, dopamine stimulated cAMP production, with an EC50 value of 220 ± 2 nM. SKF-38393 (300 nM), a D1-like receptor agonist, but not D2-like receptor agonist quinerolane (300 and 1,000 nM), stimulated cAMP production (Fig. 1, B and C). The specific D1-like receptor antagonist SKF-83566 (1 μM) abolished the

Table 1. Apparent Kd and Bmax values for D1-like and D2-like receptor binding sites labeled with [3H]Sch-23390 and [3H]YM-09151–2, respectively, in membranes from OK cells

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Kd, nM</th>
<th>Bmax, fmol/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1-like</td>
<td>0.34 ± 0.09</td>
<td>185.8 ± 15.6</td>
</tr>
<tr>
<td>D2-like</td>
<td>1.85 ± 1.20</td>
<td>326.9 ± 73.2</td>
</tr>
</tbody>
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Values are means ± SE of 5 experiments per group (4 replicate determinations per experiment). Kd, dissociation constant; Bmax, density of binding sites, OK, opossum kidney.

Fig. 1. The effect of dopamine and selective agonists on cAMP production in opossum kidney (OK) cells in the presence of 3-isobutyl-1-methylxanthine (IBMX, 100 μM). A: concentration-response relationship between cAMP production and dopamine (DA) concentration; inset shows data plotted as %basal cAMP. B: effect of SKF-38393 (300 nM) on cAMP production in OK cells in the absence and presence of SKF-83566 (1 μM). C: effect of quinerolane (300 and 1,000 nM) on cAMP production. Bars represent means of 4 independent determinations; vertical lines show SE. Significantly different from basal values (*P < 0.05) and values for agonist alone (#P < 0.05).
stimulatory effect of cAMP production by 300 nM SKF-38393 (Fig. 1B).

Effects of dopamine on steady-state pH$_i$. To test the effect of dopamine on pH$_i$, BCECF-loaded cells were perfused with dopamine. Indeed, 1,000 nM dopamine (Fig. 2A) decreased pH$_i$ by 0.082 ± 0.012 U ($n = 6$). The initial rate of acidification of dopamine was dependent on the concentration of dopamine (10–1,000 nM) in the perfusion fluid, with an EC$_{50}$ of 58 ± 2 nM (Fig. 2B). The effect of 300 nM dopamine on pH$_i$ was significantly antagonized by 1 μM SKF-83566, a D$_1$ antagonist, but not by 1 μM S-(-)-sulpiride, a D$_2$ antagonist (Fig. 2C).

Because dopamine is known to inhibit Na$^+$/K$^+$-ATPase activity (also see Na$^+$/K$^+$-ATPase activity in OK cells) leading to decreases in sodium transepithelial flux, the next series of experiments was aimed at evaluating whether the acidifying effect of dopamine depended on the inhibition of Na$^+$/K$^+$-ATPase activity. For this purpose, the effect of dopamine (1 μM) was tested in the presence of the Na$^+$/K$^+$-ATPase inhibitor ouabain (100 μM). Ouabain (100 μM) alone produced a marked acidification effect (Fig. 3A). However, pretreatment with ouabain (100 μM) markedly ($P < 0.05$) attenuated the initial rate of acidification by dopamine (Fig. 4A) but failed to prevent the net acidification induced by 1 μM dopamine (Fig. 4B). This result indicates that the acidifying effects of dopamine may result from interference with other pH regulators rather than Na$^+$/K$^+$-ATPase.

Na$^+$/H$^+$ exchanger activity. OK cells perfused with Na$^+$-free medium for 600 s responded with a marked acidification that could be reversed by the addition of Na$^+$ to the perfusion medium (Fig. 3B). Because pH recovery was obtained in an HCO$_3$-free medium, this recovery was essentially mediated by the Na$^+$/H$^+$ exchanger. To study the effects of dopamine on Na$^+$/H$^+$ exchange under conditions of maximal velocity, we used a slightly different protocol. The initial rate of pH$_i$ recovery was measured after an acid load imposed by 10 mM NH$_4$Cl, followed by removal of sodium from the Krebs-modified buffer solution (Fig. 5A). In the presence of dopamine (1 μM) during the acidification and recovery periods, the sodium-dependent recovery of pH$_i$ was markedly attenuated, and only a partial recovery in pH$_i$ was observed (Fig. 5B and Table 2). The inhibitory effect of dopamine on pH$_i$ recovery was prevented by the D$_1$-selective antagonist SKF-83566 (1 μM) (Fig. 5B and Table 2).

Na$^+$-independent HCO$_3$ transport system. In an HCO$_3$-containing medium, removal of CO$_2$/HCO$_3$ caused an initial cell alkalization as a result of CO$_2$ loss from the cell with subsequent return of pH$_i$ towards basal values. To assess whether HCO$_3$ transport in OK cells is linked to Na$^+$ and Cl$^-$ via a Cl/HCO$_3$ exchanger or an Na/HCO$_3$ cotransporter, respectively, we replaced Cl$^-$ and Na$^+$ in the perfusion medium with gluconate and choline, respectively. Removal of Na$^+$ and Cl$^-$ from the perfusion medium failed to inhibit the pH$_i$ recovery after CO$_2$ removal. However, DIDS (200 μM) significantly inhibited this recovery phase (Table 3). Taken together, these results

![Fig. 2. Effect of dopamine on steady-state intracellular pH (pHi) and initial rates of acidification measured in monolayers of OK cells loaded with the pH-sensitive fluorophore 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF). A: effect of 1,000 nM DA (solid bar) on pHi, Trace is the mean of 6 independent experiments. B: concentration-response curve to DA (10–1,000 nM) applied on a stable baseline. C: effect of 300 nM DA on initial rates of acidification in the absence and presence of SKF-83566 (1 μM) or S-(-)-sulpiride (1 μM). Bars represent means of 5 independent determinations; vertical lines show SE. Significantly different from values for DA alone (*P < 0.05).](http://ajpregu.physiology.org/Content/Full/102/3/348/F1.jpg)
suggest that OK cells are endowed with an Na\(^+\)- and Cl\(^-\)-independent HCO\(_3\)\(^-\) transport system, results that are in agreement with those reported by others (34). In an Na\(^+\)-free medium (Na\(^+\)/H\(^+\) exchanger inhibited), dopamine (1 \(\mu\)M) was devoid of effect on the pH \(_i\) recovery after CO\(_2\) removal (Table 3).

\textbf{Na\(^+\)-K\(^+\)-ATPase activity in OK cells.} Because the acidifying effects of dopamine were insensitive to 100 \(\mu\)M ouabain, it was decided to determine Na\(^+\)-K\(^+\)-ATPase activity in OK cells and evaluate its sensitivity to ouabain and dopamine. To study Na\(^+\)-K\(^+\)-ATPase activity in OK cells, we decided to use an electrophysiological method in which cell monolayers were continuously monitored for changes in \(I_{sc}\) after the addition of amphotericin B to the apical cell side to increase the sodium delivered to Na\(^+\)-K\(^+\)-ATPase to the saturating level. As shown in Fig. 6, the addition of amphotericin B produced a fast increase of \(I_{sc}\) followed by recovery to baseline. This effect is due to the transport of sodium across the basolateral membrane mediated by Na\(^+\)-K\(^+\)-ATPase, as indicated by complete prevention by ouabain (100 \(\mu\)M) and removal of sodium from medium bathing the apical side of the monolayer (Fig. 6). The increase in [Na\(^+\)]\(_i\) by amphotericin B was monitored by increases in fluorescence emission in cells loaded with SBFI (Fig. 7). The addition of the amphotericin B also decreased pH\(_i\) (Fig. 3C), which suggests that transient increases in [Na\(^+\)]\(_i\) by amphotericin B resulted in inhibition of the Na\(^+\)/H\(^+\) exchanger. Pretreatment with dopamine applied from the apical cell side significantly reduced the effect of 1.0 \(\mu\)g/ml amphotericin B on \(I_{sc}\), dopamine (1 \(\mu\)M) was devoid of effect on the pH\(_i\) recovery after CO\(_2\) removal (Table 3).

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this being prevented by the D1-like receptor antagonist SKF-83566 (1 μM) (Fig. 8). The D1-like receptor agonist SKF-38393 (30 to 1,000 nM) was also found to attenuate, in a concentration-dependent manner, the effect of amphotericin B on $I_{sc}$ (Fig. 8).

**DISCUSSION**

The data presented here show that OK cells are endowed with D1- and D2-like receptors; the activation of the former, but not the latter, is accompanied by stimulation of adenylyl cyclase, marked intracellular acidification, and attenuation of amphotericin B-induced increases in $I_{sc}$, without affecting the pHi recov-
ery after CO₂ removal. These results agree with the view that dopamine, through the activation of D₁ but not D₂-like receptors, inhibits both the Na⁺/H⁺ exchanger and Na⁺⁻K⁺-ATPase without interfering with the Na⁺-independent HCO₃⁻ transporter. Because the dopamine-induced decrease in pHe was insensitive to ouabain, it is suggested that the acidifying effect of dopamine results primarily from inhibition of the Na⁺⁻H⁺ exchanger.

Changes in pHe may result from interference with mechanisms regulating H⁺ outward transfer and Na⁺ and/or HCO₃⁻ inward and outward transporters. Despite being the major apical Na⁺ transporter, the Na⁺⁻H⁺ exchanger has as its main task extruding H⁺ from the cells (2). On the other hand, Na⁺⁻K⁺-ATPase is responsible for the maintenance of the driving force for vectorial Na⁺ transport from the apical to the basolateral membrane. Because HCO₃⁻ transport in OK cells is promoted through an Na⁺- and Cl⁻-independent HCO₃⁻ transporter (Ref. 34 and the present study), transient changes in pHe, namely those resulting from decreases in Na⁺ transepithelial flux or sensitivity to Na⁺, are believed to result mainly from changes in the activities of the Na⁺⁻H⁺ exchanger or Na⁺⁻K⁺-ATPase. Three sets of findings support this view: 1) perfusion with an Na⁺-free medium resulted in intracellular acidification, the recovery of which was obtained by the addition of Na⁺ to the perfusion medium (since pH recovery was obtained in an HCO₃⁻-free medium, this recovery was essentially mediated by the Na⁺⁻H⁺ exchanger); 2) inhibition of the Na⁺⁻K⁺-ATPase by ouabain was accompanied by intracellular acidification; and 3) the addition of the Na⁺ ionophore amphotericin B also decreased pHe. The result that both ouabain and amphotericin B produced marked decreases in pHe is compatible with the view that transient increases in intracellular Na⁺ resulted in inhibition of the Na⁺⁻H⁺ exchanger. Because dopamine is able to inhibit both the Na⁺⁻H⁺ exchanger and Na⁺⁻K⁺-ATPase, it may be difficult to ascertain the primary process responsible for intracellular acidification. Inhibition of Na⁺⁻K⁺-ATPase increases intracellular Na⁺, which in turn might lead to inhibition of the Na⁺⁻H⁺ exchanger. Similarly, inhibition of the Na⁺⁻H⁺ exchanger reduces intracellular Na⁺, which might lead to decreases in Na⁺⁻K⁺-ATPase activity. The results presented here, however, suggest that one might be able to discriminate between the actions of dopamine on the Na⁺⁻H⁺ exchanger and Na⁺⁻K⁺-ATPase. The addition of amphotericin B to the apical cell side increased the Na⁺ delivered to Na⁺⁻K⁺-ATPase to the saturating level, as indicated by the fast increase in Isc. The rapid recovery to baseline is due to the transport of Na⁺ across the basolateral membrane mediated by Na⁺⁻K⁺-ATPase, as indicated by complete prevention by ouabain and removal of Na⁺ from medium bathing the apical side of the monolayer. Because this effect of amphotericin B was accompanied by inhibition of the Na⁺⁻H⁺ exchanger, the attenuation of amphotericin B-induced increases in Isc by dopamine results most probably from direct inhibition of Na⁺⁻K⁺-ATPase. On the other hand, the finding that decreases in pHe by dopamine were of similar magnitude in the absence and presence of ouabain strongly suggest that the acidifying effect of dopamine results primarily from inhibition of the Na⁺⁻H⁺ exchanger. The argument that these effects of dopamine on pHe are secondary to inhibition of Na⁺⁻K⁺-ATPase activity would not be valid, assuming that 100 μM ouabain in OK cells produces complete inhibition of the Na⁺ pump, as shown here and as reported by others (35). Inhibition of the Na⁺⁻H⁺ exchanger secondary to elevations in intracellular Na⁺ by ouabain is not complete, as evidenced by the significant reduction of the initial rate of acidification by dopamine in cells previously exposed to ouabain. However, it still allows the

![Fig. 7. Effect of 2.5 μg/ml amphotericin B (solid bar) on steady-state intracellular Na⁺ measured in monolayers of OK cells loaded with the sodium-sensitive fluorophore sodium-binding benzofuran isophthaleate. Trace is the mean of 6 independent experiments.](image)

![Fig. 8. Effect of DA (1 μM) in the absence and presence of SKF-83566 (1 μM) and SKF-38339 (30–1,000 nM) on changes in Isc induced by amphotericin B (1.0 μg/ml) in monolayers of OK cells. Test drugs were applied from the apical cell side. Ouabain was added to the basal cell side only. Bars represent means of 4–10 independent determinations; vertical lines show SE. Significantly different from corresponding control values (*P < 0.05) or values for DA (#P < 0.05).](image)
full expression of the primary inhibitory action of dopamine on the Na\(^+\)/H\(^+\) exchanger, as shown by similar reductions of pHi in steady-state conditions. This is in agreement with the report by Gesek and Schoolwerth (17) that showed that dopamine inhibited the 5-(N-ethyl-n-isopropyl)-amiloride-suppressible \(^{22}\)Na\(^+\) uptake in conditions of Na\(^+\)-K\(^+\)-ATPase inhibition by ouabain. Because our studies on the inhibitory effect of dopamine on Na\(^+\)-K\(^+\)-ATPase were conducted in conditions that restrain the activity of the Na\(^+\)/H\(^+\) exchanger, as a result of the increase in intracellular Na\(^+\) by amphotericin B, it is concluded that dopamine reduces Na\(^+\)-K\(^+\)-ATPase by mechanisms independent from its effect on some brush-border Na\(^+\) entry mechanisms. However, the effects of dopamine on transepithelial transport of Na\(^+\) are primarily through inhibition of the Na\(^+\)/H\(^+\) exchanger, independently of its inhibitory effects upon Na\(^+\)-K\(^+\)-ATPase activity. This is in agreement with studies by Debska-Sliżien et al. (13) that suggest that endogenous dopamine appears to directly control Na\(^+\)-Pi, and Na\(^+\)/H\(^+\) transport and secondarily alter basolateral membrane Na\(^+\)-K\(^+\)-ATPase. These authors reported on an immediate increase in Na\(^+\) and Pi entry after inhibition of dopamine synthesis with carbidopa accompanied by a progressive increase in Na\(^-\)K\(^+\)-ATPase activity over the ensuing 4 h.

The effects of dopamine on the Na\(^+\)/H\(^+\) exchanger and Na\(^+\)-K\(^+\)-ATPase reported here were entirely mediated through D\(_1\)-like receptors, though OK cells were found to express both D\(_1\)- and D\(_2\)-like receptors. The inhibitory effects of dopamine on the Na\(^+\)/H\(^+\) exchanger and Na\(^+\)-K\(^+\)-ATPase were antagonized by the selective D\(_1\)-like receptor antagonist SKF-83566 and mimicked by the D\(_1\)-like receptor agonist SKP-38393. Furthermore, the D\(_2\)-like receptor agonist quinolone and the D\(_2\)-like receptor antagonist S(-)-sulpiride were devoid of effects. The type of D\(_1\)-like receptors mediating these effects of dopamine has the same characteristics that have been described by other authors, namely their coupling to adenyl cyclase and dissociation constant values in the low nanomolar range (8). The type of D\(_2\)-like receptors expressed in OK cells used in the present study has the same kinetic characteristics described by others (8). In this respect it is interesting to note that most of information in the literature indicates that activation of D\(_2\)-like receptors in different types of cells is mainly associated with stimulation of the Na\(^+\)/H\(^+\) exchanger, evidenced by increases in the rates of extracellular acidification. This has been observed in cells expressing different types of D\(_2\)-like receptors, namely D\(_2\), D\(_3\), and D\(_4\) receptors of human and nonhuman origin (9–12, 32). The most likely explanation for these findings is the possibility that these cells contained mainly the type 1 Na\(^+\)/H\(^+\) exchanger. In fact, type 1 Na\(^+\)/H\(^+\) exchanger is the amiloride-sensitive, growth-factor-activatable, and ubiquitously expressed Na\(^+\)/H\(^+\) exchanger known to regulate pHi and cellular volume (33). Type 1 Na\(^+\)/H\(^+\) exchanger is a major pH-regulating system, whereas the epithelial type 3 Na\(^+\)/H\(^+\) exchanger iso-

form specializes in transepithelial Na\(^+\) transport. In contrast to that which is observed in other types of cells, namely in nonepithelial cells, OK cells have been found to possess only the type 3 isoform (33). The brush-border Na\(^+\) absorptive process by the type 3 Na\(^+\)/H\(^+\) exchanger is acutely inhibited by activation of cAMP-dependent protein kinase, where two regulatory proteins, type 3 Na\(^+\)/H\(^+\) exchanger kinase A regulatory protein and Na\(^+\)/H\(^+\) exchanger regulatory factor, intervene and enable cAMP to inhibit type 3 Na\(^+\)/H\(^+\) exchanger (41). Because activation of D\(_1\)-like receptors was shown to result in adenyl cyclase stimulation with increases in cAMP, it is quite likely that inhibition of Na\(^+\)/H\(^+\) exchanger by dopamine is associated with activation of cAMP-dependent protein kinase. By contrast, the molecular mechanism of the stimulatory effect of the Na\(^+\)/H\(^+\) exchanger by dopamine and the molecular regulation of type 1 Na\(^+\)/H\(^+\) exchanger are both poorly understood.

In conclusion, the results presented here show that dopamine, through the action of D\(_1\)-like receptors, inhibits both the Na\(^+\)/H\(^+\) exchanger and Na\(^+\)-K\(^+\)-ATPase but its marked acidifying effects result primarily from inhibition of the Na\(^+\)/H\(^+\) exchanger, without interfering with the Na\(^+\)-independent HCO\(_3\)\(^-\) transporter and Na\(^+\)-K\(^+\)-ATPase.

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