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Dopamine D_{2L} Receptors Stimulate Na^+/K^+-ATPase Activity in Murine LTK^- Cells

IKUYO YAMAGUCHI, SCOTT F. WALK, PEDRO A. JOSE, and ROBIN A. FELDER

Department of Pathology, The University of Virginia Health Sciences Center, Charlottesville, Virginia 22908 (I.Y., S.F.W., R.A.F.), and Department of Pediatrics, Georgetown University Children's Medical Center, Washington, D.C. 20007 (P.A.J.)

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SUMMARY

Ion transport can be regulated by dopamine receptors. D_1-like receptors inhibit both Na^+/H^+ exchange (NHE) and Na^+/K^+-ATPase activity, whereas D_2-like receptors stimulate NHE. However, the effect of D_2-like receptors on Na^+/K^+-ATPase activity is controversial. In renal proximal tubular cells, where several D_1-like and D_2-like receptors are expressed, D_2 agonists have been reported either to have no effect or to act in concert with D_1 agonists to inhibit Na^+/K^+-ATPase activity. We therefore studied the effect of D_2 receptors on Na^+/K^+-ATPase activity in LTK^- cells transfected with a rat D_{2Long} receptor cDNA (maximum receptor density = 0.91 ± 0.26 pmol/mg protein, dissociation constant = 2.39 ± 0.79 nM, seven experiments). The activation of D_2 receptors in these transfected cells by the selective D_2 agonist LY171555 led to the inhibition of forskolin-stimulated cAMP accumulation. In the D_{2Long}-transfected, but not in nontransfected cells, LY171555 caused a concentration-dependent stimulation of Na^+/K^+-ATPase activity (EC_{50} = 0.55 ± 0.2 μM, E_{max} = 28 ± 6%, six experiments), which was completely blocked by the D_2-selective antagonist (−)-sulpiride. The D_2-stimulated Na^+/K^+-ATPase activity was not secondary to D_2 receptor activation of K^+ channels or NHE activity since LY171555 stimulated Na^+/K^+-ATPase activity in D_{2Long}-transfected cells, even when K^+ channels were blocked by CsCl and intracellular Na^+ was clamped by monensin. The D_2-stimulated Na^+/K^+-ATPase activity was blocked by pertussis toxin and mimicked by dideoxyadenosine. We conclude that agonist occupancy of D_{2Long} dopamine receptors stimulates Na^+/K^+-ATPase activity; this effect is mediated by the inhibition of cAMP production and is independent of intracellular Na^+ and K^+ concentration.

Dopamine is an endogenous catecholamine that modulates many cellular activities, including behavior, hormone synthesis and release, blood pressure, and transmembrane ion transport (1–4). Dopamine receptors in the brain have been classically divided into D_1 and D_2 subtypes on the basis of their interaction with the effector enzyme adenylyl cyclase; D_1 receptors stimulate adenylyl cyclase via the stimulatory protein G_s, whereas D_2 receptors inhibit this enzyme via the inhibitory G protein G_i. The cloned dopamine receptors also fall into these categories: the D_{1A} and D_{1B} (also known as D_1 and D_5 in humans), D_{1C}, and D_{1D} receptors are linked to stimulation of adenylyl cyclase, whereas the D_2, D_3, and D_4 receptors are linked to inhibition of adenylyl cyclase (1–5). Other than adenylyl cyclase, D_1-like and D_2-like receptors have been shown to interact with other signaling pathways and effector proteins, including potassium and calcium channels, phosphatidylinositol metabolism, and arachidonic acid release (1–10).

Pharmacological, biochemical, and molecular evidence also points to the existence of D_1- and D_2-like receptors in the kidney. Thus, the D_{1A}, D_{1B}, D_{2Long}, and D_3 receptor genes are expressed in specific nephron segments and in kidney cell lines (4, 11, 12). Dopamine, produced in renal proximal tubules, regulates sodium transport by inhibiting NHE activity in brush border membranes and Na^+/K^+-ATPase activity in basolateral membranes (13–17). The inhibition by dopamine of NHE activity is exerted via D_1-like receptors (14). The dopamine receptor subtype mediating its inhibitory effect on Na^+/K^+-ATPase is controversial. Several investigators have reported that both D_1-like and D_2-like receptors are necessary for dopamine-mediated inhibition of Na^+/K^+-ATPase activity (13, 15), whereas others have reported that D_1-like receptors, independent of D_2-like receptors, inhibit the sodium pump (17). The second messenger system mediating the inhibitory effect of dopamine on Na^+/K^+-ATPase in renal proximal tubules is also controversial. Some investigators have suggested involvement of cAMP and PKA (15); others have implicated PKC alone (17); and others have implicated

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ABBREVIATIONS: NHE, Na^+/H^+ exchange; PK, protein kinase; D-PBS, Dulbecco's phosphate buffered saline; $[Na^+]_i$, intracellular sodium concentration; $[K^+]_i$, intracellular potassium concentration; DDA, dideoxyadenosine.

the phospholipase A_2/cytochrome P450/arachidonic acid cascade (13).

We previously reported that in the LTK^- cell transfected with the rat D_{1A} receptor cDNA, fenoldopam, a D_1 agonist, inhibited Na^+/K^+-ATPase activity (18). The D_1-mediated inhibition of the sodium pump was mediated by PKA. In the murine LTK^- and rat C6 glioma cells transfected with the rat D_{2Short} receptor, Neve *et al.* reported that LY171555, a D_2 agonist, stimulated NHE activity independent of cAMP and G_i (8). Chio *et al.* corroborated the stimulatory effect of dopamine on NHE activity in Chinese hamster ovary cells transfected with rat D_{2A} receptor cDNA (19). However, in contrast to Neve *et al.*, Chio *et al.* showed that the stimulatory effect of D_{2A} receptor on NHE was linked to G_i, since the effect was blocked by pertussis toxin (19). Although stimulation of NHE activity should lead to an increase in $[Na]_i$ and have a secondary action of stimulating Na^+/K^+-ATPase activity (20), the effect of D_2 receptors on Na^+/K^+-ATPase activity has not been reported. D_2-like receptors can also affect Na^+/K^+-ATPase activity by stimulating K^+ channels (9). We therefore sought to determine the effect of D_2 receptors on Na^+/K^+-ATPase activity, independent of other dopamine receptors, when intracellular Na^+ and K^+ concentrations were clamped. To accomplish this, we stably transfected a murine fibroblast LTK^- cell line with a rat D_{2Long} receptor cDNA (LTK^- D_{2L} cells). LTK^- cells do not have D_1 or D_2 receptors, as determined by radioligand binding and adenylyl cyclase measurements but express a relatively ouabain-resistant Na^+/K^+-ATPase that is similar to that found in the rat kidney (21). A secondary objective of this study was to determine whether a D_{2L} receptor-mediated effect on Na^+/K^+-ATPase activity is linked to G_i and inhibition of cAMP production.

Experimental Procedures

Materials. The drugs and reagents used were obtained from the following companies: [^{125}I]iodospiperone, DuPont-New England Nuclear (Boston, MA); fenoldopam, Smith Kline Beecham (Philadelphia, PA), forskolin, (−)-sulpiride, spiperone, LY171555, Research Biochemical International (Natick, MA); G418 sulfate, GIBCO-BRL (Grand Island, NY), iodospiperone, Sumitomo Chemical Co., Ltd. (Osaka, Japan); dopamine, Sigma Chemical Co. (St. Louis, MO); and pertussis toxin, 2′,5′-dideoxyadenosine, Biomol Research Laboratories (Plymouth Meeting, PA).

Transfection and cell culture. A D_{2L} dopamine receptor cDNA (gift from Dr. M. Maral Mouradian, Genetics Pharmacology Unit, Experimental Therapeutics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD) was subcloned in the expression vector pRc/CMV (Invitrogen, San Diego, CA) between the *Hin*dIII and *Xba*I sites. The resulting construct was used to stably transfect mouse fibroblast LTK^- cells according to a modified calcium phosphate method (22). The details of the cell culture methods have been described previously (18). The transfected cells were selected in a medium containing G418 sulfate (500 mg/ml, GIBCO) and were screened by radioligand binding.

Membrane preparation and receptor assays. Both transfected and nontransfected LTK^- cells were treated with 10 mM butyrate (added to the medium for ~48 hr before each experiment). Butyrate markedly enhanced the expression of the D_{2L} receptor and generally increased Na^+/K^+-ATPase activity by 10–20% in LTK^- cells (data not shown). LTK^- cell membranes were prepared by lysis of cell monolayers in 1 mM Tris-Cl, pH 7.5, for 15 min. The cell lysates were then scraped from the dish and centrifuged at 40,000 × *g* for 15 min. The pellet was resuspended in TME buffer (75 mM Tris, pH 7.5, 12.5 mM $MgCl_2$, 1.5 mM EDTA). Radioligand binding activity was measured by specific binding of [^{125}I]iodospiperone. YM09151 (1 μM) was used to define specific binding.

cAMP accumulation. LTK^- cells were grown to subconfluence. The growth media were replaced with Dulbecco's modified Eagle's medium without fetal bovine serum and incubated at 37° for 2 hr. Thereafter, the media were aspirated, and the cells were washed twice with D-PBS. After the second wash, 200 ml of D-PBS containing 1 mM 3-isobutyl-1-methyl-xanthine was added to each well. At 10–15 min after the addition of the experimental agents, the reaction was stopped with 200 ml of ice-cold D-PBS containing 0.1 N HCl at room temperature. After 15 min, the incubates were frozen at −20° until assay. cAMP was measured by radioimmunoassay as we have previously reported (23).

Determination of Na^+/K^+-ATPase activity. Na^+/K^+-ATPase activity was assessed by measuring ouabain-inhibitable ^{86}Rb uptake (24). The advantage of this method over measurements of enzymatic activity has been discussed by Cheval and Doucet (25). The reactions were started by the addition of ~1 μCi ^{86}RbCl/well and incubated for 10–15 min at room temperature in the presence or absence of 1 mM ouabain, with or without added drugs. The reaction was terminated by aspiration of the medium, followed by three 2-ml washes with ice-cold D-PBS. The cells were solubilized with 200 ml of 0.1 N HCl. After the addition of the scintillation liquid, radioactivity was determined.

Na^+/K^+-ATPase is sensitive to changes in intracellular and extracellular K^+ and Na^+ concentrations (20). We therefore studied the effects of D_2 receptor occupancy when intracellular K^+ and Na^+ were clamped. Monensin (5 mM) is a Na^+ ionophore (24, 26, 27). Cesium inhibits voltage-sensitive K^+ channels (K_V, K_{IR}, and K_{SR}), Ca^{2+}-sensitive K^+ channels (BK_{Ca} and IK_{Ca}), ATP-sensitive K^+ channels (K_{ATP}), and some receptor-activated K^+ channels (K_{Ach} and $K_{5\text{-}HT}$) (28, 29). In our study, extracellular CsCl (5 mM) blocked K^+ flux in D_{2L}-transfected LTK^- cells by 73 ± 4% (six experiments). Therefore, we used CsCl (5 mM) and monensin (5 mM) to clamp intracellular K^+ and Na^+ concentrations (24, 26–29). In these experiments, the incubation medium was changed to Tris buffer (10 mM Tris·HCl, 140 mM NaCl, 5 mM CsCl, 1.4 mM $CaCl_2$, 1 mM $MgSO_4$, and 2.5 mM glucose, pH 7.3).

Data analysis. The data are expressed as mean ± standard error. The dissociation constant (K_d), maximum receptor density (B_{max}), and inhibition constant (K_i) were calculated using nonlinear regression (Graph PAD InPlot 4.04, Graph PAD Software, San Diego, CA). Significant differences ($p < 0.05$) were determined using Student's *t* test when two groups were compared and analysis of variance followed by the Bonferroni correction when more than two groups were compared.

Results

[^{125}I]Spiperone, a selective dopamine D_2 antagonist radioligand, was specifically bound to D_{2L}-transfected LTK^- cell membranes in a saturable fashion, with a B_{max} of 0.91 ± 0.26 pmol/mg protein (seven experiments) and a K_d of 2.39 ± 0.79 nM (seven experiments) (Fig. 1A). Specific binding was not detected in LTK^- cells either with or without the transfected rat D_{1A} receptor cDNA (data not shown). The ability of several dopaminergic ligands to compete with specific [^{125}I]spiperone binding in D_{2L}-transfected LTK^- cell membranes is shown in Fig. 1B. Spiperone was the most potent agent (K_i = 0.176 ± 0.03 nM, three experiments). The K_i for the selective D_2 agonist LY171555 and the selective D_2 antagonist (−)-sulpiride were 535.8 ± 125.5 nM (three experiments) and 12.7 ± 2.8 nM (three experiments), respectively. The K_i for dopamine was 683.9 ± 236.3 nM (three experiments). The rank

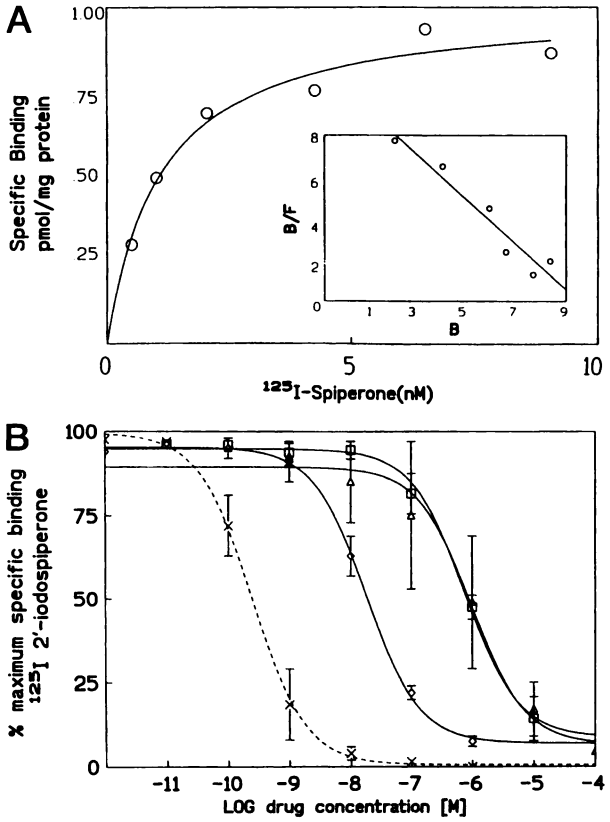

Fig. 1. The expression of the D_2 receptor in LTK$^-$ cells transfected with the rat D_{2Long} receptor cDNA. A, Saturation isotherm of the specific binding of [^{125}I]spiperone in membranes from transfected LTK$^-$ cells. *Inset*, Scatchard transformation of the specific binding data. *B*, specifically bound {Isqb}[^{125}I]spiperone; *F*, free [^{125}I]spiperone. The data are the mean values from seven experiments performed in triplicate. B, Competition between [^{125}I]spiperone and dopaminergic ligands for occupancy of specific binding sites: (×) spiperone, (◇) (−)-sulpiride, (Δ) LY171555, and (□) dopamine. The data are the mean ± standard error values from three experiments per drug performed in triplicate.

order of potency and K_i of these compounds are in agreement with those reported for D_2 receptors in brain tissue and other cell lines transfected with the rat D_{2L} cDNA (1–4).

To assess the functional association between the D_{2L} dopamine receptor and adenylyl cyclase in the D_{2L}-transfected cells, we examined the effect of the selective D_2 agonist LY171555 on forskolin-stimulated cAMP production; 1 μM LY171555 decreased cAMP production by 8.8 ± 3.2% (five experiments), whereas 1 μM forskolin increased cAMP production by 24.6 ± 5.2% (five experiments). The forskolin-induced increase in cAMP production was completely blocked by LY171555 (1 μM) ($p < 0.01$, five experiments). There was no effect of LY171555 on forskolin-stimulated cAMP production in nontransfected LTK$^-$ cells (data were not shown). These results indicate that the cloned D_{2L} receptor is linked to the inhibition of adenylyl cyclase activity, a finding that is in agreement with numerous other reports (7, 10, 19, 30). The magnitude of the decrease induced by LY171555 on forskolin-stimulated cAMP production is also consistent with these reports (19, 30).

In preliminary studies, we found that ouabain-inhibitable ^{86}Rb uptake was linear to 20 min. In other studies, we found that LY171555 stimulated ^{86}Rb uptake in D_{2L}-transfected cells in a time-related manner. The stimulatory effect was also linear to 20 min. In the D_{2L}-transfected cell, LY171555 produced a concentration-dependent stimulation of Na$^+$/K$^+$-ATPase activity, with maximal stimulation occurring at 10 μM (28 ± 6%, six experiments, Fig. 2A). The stimulatory effect on Na$^+$/K$^+$-ATPase activity by 1 μM LY171555 (25 ± 5%, six experiments) was completely blocked by the D_2-selective antagonist (−)-sulpiride (1 μM) ($p < 0.01$, four experiments) (Fig. 2B); (−)-sulpiride alone had no effect. In nontransfected cells, LY171555 did not affect Na$^+$/K$^+$-ATPase activity (data not shown).

We next determined whether the stimulatory effect of LY171555 on Na$^+$/K$^+$-ATPase activity was secondary to LY171555-induced changes in $[Na^+]_i$ and $[K^+]_i$ (20). Monensin (5 μM) has been used to clamp $[Na^+]_i$ (24, 26, 27). Cesium is a known blocker of certain K$^+$ channels (28, 29). In our study, 5 mM CsCl, added to the incubation media (in place of KCl), blocked K$^+$ flux in D_{2L}-transfected cells by 73 ± 4% (six experiments). Therefore, we used CsCl (5 mM) and monensin (5 mM) to clamp $[Na^+]_i$ and $[K^+]_i$ (24, 26–29). When K$^+$ channels were blocked by CsCl and $[Na]_i$ was clamped by monensin, LY171555 still stimulated Na$^+$/K$^+$-ATPase activity in a concentration-dependent manner in the D_{2L}-transfected cells (Fig. 2C); the stimulatory effect of LY171555 was slightly but not significantly affected by CsCl and monensin. The stimulation of Na$^+$/K$^+$-ATPase activity by 1 μM LY171555, when $[Na^+]_i$ and $[K^+]_i$ were clamped, was again completely blocked by 1 μM (−)-sulpiride (Fig. 2C). These experiments show that the D_{2L} receptor-mediated stimulation of Na$^+$/K$^+$-ATPase activity is independent of $[Na^+]_i$ and $[K^+]_i$. The inhibitory effect of forskolin, which directly stimulates adenylyl cyclase, on Na$^+$/K$^+$-ATPase activity was also independent of $[Na^+]_i$ and $[K^+]_i$ (Fig. 2C). These experiments indicate that an increase in cAMP production is involved in inhibiting Na$^+$/K$^+$-ATPase activity. We have has previously reported that the stimulation of PKA activity by 8-(4-chlorophenylthio)-cAMP decreased Na$^+$/K$^+$-ATPase activity as assessed by the hydrolysis of [γ-^{32}P]ATP (18).

To determine whether the LY171555-induced changes in Na$^+$/K$^+$-ATPase activity were related to cAMP production, we examined the effect of inhibition of cAMP production on Na$^+$/K$^+$-ATPase activity. DDA (100 μM), an inhibitor of adenylyl cyclase activity in other cells (14), also inhibited cAMP accumulation (9.7 ± 0.92%, three experiments, $p < 0.05$) and increased Na$^+$/K$^+$-ATPase activity by 31.7 ± 6.5% in nontransfected LTK$^-$ cells (Fig. 3). The greater ability of DDA to stimulate Na$^+$/K$^+$-ATPase activity compared with its inhibitory effect on cAMP accumulation was similar to the effect noted with 1 μM LY171555 (+25.3 ± 5% versus −8.8 ± 5%, respectively). In agreement with the data shown on Fig. 2C using D_{2L}-transfected cells, in nontransfected LTK$^-$ cells, forskolin (10 μM) again inhibited Na$^+$/K$^+$-ATPase activity (43.8 ± 15.3%, three experiments, $p < 0.01$ compared with basal) (Fig. 3). In D_{2L}-transfected cells, 1 μM forskolin, which increased cAMP accumulation by 25 ± 5% ($p < .05$, five experiments), modestly inhibited Na$^+$/K$^+$-ATPase activity (7 ± 1.5%, seven experiments, $p < 0.01$); this effect was blocked by 1 μM LY171555 (seven experiments, $p < 0.05$). These results, in concert with the data showing the ability of LY171555 to reverse the stimulatory effect of forskolin on cAMP production, support the hypothesis that cAMP production and Na$^+$/K$^+$-ATPase activity are reciprocally related.

To determine whether the LY171555-stimulated Na$^+$/K$^+$-

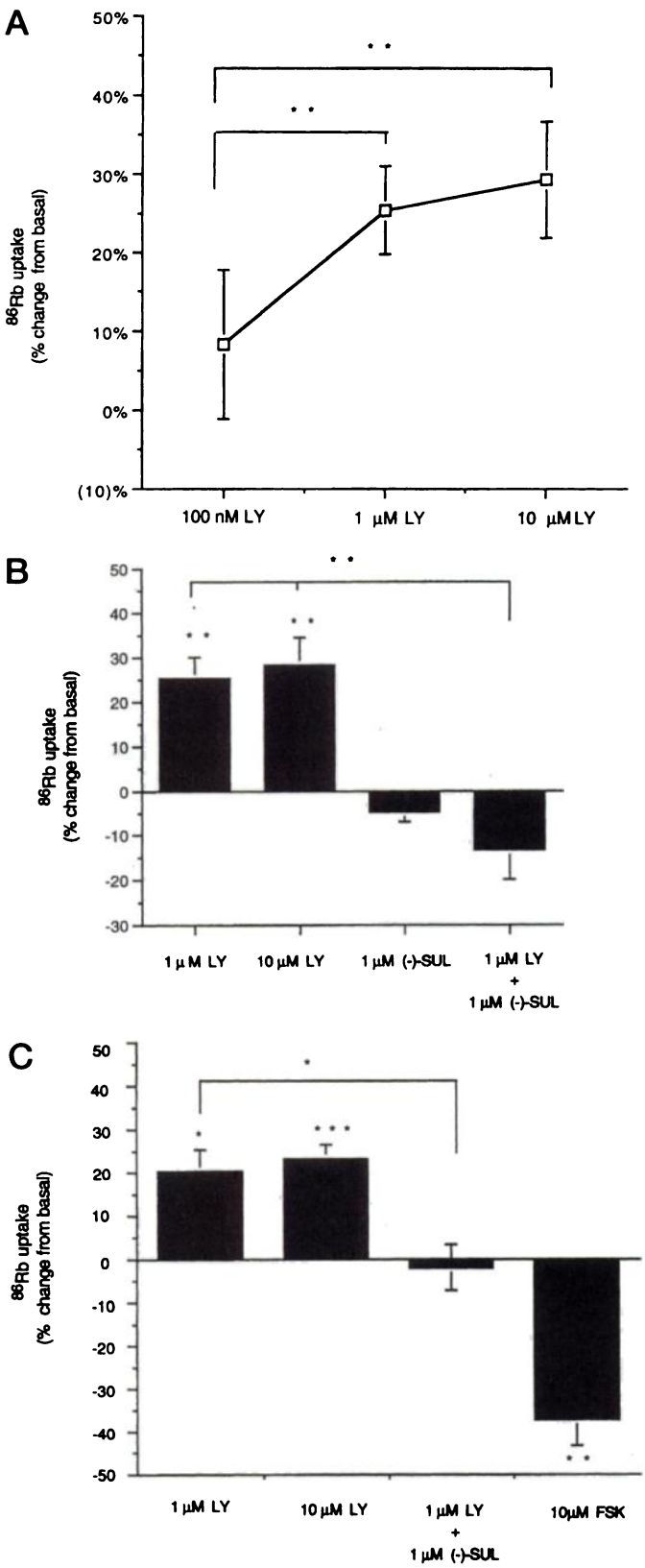

Fig. 2. The effect of dopaminergic drugs and forskolin on ouabain-inhibitable ^{86}Rb uptake in D_{2L} LTK^--transfected cells. A, The D_2 agonist LY171555 (*LY*) increased ouabain-inhibitable ^{86}Rb uptake in a concentration-dependent manner. The cells were incubated with D-PBS containing LY171555 and 1 μCi $^{86}RbCl$ for 10 min at room temperature. Data are mean ± standard error values from six experiments performed in triplicate. **, $p < 0.01$ versus basal (paired *t* test) or versus 100 nM LY171555 (analysis of variance). B, The stimulatory effect of LY171555 on ouabain-inhibitable ^{86}Rb uptake was blocked by the D_2 antagonist (−)-sulpiride (*SUL*). Data are mean ± standard error values from four experiments performed in triplicate. **, $p < 0.01$ LY171555 versus basal (paired *t* test) or versus LY171555 + (−)-sulpiride (analysis of variance). C, The stimulatory effect of LY171555 and the inhibitory effect of forskolin on ouabain-inhibitable ^{86}Rb uptake persisted even when intracellular Na^+ and K^+ concentrations were clamped by monensin (5 μM) and $CsCl_2$ (5 mM). Data are mean ± standard error values from three to six experiments performed in triplicate. *, $p < 0.05$ versus basal (paired *t* test) or versus LY171555 + (−)-sulpiride (analysis of variance); **, $p < 0.01$ versus basal (paired *t* test); ***, $p < 0.001$ versus basal (paired *t* test).

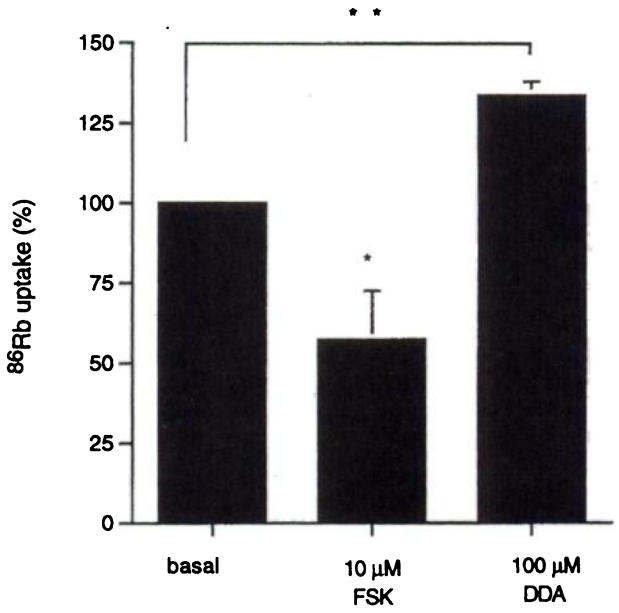

Fig. 3. The effect of forskolin (*FSK*) or an inhibitor of adenylyl cyclase, DDA, on Na^+/K^+-ATPase activity in nontransfected LTK^- cells. Forskolin (10 μM) inhibited and DDA (100 μM) stimulated Na^+/K^+-ATPase activity. Data are mean ± standard error values from three experiments performed in triplicate. *, $p < 0.05$ versus basal; **, $p < 0.01$ versus basal (paired *t* test).

ATPase activity was related to G_i proteins, we preincubated D_{2L}-transfected cells with 500 ng/ml pertussis toxin (Fig. 4). Pertussis toxin blocked the stimulatory effect of LY171555 on Na^+/K^+-ATPase activity, suggesting that a pertussis toxin-sensitive G protein, presumably G_i, was needed in the D_{2L} stimulation of Na^+/K^+-ATPase activity.

Discussion

Our results show that rat D_{2Long} receptors heterologously expressed in LTK^- cells stimulate Na^+/K^+-ATPase activity. However, Na^+/K^+-ATPase can be activated by a decrease in intracellular K^+ and an increase in intracellular Na^+ (20). Indeed, the magnitude of the D_2 agonist-induced increase in Na^+/K^+-ATPase activity is comparable to the stimulatory effect of D_2 receptors on NHE activity in LTK^- cells (8), although less than the D_2 receptor-mediated increase in NHE activity in Chinese hamster ovary cells (19). D_2 receptors have also been reported to affect K^+ channels (28, 29); a D_2 receptor-induced stimulation of K^+ channels could have led to stimulation of Na^+/K^+-ATPase activity. However, in our study, D_2 receptors expressed in LTK^- cells stimulated Na^+/K^+-ATPase activity even when K^+ channels were

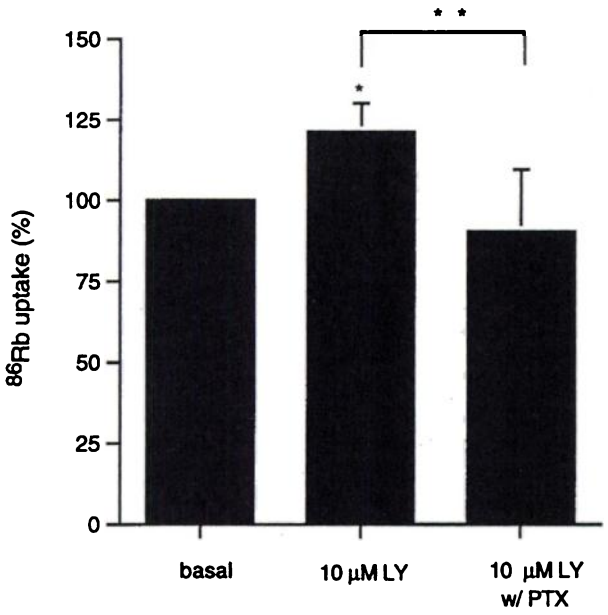

Fig. 4. The influence of pertussis toxin (*PTX*) on the stimulatory effect of LY171555 (*LY*) on Na^+/K^+-ATPase activity. Pertussis toxin blocked the stimulatory effect of 10 μM LY171555 on Na^+/K^+-ATPase activity. Data are mean ± standard error values from five experiments performed in triplicate. **, $p < 0.01$ LY171555 without pertussis toxin versus LY171555 with pertussis toxin; *, $p < 0.05$ versus basal.

blocked by CsCl and $[Na]_i$ was clamped by monensin. Thus, the stimulatory effect of D_2 receptors on Na^+/K^+-ATPase activity is independent of the D_2 receptor-mediated stimulation of either NHE activity or K^+ channels (8, 9, 19, 31, 32).

The ability of dopamine receptors to stimulate or inhibit Na^+/K^+-ATPase activity is linked to a dopamine-mediated alteration of cAMP production. We have previously shown that D_{1A} receptors expressed in LTK^- cells inhibit Na^+/K^+-ATPase activity and that this effect is mediated by PKA (18). In nontransfected LTK^- cells, forskolin, which stimulates cAMP production, and 8-(4-chlorophenylthio)-cAMP, which stimulates PKA activity, inhibit Na^+/K^+-ATPase activity. In the D_{2L}-transfected cell, LY171555, a D_2 agonist, inhibits cAMP production and stimulates Na^+/K^+-ATPase activity. Furthermore, the abilities of forskolin to stimulate cAMP production and inhibit Na^+/K^+-ATPase activity are blocked by LY171555. Moreover, in nontransfected cells, DDA inhibits cAMP production and stimulates Na^+/K^+-ATPase activity. A causal relationship between the stimulatory effect on Na^+/K^+-ATPase activity and the inhibitory effect on cAMP production by D_2 receptors is substantiated by the ability of pertussis toxin to prevent the stimulatory effect of the D_2 agonist LY171555 on the sodium pump. Whether D_2 receptors enhance Na^+/K^+-ATPase activity and NHE activity by the same mechanism is not clear. In LTK^- cells, Neve *et al.* reported that D_2 receptors stimulate NHE activity independent of cAMP and pertussis toxin-sensitive G-proteins (11). In Chinese hamster ovary cells, D_2 receptor-stimulated NHE activity was linked to pertussis toxin-sensitive G-proteins but not to cAMP (22). In some cells, PKC can stimulate NHE activity (33). Because D_2 receptor activation can increase phosphoinositide hydrolysis in LTK^- cells (10), it is possible that the stimulatory effect of D_2 receptors on NHE is linked to the phospholipase C/PKC pathway.

Stimulation of Na^+/K^+-ATPase activity by D_{2L} receptors may explain the observation that D_2 agonists enhance renal tubular sodium transport while D_2 antagonists produce the opposite effect (34–37). However, several investigators have reported that D_2-like receptors, in concert with D_1-like receptors, inhibit Na^+/K^+-ATPase activity in renal proximal tubules and neuronal cells (13, 15, 16). It is possible that D_2-like receptors interact with D_1-like receptors, resulting in an effect that may be different than that produced when stimulated independently of each other. In preliminary studies, we found that in the LTK^- cell transfected with both rat D_{1A} and D_{2Long} cDNA, D_2 agonists enhance the ability of D_1 agonists to stimulate cAMP production (38). Although neither the D_1 nor the D_2 receptor alone stimulates arachidonic acid, dopamine and other G_i-coupled receptors can amplify ATP receptor-stimulated arachidonic acid release (7, 39). Both cAMP and PKC have been reported to enhance the D_2-mediated increase in arachidonic acid release (7, 39, 40). Because PKA, PKC, and arachidonic acid by-products can inhibit Na^+/K^+-ATPase activity, a D_1/D_2 synergism in the production of cAMP, phosphoinositide, and arachidonic acid products may explain the synergism of D_1 and D_2 agonists in their inhibition of Na^+/K^+-ATPase activity. These speculations, however, remain to be proved.

In summary, we stably expressed the rat D_{2L} dopamine receptor in LTK^- cells and obtained specific ligand binding and function characteristic of the D_{2L} receptor. In the D_{2L}-transfected cells, the D_2 agonist LY171555 stimulated Na^+/K^+-ATPase activity in dose-dependent manner, even when K^+ channels were blocked by CsCl and $[Na]_i$ was clamped by monensin. The stimulatory effect of D_2 receptors on Na^+/K^+-ATPase activity was linked to a G_i-mediated inhibition of cAMP production.

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Send reprint requests to: Robin A. Felder, Ph.D., Department of Pathology, The University of Virginia Health Sciences Center, Box 168, Old Medical School, Charlottesville, VA 22908.