D₂-like receptor-mediated inhibition of Na⁺-K⁺-ATPase activity is dependent on the opening of K⁺ channels

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Gomes, Pedro, and P. Soares-da-Silva. D₂-like receptor-mediated inhibition of Na⁺-K⁺-ATPase activity is dependent on the opening of K⁺ channels. Am J Physiol Renal Physiol 283: F114–F123, 2002. First published January 29, 2002; 10.1152/ajprenal.00244.2001.—This study examined the effects of D₂-like dopamine receptor activation on Na⁺-K⁺-ATPase activity while apical-to-basal, ouabain-sensitive, amphotericin B-induced increases in short-circuit current and basolateral K⁺ (Iₛ) currents in opossum kidney cells were measured. The inhibitory effect of dopamine on Na⁺-K⁺-ATPase activity was completely abolished by either D₁- or D₂-like receptor antagonists and mimicked by D₁- and D₂-like receptor agonists SKF-38393 and quinerolane, respectively. Blockade of basolateral K⁺ channels with BaCl₂ (1 mM) or glibenclamide (10 μM), but not apamin (1 μM), totally prevented the inhibitory effects of quinerolane. The K⁺ channel opener pinacidil decreased Na⁺-K⁺-ATPase activity. The inhibitory effect of quinerolane on Na⁺-K⁺-ATPase activity was abolished by pretreatment of opossum kidney cells with pertussis toxin (PTX). Quinerolane increased Iₛ across the basolateral membrane in a concentration-dependent manner; this effect was abolished by pretreatment with PTX, S sulphuride, and glibenclamide. SKF-38393 did not change Iₛ. Both H-89 (protein kinase A inhibitor) and chelerythrine (protein kinase C inhibitor) failed to prevent the stimulatory effect of quinerolane on Iₛ. The stimulation of the D₂-like receptor was associated with a rapid hyperpolarizing effect, whereas D₁-like receptor activation was accompanied by increases in cell membrane potential. It is concluded that stimulation of D₂-like receptors leads to inhibition of Na⁺-K⁺-ATPase activity and hyperpolarization; both effects are associated with the opening of K⁺ channels.

DEPENDING ON THE PARTICULARITIES of the experimental model used and characteristics of the mechanisms under evaluation, it is difficult, on certain occasions, to define with precision the nature of the events observed. This may be the case in an analysis of ion transepithelial flux, namely, in conditions in which the integrity of the cell is maintained and several transporters are expected to operate in concert. In a recent study of monolayers of opossum kidney (OK) cells expressing dopamine D₁- and D₂-like receptors, we were able to demonstrate that dopamine and D₁-like receptor agonists inhibited Na⁺-K⁺-ATPase activity, using a methodology that consists of the measurement of ouabain-sensitive transepithelial transport of Na⁺ (18). The technique employed consists of the measurement of the transepithelial transport of Na⁺ in cell monolayers placed in Ussing chambers, with the added Na⁺ ionophore amphotericin B, from the apical cell border, which leads to increases in the Na⁺ delivered to Na⁺-K⁺-ATPase at the saturating level. Under short-circuit current (Iₛc) conditions, the observed current is an index of basolateral membrane Na⁺-K⁺-ATPase activity (14, 38–40). The advantages of this methodology over other experimental approaches are the possibility of evaluating Na⁺-K⁺-ATPase activity under in vivo conditions, avoiding freeze-thaw cell permeabilization procedures, and independently accessing the apical and basolateral cell sides. Subsequently, it was observed that stimulation of D₂-like receptors also resulted in a marked reduction in amphotericin B-induced increases in Na⁺ currents. D₂-like receptor agonists have been reported to have no effect (12, 31, 33), to act in concert with D₁-like receptor agonists to inhibit Na⁺-K⁺-ATPase activity (2, 7–9), or to stimulate the Na⁺ pump (1, 20, 23, 42). However, there are no reports showing that stimulation of D₂-like receptors leads to inhibition of Na⁺-K⁺-ATPase activity. Thus it was decided to look in more detail at the nature of the D₂-like receptor-mediated inhibition of amphotericin B-induced increases in Na⁺ currents.

Classically, an ouabain-sensitive and Na⁺-dependent amphotericin B-induced increase in Iₛc represents Na⁺ transport mediated by Na⁺-K⁺-ATPase (14). However, other mechanisms, namely, those dependent on movements of K⁺ across the cellular membranes, may be partially responsible for these alterations in electrogenic ion movement. For instance, it is now well established that luminal Na⁺ entry proceeds in concert with increases in basolateral Na⁺-K⁺-ATPase activity and increases in K⁺ conductance (6, 41). Na⁺ is extruded from the cell by the Na⁺-K⁺-ATPase at the expense of K⁺ entry; K⁺ leaves the cell again via K⁺ channels in the basolateral membrane. The increase in K⁺ conductance accompanying the stimulation of Na⁺-
K⁺-ATPase activity appears to result from the opening of ATP-sensitive K⁺ (K<sub>ATP</sub>) channels in the basolateral membrane after local reductions in ATP levels (35). The tight coordination between Na⁺-K⁺-ATPase activity and K⁺ channel activity (pump-leak coupling) is thought to be of considerable importance for cell volume and homeostasis during epithelial transport.

Taking these aspects into consideration along with the observation that stimulation of D<sub>2</sub>-like receptors, mainly in the central nervous system, has been reported to increase K⁺ conductance (11, 17, 26, 27, 32, 36), we believed it was worthwhile to evaluate in more detail the role of D<sub>2</sub>-like dopamine receptors on amphotericin B-induced I<sub>leak</sub> changes in OK cells. The present study reports on the effects of D<sub>1</sub>- and D<sub>2</sub>-like dopamine receptor activation on Na⁺ and K⁺ currents in renal OK cells and evaluated transduction pathways coupled to D<sub>2</sub>-like receptors. It is shown that activation of D<sub>2</sub>-like receptors in renal OK cells leads to stimulation of basolateral K<sub>ATP</sub> channels, which appear to be under the control of pertussis toxin (PTX)-sensitive G proteins of the G<sub>ai</sub> class.

MATERIALS AND METHODS

Cell culture. OK cells, an established cell line derived from the kidney of a female American opossum (ATCC 1840 CRL), were obtained from the American Type Culture Collection (Rockville, MD) and maintained in a humidified atmosphere of 5% CO<sub>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 streptomycin (Sigma), and 25 mM HEPES (Sigma). For subculturing, the cells were dissociated with 0.05% trypsin-EDTA (Sigma), split 1:5, and subcultured in petri dishes with a 21-cm<sup>2</sup> growth area (Costar, Badhoevedorp, The Netherlands). For electrophysiology studies, the cells were seeded onto polycarbonate filter supports (Snapwell; Costar) at a density of 13,000 cells/well. 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 days after cells reached confluence and 4 days after initial seeding, and each square centimeter contained ~100 µg of cell protein. In some experiments, cells were treated overnight from the apical cell side in the presence of agents known to interfere with signal transducing pathways, namely, G proteins, such as cholera toxin (CTX) and PTX. On the day of the experiment, culture medium containing the test agents was removed and the cells were washed with fresh medium and allowed to stabilize for at least 2 h before the start of acquisition of electrophysiological parameters.

Electrogenic ion transport in OK cells. All transport experiments were conducted under short-circuit conditions. OK cells grown on polycarbonate filters were mounted in Ussing chambers (1.0-cm<sup>2</sup> window area) equipped with water-jacketed gas lights bathed on both sides with 10 ml of Krebs-Hensleit solution, gassed with 5% CO<sub>2</sub>-95% O<sub>2</sub>, and maintained at 37°C. The standard composition of the apical and basolateral bathing Krebs-Hensleit solution was (in mM) 118 NaCl, 4.7 KCl, 25 NaHCO<sub>3</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 2.5 CaCl<sub>2</sub>, and 1.2 MgSO<sub>4</sub>; pH was adjusted to 7.4 after cells were gassed with 5% CO<sub>2</sub>-95% O<sub>2</sub>. The apical bathing Krebs-Hensleit solution contained mannitol (10 mM) instead of glucose (10 mM) to avoid entry of apical Na⁺ through the Na⁺-dependent glucose transporter. Experimental design also required modification of the bathing solution compositions for specific experiments, and these changes are indicated in Na⁺-K⁺-ATPase activity. After a 5-min stabilization, monolayers were continuously voltage clamped to zero potential differences by application of an 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·cm<sup>2</sup>) was determined by altering the membrane potential stepwise (±3 mV) and applying the ohmic relationship. Cells were allowed to stabilize for a further 25 min before permeabilization with amphotericin B; this period was also used for exposure of cells to the relevant drug treatments. The voltage-current clamp unit was connected to a PC by means of a BIOPAC MP1000 data-acquisition system (BIOPAC Systems, Goleta, CA). Data analysis was performed by using AcqKnowledge 2.0 software (BIOPAC Systems).

Na⁺-K⁺-ATPase activity. The effect of D<sub>1</sub>- and D<sub>2</sub>-like receptor agonists on Na⁺-K⁺-ATPase activity was examined in monolayers mounted in Ussing chambers bathed with the standard Krebs-Hensleit solution such that the final bath Na⁺ concentration was 145 mM on both sides of the monolayers. The apical membrane was then permeabilized by addition of amphotericin B to the apical bathing solution. Under short-circuit conditions, the resulting current is due to the transport of Na⁺ across the basolateral membrane by the Na⁺-K⁺-ATPase (14, 38). This experimental model allows the entry of apical Na⁺ and leads to inhibition of the Na⁺-/H⁺ exchanger (18). The concentration-response relationship of the I<sub>leak</sub> for bath Na⁺ was evaluated by initially bathing the apical side of the monolayers mounted in Ussing chambers with Na⁺-free Krebs-Hensleit solution (NaCl replaced with choline chloride and NaHCO<sub>3</sub> replaced with choline bicarbonate). Amphotericin B was then added to the apical bathing solution, and the I<sub>leak</sub> was continuously recorded. Thereafter, the Na⁺ concentration was incrementally increased by removing bathing medium from the apical side of the monolayers and replacing it with equal volumes of normal Krebs-Hensleit solution. Thus bath Na⁺ concentration was gradually increased over the range 0–143 mM without affecting the concentrations of other ions. Apamin, BaCl<sub>2</sub>, DIDS, glibenclamide, ouabain, and pinacidil were applied from the basolateral cell side only, whereas amiloride was applied from the apical side only. All other test drugs were applied from both apical and basolateral cell sides.

Basolateral membrane K⁺ conductance. To evaluate the effect of D<sub>1</sub>- and D<sub>2</sub>-like receptor agonists on the basolateral K⁺ conductance of OK cells, cell monolayers were mounted in Ussing chambers in the presence of an apical-to-basal K⁺ gradient (80.5 mM), while the Na⁺ concentration was maintained at 25 mM on both sides of the monolayers. NaCl in the apical bathing solution was replaced with KCl (75 mM), and NaCl in the basolateral bathing solution was replaced with choline chloride (75 mM). The modified Krebs-Hensleit solution contained (in mM) 25 NaCl, 5 KCl, 25 choline HCO<sub>3</sub>, 1.2 KH<sub>2</sub>PO<sub>4</sub>, 2.5 CaCl<sub>2</sub>, and 1.2 MgSO<sub>4</sub>; pH was adjusted to 7.4 after monolayers were gassed with 5% CO<sub>2</sub>-95% O<sub>2</sub>. After ~15 min, ouabain (100 µM) was added to the basolateral bath solution to inhibit Na⁺-K⁺-ATPase, and amphotericin B was added to the apical bath solution to permeabilize the apical plasma membrane. The resulting I<sub>leak</sub> is due to the movement of K⁺ through channels in the basolateral membrane (I<sub>bl</sub>.

cAMP measurement. cAMP was determined with an enzyme immunoassay kit (Assay Designs, Ann Arbor, MI) as...
previously described (18). OK cells were preincubated for 15 min at 37 °C in Hanks’ medium (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 Na butyrate, pH 7.4 containing 100 μM IBMX, a phosphodiesterase inhibitor. Cells were then incubated for 15 min with test compounds. 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 intracellular cAMP content.

Membrane potential assay. Changes in membrane potential were evaluated by using the bisoxonol fluorescent dye bis-(1,3-dibutylbarbituric acid)trimethine oxonol [DiBAC4(3)] as previously described (19). Cells cultured on glass coverslips were rinsed twice with assay buffer (in mM) 20 HEPES, 120 NaCl, 2 KCl, 2 CaCl2, 1.0 MgCl2, and 5 glucose, pH 7.4, at 25°C containing 5 μM DiBAC4(3) and then incubated for 30 min in 500 μl buffer solution containing 5 μM DiBAC4(3) to ensure dye distribution across the cell membrane. Thereafter, cells were mounted diagonally in a 1 × 1-cm acrylic fluorescent cuvette and placed in the sample compartment of a Fluoromax-2 spectrofluorometer (Jobin Yvon-SPEX, Edison, NJ). The cuvette volume of 2.0 ml was continually stirred and maintained at 37°C. Changes in fluorescence were monitored for 500 s by sampling every 5 s at excitation and emission wavelengths of 488 and 520 nm, respectively. Responses of drugs added to the incubation medium were corrected for any background changes in fluorescence.

Drugs. Amphotericin B, apamin, BaCl2, chelerythrine chloride, CTX, dopamine hydrochloride, forskolin, H-89, glibenclamide, IBMX, ouabain, PTX, and trypan blue were purchased from Sigma. (±)-SKF-83566 hydrochloride, H11003 (−)-SKF-38393 hydrochloride, and quinerolane H11006, sulpiride, H11001 20 HEPES, 120 NaCl, 2 KCl, 2 CaCl2, 1 MgCl2, and 5 glucose, pH 7.4; containing 100 M DiBAC4(3) and then incubated for 30 min in 500 μl buffer solution containing 5 μM DiBAC4(3) to ensure dye distribution across the cell membrane. Thereafter, cells were mounted diagonally in a 1 × 1-cm acrylic fluorescent cuvette and placed in the sample compartment of a Fluoromax-2 spectrofluorometer (Jobin Yvon-SPEX, Edison, NJ). The cuvette volume of 2.0 ml was continually stirred and maintained at 37°C. Changes in fluorescence were monitored for 500 s by sampling every 5 s at excitation and emission wavelengths of 488 and 520 nm, respectively. Responses of drugs added to the incubation medium were corrected for any background changes in fluorescence.

Results. Amphotericin B, apamin, BaCl2, chelerythrine chloride, CTX, dopamine hydrochloride, forskolin, H-89, glibenclamide, IBMX, ouabain, PTX, and trypan blue were purchased from Sigma. (±)-SKF-83566 hydrochloride, H11003 (−)-SKF-38393 hydrochloride, and quinerolane H11006, sulpiride, H11001 20 HEPES, 120 NaCl, 2 KCl, 2 CaCl2, 1 MgCl2, and 5 glucose, pH 7.4; containing 100 M DiBAC4(3) and then incubated for 30 min in 500 μl buffer solution containing 5 μM DiBAC4(3) to ensure dye distribution across the cell membrane. Thereafter, cells were mounted diagonally in a 1 × 1-cm acrylic fluorescent cuvette and placed in the sample compartment of a Fluoromax-2 spectrofluorometer (Jobin Yvon-SPEX, Edison, NJ). The cuvette volume of 2.0 ml was continually stirred and maintained at 37°C. Changes in fluorescence were monitored for 500 s by sampling every 5 s at excitation and emission wavelengths of 488 and 520 nm, respectively. Responses of drugs added to the incubation medium were corrected for any background changes in fluorescence.

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RESULTS

In conditions of 143 mM Na+ in the extracellular medium, the addition of amphotericin B to the apical cell side induced an increase in Isc; this effect was dependent on the concentration used (Fig. 1). The maximum effect was attained at 2.0 μg/ml amphotericin B; thus, in all subsequent experiments, the apical membrane was permeabilized with 1.0 μg/ml amphotericin B to increase the Na+ delivered to Na+-K+-ATPase to the half-maximal saturating level. Under these conditions, the amphotericin B (1.0 μg/ml)-induced increase in Isc was markedly (P < 0.05) attenuated by ouabain (100 μM) and by removal of Na+ from the solution bathing the apical cell border (Fig. 1B). Similarly, removal of K+ from the solution bathing the basolateral cell side (substitution by cesium chloride) markedly attenuated (87% reduction) the amphotericin B-induced increase in Isc (Fig. 1B). However, blockade of basolateral K+ channels with BaCl2 (1 mM) applied from the basolateral cell side failed to affect the amphotericin B-induced increase in Isc (Fig. 1B). The increase in Isc induced by amphotericin B (1.0 μg/ml) applied from the apical cell side was not affected by the Na+/H+ exchanger inhibitor amiloride (1 mM), the Na+-K+-Cl cotransporter inhibitor bumetanide (10 μM), or the Na+/HCO3- cotransporter inhibitor DIDS (200 μM) (Fig. 1B). The relationship between Na+-K+-ATPase activity and the concentration of intracellular Na+ was studied in another set of experiments in which the amphotericin B-induced increase in Isc was measured at increasing concentrations of extracellular Na+. In experiments performed in the presence of different concentrations of Na+, NaCl was replaced by an equimolar concentration of choline chloride. As shown in Fig. 2, amphotericin B-induced increases in Isc were saturable while approaching 100 mM Na+, with a Michaelis-Menten constant (Km) of 44.1 ± 13.7 mM and Vmax of 49.6 ± 4.8 μA/cm2.

Pretreatment with dopamine applied from the apical cell side significantly reduced the effect of 1.0 μg/ml amphotericin B on Isc, which was prevented by the D1-like receptor antagonist SKF-83566 (1 μM) or the D2-like receptor antagonist S-sulpiride (1 μM) (Fig. 1B).
3A). On the other hand, both the D₁-like receptor agonist SKF-38393 (30–1,000 nM) and the D₂-like receptor agonist quinerolane (100–1,000 nM) were found to attenuate, in a concentration-dependent manner, the amphotericin B-induced increases in \( I_{sc} \) (Fig. 3B). The magnitude of the inhibitory effect of 1 μM dopamine was similar to that produced by 300 nM SKF-38393 or 1 μM quinerolane. The inhibitory effect of SKF-38393 (300 nM) on amphotericin B-induced increases in \( I_{sc} \) was prevented by the D₁-like receptor antagonist SKF-83566 (1 μM) (Fig. 3C). Similarly, pretreatment with the D₂-like receptor antagonist S-sulpiride (1 μM) prevented the inhibitory effect of quinerolane (1 μM) on amphotericin B-induced increases in \( I_{sc} \) (Fig. 3D).

Next, we evaluated the involvement of G proteins in the regulation of Na⁺-K⁺-ATPase in OK cells. CTX and PTX ribosylate the \( \alpha \)-subunit of the Gs and Gi/o classes of G proteins, respectively. The effect of SKF-38393 (300 nM) on amphotericin B-induced increases in \( I_{sc} \) was abolished by overnight treatment of OK cells with CTX (500 ng/ml) but not with PTX (100 ng/ml) (Fig. 4). On the other hand, the effect of quinerolane (1 μM) on amphotericin B-induced increases in \( I_{sc} \) was abolished by overnight treatment of OK cells with PTX (100 ng/ml) but not with CTX (500 ng/ml) (Fig. 4). These results suggest that D₁-like receptors stimulated by SKF-38393 are coupled to CTX-sensitive G proteins of the Gs class, whereas D₂-like receptors stimulated by quinerolane are coupled to PTX-sensitive G proteins of the Gi/o class.

Previous studies have shown that D₁-like receptors in OK cells are positively coupled to adenylyl cyclase,
whereas stimulation of D2-like receptors by quinero-
lane failed to alter basal levels of cAMP (18). The next
series of experiments assessed the negative coupling
between D2-like receptors and adenylyl cyclase by mea-
suring levels of cAMP before and after stimulation of
denyyl cyclase with forskolin. Quinerolane (1 μM)
was found to affect neither the basal levels of cAMP nor
the forskolin (3 μM)-induced increase in cAMP levels
(Fig. 5). Similar results were obtained in cells pre-
treated with PTX (Fig. 5). These results strongly sug-
gest that D2-like receptors in OK cells may not be
negatively coupled to adenylyl cyclase.

Because D2-like receptors are also known to activate
K+ channel couples via G proteins, it was believed
worthwhile to explore the role of K+ channels on the
D2-like receptor-mediated inhibition of amphotericin
B-induced increases in Isc. On the other hand, it is well
known that an increase in Na\(^+\)-K\(^+\)-ATPase activity
resulting from enhanced entry of apical Na\(^+\) necess-
arily leads to a transient increase in K+ accumulation (6,
41). Some of the accumulated K\(^+\) ions are then ex-
truded out of the cell through K+ channels located in
the basolateral side of the cell. To gain further insight
into the nature of mechanisms responsible for the
quinerolane-mediated decrease in amphotericin B-in-
duced increases in Isc, the effects of BaCl2 (1 mM), a
nonselective K+ channel blocker, and glibenclamide
and apamin, selective blockers of ATP\(^-\) and calcium-
sensitive K+ channels (10, 30), respectively, were
tested. As shown in Fig. 6, both BaCl2 (1 mM) and
glibenclamide (10 μM) prevented the inhibitory effect
of quinerolane on the amphotericin B-induced increase
in Isc. By contrast, apamin (1 μM) was not found to
alter the inhibitory effect of quinerolane. These results
strongly suggest that inhibition of amphotericin B-
induced increases in Isc by quinerolane may involve the
opening of KATP channels as a result of stimulation of
D2-like receptors. To confirm this view, it was decided
to evaluate the effect of a K+ channel opener on am-
photericin B-induced increases in Isc. As shown in Fig.
7, pinacidil significantly reduced the amphotericin B-
induced increase in Isc.

Because the experimental procedure described above
does not enable one to draw definitive conclusions as to
whether stimulation of D2-like receptors leads to the

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**Fig. 4.** Effect of SKF-38393 (1 μM) and quinerolane (1 μM) on ΔIsc (%control) induced by amphotericin B (1.0 μg/ml) in the absence and presence of cholera toxin (CTX; 500 ng/ml, overnight) or pertussis
toxin (PTX; 100 ng/ml, overnight). Values are means ± SE of 3–9 experiments/group. *P < 0.05, significantly different from control; #P < 0.05 significantly different from SKF-38393 or quinerolane alone.

**Fig. 5.** Effect of quinerolane (Quin; 1 μM) on cAMP stimulated by forskolin (Forsk; 3 μM) in control- and PTX (100 ng/ml overnight)-treated OK cells. Values are means ± SE of 4 independent determinations. Basal cAMP levels in control and PTX-treated cells were 24.1 ± 2.0 and 26.3 ± 2.9 pmol/mg protein, respectively. *P < 0.05, significantly different from basal values.

**Fig. 6.** Effect of BaCl2 (1 mM), glibenclamide (Glib; 10 μM), or apamin (1 μM) on ΔIsc (%control) induced by amphotericin B (1.0 μg/ml) in the absence (control) and presence of quinerolane (1 μM). Values are means ± SE of 4–9 experiments/group. *P < 0.05, significantly different from corresponding control; #P < 0.05, significantly different from quinerolane alone.

**Fig. 7.** Effect of pinacidil (10 and 50 μM) on ΔIsc (%control) induced by amphotericin B (1.0 μg/ml). Values are means ± SE of 3–11 experiments/group. *P < 0.05, significantly different from control.
opening of K$_{\text{ATP}}$ channels or inhibition of Na$^{+}$-K$^{+}$-ATPase activity, it was decided to implement an experimental protocol that allows the measurement of $I_K$ across the basolateral membrane. $I_K$ was measured in monolayers of OK cells in conditions of an apical-to-basolateral K$^{+}$ gradient (80:5 mM) in the presence of ouabain (100 µM) (15). The addition of amphotericin B (3 µg/ml) to the apical side resulted in a rapid increase in $I_K$; this effect was markedly inhibited by the addition of the K$^{+}$ channel blocker BaCl$_2$ (1 mM) (Fig. 8A). The amphotericin B-induced $I_K$ in cells treated from the apical and basolateral cell side with quinerolane (1 µM) was greater than in controls; this was also completely abolished by the addition of BaCl$_2$ (1 mM) from the basolateral cell side (Fig. 8A). On the other hand, glibenclamide (10 µM) applied from the basolateral cell side significantly attenuated the amphotericin B-induced $I_K$ (Fig. 8A). By contrast, apamin (1 µM) applied from the basolateral cell side failed to alter the amphotericin B-induced $I_K$, whereas pinacidil increased the amphotericin B-induced $I_K$ (Fig. 8A). The potentiation by quinerolane of the amphotericin B-induced $I_K$ was a concentration-dependent effect that was abolished by the selective D$_2$-like receptor antagonist S-sulpiride (1 µM) and glibenclamide (10 µM) (Fig. 8C). By contrast, the selective D$_1$-like receptor agonist SKF-38393 (1 µM) failed to alter the amphotericin B-induced $I_K$ (Fig. 8C). Moreover, the stimulatory influence of quinerolane on the amphotericin B-induced $I_K$ was similar in the absence and presence of SKF-38393, the D$_1$-like receptor agonist (Fig. 8C). These findings agree with the view that stimulation of D$_2$-like, but not D$_1$-like, receptors may open K$_{\text{ATP}}$ channels.

Previous studies have demonstrated that second-messenger pathways thought to be involved in the renal effects of dopamine include stimulation of protein kinase A (PKA) or C (PKC) pathways (25). This is particularly true when receptors involved are of the D$_1$-like type coupled to G$_s$ and G$q/11$ proteins. By contrast, stimulation of D$_2$-like receptors coupled to G$i/o$ proteins has been demonstrated to open K$^{+}$ channels (11, 17, 26) and/or inhibit adenylyl cyclase (37). The purpose of the next series of experiments was to clarify the transduction pathways from D$_2$-like receptor activation downstream of the opening of K$_{\text{ATP}}$ channels. Again, overnight treatment of OK cells with PTX (100 ng/ml) abolished the potentiation by quinerolane of the amphotericin B-induced $I_K$ (Fig. 9), suggesting that D$_2$-like receptors stimulated by quinerolane are coupled to PTX-sensitive G proteins of the G$i/o$ class. On the other hand, selective antagonists of PKA (H-89) and PKC (chelerythrine) (4) failed to alter the potentiation by quinerolane of the amphotericin B-induced $I_K$ (Fig. 9).

Functional coupling of Na$^{+}$-K$^{+}$-ATPase activity to basolateral membrane K$^{+}$ conductance is crucial for sustaining transport in the proximal tubule. Apical Na$^{+}$ entry stimulates pump activity, lowering cytosolic ATP, which, in turn, disinhibits K$_{\text{ATP}}$ channels (28). However, this may not explain why the opening of the basolateral K$_{\text{ATP}}$ channel by pinacidil and quinerolane is also accompanied by inhibition of Na$^{+}$-K$^{+}$-ATPase activity, as indicated by the reduction in ouabain-sensitive amphotericin B-induced increases in $I_{\text{sc}}$. Because the opening of K$_{\text{ATP}}$ channels mediates hyperpolarization of the basolateral membrane (28), we believed it
was worthwhile to determine membrane potential in cells treated with quinerolane and pinacidil and to determine whether inhibition of Na\(^{+}\)-K\(^{+}\)-ATPase activity leads to changes in membrane potential. The fluorescent dye DiBAC\(_{4}(3)\) was used to monitor changes in membrane potential in OK cells treated with pinacidil, ouabain, quinerolane, and SKF-38393. As shown in Fig. 10, addition of ouabain (100 \(\mu\)M) and D\(_{1}\)-like receptor stimulation with SKF-38393 (1 \(\mu\)M) produced cell membrane depolarization, as evidenced by the time-dependent increase in fluorescence. On the other hand, pinacidil (50 \(\mu\)M) and D\(_{2}\)-like receptor stimulation with quinerolane (1 \(\mu\)M) produced rapid hyperpolarization, as evidenced by the time-dependent decrease in fluorescence (Fig. 10).

**DISCUSSION**

The present study investigated the effects of D\(_{1}\)- and D\(_{2}\)-like dopamine receptor activation on ouabain-sensitive Na\(^{+}\) currents and ouabain-insensitive K\(^{+}\) currents in renal OK cells by using an in vivo method with independent accessibility to the apical and basolateral cell sides. The results presented here show that stimulation of D\(_{1}\)-like receptors, coupled to a G\(_{s}\) class of G proteins, decreased ouabain-sensitive Na\(^{+}\) currents but failed to alter ouabain-insensitive K\(^{+}\) currents. In contrast, stimulation of D\(_{2}\)-like receptors, coupled to a G\(_{i/o}\) class of G proteins, decreased ouabain-sensitive Na\(^{+}\) currents and increased ouabain-insensitive K\(^{+}\) currents, both events most likely resulting from the opening of K\(^{+}\) channels. The stimulation of the D\(_{2}\)-like receptor was associated with a rapid hyperpolarizing effect, whereas D\(_{1}\)-like receptor activation was accompanied by increases in cell membrane potential. Transmission mechanisms set into motion during activation of D\(_{2}\)-like receptors in OK cells involve the activation of neither PKA nor PKC pathways.

Using the pore-forming antibiotic amphotericin B, we were able to isolate Na\(^{+}\) currents and assess the effects of D\(_{1}\)- and D\(_{2}\)-like receptor activation on the basolateral membrane Na\(^{+}\)-K\(^{+}\)-ATPase activity in intact OK cell monolayers. 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 \(I_{sc}\). 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 the medium bathing the apical side of the monolayer. This suggestion is also in agreement with the finding that the Na\(^{+}\)-HCO\(_{3}\) cotransport inhibitor DIDS did not alter the amphotericin B-induced increase in \(I_{sc}\). A particular advantage of this electrophysiological approach is to allow the study of Na\(^{+}\) fluxes in a “sided” preparation with independent access to the apical or basolateral cell borders. The relationship between Na\(^{+}\)-K\(^{+}\)-ATPase activity and the concentration of intracellular Na\(^{+}\) revealed that amphotericin B-induced increases in \(I_{sc}\) were saturable while the concentration approached 100 mM Na\(^{+}\), with a \(K_{m}\)

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**Fig. 9.** Effect of quinerolane (1 \(\mu\)M) on \(\Delta K_{c}\) (%control) induced by amphotericin B (3.0 \(\mu\)g/ml) in the absence and presence of PTX (100 ng/ml, overnight), H-89 (10 \(\mu\)M), or chelerythrine (1 \(\mu\)M). Values are means ± SE of 6–7 experiments/group. *P < 0.05, significantly different from quinerolane alone.

**Fig. 10.** Representative traces (A) and summarized data (B) of the effect of ouabain (100 \(\mu\)M), SKF-38393 (1 \(\mu\)M), pinacidil (50 \(\mu\)M), and quinerolane B (1 \(\mu\)M) on changes in bis-(1,3-dibutylbarbituric acid)-trimethine oxonol fluorescence in OK cells. Compounds were added after a baseline period of 5 min. Fluorescence sampling was done every 5 s. Values are means ± SE of 3 experiments/group. *P < 0.05, significantly different from baseline values.
value (44.1 ± 13.7 mM) similar to the intracellular Na⁺ concentration reported in this cell line (29). In an earlier report, we showed that OK cells expressed both D₁- and D₂-like receptors, with the activation of the former, but not the latter, being accompanied by stimulation of adenyl cyclase and marked intracellular acidification as a result of inhibition of the Na⁺/H⁺ exchanger (18). The inhibitory effects of dopamine on adenyl cyclase and the Na⁺/H⁺ exchanger were antagonized by the selective D₁-like receptor agonist SKF-83566 and mimicked by the D₁-like receptor agonist SKF-38393 (18). The D₂-like receptor agonist quinolone and the D₂-like receptor antagonist S-sulfiride were devoid of effects (18). In the present study, it is shown that dopamine significantly reduced amphotericin B-induced increases in Ösc; this was prevented by both the D₁-like receptor agonist SKF-83566 and the D₂-like receptor antagonist S-sulfiride. These effects of dopamine were mimicked, in a concentration-dependent manner, by both the D₁-like receptor agonist SKF-38393 and the D₂-like receptor agonist quinolone. The D₁-like receptor agonist SKF-83566 and the D₂-like receptor antagonist S-sulfiride prevented the inhibitory effect of SKF-38393 and quinolone, respectively. This suggests that independent stimulation of both D₁- and D₂-like receptors may lead to inhibition of Na⁺-K⁺-ATPase, as evidenced by attenuation of amphotericin B-induced increases in Ösc. The finding that inhibition of amphotericin B-induced increases in Ösc by SKF-38393 was abolished by overnight treatment of OK cells with CTX, but not with PTX, suggested that D₁-like receptors are coupled to a Gs class of G proteins. On the other hand, the finding that inhibition of amphotericin B-induced increases in Ösc by quinolone was abolished by overnight treatment of OK cells with PTX, but not with CTX, suggested that D₂-like receptors are coupled to a Gi/o class of G proteins. These results strongly suggest that attenuation of amphotericin B-induced increases in Ösc by D₁- and D₂-like receptor agonists results from stimulation of independent pathways coupled to these receptors. Because the amphotericin B-induced increases in Ösc were dependent on the ouabain-sensitive apical-to-basal Na⁺ gradient, it was assumed that the increase in current measured after the addition of amphotericin B was related to an increase in Na⁺ gradient driven by the basolateral Na⁺-K⁺-ATPase. However, when challenged with the possibility that stimulation of D₁- and D₂-like receptors could lead to the activation of independent mechanisms, we hypothesized that mechanisms set into motion during stimulation of D₂-like receptors resulted from an indirect inhibitory effect on Na⁺-K⁺-ATPase activity. Our first hypothesis considered that changes in the K⁺ gradient as a result of stimulation of D₂-like receptors could alter Na⁺-K⁺-ATPase activity and consequently the amphotericin B-induced increases in Ösc. In fact, the adequate function of Na⁺-K⁺-ATPase implies a transient increase in intracellular K⁺, which is then extruded out of the cell through K⁺ channels located in the basolateral membrane (6, 41). This view is in agreement with the finding that amphotericin B-induced increases in Ösc were abolished by substitution of KCl with cesium chloride. Another argument favoring this suggestion concerned the prevention of a quinolone-mediated decrease in amphotericin B-induced increases in Ösc by using the nonselective K⁺ channel blocker BaCl₂. The observation that glibenclamide, but not apamin, prevented the quinolone-mediated decrease in amphotericin B-induced increases in Ösc provides evidence that stimulation of D₂-like receptors may lead to the opening of KATP channels. With the use of another protocol that allows measurement of ÖK across the basolateral membrane, quinolone was also found to increase the amphotericin B-induced ÖK; this was completely abolished by the addition of BaCl₂ and glibenclamide. The potentiation by quinolone of the amphotericin B-induced ÖK was a concentration-dependent effect, which was abolished by the selective D₂-like receptor antagonist S-sulfiride. By contrast, the selective D₁-like receptor agonist SKF-38393 failed to alter the amphotericin B-induced ÖK. These findings strongly suggest that the stimulation of D₂-like, but not D₁-like, receptors leads to the opening of KATP channels. Taken together, it is suggested that stimulation of D₂-like receptors coupled to a Gi/o class of G proteins decreased ouabain-sensitive Na⁺ currents and increased ouabain-insensitive K⁺ currents; both events most likely resulted from the opening of KATP channels (Fig. 11). The positive coupling of KATP channels to D₂-like receptors is a well-known characteristic, particularly in neuronal cells, which leads to hyperpolarization and inhibition of neurotransmitter release (11, 17, 26, 27, 32, 36). To our knowledge, this is the first report on a positive coupling between D₂-like receptors and KATP channels in renal epithelial cells.

Fig. 11. Schematic representation of the signaling pathways linked to D₂-like receptor stimulation in OK cells. Stimulation of D₂-like receptors coupled to a Gi/o class of G proteins opens K⁺ channels, leading to a rapid hyperpolarizing effect that ultimately decreases the rate constant of the Na⁺-K⁺-ATPase charge translocating capacity.
Another observation in line with these findings is concerned with the stimulatory effect of dopamine on the Na+-K+-2Cl⁻ cotransport by means of actions on Ba²⁺-sensitive K⁺ channels (3). Although others have previously described the presence of D₂-like receptors in OK cells (13), their function and transduction pathways have not been reported. In the present report, we were able to demonstrate that opening of K⁺ channels during stimulation of D₂-like receptors involved the coupling to a G_{i/o} class of G proteins but lacked the involvement of transduction pathways such as PKA and PKC. On the basis of these findings, it is suggested that in OK cells, as has been demonstrated in other cell types (36), the coupling between the G protein and the K⁺ channel appears to be direct rather than mediated by intracellular soluble second messengers. In other types of cells, D₂-like receptors have been described to be negatively coupled to adenylyl cyclase (5, 24, 37). This does not appear to be the case, as evidenced by the failure of quinolinate to alter the basal and the forskolin-stimulated levels of cAMP.

The mechanisms involved in quinolinate-mediated inhibition of Na⁺-K⁺-ATPase activity during opening of the basolateral KₐTP channel may involve decreases in cell membrane potential rather than decreases in intracellular K⁺. In fact, the Na⁺ pump is activated by Na⁺ and ATP at cytoplasmic sites and by K⁺ at extracellular sites (16, 34). Furthermore, K⁺ has been shown to act as a competitive inhibitor of Na⁺ binding at cytoplasmic sites (16, 34). The suggestion that Na⁺-K⁺-ATPase activity is dependent on membrane potential agrees with the finding that cell hyperpolarization by pinacidil was accompanied by decreases in ouabain-sensitive Na⁺ currents, which fits well with the view of the voltage dependence of Na⁺-K⁺-ATPase activity (16, 22). In fact, Na⁺-K⁺-ATPase is electrogenic, implying that the membrane potential affects the rate constant of the charge-translocating step of the pump cycle (22). However, the precise relationship of Na⁺-K⁺-ATPase activity to transmembrane voltage depends on a number of as yet unknown kinetic parameters of the pump cycle and the type of Na⁺-K⁺-ATPase isoform (16). However, in renal epithelial cells that mostly contain the α₁₁ isoform, the pump current does increase with membrane potential within the −175 to −75 mV range (21). The finding that quinolinate markedly reduced membrane potential and Na⁺-K⁺-ATPase activity, which are both effects associated with the opening of K⁺ channels, strongly suggests that mechanisms that decrease membrane potential negatively affect Na⁺-K⁺-ATPase activity in OK cells (Fig. 11). This may prevent inhibition of Na⁺-K⁺-ATPase activity set into motion by other mechanisms, explaining why the magnitude of attenuation of amphotericin B-induced increases in I_{sc} by dopamine was identical to that observed during stimulation of D₁- and D₂-like receptors by selective agonists. This contrasts with that reported before on the inhibition of Na⁺-K⁺-ATPase, in which the inhibitory effect has always been referred to as a cooperative action between D₁- and D₂-like receptors, requiring the simultaneous activation of both types of receptors (8). Another discrepancy concerns reports in which activation of D₂-like receptors led to stimulation of Na⁺-K⁺-ATPase activity (1, 20, 23, 42). However, it should be stressed that inhibition of ouabain-sensitive Na⁺ currents in OK cells during D₂-like receptor stimulation most likely results from the opening of K⁺ channels. In fact, in conditions in which most K⁺ channels were blocked, D₂-like receptor stimulation failed to alter ouabain-sensitive Na⁺ currents. This would agree with reports indicating that D₂-like receptor agonists have no direct effect on Na⁺-K⁺-ATPase activity (12, 31, 33).

In conclusion, it is demonstrated that in OK cells stimulation of D₁-like receptors coupled to a Gα class of G proteins attenuates ouabain-sensitive Na⁺ currents but fails to alter ouabain-insensitive K⁺ currents, whereas stimulation of D₂-like receptors coupled to a G_{i/o} class of G proteins inhibits ouabain-sensitive Na⁺ currents and increases ouabain-insensitive K⁺ currents; both events most likely result from the opening of K⁺ channels. The coupling among D₂-like receptors, the G protein, and the K⁺ channel appears to be direct rather than mediated by intracellular soluble second messengers.

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