Parathormone promotes glycogen formation from [^{14}C]glucose in cultured osteoblast-like cells

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Parathyroid hormone stimulates [U-^{14}C]glucose incorporation into glycogen of cultured osteoblast-like calvaria cells. This effect is detectable only several hours after the addition of PTH and it is mimicked by dibutyryl cyclic AMP. In contrast to insulin (in pharmacological concentrations), PTH enhances glycogen formation only in calvaria cells, but not in fibroblasts. Insulin-like growth factor I in physiological concentrations promotes glycogen-synthesis shortly after addition.

Osteoblast-like cell *Glycogen synthesis* *Parathyroid hormone* *Cyclic AMP* *Insulin-like growth factor* *Insulin*

1. INTRODUCTION

Calvaria cells in culture are a suitable model for studying the regulation of metabolism of osteoblast-like cells in vitro [1–3]. Parathyroid hormone (PTH) increases cyclic AMP levels in bone cells. Less is known about the biological consequences thereof [4]. Some of the typical features of osteoblastic cells, such as alkaline phosphatase, collagen synthesis, and citrate decarboxylation, are reduced by PTH [1] as well as by cyclic AMP analogues and adenylate cyclase stimulating agents. All in all, the PTH-mediated effects on osteoblast-like cells are catabolic [4]. We now report that PTH enhances glycogen synthesis in cultured calvaria cells.

2. EXPERIMENTAL

2.1. *Cell culture*

Cells were cultured as in [3]. Briefly: calvaria were dissected from 30 newborn rats, cleaned from adherent connective tissue and placed in Erlenmeyer flasks containing 5 ml phosphate-buffered saline (PBS) and 2 mg collagenase/ml (Worthington Biochemical Corp., type II, lot no. 40 C 190). The material digested free, during a 30 min incubation period in a 37°C shaking water bath, was removed and discarded, and the remaining bones were incubated in an identical enzyme solution for another 60 min. The thereby released cells were collected, washed and plated at a density of 0.3 m.i.o./dish (Falcon, 35 mm diam.). Fibroblasts were obtained from the same animals by incubating the skin of the scalps in the same collagenase solution. Fibroblasts were plated at 0.2 m.i.o./dish. The culture medium was MEM containing 1 g glucose/l, 100 U penicillin/ml and 50 μg streptomycin/ml. Non-essential amino acids (1 ml/100 ml) and glutamine (final conc. 2 mM) were routinely added. Growth medium contained 5% fetal calf serum (FCS). On culture day 1 (i.e., on the day after plating) the medium was removed, and unattached cells and debris were washed away, and fresh medium containing 5% FCS was added. The medium was changed once more on day 3, and on day 5 the cells (both types) became confluent. FCS (5%) was then replaced with human serum albumin (HSA, 2 g/l) for 2 further days of culture. All experiments were carried out 7 days after the establishment of primary cultures, and the medium was always the above-mentioned HSA-supplemented MEM, in which the different test substances were dissolved. Parathyroid hormone (synthetic bovine PTH, amino acids 1–34, spec. act. 6800 IU/mg) was obtained from Beckman Instruments Inc., dibutyryl cyclic AMP from Serva. Insulin-like growth factor I (IGF I) and

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whale insulin were kindly supplied by Dr Humbel (Zürich). On the day of the experiment, parallel dishes (usually 2) were used for the determination of the no. cells/dish. After detachment with trypsin EDTA, the cells were counted in a haemocytometer. (Generally, some 0.8 m.i.o./dish for both cell types.)

2.2. *[^{14}C]Glucose incorporation into glycogen (glycogen-synthesis)*

For the measurement of glycogen formation from [^{14}C]glucose, we used the same method as in [3]. D-[U-^{14}C]Glucose (270 mCi/mmol) was from Amersham. The tracer was added to the cell culture dishes at different times after exposure to test media as indicated. The pulse was terminated by decanting the radioactive medium and quickly rinsing the dishes 3 times with cold PBS before 1 ml 20% KOH containing 5 mg glycogen (Fluka) was given to each dish and left at room temperature for 40 min. The solution (including cell lysate and carrier glycogen) was then transferred to tubes and kept for 1 h at 60°C for further hydrolysis of proteins. The tubes were cooled in ice water and 5 ml −20°C cold absolute ethanol was added. Precipitation was followed by centrifugation and 2 washes with 5 ml ethanol, 100 μl LiBr 5% and 100 μl of KOH 1 N. Glycogen was dissolved in 1 ml 0.1 N HCl and 0.5 ml was counted with 5 ml Instagel (Packard) in a β-counter (Beckman Instruments).

3. RESULTS AND DISCUSSION

Cultured calvaria cells exposed to parathyroid hormone for 4 h incorporated more [^{14}C]glucose into glycogen during the following 2 h of incubation than did cells in the absence of PTH (fig.1a). This effect is dose-dependent. PTH exerts a reproducible effect already in small concentrations. The steepest part of the dose–response curve lies between 0.1–1 nM PTH, and 5 nM stimulates maximally. With still higher concentrations of PTH, the dose–response curve begins to decline again. PTH is known to evoke a marked surge of cellular cyclic AMP in calvaria cells. This effect is caused by a rapid activation of adenylate cyclase. However, 4 and 6 h after the exposure to 1 nM PTH, an elevation of cellular cyclic AMP could no longer be detected (2 pmol/10^6 cells). Cyclic AMP

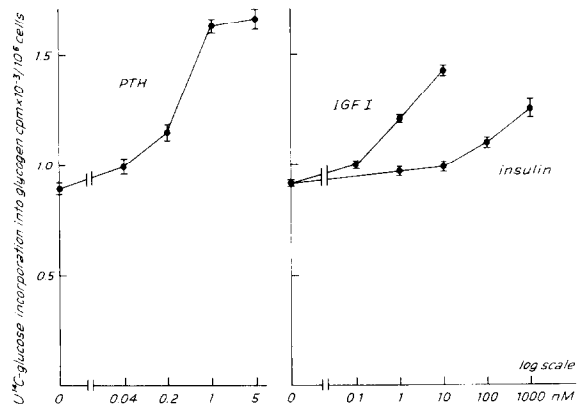

Fig.1. Stimulation of glycogen synthesis from [^{14}C]glucose in cultured rat calvaria cells by parathyroid hormone (PTH), insulin-like growth factor I (IGF I) and by insulin. Cells were cultured as described in the text: 4 h after exposure to test media, 1 μCi D-[U-^{14}C]glucose was added to each dish and the cultures were incubated for further 2 h at 37°C. [^{14}C]Glucose incorporation was determined as in section 2. Results are expressed as cpm incorporated into glycogen/10^6 cells: (a) PTH, 2 expt, n = 10; (b) IGF I and insulin, 1 expt, n = 4.

increases only slightly in cultured fibroblasts in response to PTH [3], and glycogen-synthesis (when assessed under corresponding conditions as in the calvaria cells) was not enhanced (table 1). Therefore, the effect of PTH on glycogen synthesis can be considered as target cell specific. In contrast, insulin stimulated glycogen synthesis in both cell types (table 1). The high concentration of this hormone required can be explained by our observation [3] that pharmacological insulin concentrations mimic the effect of physiological concentrations of insulin-like growth factors I and II. We have reassessed this comparison with insulin and IGF I under the conditions employed in the present experiments (fig.1b). The effect of PTH can be simulated by dibutyryl cyclic AMP (table 2). The same observation was made with another cyclic AMP analogue, 8-bromo-cyclic AMP, and with epinephrine, another stimulator of bone-cell adenylate cyclase (not shown). These findings suggest that PTH-induced glycogen-synthesis may be mediated by cyclic AMP. Time-course experiments (fig.2) revealed that enhancement of glycogen synthesis by PTH and Bt_2cAMP on one hand and by insulin and IGF I on the other hand was com-

Table 1

Effects of PTH and insulin on glycogen synthesis from [^{14}C]glucose in cultured rat calvaria cells and fibroblasts

Medium		Calvaria cells	Fibroblasts
Control		1028 ± 17	589 ± 20
PTH	0.1 nM	1335 ± 60	625 ± 16
PTH	1.0 nM	2131 ± 90	564 ± 28
Insulin	1.0 μM	1458 ± 49	743 ± 52

Conditions were as in fig.1. Results are expressed as cpm incorporated into glycogen/10^6 cells; mean ± SEM, n = 12, 3 expt

pletely different. Whereas insulin and IGF action on glycogen synthesis is early in onset, PTH and Bt_2cAMP lead to an initial slight inhibition. Only at later times calvaria cells turned to a state of increased glycogen formation from [U-^{14}C]glucose. PTH-evoked and cyclic AMP-mediated stimulation of glycogen-synthesis requires time (several hours) to develop. The different time course observed with PTH and with IGF I as well as the additivity (5 nM PTH being a maximally effective PTH concentration, which is also more effective than maximally effective concentrations of IGF) of PTH and IGF I suggest a different mechanism of action. Another possibility, though less probable, would be involvement of distinct target cells, either several cell types or the same cell type at a different stage.

Obviously, glycogen synthesis depends on the detailed circumstances under which it is studied,

Table 2

Effects of PTH and of dibutyryl cyclic AMP (Bt_2cAMP) on glycogen synthesis from [^{14}C]glucose in cultured rat calvaria cells

Medium		cpm
Control		1082 ± 35
PTH	0.1 nM	1374 ± 39
PTH	1.0 nM	1992 ± 46
Bt_2cAMP	0.1 mM	1505 ± 71
Bt_2cAMP	0.5 mM	1701 ± 98

Conditions were as in fig.1. Results are expressed as cpm incorporated into glycogen/10^6 cells; mean ± SEM, n = 12, 3 expt

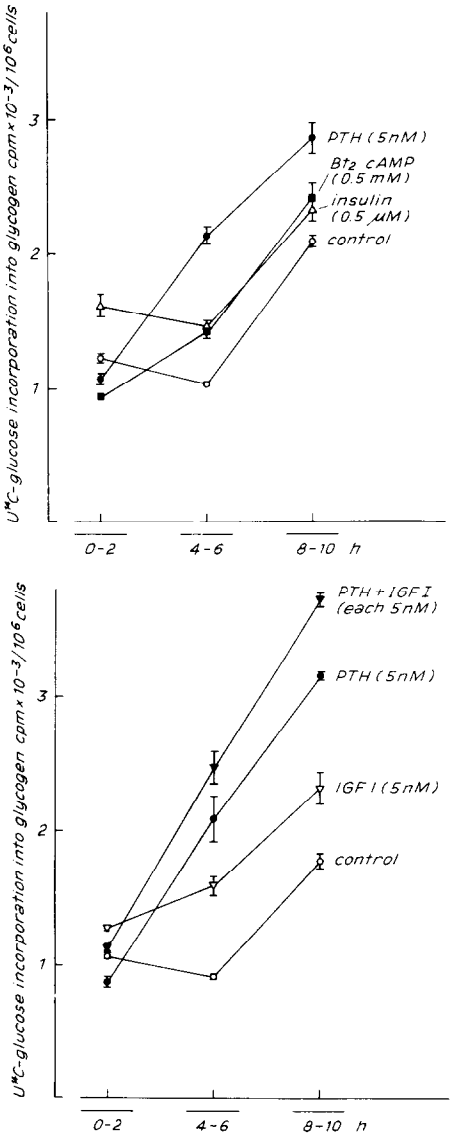

Fig.2. Effects of various stimuli on time course of glycogen synthesis from [^{14}C]glucose in cultured rat calvaria cells. Cells were cultured as described in the text. Dishes were pulsed (2 h) with 1 μCi [^{14}C]glucose immediately (0 h), 4 h and 8 h, respectively, after exposure to test media. [^{14}C]Glucose incorporation into glycogen was determined as in section 2 and is expressed as cpm incorporated into glycogen/10^6 cells. The mean ± SEM is given; n = 4, except in the samples treated with insulin or IGF I, where n = 3. Experiment: (a) control medium, parathyroid hormone (PTH, 5 nM), dibutyryl cyclic AMP (Bt_2cAMP, 0.5 mM) and insulin (0.5 μM); (b) control medium, parathyroid hormone (PTH, 5 nM), insulin-like growth factor I (IGF I, 5 nM), and a combination of both PTH and IGF I (each at 5 nM).

and especially on the glucose concentration of the cell culture medium. It was a consistent finding that under comparable conditions calvaria cells exhibited higher levels of glycogen formation than fibroblasts. The difference between calvaria cells and fibroblasts was even greater than shown in table 1 when higher glucose concentrations were used. Glycogen synthesis in the presence of higher glucose concentrations was increased far more in calvaria cells than in fibroblasts. Our experiments were always carried out with identically handled cells using a culture medium containing 1 g glucose/l; i.e., a near-physiological glucose concentration.

4. CONCLUSIONS

This is the first report on PTH regulation of glycogen metabolism in cultured osteoblast-like cells. In liver cells, PTH was shown to stimulate adenylate cyclase and glycogen breakdown [5]. The acute rise of cyclic AMP is the most extensively studied response to PTH in osteoblast-like cells. It appears to be followed, some hours later, by an increased incorporation rate of [U-^{14}C]glucose into glycogen. This effect is target cell specific (table 1), dose-dependent (fig.1a, table 1), and it is mimicked by dibutyryl cyclic AMP (table 2). PTH evokes this biological response at concentrations considerably below those required to produce easily detectable changes in total cyclic AMP levels [3]. Protein kinase in known to be activated with hormone concentrations some 2 orders of magnitude lower than those needed to get a significant increase in cellular cyclic AMP [6]. The time course of stimulation of glycogen synthesis by PTH is distinct from that observed with IGF I. Considered together with the fact that the effects of these 2 peptides are additive (fig.2b), we are prompted to propose different mechanisms of action for the 2 hormones.

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