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ALPHA-2-ADRENERGIC MODULATION OF THE PARATHYROID HORMONE-INHIBITION OF PHOSPHATE UPTAKE IN CULTURED RENAL (OK) CELLS

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SUMMARY: Parathyroid hormone enhances the formation of cAMP and decreases the Na^+-dependent uptake of phosphate in cultured renal cells derived from the American opossum (OK cells). Epinephrine, acting as an α_2-adrenergic agonist, inhibits the PTH-induced synthesis of cAMP by a pertussis toxin-sensitive mechanism and blunts the inhibition of phosphate transport by PTH. Na^+-dependent α-methylglucoside and Na^+ uptakes by the cells are unaffected by PTH and epinephrine. These findings suggest that α_2-adrenergic agonists may selectively modulate PTH-sensitive phosphate transport in the renal proximal tubule.

Both α_1 and α_2 adrenergic receptors have been identified in the renal tubule, with the apparent dominance of the α_2 receptor (1-4). In many tissues the effects of α_2 agonists appear to be mediated through interaction with G_i protein and inhibition of adenylate cyclase (5). In the isolated perfused rat kidney activation of the α_2-adrenergic receptor reverses vasopressin induced Na^+ and water retention (6), and in the cortical collecting tubule α_2-agonists blunt vasopressin-induced synthesis of cAMP and increases in water permeability (7). These findings suggest that in the kidney α_2-agonists may regulate the physiological action of vasopressin through inhibition of adenylate cyclase.

Parathyroid hormone (PTH) is a major hormonal effector of phosphate reabsorption in the renal proximal tubule (8). Administration of the hormone into the animal results in a rise in urinary cAMP followed by phosphaturia (9). Incubation, *in vitro*, of PTH with renal cortical slices causes concomitant increases in cAMP and cAMP-dependent protein kinase activity, and a decrease in phosphate uptake in brush border membranes isolated from the proximal tubules of the cortical slices (10). Whether α_2-adrenergic hormones act in the proximal tubule to modulate the action of PTH on phosphate transport has not been reported.

A continuous cell line derived from the American opossum possesses properties of the proximal tubule (11), including a PTH-inhibitable Na^+-dependent phosphate transport system (12-14) and a PTH-stimulatable adenylate cyclase system (15). In addition, recently, α_2-adrenergic receptors in the OK cell have been

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characterized (16). In this communication, we report that epinephrine, acting in OK cells as an α_2-adrenergic hormone, inhibits the PTH-induced formation of cAMP by a pertussis toxin-sensitive mechanism and blunts the PTH-induced inhibition of phosphate uptake.

METHODS

Cell culture. The original culture of OK cells was provided by Dr. Joseph Handler of the NIH. The cells were maintained in 60 mm diameter plastic petri dishes in a humidified atmosphere of 5% CO_2-95% air at 37°C for 5 to 6 days until confluent. The growth medium was low glucose (1 g/l) bicarbonate-buffered Delbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, penicillin 50 µg/ml, streptomycin 50 µg/ml, and neomycin 100 µg/ml. Cells were subcultured by trypsinization with 0.5% trypsin in Ca^{2+}-Mg^{2+}-free phosphate-buffered saline containing 1 mM EDTA. All studies were performed with cells between passages 65 and 86. For experiments, the cells were grown in 35 mm diameter dishes for 6 days to confluency. One hr prior to the addition of test compounds the medium was changed to DMEM without bicarbonate, the pH of the medium having been adjusted to 7.4 with 20 mM Hepes-KOH, and the cells preincubated at 37°C in air.

cAMP measurement. To the monolayer cell culture in 1 ml DMEM was added 10 µl of hormone solution, test compound or vehicle solution. The incubation was carried out for 30 min at 37°. The reaction was stopped by the addition of 50 µl of 60% perchloric acid. Aliquots were taken for the measurement of total cAMP. cAMP was determined by radioimmunoassay (Immuno Nuclear).

Transport studies. Cells were incubated with the test agent for 30 min at 37° as described above. The cells were washed with a medium containing 300 mM mannitol, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 4 mM KCl, and 10 mM Hepes-KOH, adjusted to pH 7.4. Phosphate uptake was initiated by adding to the monolayer 1 ml of medium containing 0.1 mM [^{32}P]-phosphate, 150 mM NaCl, 5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 2.5 mM glutamine and 10 mM Hepes-KOH, adjusted to pH 7.4. Uptake was terminated by rapid removal of the medium and washing the cells four-times with same ice-cold medium, without phosphate. The cells were solubilized with 0.5 N NaOH and radioactivity determined by liquid scintillation. Uptakes of labeled α-methylglucoside and Na^+ were carried out as described for phosphate, except that the concentrations of sugar and Na^+ were 0.05 mM and 150 mM, respectively. The results are reported on the mean ± S.E. Student's paired t-test was used for statistical analyses and a p value < 0.05 was considered significant.

Protein determination. The cell monolayer was solubilized in 0.5 N NaOH. Protein was measured by the Lowry procedure (17) using bovine serum albumin as the reference protein.

Materials. Synthetic bovine PTH (1-34) was obtained from Peninsula Laboratories. Cell culture media was purchased from GIBCO. [^{32}]-phosphoric acid (carrier-free), $^{22}Na^+$ and [^{3}H]α-methylglucoside were obtained from New England Nuclear.

RESULTS AND DISCUSSION

Fig. 1 describes the effect of incubating OK cells for 30 min with increasing concentrations of PTH on the formation of cAMP, in the absence (A) and presence (B) of epinephrine. These experiments, carried out in the absence of an added

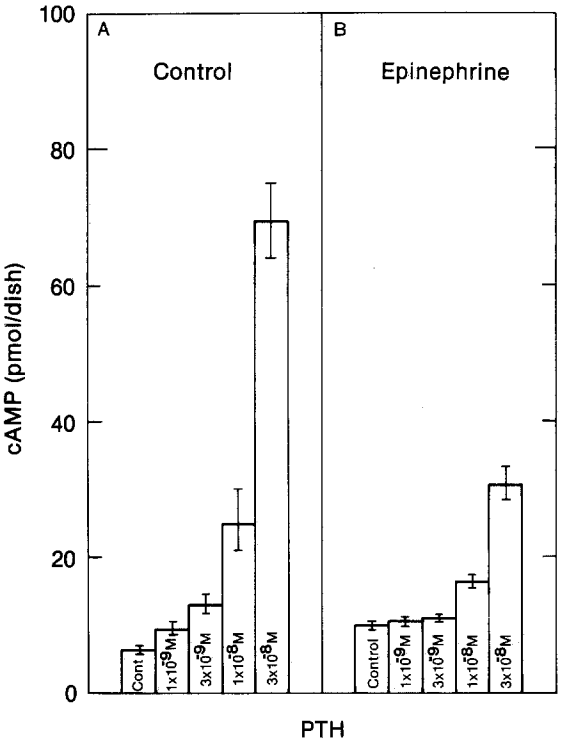

Figure 1. Effects of PTH (A) and PTH plus (-)-epinephrine (B) on total cAMP production. Cells were incubated with various concentrations of PTH, $1x10^{-5}$ M dl-propranolol, without or with $6x10^{-6}$ M epinephrine for 30 min. The data represent the mean ± SE of 6 experiments.

phosphodiesterase inhibitor and in the presence of 1×10^{-5} M dl-propranolol, showed that PTH increased cAMP production in a dose-dependent manner. cAMP was measurably increased with 1×10^{-9} M PTH and 3×10^{-8} M PTH increased cAMP more than 10-fold, from a basal value of 6.3 ± 0.6 pmol/dish to 68.0 ± 7.1 pmol/dish. When 6×10^{-6}M (-)-epinephrine was present in the incubations, cAMP formed was decreased. For example, with 3×10^{-8} M PTH, the level of cAMP was lowered from 68.0 ± 7.1 in the absence of the catecholamine to 30.8 ± 2.0 pmol/dish in its presence. These results suggested that epinephrine, acting as an α-adrenergic agonist, inhibited PTH-stimulated cAMP production in OK cultured renal cells.

To examine the type of α-adrenergic receptor that mediated the blunting of PTH-stimulated adenylate cyclase, the effects of yohimbine, an α_2-adrenergic antagonist, and prazosin, an α_1-adrenergic antagonist, were examined. As shown in Fig. 2A, yohimbine blocked the action of epinephrine in a dose-dependent manner. In these experiments, the PTH-induced cAMP level was 49.5 ± 4.6 pmol/dish and in the presence of epinephrine the cAMP level was decreased to 23.5 ± 3.0. When 6×10^{-7} M yohimbine was added, cAMP formation was 47.3 ± 7.5 pmol/dish, a level not significantly different from that found with PTH alone.

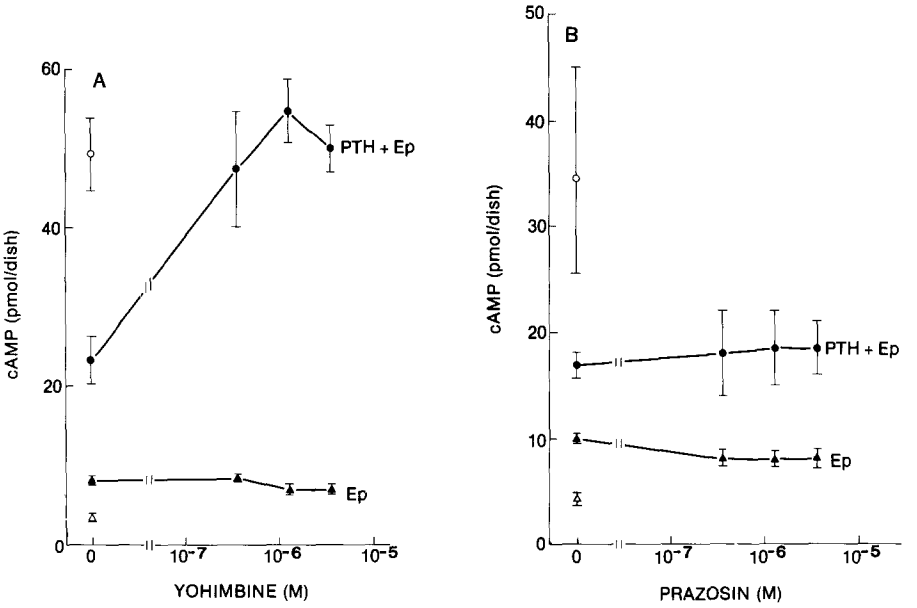

Figure 2. Effects of yohimbine and prazosin on total cAMP production. Cells were incubated without (Δ) or with $3x10^{-8}$ M PTH (o), $6x10^{-6}$ M epinephrine (▲) or PTH plus epinephrine (●) in the presence of $1x10^{-5}$ M dl-propranolol and various concentrations of yohimbine and prazosin. Each value represents the mean ± SE of 4 experiments.

Yohimbine had no effect on the cAMP produced in the absence of PTH. In contrast, prazosin at all tested concentrations had no appreciable effect on the inhibition by epinephrine of PTH-stimulated adenylate cyclase. These findings suggested that the action of epinephrine in suppressing PTH-induced elevation in cAMP in the OK cell was mediated by the α_2-adrenergic receptor, supporting conclusions from studies with other renal preparations (18-20) but differing from studies by other investigators using renal cortical homogenates or microdissected single nephron segments (21,22).

To be noted in Fig. 2 as well as in Fig. 1, epinephrine, by itself, caused a relatively small, but statistically significant increase in cAMP generation. This was not due to its action as a β-adrenergic agonist, because 1×10^{-5} M propranolol was added to all incubation mixtures and the effect of epinephrine in stimulating adenylate cyclase in the OK cell could not be mimicked by the β-agonist isoproterenol (6×10^{-6} M). Instead, this effect of epinephrine appears to be due to its weak interaction with dopamine receptors abundently present in the OK cell (to be reported separately).

Additional evidence that the blunting of the PTH-stimulated adenylate cyclase by epinephrine involved an α_2-adrenergic response mediated via G_i protein came from experiments with pertussis toxin (PT). OK cells were preincubated with activated PT (100 ng/ml) for 2 hrs. As shown in Fig. 3, PT had little effect on the basal level of cAMP formation (bar 5 vs bar 1) or on the small increment of

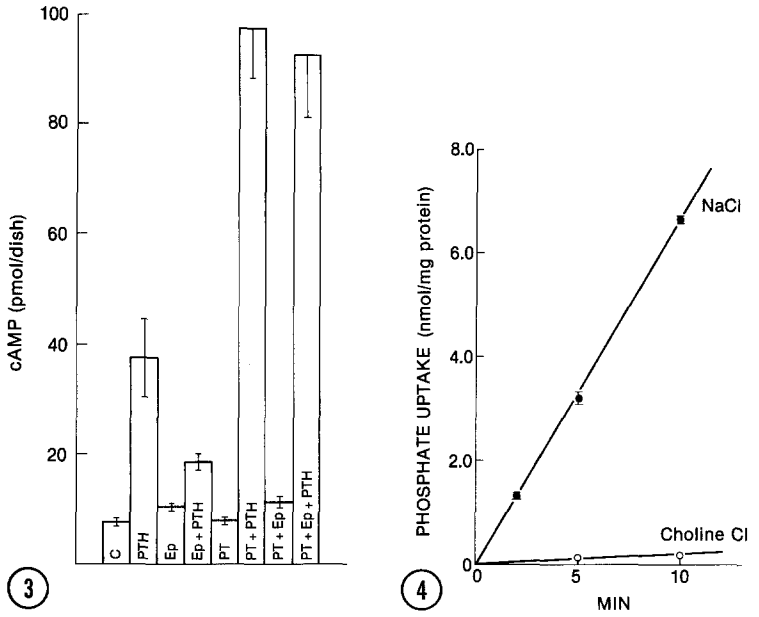

Figure 3. Effects of pertussis toxin (PT) on PTH and PTH plus epinephrine stimulated cAMP production. Cells were incubated with no toxin (control) or 100 ng/ml activated PT for 2 hr. Cells were then incubated with 3×10^{-8} M PTH or 6×10^{-6} M epinephrine or PTH plus epinephrine for 30 min. The data represent the mean ± SE for 5 experiments. PT was activated by incubation with 20 mM DTT, 50 mM Tris-Cl, pH 8.0, at room temperature for 60 min.

Figure 4. The time course of phosphate uptake by OK cells. Uptakes were measured with 150 mM NaCl (●) or choline chloride (o) in the extracellular medium. Each experiment was carried out in quadruplicate. The data represent the mean ± SE of 3 experiments. When the standard bar error is not drawn, the error is less than the illustrated value.

cAMP formed in the presence of epinephrine (bar 7 vs bar 3). However, the epinephrine-inhibition of PTH-induced cAMP generation was completely negated by pretreatment of the cells with toxin (bar 8 vs bar 4). The data in Fig. 3 also shows the substantial increase in the amount of cAMP generated when cells incubated with a saturating concentration of PTH were pretreated with PT (bar 6 vs bar 2). Since PT-catalyzed ADP-ribosylation of $G_{i\alpha}$ presumably blocked the action of the inhibitory component, this finding indicated that the G_i inhibitory pathway strongly modulated the response of the adenylate cyclase system to PTH.

Next, we determined the effect of the α_2-adrenergic agonist on PTH-inhibited phosphate uptake. Initial experiments, illustrated in Fig. 4, demonstrated that phosphate uptake into OK cells was dependent on extracellular Na^+ and was linear with time for at least 10 min. Na^+-independent uptake was very small and was neglected in subsequent experiments. Na^+-dependent uptake could be consistent with the mechanism of phosphate transport in the renal proximal tubule and reflected flux across the luminal brush border membrane (12,23,24). In subsequent determinations of the effects of hormones on phosphate uptake the

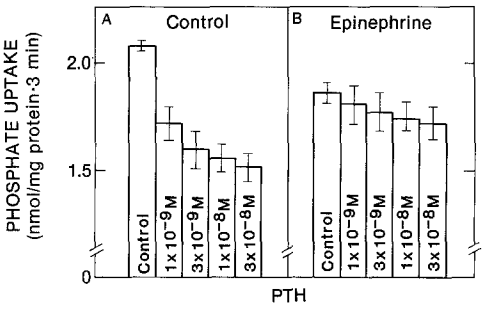

Figure 5. Inhibition of Na^+-dependent phosphate transport in OK cells as a function of PTH concentration. Cells were incubated with various concentrations of PTH, $1x10^{-5}$ M dl-propranolol, without (A) or with (B) $6x10^{-6}$ M epinephrine for 30 min. Each value represents mean ± SE of 4 experiments.

incubations were terminated after 3 min. Since the rate of uptake was linear for this period, the uptake represented the initial rate of transport and reflected the influx of phosphate into the cell.

Fig. 5 shows the effect of incubating OK cells for 30 min with increasing concentrations of PTH on Na^+-dependent phosphate uptake in the absence (A) and presence (B) of epinephrine. As shown in Fig. 5A, the inhibition of phosphate uptake by PTH was dose-dependent and in the same range of concentration that elicited formation of cAMP (Fig. 1A). With 3×10^{-8} M PTH, phosphate uptake was decreased about 25% compared to control rates, 1.52 ± 0.07 vs 2.08 ± 0.03 nmol·mg^{-1} cell protein·3 min^{-1}. Longer incubations of the cells with PTH (2 to 3 hrs) increased the inhibition of phosphate uptake to 50% or more (data not shown), but these prolonged incubations were not employed in the present study in order to compare more directly hormonal effects on both phosphate transport and cAMP formation. Epinephrine blunted the inhibition of phosphate uptake at all tested concentrations of PTH (Fig. 5B). With 1×10^{-9} M PTH, phosphate uptake was inhibited 17%; however, in the presence of 6×10^{-6} M epinephrine uptake was decreased by only 3%. With 3×10^{-8} M PTH, phosphate transport was inhibited 27 and 8% in the absence and presence of epinephrine, respectively. Also to be noted was the relatively small inhibition caused by epinephrine alone in the absence of PTH. This would be consistent with the relatively small amount of cAMP formed after the cells were incubated with the catecholamine (Figs. 1 and 2).

The extent of reversal of PTH-inhibited phosphate uptake by epinephrine was dependent on the concentration of the catecholamine. As shown in Table I, partial blunting was found with 1×10^{-6} M epinephrine and nearly complete prevention of the inhibition of PTH could be achieved with 6×10^{-5} M epinephrine.

The data in Table II demonstrate that the action of epinephrine in reversing the inhibition of phosphate uptake by PTH was mediated by the α_2-adrenergic receptor. Yohimbine negated the effect of epinephrine in reversing

TABLE I. EFFECT OF THE CONCENTRATION OF EPINEPHRINE IN BLUNTING PTH-INHIBITED PHOSPHATE UPTAKE

	% Inhibition
PTH	25 ± 3
PTH + Epinephrine (1×10^{-6} M)	19 ± 2
PTH + Epinephrine (6×10^{-6} M)	13 ± 3
PTH + Epinephrine (6×10^{-5} M)	4 ± 3

Each value represents the mean ± SE of 3 experiments. Cells were incubated with 3×10^{-8} M PTH and the indicated concentration of epinephrine for 30 min in the presence of 1×10^{-5} M dl-propranolol. The values represent the percent inhibition relative to control values obtained in the absence of PTH.

PTH-inhibited phosphate influx, 1.18 ± 0.07 vs 1.26 ± 0.07 nmol·mg^{-1}·2 min^{-1} ($p < 0.001$), decreasing phosphate uptake to the same rate found with PTH itself, 1.18 ± 0.07. In contrast, prazosin had no effect, 1.26 ± 0.07 vs 1.25 ± 0.08 nmol.mg^{-1}·2 min^{-1} (p = N.S.) when added to an incubation reaction containing PTH and epinephrine. Table II also shows that neither yohimbine nor prazosin affected phosphate uptake in the absence of PTH. In addition, determinations of phosphate uptake and cAMP formed in the same experiments revealed the strong negative correlation between the effects of the test agents on transport and cAMP. This finding supports the view that the action of α_2-adrenergic hormone in modulating the inhibition of phosphate uptake by PTH resulted from the regulation of cAMP production.

TABLE II. THE EFFECTS OF EPINEPHRINE ACTING AS AN α_2-ADRENERGIC AGONIST IN MODULATING PTH-INHIBITED PHOSPHATE UPTAKE AND PTH-STIMULATED cAMP PRODUCTION

	cAMP pmol/dish	Phosphate uptake nmol·mg^{-1}·2 min^{-1}
Control	7.1 ± 0.5	1.57 ± 0.10
PTH	42.3 ± 2.4	1.18 ± 0.07[a]
Epinephrine	10.8 ± 0.5	1.44 ± 0.07
PTH + Epinephrine	18.0 ± 1.9	1.26 ± 0.07[a,b,c]
PTH + Epinephrine + yohimbine	36.5 ± 2.9	1.18 ± 0.07[b]
Epinephrine + yohimbine	8.5 ± 0.5	1.41 ± 0.08
PTH + Epinephrine + prazosin	20.0 ± 1.9	1.25 ± 0.08[c]
Epinephrine + prazosin	9.3 ± 0.7	1.39 ± 0.09

Each value represents mean ± SE of 8 experiments. Cells were incubated with 3×10^{-8} M PTH, 6×10^{-6} M epinephrine, 6×10^{-6} M yohimbine or 6×10^{-6} M prazosin for 30 min in the presence of 1×10^{-5} M dl-propranolol and 0.2% DMSO.

[a]PTH vs PTH + Epinephrine $p < 0.001$
[b]PTH + Epinephrine vs PTH + Epinephrine + yohimbine $p < 0.001$
[c]PTH + Epinephrine vs PTH + Epinephrine + prazosin N.S.

TABLE III. THE EFFECTS OF PTH AND EPINEPHRINE ON Na^+ DEPENDENT α-METHYLGLUCOSIDE AND Na^+ UPTAKES

	α-methylglucoside uptake $nmol \cdot mg^{-1} \cdot 5\ min^{-1}$	Na^+ uptake $nmol \cdot mg^{-1} \cdot 2\ min^{-1}$
Control	0.12 ± 0.02	74 ± 1
PTH	0.12 ± 0.02	72 ± 1
Epinephrine	0.11 ± 0.01	70 ± 3
PTH + Epinephrine	0.12 ± 0.02	72 ± 1

Each value represents mean ± SE of 3-4 experiments. Cells were incubated with 3×10^{-8} M PTH, 6×10^{-6} M epinephrine or PTH plus epinephrine for 30 min in the presence of 1×10^{-5} M dl-propranolol.

To determine the specificity of the actions of PTH and epinephrine on Na^+-dependent phosphate transport in the OK cell, we examined whether Na^+-dependent α-methylglucoside uptake was affected. The data in Table III shows that the transport of the sugar was not altered by these hormones. In addition, since Na^+-dependent phosphate transport could be influenced by agents that altered the Na^+ gradient, we tested whether PTH and epinephrine changed the uptake of extracellular Na^+. It was found that Na^+ uptake was not significantly affected. Thus, the actions of PTH and epinephrine appear to be relatively specific to the phosphate transport system.

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