Parathyroid hormone increases thiol proteinase activity by activation of protein kinase C in cultured kidney tubule cells (OK)

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Chronic exposure (24 h) to parathyroid hormone (PTH) increases the intracellular proteolytic activity in cultured opossum kidney cells 2-fold at physiological PTH concentrations (10^{-12} mol/l). This increase can be blocked by E-64, an inhibitor of thiol proteinases. The phorbol ester TPA mimicks the effect of PTH, whereas the calcium ionophore A23187 reduces the intracellular proteinase activity. Forskolin and dibutyrylic cAMP do not elevate proteinase activity. The protein kinase C inhibitor staurosporine is equally effective in blocking the TPA- and PTH-induced proteinase activity increase. These data indicate that PTH increases the intracellular thiol proteinase activity by an activation of protein kinase C and not by the cAMP dependent way.

Thiol proteinase; Protein kinase C; OK cell

1. INTRODUCTION

The epithelial cells lining the proximal renal tubule have a high capacity for the uptake and intracellular breakdown of proteins (for review see [1]). This mechanism is especially important for the reabsorption of low molecular weight plasma proteins (LMWP) that are filtered to a great percentage in the glomeruli. The proximal tubule cells are highly responsive to parathyroid hormone (PTH), where it causes a decrease of phosphate reabsorption and cellular hypertrophy [2]. However, very little is known about the possible relationship between PTH and protein breakdown in this tubule segment.

We chose the cultured epithelial kidney cell line OK as a model system for the proximal tubular protein breakdown because it resembles proximal tubule cells with respect to their differentiated transport functions [3,4]. PTH activates protein kinase C (PkC) in OK cells by translocation to the cell membrane [5,6]. It has been shown that PTH inhibits phosphate uptake at very low (picomolar) PTH dosages [7,8] whereas elevation of cyclic AMP levels occurs at markedly higher concentrations ($>10^{-9}$ mol/l) [9] suggesting that PTH exerts its various effects by different second messenger pathways.

The aim of the present study was to determine if and how PTH influences the activity of intracellular proteinases in confluent differentiated epithelial monolayers of OK cells. Three main types of proteinases were assayed by specific inactivation: metallo proteinases (dependent on divalent cations; inactivated by EDTA), serine proteinases (inactivated by PMSF), and thiol proteinases (blocked by E-64).

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2. MATERIALS AND METHODS

2.1. *Chemicals*

Minimal essential medium (MEM), fetal calf serum (FCS) and EDTA were obtained from Biochrom, Berlin, FRG; azocaseine and trypsin from Serva, Heidelberg, FRG; staurosporine fom Fluka, Buchs, Switzerland; glutamine from Gibco, Grand Island, NY, USA; PTH 1-84 from Sigma, St. Louis, MO, USA; penicillin, streptomycin, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), bovine serum albumin (BSA), DMSO, A23187, L-trans-epoxysuccinyl-leucylamido-(4-guanidino)-butane (E-64), phenylmethylsulfonyl-fluoride (PMSF) and all other chemicals from Sigma, Munich, FRG.

2.2. *Cell culture*

OK cells of passages 98-112 were maintained in plastic culture flasks (Falcon, Heidelberg, FRG) and grown in MEM medium containing 10% v/v FCS, 2 mmol/l glutamine, 100 IU/ml penicillin and 10^{-7} g/l streptomycin (37°C, 5% CO_2). The cultures were split once a week after having reached confluency. For disintegration of the monolayers, cells were washed for 60 s with 10 ml of calcium- and magnesium-free Ringer solution containing (in mmol/l) NaCl 137, KCl 2.7, Na_2HPO_4 8, KH_2PO_4 1.5, EDTA 0.7, and subsequent trypsinisation (0.25 g/l).

2.3. *Experimental procedure*

OK cells designated for the experiment were grown to confluency on plastic petri dishes (6 cm diameter, Falcon, Heidelberg, FRG) under standard conditions. 24 h before measuring proteinase activity, FCS was substituted by 0.1% of BSA. The monolayers were scraped off the petri dishes with a rubber policeman and centrifuged at $500 \times g$ for 5 min 3 times with intermediate ice cold washing. The cells in the final pellet were disrupted by sonification and immediately processed for proteinase activity determination (for details see [10]).

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Briefly, 0.1 ml of the sample was incubated with 0.1 ml of a 3% solution of azocasein in Tris/HCl buffer for 45 min at 37°C. The reaction was stopped by 0.5 ml of 8% (v/v) trichloroacetic acid. The samples were centrifuged at 500 × g for 10 min and the released diazoamino acids were measured photometrically in the supernatant at 334 nm. The proteolytic activity was expressed as U/mg with 1 U representing a change of extinction of 0.001/min. The protein content of the supernatant was determined by the method of Lowry [11]. The samples were incubated with E-64 (1 mmol/l), PMSF (1 mmol/l), and EDTA (10 mmol/l) for 20 min at 37°C before the addition of azocasein to allow the formation of proteinase-inhibitor complexes. These inhibition studies were performed at the optimum pH for the respective enzymes (7.4 for PMSF, 5.4 for E-64, 7.4 for EDTA). PTH was dissolved immediately before each experiment in a low concentration of acetic acid (0.1 mmol/l) and diluted 50 times with medium. For control, PTH was inactivated by 45 min incubation in 30% (v/v) H_2O_2 (37°C), freezing and lyophilizing. Incubation with 10^{-6} mol/l inactivated PTH (24 h) did not show an effect on total proteinase activity. TPA action (10^{-7} mol/l) was tested at 2, 4, and 24 h incubation and no effect found at 2 and 4 h. Each experiment was performed 4 times; the data are usually the arithmetic means ± SE of these n = 4 experiments.

3. RESULTS AND DISCUSSION

As a pilot study we followed the time course of PTH action (10^{-6} mol/l) on total proteinase activity (Fig. 1). Because the maximum of PTH action occurred at 24 h, all subsequent experiments were performed at this incubation time. OK cells exhibit an intracellular proteinase activity of 6.4 ± 0.1 U/mg protein. This basal activity is reduced by 1 mmol/l PMSF to 2.7 ± 0.1 U/mg protein and by 10 mmol/l EDTA to 1.9 ± 0.1 U/mg protein, indicating that the major portion of basal protein breakdown occurs by enzymes that depend on divalent cations and serine. In contrast, E-64 (1 mmol/l) does not alter basal proteinase activity (6.5 ± 0.1 U/mg protein) suggesting that thiol proteinases play only a minor role in the unstimulated protein breakdown of OK cells. It must be emphasised that serum depletion affects virtually all metabolic functions of cultured cells causing a halt to many differentiated cellular functions [12].However, 24 h after the substitution of low (physiological) concentrations of PTH 1-84 the total proteinase activity is significantly stimulated

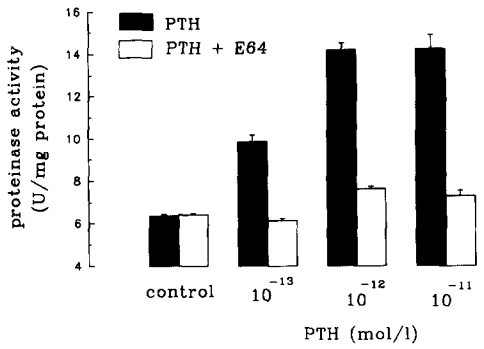

Fig.2. PTH increases proteinase activity dose-dependently. This increase is prevented by 1 mmol/l E-64.

(Fig. 2). This stimulation is maximal at 10^{-12} mol/l and is completely blocked by E-64 at all applied PTH concentrations. Hence, physiological PTH concentrations are necessary for the appearance of thiol proteinase activity that is responsible for nearly half of the total proteolytic breakdown capacity of OK cells.

We further addressed the question which mechanism of action of PTH is responsible for the evolution of this thiol proteinase activity. Possible candidates are an elevation of intracellular cAMP (the 'classical' PTH action [13]), an elevation of free intracellular Ca^{2+} [8], and the IP_3/DAG/PkC pathway [6]. Fig. 3 shows that low concentrations of the PkC activating phorbol ester TPA enhance the thiol proteinase activity in the same way as PTH. Staurosporine, a potent blocker of protein kinase C, is known to act in OK cells as an inhibitor of the desensitisation of PTH receptors [14]. When OK cells are incubated in the presence of both TPA and staurosporine (10^{-7} mol/l each) the increase in proteinase activity is abolished (4.9 ± 0.1 U/mg protein vs control 6.2 ± 0.1 U/mg protein). Similar results were obtained by incubating OK cells in the presence of both PTH and staurosporine (10^{-7} mol/l each; 5.2 ± 0.2 U/mg protein vs control 6.2 ± 0.1 U/mg protein). Staurosporine itself (10^{-7} mol/l) is capable of significantly reducing proteinase activity (3.8 ± 0.2

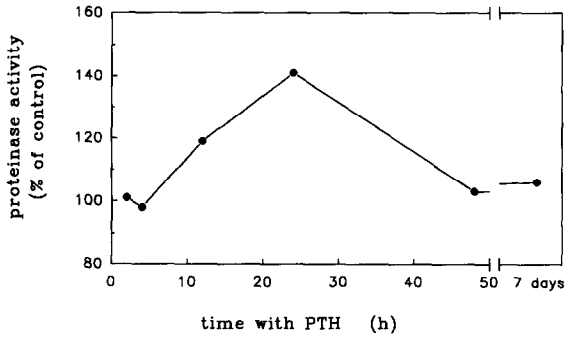

Fig.1. Time course of the effect of PTH (10^{-6} mol/l) on total proteinase activity (pH 5.4; 100% represents azocasein breakdown in the absence of PTH).

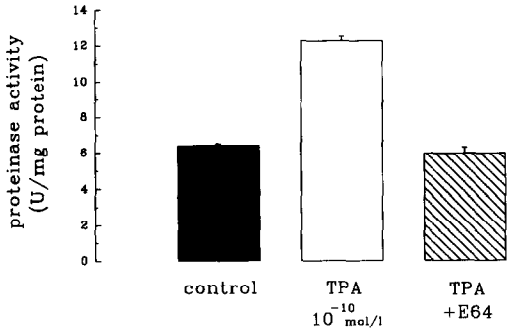

Fig.3. TPA (10^{-10} mol/l) increases proteinase activity. This increase is prevented by 1 mmol/l E-64.

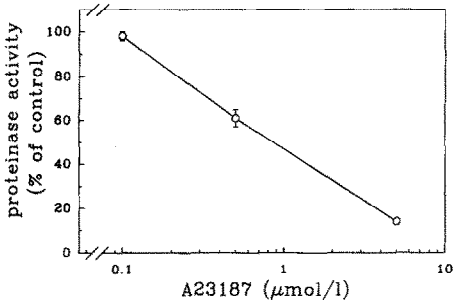

Fig.4. A23187 inhibits proteinase activity dose-dependently (100% represents azocasein breakdown without A23187).

U/mg protein vs control 5.11 ± 0.1 U/mg protein). Obviously, protein kinase C plays a crucial role in the mediation of PTH induced activation of thiol proteinases in OK cells.

In contrast to these findings, the other two possible ways of PTH action do not seem to be involved in proteinase activation: an elevation of intracellular calcium concentrations by the calcium ionophore A23187 reduces proteinase activity dose-dependently (Fig. 4). Since intracellular calcium concentrations were not directly monitored, we cannot fully exclude a possible positive effect of Ca^{2+} elevation in the nanomolar range on proteinase activity that is reversed at unphysiologically high Ca_i^{2+}. However, our experiments do not give any hint for such a biphasic effect of calcium.

In a further set of experiments we elevated the intracellular concentrations of cAMP both by incubating OK cells with the membrane permeable compound dibutyrylic cAMP (dbcAMP) and forskolin [9]. Neither substance elevated the total or the E-64 blockable proteinase activity. At high concentrations (10^{-4} mol/l) dbcAMP even reduced proteinase activity to 68 ± 3% of control. Forskolin did not alter total proteinase activity in a range between 10^{-7} and 10^{-5} mol/l (n = 6).

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