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Dopamine D_2-like receptor-mediated opening of K^+ channels in opossum kidney cells

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1 This study examined the effects of dopamine D_1- and D_2-like receptor activation upon basolateral K^+ (I_K) currents and changes in membrane potential in opossum kidney (OK) cells.

2 The addition of amphotericin B (3 μg ml^{-1}) to the apical side resulted in a rapid increase in I_K, this effect being markedly inhibited by the addition of the K^+ channel blockers barium chloride (1 mM) or glibenclamide (10 μM), but not apamin (1 μM). The K^+ channel opener pinacidil increased the amphotericin B-induced I_K. The selective D_2-like receptor agonist quinerolane increased, in a concentration dependent manner ($EC_{50}=136$ nM), I_K across the basolateral membrane, this effect being abolished by pre-treatment with pertussis toxin (PTX), S-sulpiride (selective D_2-like receptor antagonist) and glibenclamide. The selective D_1-like receptor agonist SKF 38393 did not change I_K. Both H-89 (PKA inhibitor) and chelerythrine (PKC inhibitor) failed to prevent the stimulatory effect of quinerolane upon I_K.

3 Quinerolane did not change basal levels of cyclic AMP and also failed to affect the forskolin-induced increase in cyclic AMP levels.

4 The stimulation of 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. The hyperpolarizing effect of quinerolane ($EC_{50}=129$ nM) was prevented by pre-treatment with PTX, S-sulpiride and glibenclamide.

5 It is concluded that stimulation of dopamine D_2-like, but not D_1-like, receptors coupled to PTX-sensitive G proteins of the $G_{i/o}$ class produce membrane hyperpolarization through opening of K_{ATP} channels.

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Abbreviations: AC, adenylyl cyclase; $DiBAC_4(3)$, bis-(1,3-dibutylbarbituric acid)trimethine oxonol; IBMX, 3-isobutyl-1-methylxanthine; I_K, basolateral K^+ current; K_{ATP} channels, ATP-sensitive K^+ channels; OK, opossum kidney; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PTX, pertussis toxin

Introduction

Na^+-K^+-ATPase is a target for the action of dopamine, a mechanism by which the amine affects tubular sodium absorption and produces natriuresis (Aperia, 2000; Jose *et al*., 2000). In hypertension there may be a primary defect in D_1-like receptors and an altered signalling system in the proximal tubules that leads to reduced dopamine-mediated effects on renal sodium excretion (Hussain & Lokhandwala, 1998; Jose *et al*., 1998). The process from the activation of D_1-like dopamine receptors to the inhibition of Na^+-K^+-ATPase activity involves multiple intracellular signalling pathways that despite a great deal of work appear to be complex and are yet to be completely understood (Aperia, 2000; Hussain & Lokhandwala, 1998; Jose *et al*., 1998). There is evidence that signalling pathways linked to D_1-like receptors include the adenylyl cyclase–protein kinase A (AC–PKA) and the phospholipase C-protein kinase C (PLC–PKC) systems, through the coupling of, respectively, $G_s\alpha$ and $G_{q/11}\alpha$ proteins (Hussain & Lokhandwala, 1998; Jose *et al*., 1998). D_2-like receptor agonists have been reported either to have no effect (Chen & Lokhandwala, 1993; Shahedi *et al*., 1995; Takemoto *et al*., 1992), to act in concert with D_1-like receptor agonists to inhibit Na^+-K^+-ATPase activity (Aizman *et al*., 2000; Bertorello & Aperia, 1988; 1990; Bertorello *et al*., 1990) or to stimulate the Na^+ pump (Abu-Jayyab & Mahgoub, 1987; Guerrero *et al*., 2001; Hussain *et al*., 1997; Yamaguchi *et al*., 1996).

It is now well established that luminal Na^+ entry in renal proximal tubules proceeds in concert with increases in basolateral Na^+-K^+-ATPase activity and increases in K^+ conductance (Beck *et al*., 1994; Welling, 1995). Na^+ is extruded from the cell by the Na^+-K^+-ATPase at the expenses of K^+ entry; K^+ leaves the cell again *via* K^+ channels in the basolateral membrane. The increase in K^+ conductance accompanying the stimulation Na^+-K^+-ATPase activity appears to result from the opening of ATP-sensitive K^+ (K_{ATP}) channels in the basolateral membrane, following local reductions in ATP levels (Tsuchiya *et al*., 1992). The tight co-ordination 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. However, despite the observation that stimulation of D_2-like receptors in the central nervous

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system leads to increased K^+ conductance (Castelletti *et al*., 1989; Freedman & Weight, 1988; Lacey *et al*., 1987; Liu *et al*., 1999; Sun *et al*., 2000; Uchida *et al*., 2000), there is scarce information on the effects of dopamine and D_1- and D_2-like receptor agonists on K^+ conductance in renal tubular epithelial cells.

The present study investigated whether K^+ channels in polarized opossum kidney (OK) cells are target entities for the actions of dopamine. This cell line is frequently used as a model of the tubular proximal epithelium and express characteristics that make them useful for the study of the renal dopaminergic system (Baines & Drangova, 1998; Bates *et al*., 1991; Chibalin *et al*., 1998; Glahn *et al*., 1993; Gomes *et al*., 2001; Guimaraes *et al*., 1997; Nash *et al*., 1993; Pedemonte *et al*., 1997; Vieira-Coelho *et al*., 2001; Vieira-Coelho & Soares-da-Silva, 1997). Recently, it was shown that OK cells expressed both D_1-like and D_2-like dopamine receptors (Gomes *et al*., 2001) and inhibition of Na^+-K^+-ATPase activity following stimulation of D_1-like receptors involves both the AC–PKA and the PLC–PKC systems (Gomes & Soares-da-Silva, 2002). The results reported here indicate that activation of D_2- like receptors, but not D_1-like receptors, in renal OK cells lead to stimulation of basolateral K_{ATP} channels, which appear to be under the control of pertussis toxin (PTX) sensitive G proteins of the $G_{i/o}$ class.

Methods

Cell culture

OK cells, an established cell line derived from the kidney of a female American opossum, were obtained from the American Type Culture Collection (ATCC 1840 CRL Rockville, MD, U.S.A.) and maintained in a humidified atmosphere of 5% CO_2–95% air at 37°C. OK cells were grown in minimum essential medium (Sigma Chemical Co, MO, U.S.A.) supplemented with 10% foetal bovine serum (Sigma), 100 U ml^{-1} penicillin G, 0.25 μg ml^{-1} amphotericin B, 100 μg ml^{-1} streptomycin (Sigma) and 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanosulphonic acid (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^2 growth area (Costar, Badhoevedorp, The Netherlands). For electrophysiology studies, the cells were seeded onto polycarbonate filter supports (Snapwell, Costar) at a density 13,000 cells per well. The cell medium was changed every 2 days, and the cells reached confluence after 3–5 days of initial seeding. For 24 h prior to each experiment, the cell medium was free of foetal bovine serum. Experiments were generally performed 2 days after cells reached confluence and 4 days after initial seeding, and each cm^2 contained about 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 and pertussis toxin. On the day of the experiment, culture medium containing the test agents was removed, the cells washed with fresh medium and allowed to stabilize for at least 2 h before the start of acquisition of electrophysiological parameters.

Eletrogenic ion transport in OK cells

All transport experiments were conducted under short-circuit conditions. OK cells grown on polycarbonate filters (Snapwell, Costar) were mounted in Ussing chambers (window area 1.0 cm^2) equipped with water-jacketed gas lifts bathed on both sides with 10 ml of Krebs–Hensleit solution, gassed with 95% O_2 and 5% CO_2 and maintained at 37°C. The standard composition of the apical and basolateral bathing Krebs–Hensleit solution was (in mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2; pH was adjusted to 7.4 after gassing with 5% CO_2 and 95% O_2. 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 below. After 5 min stabilization, 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, U.S.A.). Transepithelial resistance ($\Omega.cm^2$) was determined by altering the membrane potential stepwise ($\pm$3 mV) and applying the Ohmic relationship. Cells were allowed to stabilize for 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 *via* a BIOPAC MP1000 data acquisition system (BIOPAC Systems, Inc., Goleta, CA, U.S.A.). Data analysis was performed using Acq*Knowledge* 2.0 software (BIOPAC Systems).

Basolateral membrane K^+ conductance

To evaluate the basolateral K^+ conductance of OK cells, cell monolayers were mounted in Ussing chambers in the presence of an apical-to-basolateral K^+ gradient (80 : 5 mM), while the Na^+ concentration was maintained at 25 mM on both sides of the monolayers (DuVall & O'Grady, 1993). 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): NaCl 25, KCl 5, choline HCO_3 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgSO_4$ 1.2; pH was adjusted to 7.4 after gassing with 5% CO_2 and 95% O_2. After $\sim$25 min, amphotericin B was added to the apical bath solution to permeabilize the apical plasma membrane, this being preceded (5 min) by the addition of ouabain (100 μM) to the basolateral bath solution to inhibit the Na^+-K^+-ATPase. The resulting I_{sc} is due to the movement of K^+ through channels in the basolateral membrane (I_K). Test drugs were usually added to the bath solution 20–30 min before the addition of amphotericin. Apamin, glibenclamide and pinacidil, were applied from the basolateral cell side only, whereas D_1- and D_2-like receptor agonists were applied from both the apical and the basolateral cell side. The D_2-like receptor antagonist S-sulpiride and glibenclamide were applied 10 min before the addition of the D_2-like receptor agonist. Barium chloride was usually added after obtaining the maximal response to amphotericin B. In some experiments, glibenclamide was also applied after obtaining the maximal response to amphotericin B.

Cyclic AMP measurement

Cyclic AMP was determined with an enzyme immunoassay kit (Assay Designs, Inc., Ann Arbor, MI, U.S.A.), as previously described (Gomes *et al*., 2001). OK cells were preincubated for 15 min at 37°C in Hanks' medium (medium composition, in mM: NaCl 137, KCl 5, $MgSO_4$ 0.8, Na_2HPO_4 0.33, KH_2PO_4 0.44, $CaCl_2$ 0.25, $MgCl_2$ 1.0, Tris HCl 0.15 and sodium butyrate 1.0, pH = 7.4), containing 100 μM 3-isobutyl-1-methylxanthine (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 cyclic AMP content.

Membrane potential assay

Changes in membrane potential were evaluated using the bisoxonol fluorescent dye $DiBAC_4(3)$ (bis-(1,3-dibutylbarbituric acid)trimethine oxonol), as previously described (Gopalakrishnan *et al*., 1999). Cells cultured on glass coverslips were rinsed twice with assay buffer (mM HEPES 20, NaCl 120, KCl 2, $CaCl_2$ 2, $MgCl_2$ 1, glucose 5, pH 7.4, at 25°C) containing 5 μM $DiBAC_4(3)$ and then incubated for 30 min in 500 μl buffer solution containing 5 μM $DiBAC_4(3)$ to ensure dye distribution across the cell membrane. Thereafter, 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, U.S.A.). The cuvette volume of 2.0 ml was constantly 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.

Protein assay

The protein content of monolayers of OK cells was determined by the method of Bradford (1976), with human serum albumin as a standard.

Data analysis

Arithmetic means are given with s.e.mean or geometric means with 95% confidence values. Statistical analysis was done with a two-way analysis of variance (ANOVA) followed by Newman–Keuls test for multiple comparisons. A P value less than 0.05 was assumed to denote a significant difference.

Drugs

Amphotericin B, apamin, barium chloride, chelerythrine chloride, cholera toxin, dopamine hydrochloride, forskolin, H-89, glibenclamide, isobutylmethylxanthine, ouabain, pertussis toxin and trypan blue were purchased from Sigma Chemical Company, St. Louis, MO, U.S.A. (±)-SKF 83566 hydrochloride, S(−)-sulpiride, (±)-SKF-38393 hydrochloride and quinerolane hydrochloride were obtained from Research Biochemicals International (Natick, MA, U.S.A.). $DiBAC_4(3)$ (bis-(1,3-dibutylbarbituric acid)trimethine oxonol was purchased from Molecular Probes (Eugene, OR, U.S.A.). Pinacidil was a kind gift from Leo Pharmaceuticals (Denmark).

Results

K^+ currents (I_K) across the basolateral membrane were 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). The addition of amphotericin B (3 μg ml^{-1}) to the apical side resulted in a rapid increase in I_K, this effect being markedly inhibited (78 ± 2% reduction, $n = 3$) by the addition of the non-specific K^+ channel blocker barium chloride (1 mM) (Figure 1A). Pretreatment with the K^+ channel opener pinacidil (50 μM) significantly increased the amphotericin B-induced I_K (Figure 1B). On the other hand, pretreatment with the ATP-sensitive K^+ channel blocker glibenclamide (10 μM) significantly attenuated the amphotericin B-induced I_K (Figure 1A,B), whereas pretreament with the Ca^{2+}-sensitive K^+ channel blocker apamin (1 μM) failed to alter the amphotericin B-induced I_K (Figure 1B). As shown in Figure 1C, glibenclamide (10 μM) was also found to significantly inhibit (30 ± 3% reduction, $n = 3$) I_K across the basolateral membrane after obtaining the maximal response to amphotericin B.

The selective D_1-like receptor agonist SKF 38393 (1 μM) failed to alter the amphotericin B-induced I_K (Figure 2A), whereas the selective D_2-like receptor agonist quinerolane (1 μM) significantly increased the amphotericin B-induced I_K (Figure 2A). In fact, the amphotericin B-induced I_K in cells treated with quinerolane (1 μM) was greater than in controls, this being also completely abolished by the addition of barium chloride (1 mM) from the basolateral cell side (Figure 2B). Moreover, the stimulatory effect of quinerolane on the amphotericin B-induced I_K was similar in the absence and the presence of SKF 38393, the D_1-like receptor agonist (Figure 2A). The potentiation by quinerolane of the amphotericin B-induced I_K was a concentration dependent effect with an EC_{50} (geometric mean with 95% confidence interval) of 136 (108, 171) nM (Figure 3A). The stimulatory effect of quinerolane (1 μM) on amphotericin B-induced I_K was abolished by the selective D_2-like receptor antagonist S-sulpiride (1 μM) and glibenclamide (10 μM) (Figure 3B). These findings agree with the view that stimulation of D_2-like, but not D_1-like, receptors may open K_{ATP} channels.

The next series of experiments was aimed at clarifying the transduction pathway(s) from D_2-like receptors activation downstream to opening of K_{ATP} channels. Selective antagonists of PKA (H-89) and PKC (chelerythrine) (Azarani *et al*., 1995) failed to alter the potentiation by quinerolane of the amphotericin B-induced I_K (Table 1). H-89 and chelerythrine effectively prevented PKA-mediated and PKC-mediated inhibition of Na^+-K^+-ATPase activity in OK cells (Gomes & Soares-da-Silva, 2002). On the other hand, overnight treatment of OK cells with pertussis toxin (PTX, 100 ng ml^{-1}) abolished the potentiation by quinerolane of the amphotericin B-induced I_K (Table 1), suggesting that D_2-like receptors stimulated by quinerolane are coupled to PTX-sensitive G proteins of the $G_{i/o}$ class. Since dopamine D_2-like receptors have been demonstrated to inhibit adenylyl cyclase (Vallar & Meldosi, 1989), it was believed worthwhile to assess in OK cells the coupling of D_2-like receptors to adenylyl

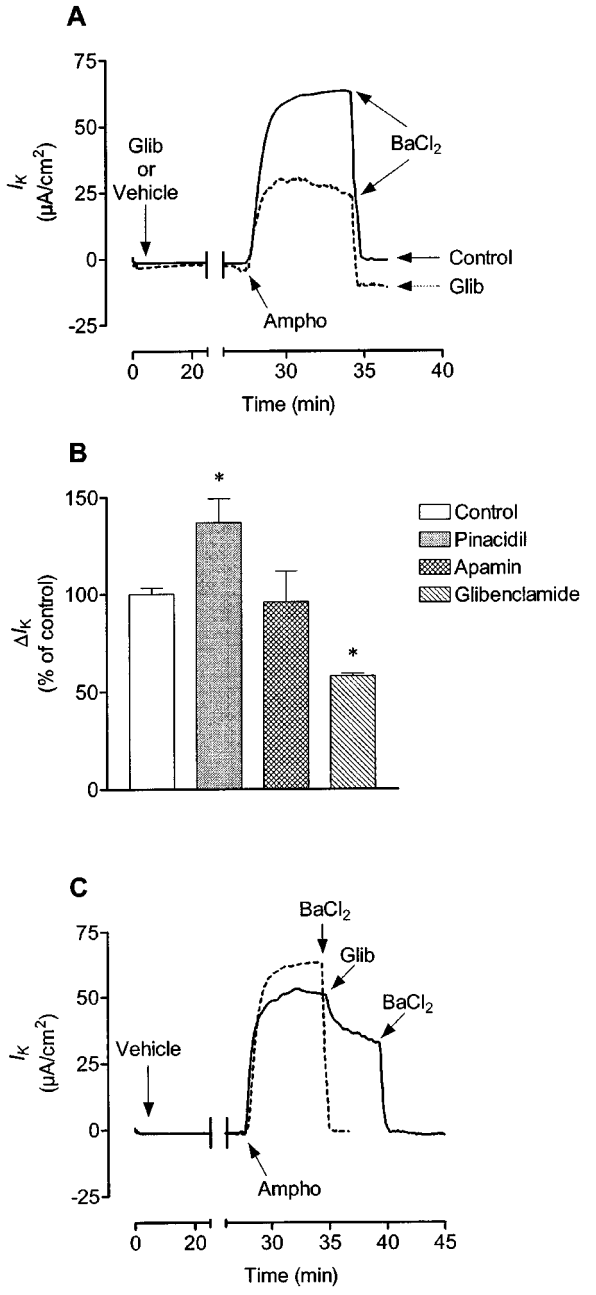

Figure 1 (A) Representative traces of the effect of amphotericin B (Ampho; 3.0 μg ml^{-1}) upon K^+ currents (ΔI_K, μA/cm^2) across OK cell monolayers, in the absence (Control) and the presence of glibenclamide (Glib; 10 μM); barium chloride (1 mM) was added after obtaining the maximal effect of amphotericin B. (B) Effect of pinacidil (50 μM), apamin (1 μM) and glibenclamide (10 μM) upon changes in K^+ currents (ΔI_K, % of control) induced by amphotericin B (3.0 μg ml^{-1}); vehicle or inhibitors were applied 20 min before amphotericin B. Columns represent means of 3–7 experiments per group and vertical lines show s.e.mean. Significantly different from control value ($^*P<0.05$). (C) Representative traces of the effect of glibenclamide (10 μM) upon amphotericin B (Ampho; 3.0 μg ml^{-1}) induced increases in K^+ currents (ΔI_K, μA/cm^2) across OK cell monolayers.

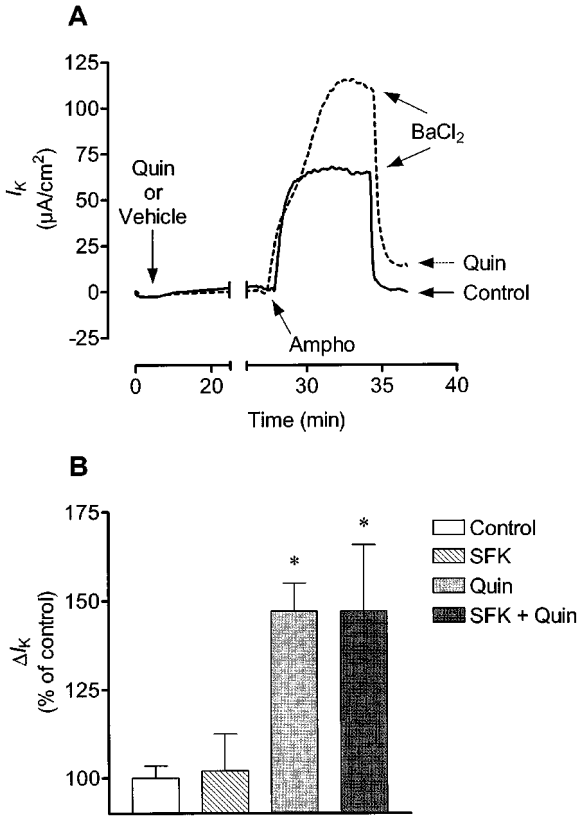

Figure 2 (A) Representative traces of the effect of amphotericin B (Ampho; 3.0 μg ml^{-1}) upon K^+ currents (ΔI_K, μA/cm^2) across OK cell monolayers, in the absence (Control) and the presence of quinerolane (Quin; 1 μM); barium chloride (1 mM) was added after obtaining the maximal effect of amphotericin B. (B) Effect of SKF 38393 (1 μM), quinerolane (1 μM) and quinerolane plus SKF 38393 (both at 1 μM), upon changes in K^+ currents (ΔI_K, % of control) induced by amphotericin B (3.0 μg ml^{-1}); vehicle, SKF 38393 or quinerolane were applied 20 min before amphotericin B. Columns represent means of four experiments per group, and vertical lines show s.e.mean. Significantly different from control values ($^*P<0.05$).

cyclase. As observed in previous studies (Gomes *et al.*, 2001), stimulation of D_1-like receptors in OK cells with SKF 38393 (1 μM) resulted in increased accumulation of cyclic AMP (Table 2). By contrast, stimulation of D_2-like receptors by quinerolane (1 μM) failed to alter basal levels of cyclic AMP. Quinerolane (1 μM) also failed to affect the forskolin (3 μM)-induced increase in cyclic AMP levels (Table 2). Similar results were obtained in cells pre-treated with pertussis toxin (Table 2). These results suggest that D_2-like receptors in OK cells are not negatively or positively coupled to adenylyl cyclase.

Since the opening of K_{ATP} channels mediates hyperpolarization of the basolateral membrane (Mauerer *et al.*, 1998), we determined the membrane potential in cells treated with the K^+ channel opener, dopamine and the selective D_1-like and D_2-like receptor agonists. The fluorescent dye $DiBAC_4(3)$ was used to monitor changes in membrane potential in OK cells treated with pinacidil, dopamine, quinerolane and SKF 38393. As shown in Figure 4, dopamine (1 μM) produced rapid hyperpolarization, as evidenced by the time-dependent decrease in fluorescence (Figure 4). By contrast, stimulation of dopamine D_1-like receptors with SKF 38393 (1 μM) produced cell membrane depolarization, as evidenced by the time-dependent increase in fluorescence. Stimulation of dopamine D_2-like receptors with quinerolane (1 μM) and opening of K_{ATP} channels with pinacidil (50 μM) produced

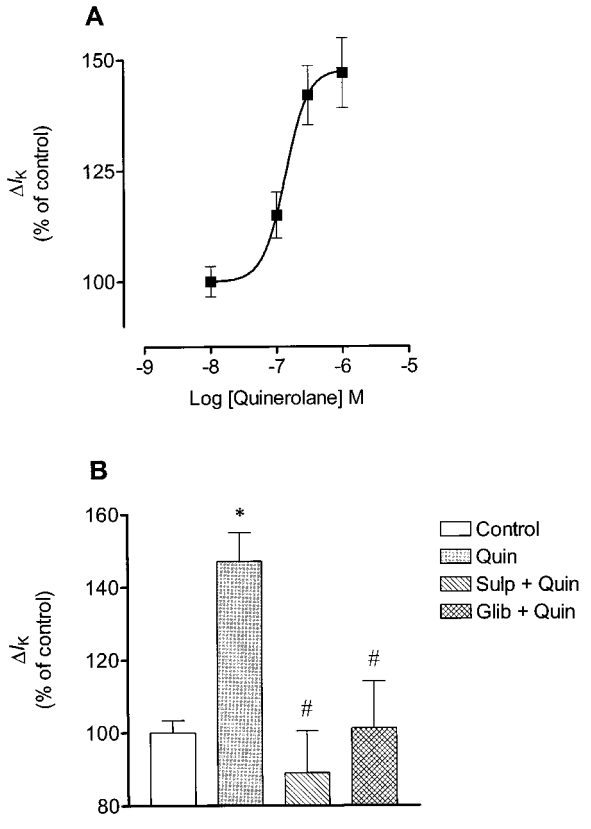

Figure 3 (A) Effect of quinerolane (0.1–1.0 μM) upon changes in K^+ currents (ΔI_K, % of control) induced by amphotericin B (3.0 μg ml^{-1}). (B) Effect of quinerolane (1 μM), quinerolane plus (S)-sulpiride (both at 1 μM) and quinerolane (1 μM) plus glibenclamide (10 μM) upon changes in K^+ currents (ΔI_K, % of control) induced by amphotericin B (3.0 μg ml^{-1}); (S)-sulpiride and glibenclamide were added 10 min prior to quinerolane, which was applied 20 min before amphotericin B. Symbols or columns represent means of 4–7 experiments per group and vertical lines show s.e.mean. Significantly different from control values ($*P<0.05$) and values for quinerolane alone ($\#P<0.05$).

Table 1 Effect of quinerolane (1 μM) upon changes in K^+ currents (ΔI_K), % of control) induced by amphotericin B (3.0 μg ml^{-1}), in the absence and the presence of H-89 (10 μM), chelerythrine (1 μM) or pertussis toxin (100 ng ml^{-1}, overnight).

Treatment	*Vehicle*	*Quinerolane*
Vehicle	100±3	147±8*
H-89	100±6	144±10*
Chelerythrine	100±6	155±17*
PTX	100±3	103±11†

Values are mean±s.e.mean of 6–7 experiments per group. Significantly different from corresponding control values ($*P<0.05$) and values for quinerolane alone ($\dagger P<0.05$).

rapid hyperpolarization. The hyperpolarizing effect of dopamine was less pronounced than that produced by the quinerolane. As shown in Figure 5, immediately after the addition of pinacidil (Figure 5A) or quinerolane (Figure 5B) to the superfusion fluid there was a marked decrease in membrane potential that depend on the concentration applied. In fact, the hyperpolarizing effects of both pinacidil and quinerolane were dependent on the concentration applied to the bath with EC_{50} values (geometric means with 95% confidence interval) of, respectively, 7.0 (1.6, 30.3) μM and 129 (75, 221) nM (Figure 5C). The hyperpolarizing effect of quinerolane (1 μM) was markedly attenuated by glibenclamide (10 μM) and overnight exposure to PTX (100 ng ml^{-1}) (Figure 6). These findings agree with the view that stimulation of dopamine D_2-like, but not D_1-like, receptors coupled to PTX-sensitive G proteins of the $G_{i/o}$ class produce membrane hyperpolarization through opening of K_{ATP} channels.

Discussion

The present study investigated the effects of D_1- and D_2-like dopamine receptor activation upon ouabain-insensitive K^+ currents and membrane potential in renal OK cells. The results presented here show that stimulation of D_2-like receptors, coupled to a $G_{i/o}$ class of G proteins, increased ouabain-insensitive K^+ currents, which most likely results from the opening of K^+ channels. In contrast, stimulation of D_1-like receptors failed to alter ouabain-insensitive K^+ currents. The stimulation of 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. Transduction mechanisms set into motion during activation of D_2-like receptors in OK cells involve neither the activation of PKA nor PKC pathways.

In an earlier report we showed that OK cells expressed both D_1- and D_2-like receptors, the activation of the former, but not the latter, being accompanied with stimulation of adenylyl cyclase and marked intracellular acidification, as a result of inhibition of the Na^+/H^+ exchanger (Gomes *et al.*, 2001). The inhibitory effects of dopamine upon adenylyl cyclase and the Na^+/H^+ exchanger were antagonized by the selective D_1-like receptor antagonist SKF 83566 and mimicked by the D_1-like receptor agonist SKF 38393 (Gomes *et al.*, 2001). The D_2-like receptor agonist quinerolane and the D_2-like receptor antagonist S-sulpiride were devoid of effects (Gomes *et al.*, 2001). In the present study, it is shown that the D_2-like receptor agonist quinerolane significantly increased amphotericin B-induced increases in I_K, this being completely abolished by the addition of barium chloride and glibenclamide. The potentiation by quinerolane of the amphotericin B-induced I_K was a concentration dependent effect, being abolished by the selective D_2-like receptor antagonist S-sulpiride. By contrast, the selective D_1-like receptor agonist SKF 38393 did not affect K^+ currents or altered the quinerolane-induced increase in K^+ conductance. These findings strongly suggest that the stimulation of D_2-like, but not D_1-like, receptors leads to the opening of K_{ATP} channels. The positive coupling of K_{ATP} channels to D_2-like receptors is a well-known characteristic, particularly in neuronal cells, which leads to hyperpolarization and inhibition of neurotransmitter release (Castelletti *et al.*, 1989; Freedman & Weight, 1988; Lacey *et al.*, 1987; Liu *et al.*, 1999; Sun *et al.*, 2000; Uchida *et al.*, 2000). To our knowledge this is the first report on a positive coupling between D_2-like receptors and K_{ATP} channels in renal epithelial cells. Another observation in line with these findings is concerned with the stimulatory effect of dopamine

Table 2 Changes in cyclic AMP levels in OK cells in control conditions and after exposure to SKF38393 (1 μM), quinerolane (1 μM), forskolin (3 μM) and pertussis toxin (100 ng ml^{-1}, overnight exposure).

Treatment	*cyclic AMP* (% of control)
Control	100±8
SKF 38393	217±24*
Quinerolane	107±23
Forskolin	277±14*
Quinerolane + Forskolin	225±8*
PTX	100±11
PTX + Forskolin	257±33*
PTX + Quinerolane + Forskolin	225±21*

Values (mean±s.e.mean) are per cent of control for accumulation of cyclic AMP. The basal cyclic AMP levels in control and pertussis toxin-treated cells were 24.1±2.0 and 26.3±2.9 pmol mg $protein^{-1}$, respectively. Significantly different from corresponding control values (*$P<0.05$).

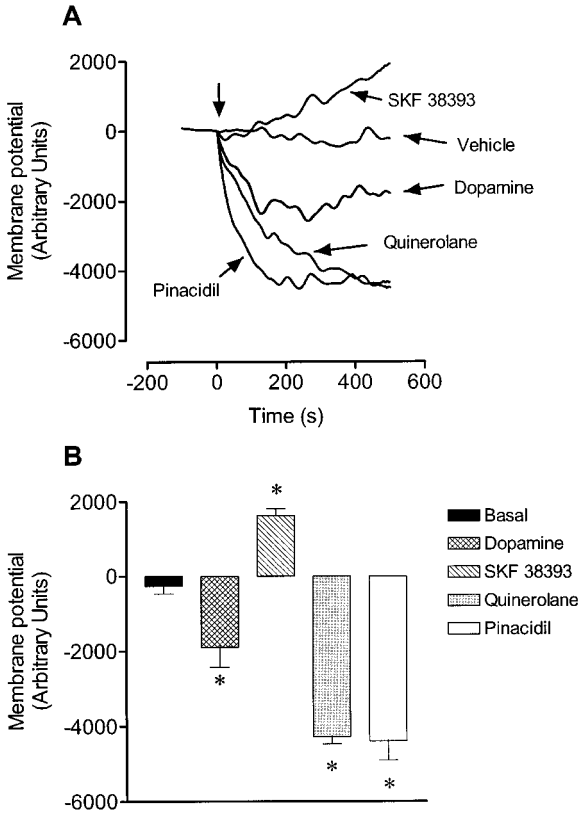

Figure 4 Representative traces (A) and summarized data (B) of the effect of dopamine (1 μM), SKF 38393 (1 μM), quinerolane (1 μM) and pinacidil (50 μM) on changes in $DiBAC_4(3)$ fluorescence in OK cells. Compounds were added after a baseline period of 5 min. Fluorescence sampling was done every 5 s. Columns represent means of four experiments per group and vertical lines show s.e.mean. Significantly different from baseline values (*$P<0.05$).

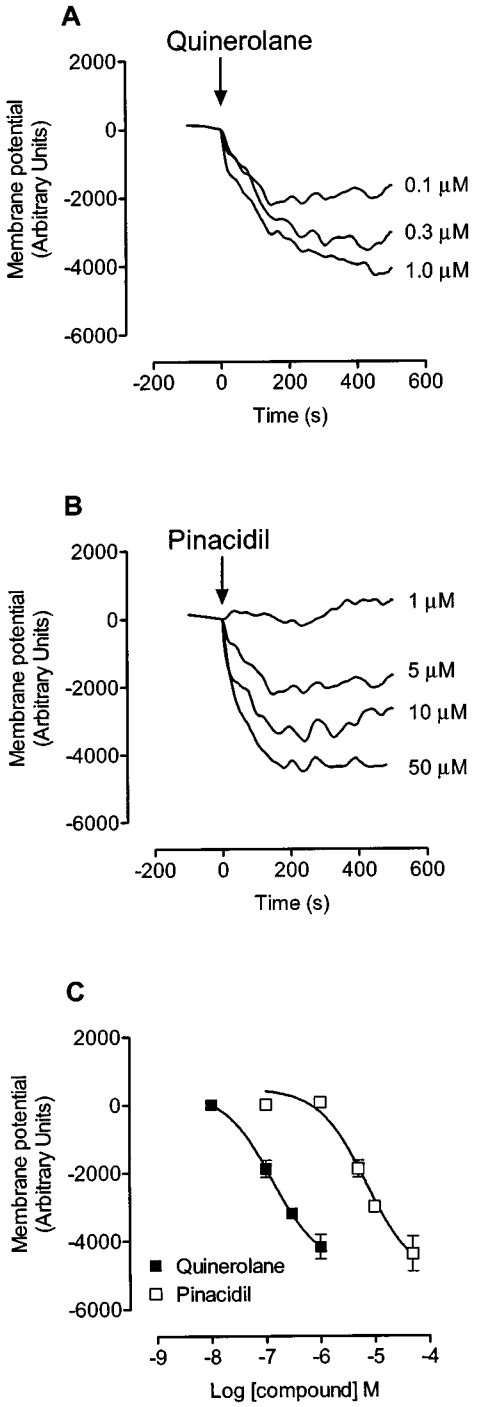

Figure 5 Representative traces (A and B) and summarized data (C) of the effect of (A) quinerolane (0.1–1.0 μM) and (B) pinacidil (1–50 μM) on changes in $DiBAC_4(3)$ fluorescence in OK cells. Compounds were added after a baseline period of 5 min. Fluorescence sampling was done every 5 s. Columns represent means of four experiments per group and vertical lines show s.e.mean.

upon the Na^+-K^+-$2Cl^-$ cotransport *via* actions on Ba^{2+}-sensitive K^+ channels (Aoki *et al*., 1996). Though others have described before the presence of D_2-like receptors in OK cells (Cheng *et al*., 1990), its 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 D_2-like receptors involved the coupling to a PTX-sensitive class of G proteins, but apparently lacked the involvement of transduction pathways such as PKA and PKC. Based on these findings it is suggested that in OK cells, as has been demonstrated in other cell types (Uchida *et al*., 2000), the coupling between the G protein and the K^+ channel appears

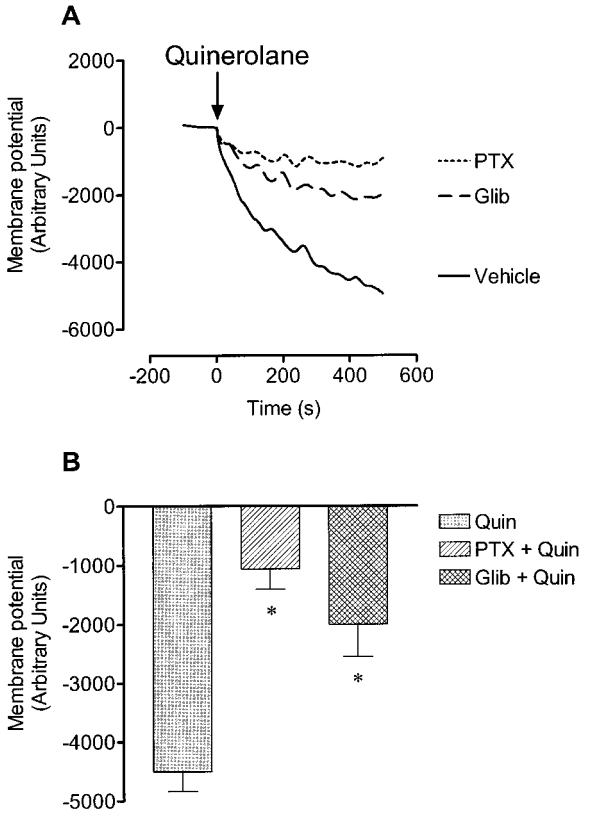

Figure 6 Representative traces (A) and summarized data (B) of the effect of quinerolane (1 μM) alone and after treatment with glibenclamide (10 μM) or pertussis toxin (PTX, 100 ng ml^{-1} overnight exposure) on changes in $DiBAC_4(3)$ fluorescence in OK cells. Quinerolane was added after a baseline period of 5 min. Fluorescence sampling was done every 5 s. Columns represent means of four experiments per group and vertical lines show s.e.mean. Significantly different from baseline values ($^*P<0.05$).

to be a direct one, rather than mediated by intracellular soluble second messengers. In other types of cells, D_2-like receptors have been described to be negatively coupled to adenylyl cyclase (Bates *et al*., 1991; Johansson & Westlind-Danielsson, 1994; Vallar & Meldosi, 1989). This does not appear to be the case, as evidenced by the failure of quinerolane to alter the basal and the forskolin-stimulated levels of cyclic AMP.

The reduction in membrane potential by pinacidil, as evidenced by decreases in $DiBAC_4(3)$ fluorescence, fits well the observation that opening of K_{ATP} channels results in membrane hyperpolarization (Mauerer *et al*., 1998). The finding that glinbeclamide prevented quinerolane-induced decreases decreases in $DiBAC_4(3)$ fluorescence strongly suggest that stimulation of D_2-like dopamine receptors produces membrane hyperpolarization as a result of opening of K_{ATP} channels. In this respect, it is interesting to underline the finding that the predominant effect of dopamine was a decrease in membrane potential *via* stimulation of D_2-like dopamine receptors, whereas the effect of the D_1-like dopamine receptor agonist SKF 38393 was an increase in membrane potential. The nature of the phenomenon responsible for membrane depolarization following D_1-like dopamine receptor stimulation is not apparent from this series of experiments, since SKF 38393 was devoid of effects on ouabain-insensitve K^+ currents. However, it is likely that increases in membrane potential by SKF 38393 may result from inhibition of Na^+-K^+-ATPase activity or a decrease in K^+ conductance. In fact, SKF 38393 was found in OK cells to markedly inhibit Na^+-K^+- ATPase activity (Gomes & Soares-da-Silva, 2002) and in rabbit renal cortical collecting ducts D_1-like dopamine receptor stimulation was found to produce membrane depolarization and decreases in Na^+ transepithelial flux (Saito *et al*., 2001). On the other hand, it has been reported in neuronal cells, podocytes and in salivary ducts that D_1-like dopamine receptor mediated depolarization may result from decreases in K^+ conductance (Bek *et al*., 1999; Kim *et al*., 1997; Lang & Walz, 2001).

The functional consequences resulting from the opening of K_{ATP} channels during stimulation of D_2-like receptors at the kidney level may not be straightforward. In the renal tubular system, K_{ATP} channels are found in the proximal tubule, the thick ascending limb of Henle's loop and the cortical collecting duct (Quast, 1996). Under physiological conditions, K_{ATP} channels have a high open probability and play an important role in the reabsorption of electrolytes and solutes as well as in K^+ homeostasis (Quast, 1996). Blockers and openers of K_{ATP} channels , exemplified by glibenclamide and levcromakalim, show a wide spectrum of affinities towards the different types of K_{ATP} channels. However, the renal effects of these compounds have not been examined in detail. There is evidence that glibenclamide evoked natriuresis and diuresis, without changes in potassium excretion (Bailey & Walter, 1998; Clark *et al*., 1993). However, the natriuresis resulting from glibenclamide administration may be a consequence of blockade of potassium channels in the apical membrane of the thick ascending limb of Henle's loop. It is likely that both drugs may interfere with reabsorption of Na^+ by blocking K_{ATP} channels and thereby interrupting K^+ recycling and Na^+-$2Cl^-$-K^+ cotransport in the loop of Henle (Bailey & Walter, 1998; Clark *et al*., 1993). In the renal proximal tubule there is a tight co-ordination between Na^+-K^+-ATPase activity and K^+ channel activity (pump-leak coupling) that is thought to be of considerable importance for cell volume and homeostasis during epithelial transport (Beck *et al*., 1994; Welling, 1995). However, blockade of K_{ATP} channels has been found not to affect the fractional reabsorption of Na^+ in the proximal convoluted tubule (Bailey & Walter, 1998). It will be interesting to examine to which extent the opening of K_{ATP} channel by D_2-like receptor agonists alters the proximal tubular function, namely the co-ordination between Na^+ and K^+ transepithelial transporters.

In conclusion, it is demonstrated that in OK cells stimulation of D_2-like receptors, coupled to a PTX-sensitive class of G proteins, increases ouabain-insensitive K^+ currents and produces membrane hyperpolarization, both events most likely resulting from the opening of K^+ channels, whereas stimulation of D_1-like receptors fail to alter ouabain-insensitive K^+ currents. The coupling between D_2-like receptors, the G protein and the K^+ channel appears to be a direct one, rather than mediated by intracellular soluble second messengers.

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