J. Membrane Biol. 124, 227–237 (1991)

The Journal of
Membrane Biology
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Apical and Basolateral Effects of PTH in OK Cells: Transport Inhibition, Messenger Production, Effects of Pertussis Toxin, and Interaction with a PTH Analog

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Summary. The cellular distribution (apical *vs.* basolateral) of parathyroid hormone (PTH) signal transduction systems in opossum kidney (OK) cells was evaluated by measuring the action of PTH on apically located transport processes (Na/P_i cotransport and Na/H exchange) and on the generation of intracellular messengers (cAMP and IP_3).

PTH application led to immediate inhibition of Na/H-exchange without a difference in dose/response relationships between apical and basolateral cell-surface hormone addition (half-maximal inhibition at $\approx 5 \times 10^{-12}$ M). PTH required 2–3 hr for maximal inhibition of Na/P_i cotransport with a half-maximal inhibition occurring at $\approx 5 \times 10^{-10}$ M PTH for basolateral application and $\approx 5 \times 10^{-12}$ M for apical application. PTH addition to either side of the monolayer produced a dose-dependent production of both cAMP and IP_3. Half-maximal activation of IP_3 was at about 7×10^{-12} M PTH and displayed no differences between apical and basolateral hormone addition, while cAMP was produced with a half maximal concentration of 7×10^{-9} M for apical PTH application and 10^{-9} M for basolateral administration.

The PTH analog [nle^{8,18},tyr^{34}]PTH(3–34), (nlePTH), produced partial inhibition of Na/P_i cotransport (agonism) with no difference between apical and basolateral application. When applied as a PTH antagonist, nlePTH displayed dose-dependent antagonism of PTH inhibition of Na/P_i cotransport on the apical surface, failing to have an effect on the basolateral surface. Independent of addition to the apical or basolateral cell surface, nlePTH had only weak stimulatory effect on production of cAMP, whereas high levels of IP_3 could be measured after addition of this PTH analog to either cell surface. Also an antagonistic action of nlePTH on PTH-dependent generation of the internal messengers, cAMP and IP_3, was observed; at the apical and basolateral cell surface nlePTH reduced PTH-dependent generation of cAMP, while PTH-dependent generation of IP_3 was only reduced by nlePTH at the apical surface.

Pertussis toxin (PT) preincubation produced an attenuation of both PTH-dependent inhibition of Na/P_i cotransport and IP_3 generation while producing an enhancement of PTH-dependent cAMP generation; these effects displayed no cell surface polarity, suggesting that PTH action through either adenylate cyclase or phospholipase C was transduced through similar sets of G-proteins at each cell surface.

It is concluded that apparent receptor activities with high and low affinity for PTH exist on both cell surfaces; those with apparent high affinity seem to be coupled preferentially to phospholipase C and those with apparent low affinity to adenylate cyclase. The differences in apparent affinity of receptor events coupled to adenylate cyclase and the differences in PTH/nlePTH interaction on the two cell surfaces are suggestive of the existence of differences in apparent PTH-receptor activities on the two cell surfaces.

Key Words cell polarity · pertussis toxin · parathyroid hormone (PTH) · PTH-analogs · internal messengers

Introduction

Parathyroid hormone (PTH) is an important regulator of renal proximal tubular reabsorption of phosphate (P_i) and bicarbonate, and its action involves an inhibition of apically located Na/P_i cotransport and Na/H exchange, respectively (Alpern, 1990; Murer et al., 1991). Cell model systems, particularly that from the opossum kidney (OK), have contributed to the understanding of the mechanisms involved in the regulation of these transport processes by PTH. Studies with OK cells cultured on plates (impermeant supports) and measuring the dose/response PTH relationships between intracellular messenger production, the stimulation of PKA and/or PKC activity and membrane protein phosphorylation patterns have suggested that PTH action may be mediated by both protein kinase A and C; this conclusion was also supported by studies of pharmacological activation of protein kinases (Cole et al., 1987; Malmström, Stange & Murer, 1988; Quamme, Pfeilschifter & Murer, 1989; Dunlay & Hruska, 1990; Murer et al., 1991; Reshkin & Murer, 1991). These studies might suggest that, in OK cells, PTH concentrations below 10^{-10} M function preferentially through the PKC pathway, while above 10^{-10} M both signal transduction systems function together

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(Murer et al., 1991). Furthermore, studies with agonist/antagonist-PTH analogs (Cole et al., 1987, 1988, 1989; Caulfield & Rosenblatt, 1990; Muff, Caulfield & Fischer, 1990) and receptor studies (*see* Nissenson & Klein, 1987; Caulfield & Rosenblatt, 1990; Dunlay & Hruska, 1990) have suggested that there may be separate classes of PTH receptors linked to the different regulatory cascades.

In most of the previous studies using the OK-cell system, monolayers were grown on impermeant supports; thus, it has to be assumed that the signal transducing mechanisms were accessible at the apical cell surface. However, recent use of OK-cell monolayers grown on permeant filters has permitted the elucidation of both the distribution of PTH-responsive transport systems and the 'polarity' of PTH-dependent signal transduction systems (Reshkin, Forgo & Murer, 1990*a*,*b*; Helmle-Kolb, Montrose & Murer, 1990*a*). Although PTH inhibits only transport systems located on the apical cell surface (Na/P_i cotransport, NaH exchange), PTH action can be initiated by hormone addition to either cell surface, suggesting the existence of receptors on both cell surfaces of the monolayer. Though both transport systems displayed equal concentration dependence for inhibition by apical PTH application, the Na/H exchanger was about a 100-fold more sensitive to basolateral PTH than was the Na/P_i cotransporter (Helmle-Kolb et al., 1990*a*; Reshkin et al., 1990*b*). These data suggest that there may be distributional and/or functional differences between the PTH receptors located on the two cell surfaces.

Although much has been learned about the PTH-dependent secondary messenger pathways from the above described studies of cells grown on impermeant supports, nothing is known about the intermediary steps from basolateral PTH/receptor interaction to final physiological event at the apical membrane. In the present report we describe an analysis of 'polarity' (apical *vs.* basolateral) of PTH-dependent signal transducing mechanisms. Although we observe receptor-linked events with high affinity, most likely coupled to phospholipase C, and those with low affinity, coupled to adenylate cyclase, on both cell surfaces, the use of the PTH analog [$nle^{8,18}$,tyr^{34}]PTH(3–34) as agonist/antagonist suggests some differences between apically and basolaterally located signal transduction systems. The use of pertussis toxin disclosed that there were no differences between the apical and basolateral cell surfaces in the involvement of G-proteins in PTH-dependent signal transduction.

Materials and Methods

All cell culture supplies were obtained from Amimed, Basel, Switzerland, or GIBCO, Basel, Switzerland. Bovine PTH-(1–34) and [$Nle^{8,18}$, Tyr^{34}]PTH-(3–34) was purchased from Calbiochem (Basel, Switzerland). 2-^{3}[H]-inositol(1,4,5)trisphosphate (20–60 Ci/mmol), ^{3}[H]-cAMP (20–30 Ci/mmol) and their unlabeled analogs were purchased from Amersham International. All salts were analytical grade and purchased from commercial sources.

Cell Culture

OK cells, an established continuous cell culture from opossum kidney (Koyama et al., 1978; Malmström & Murer, 1986) were grown in monolayer culture in Dulbeccos modified Eagle's medium (DMEM/Hams F12) (1 : 1) supplemented with 10% fetal calf serum (FCS), 22 mM $NaHCO_3$, 20 mM HEPES, 2 mM L-glutamine, 50 IU/ml penicillin and 50 μg/ml streptomycin in a humidified atmosphere of 5% CO_2, 95% air at 37°C. Subcultures were prepared by trypsinizing and reseeding at high density (approx. 5×10^5 cells/ml) in either 75 cm^2 flasks or on filters (Malmström & Murer, 1986; *see also* below).

Monolayers on permeable filter support were grown on Millicell®-CM filter inserts (Millipore, 12 mm diameter, 0.45 μm pore size) coated with a very thin film of rat-tail collagen (R-type, Serva; 0.5 mg/ml in 50% EtOH). Cells from confluent monolayers were trypsinized and seeded at approximately $1–2 \times 10^5$ cells/filter in the above growth medium under a 5% CO_2 atmosphere. The medium was changed after 1–2 hr of seeding, and then cells were refed fresh medium every 12 hr. Monolayers just reached confluency in 24 hr and transport or messenger level studies were commenced 36 hr after seeding (Reshkin et al., 1990*a*).

Transport Assays

Transport measurements were performed in uptake media consisting of (in mM) 137 NaCl (replaced by TMA-Cl for Na-independent transport); 5.4 KCl; 2.8 $CaCl_2$; 1.2 $MgSO_4$ and 10 HEPES/Tris (pH 7.4). For Na/P_i-cotransport transport assays, growth medium was aspirated and both sides of the monolayer were gently rinsed twice in substrate-free TMA uptake solution at 25°C (Reshkin et al., 1990*a*). For Na/H-exchange assays, monolayers were first acidified by the ammonium-prepulse method as described by Haggerty et al. (1990) and then quickly treated as in the P_i-transport assay. For uptake assay, filter insert monolayers were then placed in a 24-well culture plate (Nunclon). TMA-Cl solution was added to the compartment opposite that in which transport would be measured. Transport was initiated by adding 500 μl of the NaCl uptake solution containing the desired radioactive substrate to the opposite filter insert compartment. Na/P_i cotransport was measured at 0.1 mM $^{32}P_i$ and Na/H exchange at 2 mM ^{22}Na. At the end of the incubation period uptake was stopped and nonspecifically bound radioactivity effectively removed (*data not shown*) by rapid aspiration of the uptake solution and careful rinsing (6×) of the filter and filter insert in an ice-cold isotonic solution containing 100 mM of the cold substrate. Nonspecific binding (blanks) was assessed measuring zero time uptake by starting uptake, and immediately aspirating the uptake solution and processing the filters as above. Nonspecific binding was below 10% of radioactivity associated with any experimental point. Total radioactivity incorporated into the monolayer was measured by liquid scintillation counting of the whole filter insert in 10 ml of Packard® 399 scintillation fluid in a Kontron® counter.

Cyclic AMP Assay

Cyclic-AMP (cAMP) formation in response to hormones was measured essentially as previously described (Malmström & Murer, 1986; Malmström et al., 1988). Briefly, cell monolayers

that had been treated with hormone (±1 mM IBMX depending on the experiment) were rinsed twice with ice-cold assay buffer A (in mM: 50 Tris/HCl, 16 β-mercaptoethanol, 8 theophylline, pH 7.4). The reaction was stopped by dipping the monolayer directly into liquid nitrogen and the filter apparatus was stored at −20°C until assayed (generally within 24 hr). The monolayer was cut out of the filter apparatus and placed into an Eppendorf tube containing 100 μl of the assay buffer plus 10 μl 0.1 N HCl, and the cells were disrupted by sonication with three pulses of 1 sec using a Labsonic 1510 (Braun, Zurich, Switzerland) at approximately 200 watts/pulse. The sample was then neutralized with 10 μl 0.1 N NaOH, and the filter/disrupted cells were removed by rapid centrifugation in an Eppendorf centrifuge (14000 rpm for 15 sec). The cAMP was determined in a 50 μl aliquot of the supernatent by a competitive protein binding assay as described by Brown, Ekins and Albano (1972).

Inositol (1,4,5)-Trisphosphate (IP_3) Assay

Previous to experimental manipulation, monolayers were preincubated with growth media containing 20 μM LiCl for 20 min in order to inhibit the inositol phosphate phosphomonoesterases (Dean & Beaven, 1989). After treatment for the prescribed time with the various effectors, the monolayers were quickly rinsed with the above assay buffer and dipped into liquid nitrogen to stop the reaction. Filters were stored at −70°C until analysis (generally within 24 hr). The filter was removed as above from the filter insert and placed in 50 μl 10% TCA in an Eppendorf tube. The monolayer was extracted for 20 min at 4°C, spun 2 min at 14000 rpm, the supernatent removed and the filter rinsed with 50 μl H_2O, spun again and the two supernatents were added together. The pH of this solution (5% TCA) was adjusted to approximately 8 with 1 N NaOH. The IP_3 concentration in this solution was determined by a competition protein binding assay, utilizing binding protein obtained from the membrane fraction of bovine adrenal cortex (Bredt, Mourey & Snyder, 1989; Nibbering et al., 1990). Briefly, 20 μl of the above extract was incubated with 20 μl assay buffer B (in mM): 50 Tris/HCl, 2 EGTA, 1 β-mercaptoethanol, pH 8.2), 50 μl ^{3}H-IP_3 (≈4500 cpm) and 20 μl binding protein (≈15 mg/ml) for 20 min on ice. The sample was centrifuged for 4 min at 14000 rpm in an Eppendorf Centrifuge (4°C) to separate bound from unbound IP_3. The pellet was suspended in 100 μl assay buffer B and counted. The IP_3 content of each sample was determined from the standard curve.

Experimental Repetitions

All experiments were repeated at least three times with qualitatively identical behavior. Between individual experiments absolute values varied by a factor of up to 2. Throughout the paper we show single experiments performed in replicates.

Statistics

Significance was determined using a two-tailed t test for paired or unpaired means. A value of $P < 0.05$ was accepted as significant. All values are presented as means ± SE.

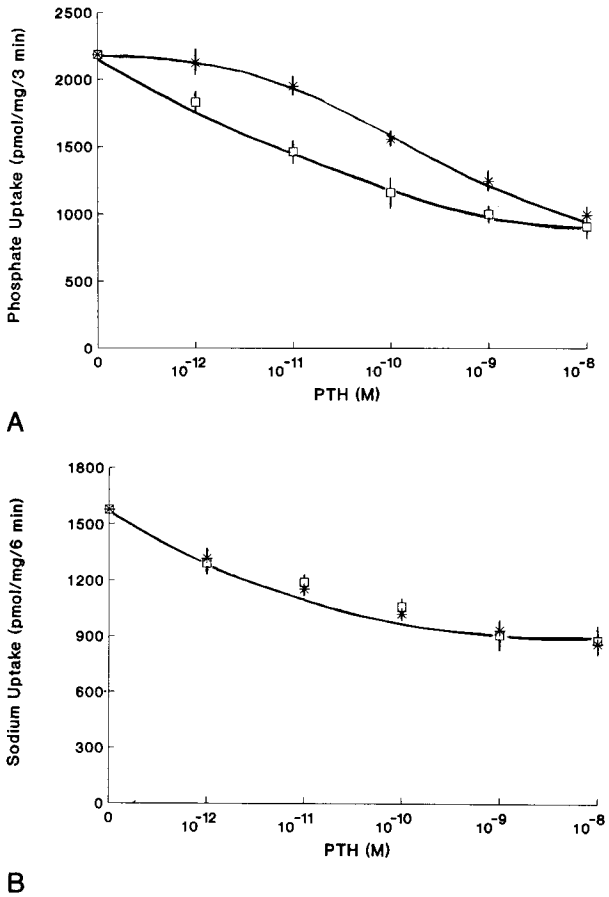

Fig. 1. Concentration dependence of either apical (squares) or basolateral (stars) application of PTH on apical Na/P_i cotransport (*A*) and apical Na/H exchange (*B*). Monolayers were incubated with PTH for 3 hr for Na/P_i cotransport and 5 min for Na/H exchange, and then transport was measured as described in Materials and Methods. Values are the mean ± SE for three replicates. The values for inhibition of Na/P_i cotransport (*A*) are significantly different ($P < 0.05$) between apical and basolateral hormone addition at PTH concentrations between 10^{-12} and 10^{-9} M.

Results

Basic Transport Studies

The concentration dependence of PTH inhibition of Na/P_i cotransport and Na/H exchange are shown in Fig. 1*A* and *B*, respectively. Similar to our previous findings, the apical and basolateral dose/response curves were strikingly different for PTH inhibition of Na/P_i cotransport (Fig. 1*A*) (Reshkin et al., 1990*b*) while displaying no difference for Na/H exchange (Fig. 1*B*) (Helmle-Kolb et al., 1990*a*). The apical half-inhibition value (K_d) for both transport systems was about 5×10^{-12} M PTH, as was also the case for the basolateral inhibition of Na/H exchange while the K_d of basolateral inhibition of Na/P_i cotransport was about a 100-fold higher (5×10^{-10} M).

Table 1. Time dependence and polarity of PTH-dependent inhibition of apical Na/P_i cotransport and of apical Na/H exchange

Time of exposure to PTH (min)	Apical PTH addition (pmol/mg/5 min)	Basolateral PTH addition (pmol/mg/5 min)
a) Na/P_i-cotransport		
0 min	3410	3410
20 min	2321	2436
45 min	2008	1986
90 min	1492	1524
180 min	1408	1436
b) Na/H-exchange		
0	1248	1248
2	976	912
4	712	706
5	402	411

Monolayers were incubated with 10^{-9} M PTH for the time indicated and then transport was measured as described in Materials and Methods. Values are the means of three replicates with SE values below 7%.

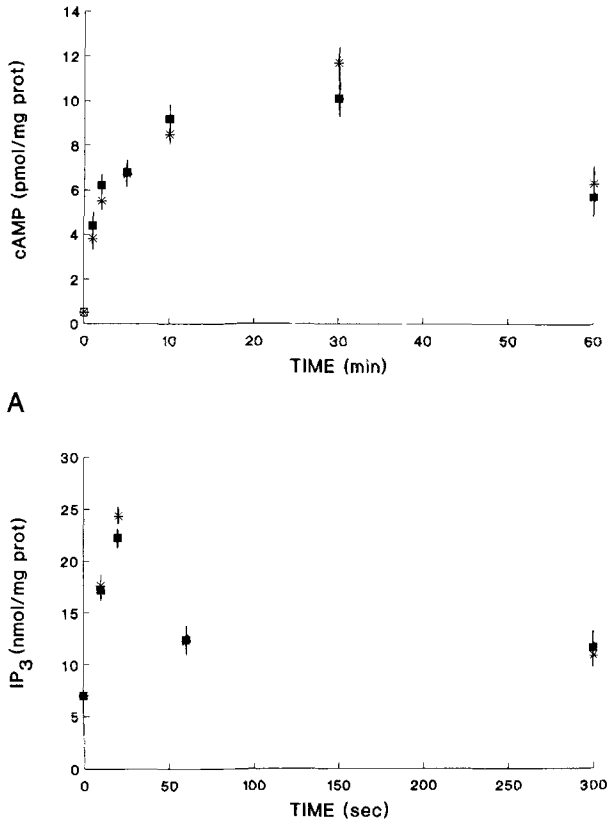

Fig. 2. Time-course of PTH stimulation of generation of intracellular cAMP (*A*, without IBMX) and IP_3 (*B*) applied to the apical (squares) or basolateral (stars) cell surface. PTH (10^{-8} M) was applied to the monolayer for the indicated time and cAMP or IP_3 measured as described in Materials and Methods. Values are mean ± SE for three replicates of a representative experiment. Only one symbol is included if values are identical after apical or basolateral application.

It should be noted that for the two transport systems the time required for inhibition after application of PTH was very different (Table 1). As has been previously reported, Na/H exchange was maximally inhibited in a few minutes (Table 1*b*) (Helmle-Kolb et al., 1990*b*), while more than 2 hr were necessary for maximal inhibition of Na/P_i cotransport (Table 1*a*) (Malmström & Murer, 1986).

The above different pattern of PTH inhibition of two apically located transport systems suggests some differences in PTH-dependent signal transduction systems on the two cell surfaces. Therefore, we have analyzed in more detail the 'polarity' (apical *vs.* basolateral PTH addition) of the PTH-dependent generation of the messengers cAMP and IP_3, the 'polarity' of interaction with a PTH-analog agonist/antagonist on transport inhibition and messenger generation (for review *see* Caulfield & Rosenblatt, 1990) and, finally, the 'polarity' of the action of the G-protein ribosyltransferase, pertussis toxin, on transport inhibition and messenger generation.

Basic Intracellular Messenger Studies

The time course of messenger generation was measured at 10^{-8} M PTH, a concentration giving maximal generation of both cAMP and IP_3 (Fig. 2*A* and *B*; *compare* to Fig. 3). These experiments showed that (i) at this PTH concentration there was no apparent 'polarity' in the time-dependent generation of internal messengers that could explain the differences in time required for transport inhibition and (ii) for both messengers there was a decrease in messenger levels some time after PTH stimulation. From these measurements the time of incubation for further experiments on second messenger production was chosen as 20 sec for IP_3 and 5 min for cAMP.

The levels of the intracellular messengers cAMP and IP_3 were then determined as a function of the PTH concentration given either to the apical or basolateral side of the monolayers (Fig. 3). As has been reported from OK-cell monolayers grown on impermeant supports (Quamme et al., 1989), IP_3 was produced at much lower PTH levels after administration to the apical side than was cAMP, with a half-maximal response concentration of approximately 7×10^{-12} M PTH for IP_3 generation. The PTH-dependent generation of IP_3 after administration of the hormone to the basolateral cell surface was not different from

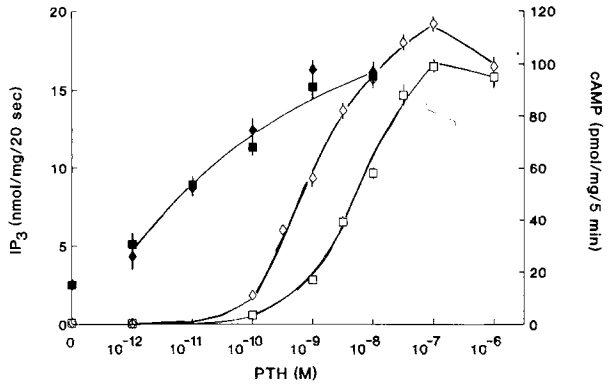

Fig. 3. PTH dose-response curves for cAMP (open symbols) and IP_3 (filled symbols) when applied to either the apical (squares) or basolateral (diamonds) cell surface. PTH was applied to the monolayer at the indicated concentration for either 5 min for the cAMP determination (in the presence of 1 mM IBMX) or 20 sec for the IP_3 determination and then the level of messenger measured as described in Materials and Methods. Values are mean ± SE for three replicates of a representative experiment. For the cAMP determinations values are significantly different between apical and basolateral PTH addition at concentrations between 10^{-10} and 10^{-7} M ($P < 0.05$). Only one symbol is included if values are identical after apical or basolateral application.

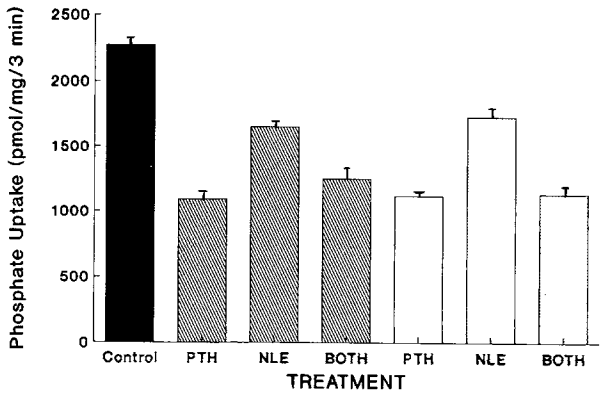

Fig. 4. Effect of nlePTH(3–34) as an agonist and antagonist on PTH-dependent inhibition of Na/P_i cotransport when applied to either the apical (slant bars) or basolateral (stippled bars) cell surface. Solid bar represents the control value of Na/P_i cotransport measured as in Materials and Methods. *PTH* represents transport values after 3 hr incubation with 10^{-9} M PTH, *NLE* represents transport values after 3 hr incubation with 10^{-6} M nlePTH (agonism) and *BOTH* is transport after 3 hr joint incubation of both at the above concentrations (antagonism). The statistical treatment of values obtained in a similar set of experiments are reported in the legend to Table 2.

that after addition to the apical side, while cAMP generation was more sensitive to basolateral PTH administration as compared to apical administration ($K_d \approx 10^{-9}$ M *vs.* 7×10^{-9} M, respectively).

The Action of [nle^{8,18},Tyr^{34}]PTH(3–34)

In OK-cell monolayers cultured on impermeant support, the PTH analog [nle^{8,18},Tyr^{34}]PTH(3–34) (nlePTH) has been reported to act as a partial agonist on inhibition of Na/P_i cotransport without stimulating cAMP formation and as an antagonist of PTH-stimulated adenylate cyclase activity (Cole et al., 1987, 1988, 1989; Muff et al., 1990). To determine the 'polarity' of agonist and antagonist properties of nlePTH(3–34) on Na/P_i cotransport, this analog was added to either side of the monolayer in the absence (agonism) or presence (antagonism) of 10^{-8} M PTH. Figure 4 displays the data for one experiment conducted at 5×10^{-6} M nlePTH, and data for its action at three concentrations are shown in Table 2 as either the percent (%) of the maximum PTH inhibition (agonism) or as the percent reduction of PTH inhibition of Na/P_i cotransport activity (antagonism). The analog displayed a concentration-dependent agonist inhibition of Na/P_i cotransport (maximal effect of the PTH analog was at 10^{-5} M); this inhibitory potency was equal on both cell surfaces and ≈46% of the inhibition by 10^{-8} M PTH. As a PTH antagonist, nlePTH displayed very different effects: It had a strong concentration-dependent antagonist effect only from the apical cell surface. Thus, although both apical and basolateral receptor systems were equally sensitive to the agonist properties of nlePTH, only the apical receptor systems had any sensitivity to antagonism by this analog.

The agonist and PTH-antagonist properties of nlePTH on the levels of the intracellular messengers cAMP and IP_3 were next determined. This analog had only a small effect on cAMP levels, if compared to PTH stimulation (Fig. 5*A*, *compare* to Fig. 3) but stimulated the production of IP_3 to levels comparable to PTH stimulation (Fig. 5*B*, *compare* to Fig. 3). These data are supportive of earlier suggestions that nlePTH must function as an agonist through IP_3-linked receptors (Cole et al., 1987, 1988, 1989). As was found above for the agonist effect in inhibition of Na/P_i cotransport (Fig. 4), there was no 'polarity' of the agonist action of nlePTH on IP_3 generation.

The antagonistic action of nlePTH on PTH-dependent generation of the messengers was more complex with different characteristics on the two cell surfaces (Fig. 6*A* and *B*). The application of 10^{-8} M PTH to the basolateral surface stimulated greater cAMP production than apical hormone application, as was also found in the concentration curve (Fig. 3). While the higher concentration of nlePTH (5×10^{-6} M) severely attenuated the PTH-dependent cAMP increase on both cell surfaces, the lower concentration of analog used (5×10^{-7} M) actually fur-

Table 2. 'Polarized' effect of [nle^{8,18},Tyr^{34}]bPTH(3–34) on apical Na/P_i cotransport and on bPTH(1–34)-dependent inhibition of apical Na/P_i cotransport

nlePTH(3–34) (M)	Side of hormone addition	Agonist property: nlePTH(3–34) inhibition of Na/P_i cotransport (% of maximum of PTH effect)	Antagonist property: nlePTH(3–34) reduction of PTH activity (% of reduction of PTH effect)
10^{-6}	Basolateral	36 ± 6	3.5 ± 2
	Apical	38 ± 5	13 ± 3
5×10^{-6}	Basolateral	38 ± 7	2 ± 1
	Apical	44 ± 5	21 ± 4
10^{-5}	Basolateral	43 ± 4	−4 ± 2
	Apical	49 ± 7	33 ± 4

Values are mean ± SE. PTH (10^{-8} M) and/or analog were added to culture medium and monolayers incubated for 3 hr. The effect of the analog (agonist property) was calculated as percent of the PTH inhibition of Na/P_i cotransport at 10^{-8} M PTH. The effect of the analog on PTH-dependent inhibition of Na/P_i cotransport (antagonist property) is reported as the percent suppression of the PTH-inhibitory effect. All values, with the exception of the antagonist property after basolateral application on the PTH analog, are significantly different from the control values ($P < 0.05$).

ther augmented the apical PTH-stimulated increase on the apical and not on the basolateral cell surface.

This same protocol was also used to examine the effect of nlePTH(3–34) on the PTH-dependent stimulation of IP_3 (Fig. 6*B*). As in Fig. 3, there was no difference between the two cell surfaces in the stimulation of IP_3 by 10^{-11} M PTH. On the apical cell surface the analog was found to antagonize fairly strongly this PTH-stimulation of IP_3 cell levels while having no effect on PTH stimulation of IP_3 from the basolateral cell surface. The same relationships were observed at 10^{-8} M PTH, a concentration at which both internal messengers were produced (*data not shown*).

The Action of Pertussis Toxin (PT)

Monolayers were preincubated overnight in the absence or presence of 5 or 50 ng/ml of PT and then assayed for inhibition by PTH of Na/P_i cotransport and stimulation by PTH of internal messenger production. Figure 7 shows that PT pretreatment partially released the inhibition observed with PTH alone; this effect of PT was observed after apical administration of PTH (Fig. 7*A*) and basolateral application (Fig. 7*B*). At the lower PTH concentration (10^{-11} M) the attenuation was increasing with increasing PT concentration ($P < 0.05$), while at the higher PTH (10^{-8} M) 5 ng/ml PT attenuated slightly the PTH-dependent transport inhibition ($P < 0.05$), with no significant effect at 50 ng/ml PT.

To determine the effect of PT on PTH-dependent production of cAMP, PT-pretreated monolayers were incubated for 5 min with low PTH (Fig. 8*A*: basolateral, 5×10^{-10} M; apical, 5×10^{-9} M) and medium PTH (Fig. 8*B*: basolateral, 10^{-9} M; apical, 10^{-8} M). At the low PTH, 5 ng/ml PT induced a higher enhancement of PTH-stimulated cAMP production than 50 ng/ml PT (Fig. 8*A*), while at the intermediate PTH concentration the enhancement of PTH stimulation of cAMP was greater with greater PT concentration (Fig. 8*B*). The PT enhancement at 10^{-7} M PTH was also measured and was identical in form but proportionally less than that seen at intermediate PTH (*data not shown*).

After PT pretreatment monolayers were also treated for 20 sec with 10^{-11} M PTH, a concentration with no measurable PTH-dependent cAMP generation (*see* Fig. 3). As can be seen in Fig. 8*C*, increasing PT resulted in increasing attenuation of the PTH-stimulated IP_3 signal, equally for both sides of hormone addition. An identical pattern was observed when monolayers were incubated at 10^{-8} M PTH and the intracellular concentration of IP_3 was measured (*data not shown*).

Discussion

In the last decade there have been substantial advances in our understanding of the cellular mechanisms of PTH-dependent regulation of the transport of inorganic phosphate (P_i) and protons in renal epithelia (proximal tubule). At present, it might be suggested, primarily based on experiments with cultures of established cell lines (OK cells) grown on

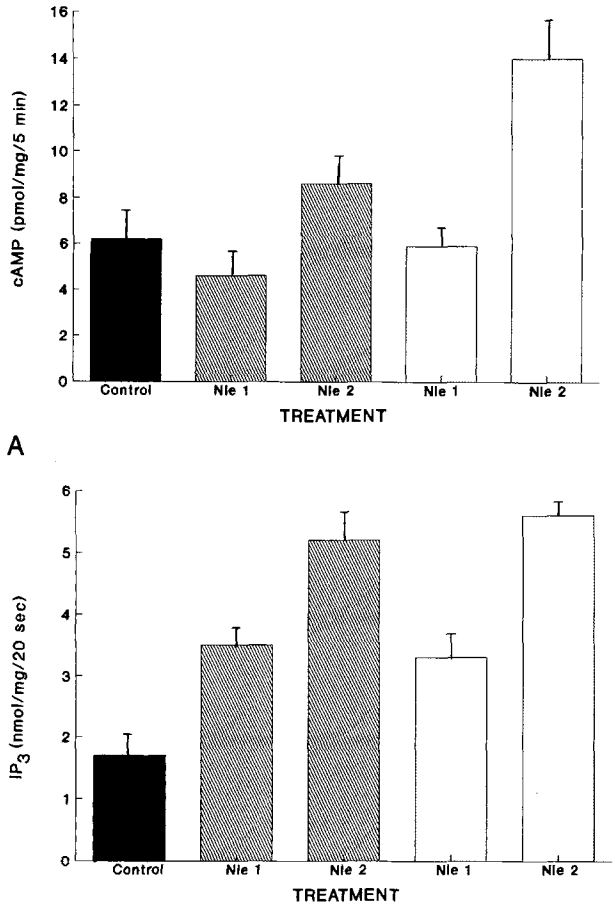

Fig. 5. Effect of nlePTH(3–34) as an agonist on cAMP (*A*) and IP_3 (*B*) generation when applied to either the apical (slanted bars) or basolateral (stippled bars) cell surface. NlePTH was added at either 5×10^{-7} M (*Nle 1*) or 5×10^{-6} M (*Nle 2*) for 5 min for cAMP (in the presence of 1 mM IBMX) or 20 sec for IP_3. Internal messengers were then measured as described in Materials and Methods. Values are mean ± SE for three replicates of a representative experiment. For cAMP determinations (*A*) the high concentration of the PTH analog and only basolateral addition resulted in a significant increase ($P < 0.05$). For IP_3 determination (*B*) all different conditions resulted in significant increases ($P < 0.05$).

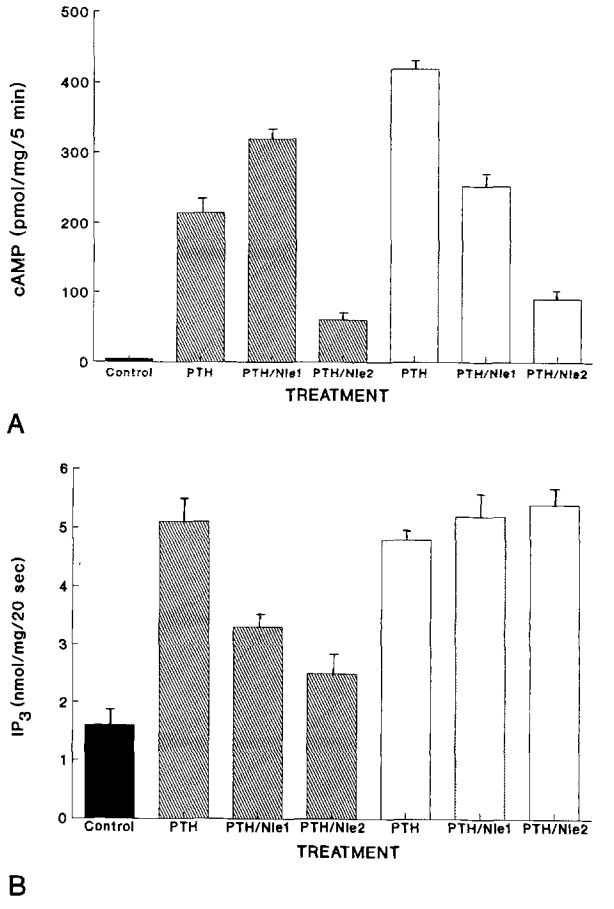

Fig. 6. Effect of nlePTH(3–34) as a PTH antagonist on cAMP (*A*) and IP_3 (*B*) generation when applied to apical (slanted bars) or basolateral (stippled bars) cell surface. PTH was applied to the monolayers (10^{-8} for cAMP and 10^{-11} for IP_3) for the same time periods as in Fig. 3 without added nlePTH or with 10^{-7} M (*Nle1*) or 10^{-6} M (*Nle2*) nlePTH. cAMP and IP_3 were measured as described in Materials and Methods. Values are mean ± SE of three replicates of a representative experiment. For cAMP determinations the addition of the PTH analog produced significant alteration in comparison to cAMP generation observed in the presence of PTH alone ($P < 0.05$). For IP_3 determinations only apical administration of the analog produced significant alterations in PTH-dependent generation of intracellular messengers ($P < 0.05$).

impermeant supports, that PTH action at low concentrations ($< 10^{-10}$ M) is transduced preferentially through activation of the phospholipase C/protein kinase C pathway, while higher PTH concentrations additionally lead to activation of the adenylate cyclase/protein kinase A pathway (for review *see* Dunlay & Hruska, 1990; Murer et al., 1991). Evidence suggests that at the higher concentrations both transduction systems work together to produce full PTH-dependent inhibition and that both cascade systems must be functional for complete PTH action (Quamme et al., 1989; Miyauchi et al., 1990; Segal & Pollock, 1990). Work on both the agonist and antagonist action of PTH analogs, primarily nlePTH, on inhibition of Na/P_i cotransport and on adenylate cyclase stimulation/cAMP production has suggested that on the apical surface there may exist multiple PTH-dependent signal transduction systems (Cole et al., 1987, 1988, 1989; Caulfield & Rosenblatt, 1990; Muff et al., 1990).

The recent use of OK cells grown on filters has demonstrated the presence of PTH receptors on both cell surfaces capable of initiating regulatory cascades involved in inhibition of apically located Na/H exchange and Na/P_i cotransport; interest-

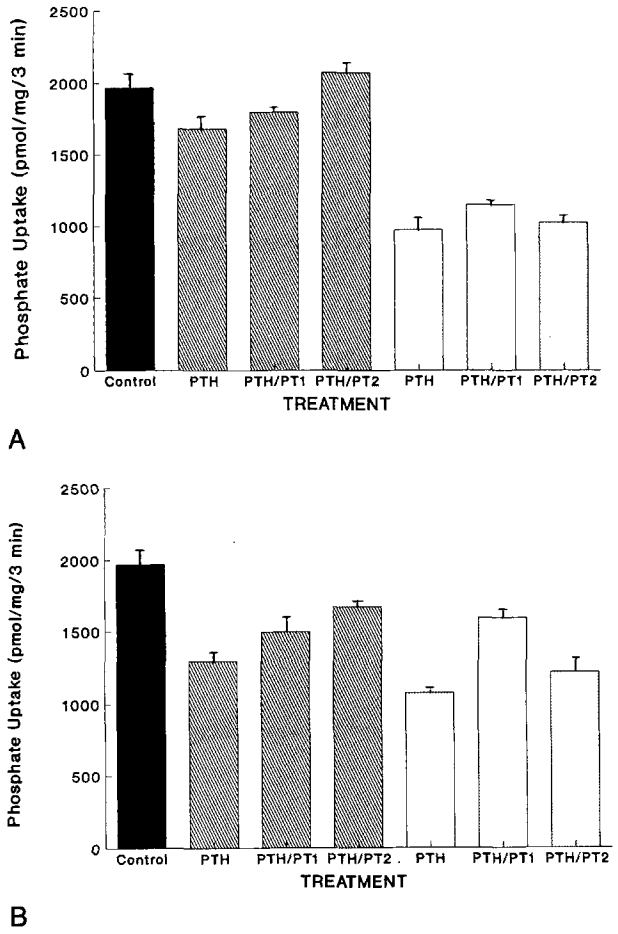

Fig. 7. Effect of pertussis toxin (PT) on PTH-dependent inhibition of Na/P_i cotransport applied to either the apical (*A*) or basolateral (*B*) cell surface. Monolayers were incubated 16 hr in growth media with either 5 ng PT/ml (*PT1*) or 50 ng PT/ml (*PT2*). The monolayers were then incubated with either 10^{-11} M PTH (slanted bars) or 10^{-8} M PTH (stippled bars) for 3 hr. Na/P_i cotransport was then measured as described in Materials and Methods. Values are mean ± SE for three replicates of a representative experiment. At the lower PTH concentration addition of pertussis toxin attenuated PTH-dependent transport inhibition significantly after either apical or basolateral hormone addition ($P < 0.05$). At the higher PTH concentration only the lower concentration of pertussis toxin produced a significant alteration of the transport inhibition ($P < 0.05$).

ingly, differences in time and concentration required for hormone-dependent inhibition of the transport systems were observed for the two cell surfaces (Reshkin et al., 1990; Helmle-Kolb et al., 1990*a*,*b*). In contrast to the study with OK cells grown on impermeant supports, where predominantly apical events were analyzed, nothing is known about distributional or functional identification of the PTH receptors at the two cell surfaces in cells grown on permeant supports, e.g. as a function of the PTH-dependent activation of the internal signal cascade systems or of the interaction of these receptors with G-proteins, or with PTH analogs.

The time-course of PTH inhibition displayed a rapid onset of inhibition for Na/H exchange (minutes) and a slower onset for inhibition of Na/P_i cotransport (hours), without any observable polarity in development of inhibition for either transport system. Application of PTH to either cell surface resulted in a dose-dependent decrease in the transport rate of either Na/P_i cotransport or Na/H exchange and an increase in the intracellular levels of IP_3 and cAMP (Figs. 1–3). As previously reported for the 'polarity' of the effect of PTH on the transport systems, Na/P_i cotransport displayed a differential sensitivity to PTH with the apical surface half-inhibition constant being about a 100-fold less than that for application of the hormone at the basolateral cell surface, while PTH inhibition of Na/H exchange was equally sensitive from the two cell surfaces. These effects of PTH on transport properties and production of second messengers might imply that there could be some sort of cell surface 'polarity' with respect to PTH interaction with receptors present on the apical and basolateral surfaces, respectively. This 'polarity' could be: (i) of the receptors themselves, e.g. in sensitivity to PTH including PTH analogs; (ii) of their time dependence with regard to generation of second messengers; or (iii) in properties of the receptor-messenger link after the hormone/receptor step, e.g. in G-protein interaction. 'Polarity' in any or all of these properties could result in the difference observed between the two cell surfaces in the pattern of final inhibition of the apical transport systems. To learn more about these processes, we have first measured the 'polarity' of the basic characteristics of the receptor/internal messenger generation and then the interaction of a PTH-analog agonist/antagonist (nlePTH) and a G-protein ribosyltransferase (pertussis toxin) with both PTH-dependent inhibition of Na/P_i cotransport and generation of second messengers (cAMP and IP_3).

The PTH-dependent generation of IP_3 was very sensitive to PTH concentration and showed no 'polarity' with respect to PTH application. For hormone application to both cell surfaces the generation of IP_3 mirrored closely the PTH-dependent inhibition of Na/H exchange and inhibition of Na/P_i cotransport after apical PTH addition, consistent with previous reports on cells grown on impermeant supports (Quamme et al., 1989), or for the apical cell surface in cells grown on permeant supports (Reshkin et al., 1990*b*). Interestingly, PTH-dependent cAMP generation after basolateral hormone administration was more sensitive than after apical hormone addition (Fig. 3).

The PTH analog nlePTH(3–34) has been used to

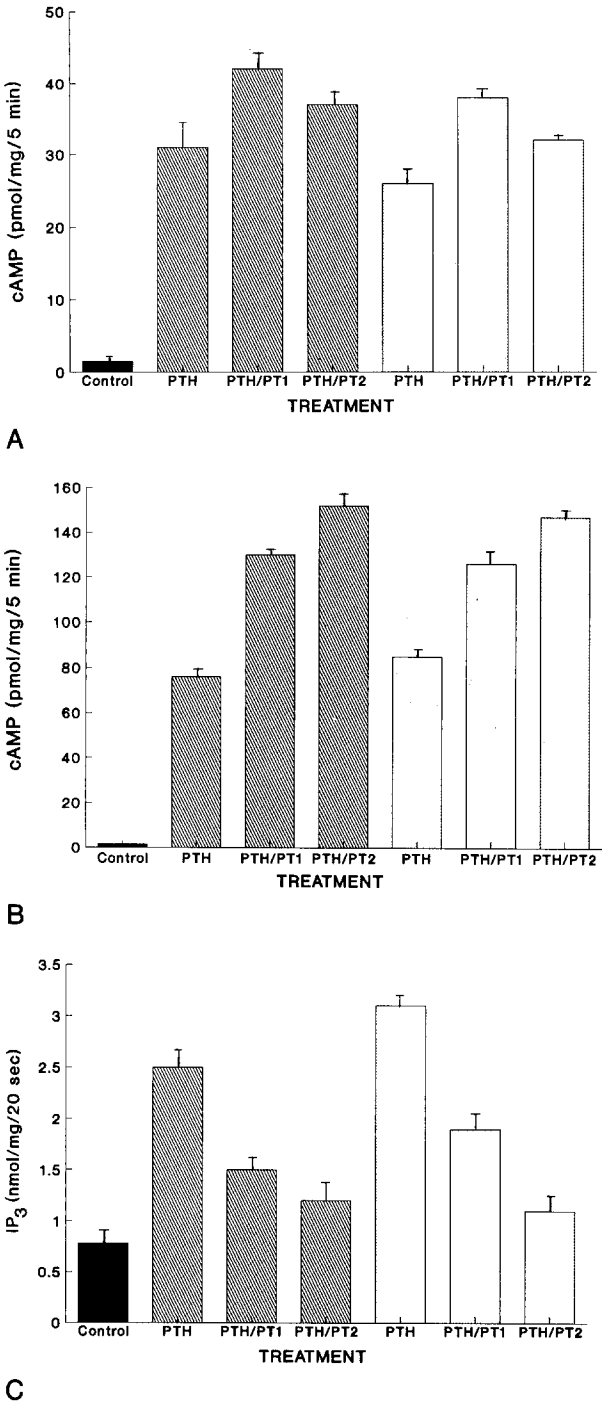

Fig. 8. Effect of pertussis toxin (PT) on PTH-dependent generation of cAMP and IP_3 when applied to the apical (slanted bars) or basolateral (stippled bars) cell surface. Monolayers were treated as in Fig. 7. For cAMP determination monolayers were incubated for 5 min in the presence of 1 mM IBMX in the following PTH concentrations: (*A*) basolateral 5×10^{-10} M, apical 5×10^{-9} M; (*B*) basolateral 10^{-9} M apical 10^{-8} M. For the IP_3 determination (*C*) monolayers were incubated in 10^{-11} M PTH for 20 sec. Levels of internal messengers were determined as described in Materials and Methods. Values are mean ± SE for three replicates of a representative experiment. At the lower PTH concentration (*A*) 5 ng PT/ml (*PT1*) produced a significantly higher increase in PTH-dependent generation of cAMP than 50 ng PT/ml (*PT2*); at both concentrations of pertussis toxin cAMP generation was increased as compared to the absence of pertussis toxin. At the higher concentration of PTH (*B*), both pertussis toxin conditions increased PTH-dependent cAMP generation with the effect being higher at higher toxin concentrations. For IP_3 determinations pertussis toxin attenuated the PTH-dependent generation of IP_3 with significantly different effects between high and low toxin concentration only for the basolateral cell surface.

further characterize the interaction of PTH receptors and their associated secondary messenger system(s) (for review: Caulfield & Rosenblatt, 1990). In OK cells grown on impermeant support, nlePTH inhibited apical Na/P_i cotransport with a K_d approximately four orders-of-magnitude greater than PTH without a concurrent stimulation of cAMP production (Cole et al., 1987, 1988, 1989; Muff et al., 1990). As an antagonist, nlePTH completely antagonized PTH-stimulated cAMP production (Cole et al., 1988, 1989; Muff et al., 1990) while antagonizing PTH-dependent inhibition of Na/P_i cotransport only about 20% (Muff et al., 1990). These data led these authors to conclude that, on the apical membrane, nlePTH acts as a partial agonist at receptors not related to cAMP generation (i.e., IP_3 production), while acting as an antagonist primarily at receptors coupled to cAMP generation. This differential activity at putatively different PTH-dependent signal transduction systems led us to utilize nlePTH as a probe to further analyze the two cell surfaces for potential differences in PTH-dependent effects on transport and messenger-generation. As can be seen in Fig. 4 and Table 2, nlePTH showed identical partial agonist activity with respect to inhibition of Na/P_i cotransport on both cell surfaces, while displaying antagonism of PTH (10^{-8} M)-dependent inhibition of Na/P_i cotransport only on the apical surface. The level of transport antagonism on the apical surface was similar to that reported by Muff et al., 1990).

The effect of nlePTH on the production of internal messengers (agonism) and on their PTH-dependent generation (antagonism) also demonstrated differences between apical and basolateral application of hormone and hormone analog, respectively. In the present study, nlePTH functioned as an agonist through IP_3 generation, supporting this suggestion by previous workers (Cole et al., 1987). As has been reported for the apical membrane (Cole et al., 1987, 1988, 1989; Muff et al., 1990), nlePTH at the higher concentration effectively antagonized the PTH-stimulated cAMP production at both cell surfaces. However, at the apical cell surface the lower nlePTH concentration used actually augmented the PTH-

stimulated increase in cAMP generation. A similar apical cell surface stimulation of cAMP production by a very low level of a hormone antagonist of vasopressin stimulation has been reported for primary cultures of rat inner medullary collecting tubule cells (Teitelbaum, Strasheim & Berl, 1989).

Previous authors have concluded from similar studies on the use of this PTH analog (Cole et al., 1987, 1988, 1989) that the difference between nlePTH antagonism on transport and cAMP generation implied that nlePTH has no antagonistic effect on IP_3 generation. In analogy, from the present data on polarity of nlePTH antagonism of PTH-dependent transport and cAMP production, it could be expected that IP_3 should move in parallel with inhibition of Na/P_i cotransport. That is, at the apical surface nlePTH should have some antagonizing effect on PTH stimulation of IP_3, but that at the basolateral surface no effect should be observed. As seen in Fig. 6, nlePTH, in fact, had no antagonist properties when applied to the basolateral surface together with either 10^{-8} M PTH, as shown, or at 10^{-11} M PTH (*data not shown*). However, on the apical surface, nlePTH was able to antagonize PTH-dependent IP_3 production, implying that the dynamics of PTH action are not simple and that there might be differences in receptor properties between cells grown on permeant supports and cells grown on impermeant supports.

The data of experiments involving the use of the PTH analog suggest some differences between PTH-dependent signal transduction systems localized at the two cell surfaces. The agonist activity on both cell surfaces at only the receptor or the equivalent component of a dual receptor coupled to IP_3 production, together with the lack of agonist 'polarity' (while displaying different polarity as an antagonist), provides evidence for the existence of differences in transmembrane signaling mechanisms. This was particularly true for the very different low nlePTH concentration interaction with PTH-dependent cAMP- and IP_3-generation.

An additional cause of the variation observed at the two cell surfaces in PTH action in both transport inhibition and internal messenger generation could be due to different couplings of (a) PTH receptor(s) to the effector(s) via G-proteins (Spiegel, 1990). The pre-application of pertussis toxin (PT), an ADP ribosyltransferase, has been shown to attenuate the PTH-dependent inhibition of Na/P_i cotransport (Garcia et al., 1989) and IP_3 (Miyauchi et al., 1990) and enhance cAMP generation in OK cells (Rizzoli & Bonjour, 1988) and, recently, to attenuate the Epidermal Growth Factor-stimulated IP_3 signal in mouse collecting duct cells (Teitelbaum, Strasheim & Berl, 1990). In OK-cell monolayers, we found a complex relationship of pertussis toxin application relevant to concentration of PT and PTH but not in relation to cellular 'polarity'. That is, PT was observed to attenuate both the PTH-dependent inhibition of Na/P_i cotransport (Fig. 7) and stimulation of IP_3 production (Fig. 8*C*), while augmenting the PTH-dependent stimulation of cAMP (Fig. 8*A* and *B*). Although these data suggest that PTH receptors can interact with both G_i and G_s, the observed parallelism of PT effect on PTH-dependent transport and IP_3 production but not cAMP production would suggest that PTH-dependent inhibition of Na/P_i cotransport may be preferentially transduced via the G_s-linked protein kinase C pathway. These effects of PT-dependent ADP ribosylation on PTH action were found to be equal at both cell surfaces.

Until recently it has generally been thought that the receptor for PTH is located on the basolateral membrane and is coupled to adenylate cyclase (*see* Nissenson & Klein, 1987). Studies to date have not found evidence for high affinity PTH radioligand binding (circa 10^{-11}–10^{-12} M) on this membrane and had not searched for binding on the apical membrane (Nissenson & Klein, 1987). With the accumulating evidence in cell culture for high affinity PTH receptors and for apical location of PTH receptors (this study and *see* Introduction) it becomes important to corroborate these cell culture data with *in situ* data from the proximal tubule. Furthermore, these data necessitate that PTH be present in the primary proximal filtrate in concentrations compatible with the function of the apical system. Although this is not known for PTH in kidney, there are data demonstrating that parathyroid hormone may not be unique in having functionally active receptors on both cell surfaces of the renal epithelia. Angiotensin II-dependent regulation of bicarbonate reabsorption in the proximal tubule was recently demonstrated to be active from both the apical and basolateral cell surfaces (Liu & Cogan, 1988).

In conclusion, evidence is presented here for the existence of different 'PTH-receptor' subtypes on the apical and basolateral cell surfaces of OK-cell monolayers. The spatial separation of the receptor groups on the two cell surfaces could further facilitate their participation in a postulated dual-messenger mechanism (Cole et al., 1987; Malmström et al., 1988; Quamme et al., 1989; Dunlay & Hruska, 1990; Murer et al., 1991). Such a spatial separation of the two receptor classes could permit the epithelial cells to respond to PTH from two separate but functionally connected cellular 'pools', i.e., the cells could respond to PTH present either in the basolateral compartment (plasma) or in the apical compartment (primary filtrate).

We are happy to thank the Geigy Jubiläumsstiftung and the Roche Research Foundation for providing a visiting scientist award to

SJR, and the Swiss National Science Foundation for financial support (grant 3.854.088); also financial support from the "Jubiläumsspende für die Universität Zürich" is acknowledged.

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Received 10 May 1991; revised 22 July 1991