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Parathyroid hormone regulates phosphate transport in OK cells via an irreversible inactivation of a membrane protein

Kerstin Malmström and Heini Murer

Institute of Physiology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

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In a cultured, renal epithelial cell line, OK, parathyroid hormone (PTH) reduces Na/phosphate cotransport and upon removal of the hormone the activity is regained. Cycloheximide, an inhibitor of protein synthesis, did not interfere with the PTH action, but prevented the reduced phosphate transport from regaining its original activity. Drugs, such as colchicine, that disrupt the microtubular network, lessened both the action of PTH and prevented the recovery of activity. The results are consistent with irreversible inactivation of a plasma membrane protein necessary for full activity of the Na/phosphate cotransport.

Phosphate transport; Parathyroid hormone; Membrane transport; Protein synthesis; Cell culture; (Proximal tubule)

1. INTRODUCTION

The OK cell line, derived from an American opossum kidney, has been used successfully for studying the regulation of Na/phosphate cotransport by parathyroid hormone (PTH) [1,2]. The cells respond to PTH by an immediate transient increase in intracellular Ca^{2+} and diacylglycerol (DAG) [3], rapid stimulation of adenylate cyclase [1,2,4], followed by decreased Na/H exchange activity [5], and decreased Na/phosphate cotransport activity [1,2]. It is generally believed that cyclic AMP is a mediator in the regulation of Na/phosphate cotransport activity in OK cells [1,2], whereas the role of Ca^{2+} remains unclear [3]. This study describes that the actual mechanism responsible for the decreased Na/phosphate cotransport is the PTH-induced inactivation of a protein. The protein is inactivated irreversibly, most likely by lysosomal degradation after having being endocytosed from the plasma membrane.

Correspondence address: K. Malmström, Institute of Physiology, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland

2. MATERIALS AND METHODS

2.1. *Materials*

The OK cells were a kind gift from Dr D. Warnock, San Francisco, CA. All cell culture reagents were purchased from Amimed (Basle); cycloheximide, colchicine and lumicolchicine from Sigma (St. Louis, MO) and the radiolabelled phosphate from New England Nuclear (Boston, MA).

2.2. *Methods*

Cells were cultured as described in [2]. For experiments, cells were plated at a density of $1-2\times 10^5$ cells/35 mm dish (Nunc, Denmark), and used after 5 days when confluency was reached. The culture medium was replaced by serum-free medium 12–15 h before experiments. PTH and all other additions were made in aliquots directly to the medium. Na/phosphate uptake was measured in cell monolayers after incubation in a (37°C) medium containing (in mM): 137 NaCl, 5.4 KCl, 2.8 $CaCl_2$, 1.2 $MgSO_4$, 10 Hepes-Tris, pH 7.4, 0.1 $KH_2{}^{32}PO_4$ (0.5 μCi/ml). The Na-independent phosphate uptake in OK cells is negligable (<1% of the Na/phosphate uptake) and was therefore

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routinely not measured [2]. The uptake was stopped after 3 min by washing the monolayers 4 times with ice-cold stop solution containing (in mM): 137 NaCl, 14 Tris-HCl, pH 7.4. The monolayer was solubilized in 1 ml of 0.5% Triton X-100 and aliquots were used for determining the accumulated phosphate by scintillation counting.

3. RESULTS AND DISCUSSION

Incubation of confluent cell monolayers with PTH and with PTH plus cycloheximide [6] resulted in identical reduction of the Na/phosphate cotransport (table 1). Similar results have been reported by Caverzasio et al. [1]. Inhibition of Na/phosphate cotransport is thus not caused by de novo synthesis of an inhibitory protein. Furthermore, PTH does not prevent the synthesis of a regulatory protein required for full transport activity (negative control). If a constant turnover of this putative protein is assumed, PTH would, by suppressing the resynthesis, inhibit Na/phosphate cotransport. However, incubation with cycloheximide for as long as 4 h did not reduce the Na/phosphate cotransport significantly in control cells (table 1).

Cell monolayers which had been exposed to PTH exhibited reduced Na/phosphate cotransport. After removing the hormone the Na/phosphate cotransport started to regain its activity (recovery phase), and 5–7 h later the original activity had returned [2]. Inhibitors of protein synthesis, e.g. cycloheximide [6], prevented the recovery (fig.1). Actinomycin D [7], which interferes with the transcriptional step, had no effect on the recovery phase even at high concentrations (2 μg/ml) (not shown). 3'-Deoxyadenosine (cordycepin), which affects messenger RNA polyadenylation [8], also delayed the recovery phase (fig.1). PTH induces most likely irreversible inactivation of a protein that is necessary for full activity of Na/phosphate cotransport. The immediate control appears to be at the level of mRNA and the translation step. The necessity of de novo synthesis in the recovery phase was further documented by the effect of colchicine (fig.2). Colchicine, which disrupts the microtubular network, an important component for the intracellular routing of secretory and plasma membrane proteins [9], also prevented the recovery of phosphate transport.

The mechanism of the irreversible inactivation was investigated by considering two possibilities: Ca^{2+}-dependent proteolysis at the plasma membrane, and internalization of the protein by endocytosis followed by lysosomal degradation.

Table 1

Effect of cycloheximide on Na/phosphate cotransport in OK cells

Condition	Phosphate uptake (nmol phosphate · mg protein^{-1} · 3 min^{-1}	
	Control	PTH
No addition	15.9 ± 1.5	7.2 ± 1.1
Cycloheximide	15.9 ± 0.6	8.2 ± 0.2

Incubation time with cycloheximide and PTH was 4 h; concentration of cycloheximide was 20 μM and of 1-34b PTH, 10^{-10} M. Data are means ± SD from a representative experiment performed in triplicate. Similar results were obtained in at least 3 other experiments

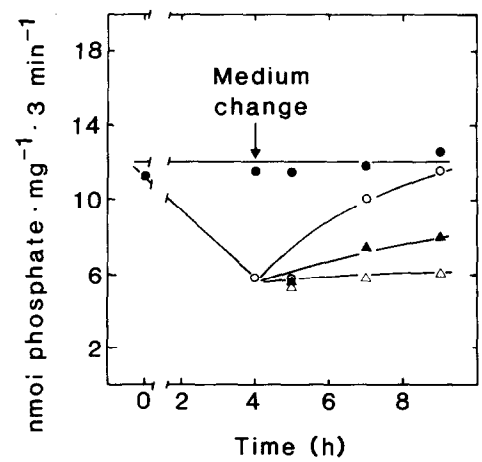

Fig.1. Effect of protein synthesis inhibitors on the recovery of Na/phosphate cotransport after PTH removal. After 4 h incubation with 10^{-10} M 1-34b PTH, the culture medium containing hormone was removed and replaced with medium (○—○), medium containing 20 μM cycloheximide (△—△), or medium containing 200 μM cordycepin (▲—▲); control, no PTH (●—●). The phosphate uptake was measured as described in section 2. Data are from a representative experiment performed in triplicate. Similar results were observed in at least 3 other experiments.

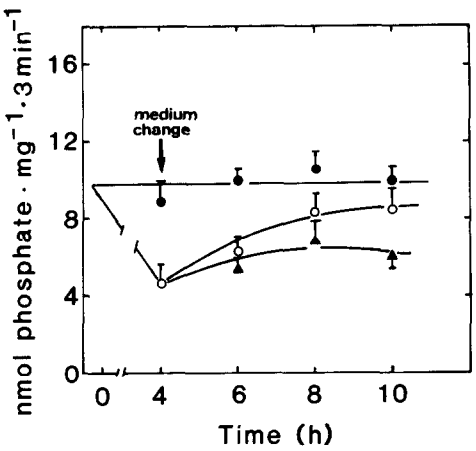

Fig.2. Effect of colchicine and cytochalasin B on the recovery of Na/phosphate cotransport after PTH removal. For experimental details see section 2 and the legend to fig.1. Cell monolayers were left to recover in mdium (○—○), medium containing 10 μM colchicine (▲—▲); control, no PTH (●—●). Data are means ± SD of two independent experiments performed in triplicate.

Ca^{2+}-dependent proteases may be activated in response to a transient increase in cytosolic Ca^{2+} [10]. To prevent the action of calpains we used antipain and leupeptin [11] at millimolar concentration, but the action of PTH was not reduced (not shown). Furthermore, by selectively increasing the intracellular Ca^{2+} transiently by Ca^{2+} ionophores (ionomycin or A23187), only a minor reduction of Na/phosphate cotransport (< 10% after 4 h) is produced (not shown). Selective inactivation of a regulatory protein by Ca^{2+}-dependent proteolysis was considered unlikely.

Endocytosis followed by lysosomal degradation requires an intact microtubular network. Cell monolayers were exposed to colchicine and to the inactive analogue β-lumicolchicine [9]. In colchicine-treated cells the PTH effect on Na/phosphate was reduced by a factor of 2, whereas β-lumicolchicine had no effect (table 2). Similar results were obtained with other microtubule-disruptive drugs such as vinblastine (not shown). It is proposed that the reduction of Na/phosphate cotransport occurs via internalization of a protein by endocytosis.

In conclusion, we provide evidence that the recovery from the PTH-dependent inhibition of phosphate transport requires protein synthesis at the translational level, and an intact cytoskeletal system. The inhibition is most likely due to the irreversible inactivation of a protein, necessary for full transport activity. The experimental evidence supports the internalization of a membrane protein. Hormone-induced down-regulation of transport systems has not been described so far, although up-regulation, e.g. vasopressin-stimulated water transport [12] and insulin-stimulated glucose transport [13], is known. More knowledge is required about the molecular structure of the Na/ phosphate carrier before it will be possible to distinguish whether the endocytosed protein is the Na/phosphate carrier or an activator protein.

Table 2

Effect of colchicine and β-lumicolchicine on the Na/phosphate cotransport in OK cells

Condition	Phosphate uptake (nmol phosphate · mg protein^{-1} · min^{-1})		
	Control	PTH	% uptake
No addition	11.0 ± 1.6	3.0 ± 0.3	27
Colchicine	8.7 ± 1.0	4.9 ± 0.8	57
Lumicolchicine	10.6 ± 0.7	2.9 ± 0.3	27

Prior to determining the uptake, cell monolayers were incubated with the drugs for 4 h (control) or 1 h preincubation with the drugs and 3 h together with PTH. Concentrations of drugs were 10 μM and of PTH 10^{-10} M 1-34b PTH. Data are means ± SD from three independent experiments performed in triplicate. % uptake indicates uptake after PTH incubation as compared to that in control cells

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