Dopamine Regulates Phosphate Uptake by Opossum Kidney Cells Through Multiple Counter-Regulatory Receptors

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Abstract. The purpose of this study was to determine the mechanisms of dopamine regulation of phosphate uptake in opossum kidney (OK) cells, a model of proximal renal tubules. Dopamine stimulated cAMP generation and inhibited radiolabeled phosphate uptake into OK cell monolayers by 14.4 ± 1.8%. The effect of dopamine was transient, as phosphate uptake returned toward control level by 3 h despite the continued presence of dopamine. Pretreatment with pertussis toxin increased dopamine inhibition of phosphate uptake to 25 ± 3%, increased the duration of the dopamine effect to at least 3 h, and enhanced cAMP generation. In an OK cell clone that overexpressed cAMP phosphodiesterase, dopamine did not inhibit phosphate uptake, but pharmacologic inhibition of protein kinase A activation did not prevent dopamine inhibition of phosphate uptake. A DA1 receptor agonist inhibited phosphate uptake more potently than dopamine (29.5 ± 1.1%) or a DA2 receptor agonist (7.9 ± 2%). However, both DA1 and DA2 receptor antagonists completely blocked dopamine inhibition of phosphate uptake. DA1, but not the DA2, antagonists blocked dopamine-stimulated cAMP generation. Treatment with α-adrenergic receptor antagonists potentiated dopamine inhibition of phosphate uptake to the same extent as pertussis toxin and was not additive with pertussis toxin. It is concluded that dopamine inhibits phosphate uptake through DA1 and DA2 receptor stimulation by cAMP-dependent and -independent pathways and activates a pertussis toxin-sensitive counter-regulatory pathway that attenuates this response through α-adrenergic receptor stimulation. (J Am Soc Nephrol 9: 975–985, 1998)

Sodium-coupled phosphate transport in the proximal renal tubule is regulated by numerous dietary and hormonal factors (1). Parathyroid hormone (PTH), the most extensively studied hormonal regulator, inhibits sodium-phosphate cotransport through interaction with G protein-coupled receptors located on the basolateral membrane of proximal tubule cells and subsequent activation of protein kinase A and protein kinase C (2). The specific roles of these two kinases in producing the inhibitory effect on phosphate transport remain controversial. Studies of the sodium-hydrogen exchanger, another PTH-regulated sodium-coupled transporter, suggest an independent effect of each kinase (3). Other studies of sodium-phosphate cotransport argue for a paramount effect of either protein kinase A (4,5) or protein kinase C (6,7).

Dopamine, an autocrine neurotransmitter (8,9), also stimulates G protein-coupled receptors that activate protein kinase A and protein kinase C in proximal renal tubule cells (10–13). Several lines of evidence support a physiologically significant role for dopamine in the regulation of phosphate transport. First, both DA1 and DA2 dopamine receptors have been described on proximal renal tubule cells (12). Stimulation of DA1 receptors, which are similar to the D1 receptors characterized in the central nervous system (14,15), results in activation of adenylyl cyclase and phospholipase C in rat proximal renal tubule cells (10,13). DA2 receptors, peripheral tissue analogues of the D2 receptors in the central nervous system, inhibit cAMP generation in rat renal cortex (16). Second, proximal tubule cells synthesize dopamine in response to high phosphate intake (8). Third, inhibition of intrinsic dopamine production by carbopoda, an inhibitor of aromatic L-amino acid decarboxylase (8,17), or blockage of dopamine receptors by antagonists (9) results in reduced fractional excretion of phosphate. Fourth, exogenous dopamine inhibits phosphate transport in isolated perfused rabbit proximal renal tubules (18) and in intact rats (19).

Despite activating similar signaling pathways, dopamine inhibits phosphate uptake less effectively than PTH (20). The purpose of the present study was to identify the cellular mechanisms of dopamine regulation of phosphate transport by comparing dopamine and PTH inhibition of phosphate uptake in opossum kidney (OK) cells, a model for proximal renal tubules (21). These cells express PTH receptors coupled by G proteins to adenylyl cyclase and phospholipase C. Activation of these receptors by PTH results in inhibition of sodium-dependent phosphate uptake. Dopamine DA1 receptors coupled to adenylyl cyclase have also been demonstrated on OK cells (10,15). The results of this study show that dopamine and PTH inhibition of phosphate uptake differ in magnitude, duration, and pertussis toxin sensitivity. Furthermore, dopamine modulates phosphate uptake through stimulation of counter-regulatory signal transduction pathways.
Materials and Methods

Wild-type OK cells were a generous gift of Dr. Steve Scheinman (State University of New York, Syracuse, NY). An OK cell clone that overexpresses cAMP phosphodiesterase was a generous gift of Dr. John Raymond (Medical University of South Carolina, Charleston, SC). Bovine 1-34 PTH was obtained from Bachem (Philadelphia, PA). Dopamine, yohimbine, and prazosin were purchased from Sigma Chemical Co. (St. Louis, MO). R(+)-SKF-38393 HC1 (D1 dopamine receptor agonist), SCH-23390 (D1 dopamine receptor antagonist), quinpirole (D2 receptor agonist), and S-sulpiride (D2 receptor antagonist) were obtained from Research Biochemicals International (Natick, MA). [32P]Phosphoric acid was obtained from ICN Biomedicals (Irvine, CA). Pertussis toxin was obtained from List Biochemicals (Campbell, CA). Peroxidase-labeled goat anti-rabbit IgG was obtained from Vector Laboratories (Burlingame, CA).

Cell Culture Technique

OK cells were grown to confluence as monolayers in 175-cm² flasks in culture medium consisting of Eagle’s with Earle’s salts (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum, 4 mM glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin, pH 7.4. Cells were maintained in a humidified atmosphere with 5% CO2 at 37°C. The medium was changed three times per week, and the cells were split 1:3 or 1:4 once per week by brief trypsinization and dispersal. Cells from passages 81 to 88 were used for experiments at 100% confluence, 4 to 5 d after seeding.

Phosphate Uptake

Sodium-dependent phosphate uptake was measured by uptake of radiolabeled phosphate into OK cell monolayers as described previously (22). Cells seeded onto 96-well tissue culture plates were serum-deprived for 24 h before the uptake experiments. Uptake experiments were initiated by aspiration of serum-free medium and addition of serum-free medium containing agonist. The cells were incubated with agonist at 37°C for 2 h unless indicated otherwise and washed three times with transport medium containing radiolabeled [32P]phosphoric acid (2 μCi/ml), pH 7.4, at room temperature. The uptake experiments were designed specifically to pair sodium-independent phosphate uptake and response to agonists; however, on a single given day, both parameters varied little between separate wells or trays of cells. Therefore, the experiments were designed specifically to pair the data by the day on which the experiments were performed. Agonist dose-response curves were fitted with computer assistance (SigmaPlot), and maximal response and half-maximal concentrations balanced salt solution containing agonist and 100 μM RO 20-1724 (a cAMP-specific phosphodiesterase inhibitor) and terminated after 30 min (unless indicated otherwise) by aspiration of the overlying medium, addition of 100 μl of 0.2N HCl to the cell monolayer, and snap-freezing in liquid nitrogen. The assays were defrosted for 1 h at 4°C to disrupt the cells, and neutralized with 75 μl of 0.05 M Na2HPO4. cAMP was measured by an RIA kit (DuPont-New England Nuclear, Boston, MA) according to the manufacturer’s instructions. Each assay was run in duplicate, and the results were averaged and expressed as fmol cAMP/mg protein.

Peptide Antibody Production

A peptide identical to the carboxy-terminal 12 amino acid sequence of NaPi-4 (CGVLSQHNATRL) was generated and conjugated to keyhole limpet hemocyanin (Genosys Biotechnologies, Woodlands, TX). One hundred micromicrograms of peptide was mixed in Freund’s complete adjuvant and injected subcutaneously into New Zealand White rabbits. The rabbits were given booster injections in incomplete Freund’s adjuvant on a monthly basis. Before the first injection, 50 ml of blood was drawn, and serum was separated and frozen at −70°C to be used for preimmune testing. After immunization, 50 ml of blood was drawn, and the serum was separated and frozen three times monthly.

Membrane Preparation

OK cells grown in 6-well trays were washed with phosphate-buffered saline without Mg2+ or Ca2+ and once with Hepes-KOH (5 mM Hepes, pH 7.4). A total of 1.5 ml of Hepes-KOH containing 4 mM ethylenediaminetetra-acetic acid and 1 mM phenylmethylsulfonyl fluoride was added to each well, and the cells were scraped into separate microcentrifuge tubes. The cells were homogenized by repetitive aspiration through a 21-gauge needle, then centrifuged at 2000 rpm for 10 min at 4°C. The supernatant was aspirated into another microcentrifuge tube and centrifuged at 16,000 rpm for 40 min at 4°C. The supernatant was discarded, and the pellet was resuspended in 75 μl of 50 mM mannitol with 10 mM Hepes-Tris, pH 7.2. A total of 25 μl of 4X Laemmli sample buffer was added, and the specimen was boiled for 5 min and then cooled. The samples were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred electrophoretically to polyvinylidene difluoride (PolyScreen, DuPont-New England Nuclear). The membrane was incubated in 5% milk in 20 mM Tris, 50 mM NaCl, and 0.05% Tween 20 (TTBS) for 1 h to block nonspecific binding, then incubated in a 1:2000 dilution of NaPi-4 antibody in 5% milk in TTBS overnight at 4°C. The blot was washed three times with TTBS, incubated in a 1:10,000 dilution of goat anti-rabbit IgG for 1 h at room temperature, then washed again with TTBS. Labeled bands were identified by chemiluminescence (Renaiissance, DuPont-New England Nuclear). The films were scanned using a Personal Densitometer SI (Molecular Dynamics, Sunnyvale, CA), and the areas of interest were outlined and quantified by integrated software (ImageQUANT, Molecular Dynamics).

Statistical Analyses

The model system demonstrated day-to-day variability in baseline phosphate uptake and response to agonists; however, on a single given day, both parameters varied little between separate wells or trays of cells. Therefore, the experiments were designed specifically to pair the data by the day on which the experiments were performed. Agonist dose–response curves were fitted with computer assistance (SigmaPlot), and maximal response and half-maximal concentrations.
were determined. Experiments were analyzed by paired t test or by ANOVA as indicated. The 95% confidence limits were \textit{a priori} determined to be statistically significant.

Results

The effect of dopamine on sodium-phosphate cotransport was examined by measuring sodium-dependent phosphate uptake in OK cell monolayers. Figure 1A depicts the dose–response curve for dopamine inhibition of phosphate uptake after a 2-h incubation. Inhibition of phosphate uptake was significant at $10^{-6}$ M dopamine. Maximal inhibition of phosphate uptake was $14.6 \pm 3.3\%$ at $10^{-5.5}$ M dopamine, from $396 \pm 39.6$ pmol Pi/mg protein to $338.2 \pm 11.2$ pmol Pi/mg protein. As the concentration of dopamine increased above $10^{-5}$ M, inhibition of phosphate uptake tended to decrease, but phosphate uptake remained significantly less than in untreated cells. PTH inhibition of phosphate uptake in OK cells requires 3 to 4 h to reach a maximum effect. To determine whether maximal dopamine inhibition of phosphate uptake also required a prolonged period of agonist exposure, a time course of dopamine inhibition of phosphate uptake was performed. The results are shown in Figure 1B. Inhibition of phosphate uptake by $10^{-5}$ M dopamine reached a maximum of $14.7 \pm 3.2\%$ at 120 min of incubation and subsequently fell to $6.8 \pm 2.8\%$ by 3 h.

Dopamine stimulates cAMP generation through interaction with DA$_1$ receptors (10). Figure 2 shows the dose–response curve for dopamine-stimulated cAMP. After 30 min of incubation, maximal stimulation of cAMP generation was $1880 \pm 203$ fmol/mg protein at $10^{-5}$ M dopamine compared with a control cAMP value of $43.7 \pm 4.3$ fmol/mg protein. At higher concentrations of dopamine, cAMP generation fell, paralleling the dose–response curve for dopamine inhibition of phosphate uptake. A time course of dopamine-stimulated cAMP showed that dopamine ($10^{-5}$ M) stimulated an increase in cAMP generation from a control level of 129.7 fmol/mg protein to a peak of 3517.9 fmol/mg protein at 10 min, falling to 1545.2 fmol/mg protein by 90 min.

To determine whether activation of adenyl cyclase was essential for dopamine inhibition of phosphate uptake, phosphate uptake was measured in a clone of OK cells stably transfected with the cDNA for a rat cAMP phosphodiesterase. These cells, which have been characterized by Bates \textit{et al.} (23), overexpress cAMP phosphodiesterase, effectively preventing activation of protein kinase A by hormone-stimulated cAMP. As with wild-type OK cells, the cells of this clone express sodium-phosphate cotransporters that are regulated by protein kinase A and protein kinase C. As shown in Figure 3A, 8-bromo-cAMP (8-Br-cAMP), a phosphodiesterase-resistant cAMP analogue that directly activates protein kinase A, and phorbol myristate acetate, which directly activates protein kinase C, inhibited phosphate uptake in this clonal line. PTH also inhibited phosphate uptake, presumably by activation of protein kinase C. Neither dopamine nor the specific DA$_1$ agonist SKF-38393 inhibited phosphate uptake. Figure 3B shows that the addition of the specific cAMP phosphodiesterase inhibitor

![Figure 1. Dopamine inhibition of phosphate uptake. Opossum kidney (OK) cell monolayers were incubated overnight in serum-free unsupplemented medium. Phosphate uptake was measured by liquid scintillation spectrometry of solubilized cells after incubation in unsupplemented medium containing increasing concentrations of dopamine at 37°C followed by 10-min uptake of radiolabeled phosphate at room temperature. (A) Dose–response. Phosphate uptake was performed after 2 h of incubation with increasing concentrations of dopamine. Results, expressed as percent inhibition phosphate uptake compared to cells treated with vehicle, are means \pm SEM of seven separate experiments. *P < 0.01, treated versus untreated cells. (B) Time course. Phosphate uptake was measured after incubation with $10^{-5}$ M dopamine for the designated times at 37°C followed by 10-min uptake of radiolabeled phosphate at room temperature. Results, expressed as percent inhibition phosphate uptake compared to cells treated with vehicle, are means \pm SEM of six separate experiments. *P < 0.01, treated versus untreated cells.](image-url)
Figure 2. Dopamine-stimulated cAMP generation. Intracellular cAMP was measured by RIA after incubation of the cells with or without agonist for the designated time at 30°C. cAMP was measured after a 30-min treatment with increasing concentrations of dopamine at 30°C. Results are means ± SEM of six separate experiments. *P < 0.05, treated versus untreated cells.

RO 20-1724 (100 μM) restored dopamine inhibition of phosphate uptake (34.5 ± 3.2% inhibition in the presence of RO 20-1724 versus 4.8 ± 5.1% in the absence of RO 20-1724). RO 20-1724 alone inhibited phosphate uptake by 17.5 ± 3.2%. These data suggest that dopamine-mediated inhibition of phosphate uptake is dependent on the generation of cAMP.

We also investigated the dependence of dopamine inhibition of phosphate uptake on cAMP production by measuring dopamine inhibition of phosphate uptake in the presence of the specific protein kinase A inhibitors H89 and RpcAMPS. The concentrations of H89 and RpcAMPS that prevented 8-Br-cAMP-stimulated protein kinase A activation were determined in separate experiments. After pretreatment of OK cells with 10^{-6} M H89 for 1 h, followed by incubation with 10^{-5} M dopamine for 2 h, inhibition of phosphate uptake was 27.7 ± 1.1% compared with 34.1 ± 1.4% in untreated cells (n = 8, P < 0.003). After pretreatment of OK cells with 10^{-6} M RpcAMPS for 1 h, followed by incubation with dopamine for 2 h, inhibition of phosphate uptake was 22.2 ± 2.3% compared with 20.1 ± 2.3% in untreated cells (n = 8, P = NS). These data suggest that dopamine can inhibit phosphate uptake in OK cells by non-cAMP-related pathways.

In addition to DA_1 receptors, proximal renal tubule cells also express DA_2 receptors. There is also evidence suggesting that OK cells may express DA_2 receptors (24). Because DA_2 receptors inhibit adenyl cyclase through pertussis toxin-sensitive G proteins, the effect of pertussis toxin pretreatment on dopamine inhibition of phosphate uptake and dopamine stimulation of cAMP in OK cells was examined. As shown in Figure 4A, 10^{-5.5} M dopamine decreased phosphate uptake in pertussis toxin-treated cells by 22.6 ± 2.6%, from 406.3 ± 33 to 314.5 ± 8.2 pmol Pi/mg protein. This inhibition of phosphate uptake by dopamine was significantly greater than in untreated cells and persisted at higher dopamine concentrations (26.8 ± 2.7% at 10^{-4} M dopamine). Pretreatment with pertussis toxin (30 ng/ml) also prevented the time-dependent decrease in dopamine inhibition of phosphate uptake (Figure
Dopamine stimulated a significantly greater cAMP generation in pertussis toxin-treated cells compared with untreated cells, from a basal level of 40.5 ± 7.4 to 2671 ± 266 fmol/mg protein at $10^{-5}$ M dopamine (Figure 4C). The time course for dopamine-stimulated cAMP in pertussis toxin-treated cells was similar to untreated cells. cAMP generation rose from 115.1 to 4303.6 fmol/mg protein by 10 min and fell to 1962.5 fmol/mg protein by 90 min ($n = 2$).

We interpret these findings to indicate that dopamine regulates phosphate transport through activation of two pathways, one that stimulates adenylyl cyclase and one that inhibits adenylyl cyclase through a pertussis toxin-sensitive mechanism. To identify which receptors were activated by dopamine to stimulate these counter-regulatory pathways, we first examined the effect of relatively specific DA₁ and DA₂ receptor agonists on inhibition of phosphate uptake. Figure 5A shows a dose–response curve for inhibition of phosphate uptake by the DA₁ agonist SKF-38393 and the DA₂ agonist quinpirole. After a 2-h incubation, SKF-38393 produced a dose-dependent inhibition of phosphate uptake that reached 35.6 ± 1.1% at $10^{-4}$ M, whereas $10^{-4}$ M quinpirole inhibited phosphate uptake by 18.5 ± 2.1%. In side-by-side experiments (Figure 5B), $10^{-5}$ M SKF-38393 inhibited phosphate uptake to a greater extent than $10^{-5}$ M dopamine (29.5 ± 1.1% versus 15.7 ± 1.9%). Quinpirole at $10^{-5}$ M inhibited phosphate uptake by only 7.9 ± 2%. In contrast to the results with dopamine, SKF-38393 inhibition of phosphate uptake was stable over 3 h time ($n = 4$, data not shown). Pretreatment with pertussis toxin did not alter the inhibition of phosphate uptake by either SKF-38393 or quinpirole (Figure 5C). These data suggest that dopamine inhibits phosphate uptake primarily from activation of DA₁ receptors and that neither DA₁ nor DA₂ receptors regulate phosphate transport through a pertussis toxin-sensitive pathway.

To confirm that dopamine inhibition of phosphate uptake was mediated by activation of DA₁ receptors, the effect of temperature. Results, expressed as percent inhibition phosphate uptake compared with control cells, are means ± SEM of seven separate experiments. *$P < 0.01$, compared with control; **$P < 0.01$, inhibition of phosphate uptake in pertussis toxin-treated cells compared with cells not treated with pertussis toxin. (B) Time course of dopamine-mediated inhibition of phosphate uptake in the presence of pertussis toxin. Phosphate uptake was measured after incubation with $10^{-5}$ M dopamine for the designated times at 37°C followed by 10-min uptake of radiolabeled phosphate at room temperature. Results, expressed as percent inhibition phosphate uptake compared to cells treated with vehicle, are means ± SEM of six separate experiments. *$P < 0.01$, compared to cells treated with vehicle. (C) Dopamine stimulation of cAMP in the presence of pertussis toxin. The cells were washed, incubated with increasing concentrations of dopamine for 30 min at 30°C, followed by determination of intracellular cAMP by RIA. Results are means ± SEM of six separate experiments. *$P < 0.01$, compared to unstimulated cells; **$P < 0.01$, stimulation of cAMP generation in pertussis toxin-treated cells compared to cells not treated with pertussis toxin.

Figure 4. Effect of pertussis toxin on dopamine inhibition of phosphate uptake and cAMP generation. OK cell monolayers were incubated overnight in serum-free medium in the presence or absence of 50 ng/ml pertussis toxin overnight. (A) Concentration-dependent inhibition of phosphate uptake in the presence of pertussis toxin. The cells were washed, incubated with increasing concentrations of dopamine for 2 h at 37°C, followed by a 10-min phosphate uptake at room temperature. Results, expressed as percent inhibition phosphate uptake compared with control cells, are means ± SEM of seven separate experiments. *$P < 0.01$, compared with control; **$P < 0.01$, inhibition of phosphate uptake in pertussis toxin-treated cells compared with cells not treated with pertussis toxin. (B) Time course of dopamine-mediated inhibition of phosphate uptake in the presence of pertussis toxin. Phosphate uptake was measured after incubation with $10^{-5}$ M dopamine for the designated times at 37°C followed by 10-min uptake of radiolabeled phosphate at room temperature. Results, expressed as percent inhibition phosphate uptake compared to cells treated with vehicle, are means ± SEM of six separate experiments. *$P < 0.01$, compared to cells treated with vehicle. (C) Dopamine stimulation of cAMP in the presence of pertussis toxin. The cells were washed, incubated with increasing concentrations of dopamine for 30 min at 30°C, followed by determination of intracellular cAMP by RIA. Results are means ± SEM of six separate experiments. *$P < 0.01$, compared to unstimulated cells; **$P < 0.01$, stimulation of cAMP generation in pertussis toxin-treated cells compared to cells not treated with pertussis toxin.
selective DA₁ and DA₂ receptor antagonists on dopamine inhibition of phosphate uptake was determined. Dopamine inhibition of phosphate uptake was completely prevented by pretreatment with the DA₁ receptor antagonist SCH-23390 (10⁻⁵ M), as shown in Figure 6A. However, the DA₂ receptor antagonist sulpiride (10⁻⁵ M) also prevented dopamine inhibition of phosphate uptake. Neither SCH-23390 nor sulpiride alone had any effect on phosphate uptake (data not shown). Sulpiride also decreased inhibition of phosphate uptake by 10⁻³ M SKF-38393 from 32.6 ± 1.2% to 21.5 ± 1.6% (n = 8, P < 0.0007). This finding suggests either that sulpiride at this relatively high concentration could be an antagonist for both DA₁ and DA₂ receptors (25) or that sulpiride could block phosphate uptake at a more distal site in the signaling pathway.

To examine the possible mechanisms by which the DA₁ and DA₂ receptor antagonists blocked dopamine inhibition of phosphate uptake, the effect of SCH-23390 and sulpiride on dopamine stimulation of cAMP was measured. As shown in Figure 6B, 10⁻⁵ M SCH-23390 completely blocked dopamine stimulation of cAMP, consistent with its effects on dopamine inhibition of phosphate uptake. However, despite the nearly complete prevention of dopamine inhibition of phosphate uptake, sulpiride did not significantly inhibit dopamine stimulation of cAMP. Perrichot and colleagues have shown that at lower concentrations, sulpiride enhances dopamine inhibition of phosphate uptake, presumably by blocking DA₂ receptor inhibition of adenylyl cyclase (24). Therefore, to further exclude the possibility that at 10⁻⁵ M sulpiride blocked dopamine inhibition of phosphate uptake simply by acting as a DA₁ receptor antagonist, we examined dopamine inhibition of phosphate uptake in the presence of 10⁻⁵ M SCH-23390 or 10⁻⁷ M sulpiride, concentrations at which these antagonists are highly selective for their respective receptors (25). Dopamine alone inhibited phosphate uptake by 16.2 ± 1.1% after 2 h of incubation. SCH-23390 (10⁻⁵ M) completely blocked dopamine inhibition of phosphate uptake. Sulpiride (10⁻⁷ M) partially but significantly prevented dopamine inhibition of phosphate uptake, albeit to a much lesser extent than at 10⁻⁵ M. These results suggest that at high concentrations sulpiride could act as a DA₁ receptor antagonist, but that DA₂ receptor activation could play a significant role in dopamine inhibition of phosphate uptake. To exclude the possibility that sulpiride acts at a site distal to cAMP generation, we measured inhibi-

![Figure 5. Effect of specific DA₁ and DA₂ agonists on phosphate uptake. (A) Dose–response curves for DA₁ and DA₂ agonist inhibition of phosphate uptake. Phosphate uptake was measured by liquid scintillation spectroscopy of solubilized cells after treatment with increasing concentrations of SKF-38393 or quinpirole for 2 h at 37°C, followed by 10-min uptake of radiolabeled phosphate at room temperature. Results are means ± SEM of four separate experiments. (B) Comparison of DA₁ and DA₂ agonists on phosphate uptake. Phosphate uptake was measured by liquid scintillation spectroscopy of solubilized cells after treatment with 10⁻⁵ M dopamine, 10⁻⁵ M SKF-38393, or 10⁻⁵ M quinpirole for 2 h at 37°C, followed by 10-min uptake of radiolabeled phosphate at room temperature. Results are means ± SEM of eight separate experiments. **P < 0.01, compared with dopamine. *P < 0.01, compared with SKF-38393. (C) Effect of pertussis toxin on inhibition of phosphate uptake by DA₁ and DA₂ agonists. OK cell monolayers were incubated overnight in unsupplemented medium in the absence or presence of pertussis toxin (50 ng/ml). Phosphate uptake was measured after treatment with SKF-38393 or quinpirole for 2 h at 37°C followed by 10-min uptake of radiolabeled phosphate at room temperature. Results are means ± SEM of eight separate experiments.](image-url)
cAMP inhibited phosphate uptake by 34 ± 1.5% in the absence of sulpiride. After a 2-h incubation, 10^{-5} M 8-Br-cAMP inhibited phosphate uptake by 34 ± 1.5% in the absence and by 35 ± 1.4% in the presence, of 10^{-5} M sulpiride (n = 4, data not shown). This finding suggests that sulpiride does not attenuate dopamine inhibition of phosphate uptake through an effect distal to cAMP generation.

We next compared the ability of dopamine, SKF-38393, and quinpirole to stimulate cAMP. After 30 min of incubation, 10^{-5} M dopamine stimulated an increase in cAMP from 31.6 ± 3.8 to 1326.2 ± fmol/mg protein, whereas 10^{-5} M SKF-38393 stimulated an increase to 163.6 ± 7.5 fmol/mg protein cAMP, and 10^{-5} M quinpirole did not stimulate cAMP (40.3 ± 6.7 fmol/mg protein) (n = 6). Pretreatment with pertussis toxin increased dopamine-stimulated cAMP generation from 1326.2 ± 42.8 to 1876.6 ± 38.1 fmol/mg protein but had no effect on cAMP generation by either SKF-38393 (127.1 ± 5.5 fmol/mg protein) or quinpirole (32.7 ± 3.7 fmol/mg protein). To determine whether activation of DA_2 receptors would decrease dopamine stimulation of cAMP generation, the ability of 10^{-5} M dopamine to stimulate cAMP was measured in the presence or absence of 10^{-5} M quinpirole. After 30 min of incubation, dopamine stimulated 1192.8 ± 42.9 fmol/mg protein from a basal rate of 26.9 ± 0.9 fmol/mg protein. In the presence of 10^{-5} M quinpirole, dopamine stimulation of cAMP was 1460 ± 5.8 fmol/mg protein, not different from dopamine alone (n = 6). After treatment with pertussis toxin, dopamine stimulation of cAMP generation was 1758 ± 24.4 fmol/mg protein in the absence, and 1473.6 ± 32.9 fmol/mg protein in the presence, of quinpirole (P = NS, n = 6).

Dopamine can stimulate α-adrenergic receptors (26), as well as dopamine receptors, both of which are present on OK cells and proximal renal tubule cells (27). Stimulation of α-adrenergic receptors has been shown to decrease PTH-stimulated cAMP generation and inhibition of phosphate uptake (27). Therefore, the effect of α-adrenergic receptor antagonists on dopamine inhibition of phosphate uptake was examined. Neither prazosin nor yohimbine alone had a significant effect on phosphate uptake (1.4 ± 2.4% inhibition by 10^{-5} M prazosin, −0.8 ± 2.4% inhibition by 10^{-5} M yohimbine). However, as shown in Figure 7A, both prazosin and yohimbine enhanced dopamine inhibition of phosphate uptake to the same degree as pretreatment with pertussis toxin. Furthermore, after pretreatment with pertussis toxin, dopamine-stimulated cAMP generation was not significantly different between control cells (3013 ± 616 fmol/mg protein) and those treated with either prazosin (3364 ± 748 fmol/mg protein) or yohimbine (2503 ± 342 fmol/mg protein, n = 8, P = 0.2) (Figure 7B).

One common mechanism for regulation of phosphate transport in proximal tubule cells is by altering the apical membrane expression of the sodium-phosphate cotransporter protein (28). To determine whether dopamine inhibition of phosphate uptake corresponds with a decrease in the number of sodium-phosphate cotransporters, we measured the membrane expression of NaPi-4, the sodium-phosphate cotransport in OK cells by immunoblot, using antisera directed against a C-terminal peptide of the full protein. We have demonstrated previously that these antisera recognize NaPi-4 (29,30). Incubation of OK cells with 10^{-5} M dopamine for 2 h reduced membrane ex-
A

![Graph A]

**Figure 7.** Effect of α-adrenergic receptor antagonists on dopamine inhibition of phosphate uptake and stimulation of cAMP generation. (A) Effect of α-adrenergic receptor antagonists on dopamine inhibition of phosphate uptake. Phosphate uptake was measured by liquid scintillation spectroscopy of solubilized cells after treatment with 10^{-5} M dopamine, dopamine plus 10^{-5} M prazosin, or dopamine plus 10^{-6} M yohimbine for 2 h at 37°C, followed by 10-min uptake of radiolabeled phosphate at room temperature. Results are means ± SEM of seven separate experiments with prazosin and eight separate experiments with yohimbine. *P < 0.001, dopamine versus dopamine + antagonist. (B) Effect of α-adrenergic receptor antagonists on dopamine stimulation of cAMP. OK cell monolayers were incubated overnight in unsupplemented medium with pertussis toxin. Intracellular cAMP was measured by RIA after incubation with 10^{-5} M dopamine, dopamine plus 10^{-5} M prazosin, or dopamine plus 10^{-6} M yohimbine for 30 min at 30°C. Results are means ± SEM of eight separate experiments.

Discussion

PTH and dopamine are physiologic regulators of proximal renal tubule phosphate transport (8,10). As a model for proximal renal tubule, OK cells have been used extensively to investigate sodium-dependent phosphate transport and PTH signaling mechanisms (1,21). We have demonstrated previously that PTH (10^{-11} to 10^{-6} M) inhibits phosphate uptake and stimulates cAMP generation in a dose-dependent manner, with a maximal inhibition of phosphate uptake of 35% and maximal cAMP generation of 40-fold at PTH 10^{-7} M (22). The present study confirms that OK cells are also a model for the study of dopamine signaling and regulation of phosphate uptake. Dopamine produced a time- and dose-dependent stimulation of cAMP and inhibition of phosphate uptake with a peak effect of approximately 15% inhibition of phosphate uptake at a dopamine concentration of 10^{-5.5} M after 90 to 120 min of incubation. The effect of dopamine waned thereafter, with a 50% loss of inhibitory activity by 3 h. The reduced inhibition of phosphate uptake by dopamine compared to PTH in OK cells correlates with the reduced phosphaturic response seen in rats infused with dopamine compared to PTH (19,20), further validating the model. The mechanism by which dopamine inhibits sodium-dependent phosphate uptake appears to be similar to that of PTH, i.e., by downregulation of the apical membrane expression of NaPi-4, the type II sodium-phosphate cotransporter in OK cells.

Dopamine stimulates cAMP less potently than PTH, suggesting that the decreased inhibition of phosphate uptake by dopamine may be due to differences in PTH and dopamine-stimulated adenylyl cyclase. Previous studies have shown that PTH inhibition of phosphate uptake is not affected by pertussis toxin. Our data demonstrate that pretreatment with pertussis toxin potentiated dopamine-stimulated cAMP generation and enhanced the magnitude and duration of dopamine inhibition of phosphate uptake. These findings suggest that dopamine activates a pertussis toxin-sensitive pathway that attenuates dopamine inhibition of phosphate uptake. We constructed three hypotheses to explain activation of this counter-regulatory pathway, based on the ability of dopamine to stimulate multiple dopamine receptor subtypes and α-adrenergic receptors (2,10,26,27). First, dopamine could stimulate a single dopamine receptor subtype that activates two pathways, one that inhibits phosphate uptake and another that attenuates this response. Second, dopamine could stimulate two dopamine receptor subtypes that activate pathways exerting opposing effects on phosphate uptake. Third, dopamine could stimulate dopamine receptors coupled to a pathway that inhibits phosphate uptake and α-adrenergic receptors coupled to a counter-regulatory pathway.

Initially, we tested these hypotheses by examining the effect of specific DA_1 and DA_2 dopamine receptor agonists and antagonists on sodium-dependent phosphate uptake. These experiments demonstrated that dopamine stimulates cAMP and inhibits phosphate uptake primarily through activation of DA_1 receptors. Dopamine inhibition of phosphate uptake and stimulation of cAMP were completely abolished by pretreatment...
with a DA₁ receptor antagonist. Inhibition of phosphate uptake by the specific DA₁ agonist SKF-38393 was greater than inhibition by dopamine, but was similar to inhibition seen after pertussis toxin pretreatment of dopamine-stimulated cells, and was not increased by pretreatment with pertussis toxin. Thus, stimulation of DA₁ receptors alone is sufficient for inhibition of phosphate uptake. The lack of effect of pertussis toxin on DA₁ receptor-mediated inhibition of phosphate uptake and cAMP generation indicates that DA₁ receptors are not coupled to a pertussis toxin-sensitive pathway. Therefore, dopamine activates more than one type of receptor.

Although stimulation of DA₁ receptors alone appears to be sufficient for inhibition of phosphate uptake, our data suggest that DA₂ receptors may also contribute to dopamine inhibition of phosphate uptake. The DA₂ receptor agonist quinpirole inhibited phosphate uptake, albeit to a far lesser degree than either dopamine or SKF-38393. Quinpirole predictably did not stimulate cAMP generation, and the effect of quinpirole was not altered by pertussis toxin pretreatment. At high concentrations, the DA₂ receptor antagonist sulpiride completely blocked dopamine inhibition of phosphate uptake and partially prevented inhibition of phosphate uptake by SKF-38393. Even at a lower concentration more selective for DA₂ receptor antagonism (25), sulpiride still partially blocked dopamine inhibition of phosphate uptake. Although DA₁ and DA₂ receptor activation ostensibly have mutually antagonistic effects on adenylyl cyclase activation, a synergistic interaction between DA₁ and DA₂ receptors has also been demonstrated for dopamine regulation of renal cortical Na-K-ATPase by Bertorello and Aperia (31) and for dopamine-stimulated responses in neural tissue (32). The mechanism for the antagonistic effect of sulpiride on dopamine inhibition of phosphate uptake cannot be determined from our data, because sulpiride did not block dopamine-stimulated cAMP generation. However, the ability of sulpiride to block a physiologic response of dopamine despite a failure to inhibit dopamine-stimulated cAMP has been reported previously (33).

Our demonstration that 10⁻⁷ M sulpiride still partially prevents dopamine inhibition of phosphate uptake is at variance with the report of Perrichot et al. (24), who demonstrated that 10⁻⁷ M sulpiride enhanced dopamine inhibition of phosphate uptake. Because DA₂ receptors are coupled to a pertussis toxin-sensitive pathway leading to inhibition of adenylyl cyclase, the authors postulated and demonstrated that sulpiride enhanced dopamine-stimulated cAMP generation. The precise reasons for the discrepancy in the results of these two studies cannot be resolved; however, the techniques for activation of dopamine receptors differed significantly in the two studies. Perrichot and colleagues activated dopamine receptors by treating the OK cells with l-dopa, from which the cells made dopamine endogenously. In the present study, exogenous dopamine was added directly to the cells. The differences in the two techniques may have resulted in disparate effects on receptor subtype expression or activation. Another potential explanation is that the two populations of OK cell lines may differ in the expression of dopamine receptor subtypes or components of the dopamine-stimulated signaling pathways.

Because we could not demonstrate activation of a pertussis toxin-sensitive pathway coupled to cAMP generation by DA₁ or DA₂ receptors, we examined the possibility that dopamine-stimulated α-adrenergic receptors. We postulated that stimulation of α-adrenergic receptors would inhibit adenylyl cyclase through a pertussis toxin-sensitive pathway and that treatment of the cells with α-adrenergic receptor antagonists would potentiate dopamine inhibition of phosphate uptake and dopamine stimulation of cAMP. As predicted, treatment with α-adrenergic receptor antagonists increased dopamine inhibition of phosphate uptake. Pretreatment with pertussis toxin did not further potentiate either dopamine inhibition of phosphate uptake or dopamine stimulation of cAMP in the presence of α-adrenergic antagonists, suggesting that the α-adrenergic receptors stimulated by dopamine are coupled to a pertussis toxin-sensitive pathway. Our data do not allow us to state with certainty which α-adrenergic subtype is activated by dopamine. OK cells express α₁- and α₂-adrenergic receptors that were initially cloned from OK cells and that exhibit a unique pharmacologic profile (34). The equivalent ability of both prazosin and yohimbine, albeit at relatively high concentrations, to enhance dopamine inhibition of phosphate uptake is consistent with the pharmacology of α₁- and α₂-adrenergic receptors.

Our data suggest that dopamine-stimulated cAMP is neither essential nor sufficient for mediating dopamine inhibition of phosphate uptake in OK cells. Dopamine stimulation of cAMP and inhibition of phosphate uptake tend to parallel one another, and both are enhanced by pertussis toxin. cAMP generation and inhibition of phosphate uptake by SKF-38393 are both abolished by DA₁ receptor antagonism. The inability of dopamine to inhibit phosphate uptake in the cAMP phosphodiesterase overexpression clone is restored by treatment with a cAMP-specific phosphodiesterase inhibitor. However, several findings suggest that dopamine inhibits phosphate uptake through activation of other signaling pathways. SKF-38393 more potently inhibited phosphate uptake than dopamine despite inferior cAMP generation. In addition, the DA₂ antagonist sulpiride blocked dopamine inhibition of phosphate uptake without affecting cAMP generation. Also, inhibition of protein kinase A activation by H89 and RpAMPs had little or no effect on dopamine inhibition of phosphate uptake. These latter data appear to contradict the findings in the OK cells overexpressing phosphodiesterase, because presumably in both conditions hormone-stimulated protein kinase A activation would be inhibited. It is possible that the two populations of OK cells express different dopamine receptors or different signal transduction pathways. Alternatively, inhibition of cAMP phosphodiesterase may have effects other than simply prevention of protein kinase A activation, or the protein kinase A inhibitors may have other pharmacologic effects as well.

We conclude that dopamine regulates phosphate transport in OK cells in a complex manner, by activating several interacting signal pathways. Through stimulation of DA₁ and DA₂ receptors, dopamine inhibits sodium-dependent phosphate uptake by downregulation of the membrane expression of NaPi-2A. Activation of adenylyl cyclase is sufficient but not essential for this response. DA₁ or DA₂ receptors may activate another, as...
yet unidentified, signaling system that also inhibits phosphate transport. Additionally, through stimulation of α-adrenergic receptors, dopamine stimulates a pertussis toxin-sensitive pathway that attenuates dopamine-mediated inhibition of phosphate uptake and cAMP generation.

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