Stimulation by defined parathyroid hormone fragments of cell proliferation in skeletal-derived cell cultures

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We have reported previously that parathyroid hormone (PTH) acts on cultured bone cells to stimulate creatine kinase (CK) activity and [³H]thymidine incorporation into DNA via phosphoinositide turnover, in addition to its other actions via increased cyclic AMP production. We also found that mid-region fragments of PTH stimulate [³H]thymidine incorporation into avian chondrocytes. In the present study of mammalian systems, we demonstrate differential effects of defined synthetic PTH fragments on CK activity and DNA synthesis, as compared with cyclic AMP production, in osteoblast-enriched embryonic rat calvaria cell cultures, in an osteoblast-like clone of rat osteosarcoma cells (ROS 17/2.8) and in chondroblasts from rat epiphyseal cartilage cell cultures. Unlike full-length bovine (b)PTH-(1–84) or the fully effective shorter fragment human (h)PTH-(1–34), fragments lacking the N-terminal region of the hormone did not increase cyclic AMP formation, whereas they did stimulate increases in both DNA synthesis and CK activity. Moreover, the PTH fragment hPTH-(28–48) at 10 μM inhibited the increase in cyclic AMP caused by 10 nM-bPTH-(1–84). The increase of CK activity in ROS 17/2.8 cells caused by bPTH-(1–84) or hPTH-(28–48) was completely inhibited by either cycloheximide or actinomycin D, as was shown previously for rat calvaria cell cultures. These results indicated the presence of a functional domain of PTH in the central part of the molecule which exerts its mitogenic-related effects on osteoblast- and chondroblast-like cells in a cyclic AMP-independent manner. Since cyclic AMP formation by PTH leads to bone resorption, specific mid-region fragments of PTH might prove suitable for use *in vivo* to induce bone formation without concomitant resorption.

INTRODUCTION

In conjunction with its regulation of calcium homeostasis, parathyroid hormone (PTH) can stimulate both net bone formation and resorption, depending on the conditions of application. Increased levels of cyclic AMP [1] are considered to mediate the action of PTH in causing bone resorption [2] and stimulation of ornithine decarboxylase activity [3,4]. On the other hand, PTH has also been shown to exert proliferative and anabolic effects, via changes in [Ca²⁺] [5–9], which include increased cell proliferation *in vitro* [10]. PTH stimulates DNA synthesis in osteoblast cultures of chicken [11] and rat calvaria [12] and growth of organ cultures of chicken embryonic cartilage [13]. In a clinical study, the apposition rate of bone was increased by the combination of PTH and vitamin D [14].

We have shown that the mitogenic effect of PTH is mimicked in osteoblast-like cells [12] by phospholipase C treatment or by protein kinase C activation by phorbol ester, which implicated phosphoinositide turnover in the pathway of PTH action, as confirmed directly in more recent work [15]. Moreover, we have also shown that, in chicken sternum chondroblasts, the central region of the PTH molecule is sufficient to stimulate thymidine incorporation into DNA [16]. In the present paper, we used two osteoblast-like cell cultures and one chondroblast-like cell culture of rat cells to demonstrate that the same functional domain of the PTH molecule which is active in an avian system is responsible for the mitogenic response in the rat. For this work, we used two assay systems: (1) the incorporation of [³H]thymidine, a measure of DNA synthesis, and (2) the stimulation of the specific activity

of the brain-type isoenzyme of creatine kinase (CK [17–19]), the enzyme that regulates intracellular levels of ATP and ADP [20]. Whereas [³H]thymidine incorporation is a relatively late response to a mitogen, the increased activity of CK reflects a very early response to mitotic stimulation, and its rapid and sensitive assay makes CK a convenient enzyme marker for hormone action [12,17,19,21]. In this paper we also take the first steps towards defining the molecular mechanism of CK stimulation by PTH using inhibitors of RNA and protein synthesis.

MATERIALS AND METHODS

Biochemicals

Bovine parathyroid hormone [bPTH-(1–84), trichloroacetic acid powder from bovine parathyroid], collagenase type I, cycloheximide and actinomycin D were obtained from Sigma (St. Louis, MO, U.S.A.). Human parathyroid hormone (hPTH) fragments hPTH-(1–34) and hPTH-(28–48) were from Bachem Co. (Torrance, CA, U.S.A.). All other fragments were synthesized at the Department of Genetics of the Gesellschaft für Biotechnologische Forschung, Germany [16]. All biochemicals used were of analytical reagent grade.

Cell cultures

Osteoblast-enriched cultures were prepared from calvaria of 19–21-day-old Wistar-derived rat embryos [18]. Calvaria were excised and cells were released by digestion with trypsin/EDTA. Cells were plated (3 × 10⁵ cells/35 mm-diam. culture dish) and

Abbreviations used: PTH, parathyroid hormone (the prefixes 'h' and 'b' refer to human and bovine respectively); CK, creatine kinase; PBS, phosphate-buffered saline.

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incubated in 2 ml of BGJ₀ medium without Ca²⁺ [22] supplemented with 10% fetal calf serum at 37 °C in 5% CO₂. Cultures were used when subconfluent.

ROS 17/2.8 cells (a subclone of a rat osteogenic sarcoma line [23]) were cultured in 35 mm-diam. culture dishes in 2 ml of Dulbecco's modified Eagle's medium/Ham's F12 medium (1:1) [24] containing 10% fetal calf serum [18] at 37 °C in 8% CO₂. Cells were used when subconfluent.

Rat epiphyseal cartilage cells were obtained from 19–21-day-old rat embryos, which have a wider epiphyseal cartilage zone than older rats. Epiphyseal cartilage plates were isolated under a binocular dissecting microscope. Cells were released by digestion with 0.25% (w/v) collagenase in phosphate-buffered saline (PBS; 0.05 M-sodium phosphate/0.154 M-NaCl, pH 7.4) for 60 min at 37 °C and were cultured as described above for calvaria cells, but in standard BGJ₀ medium containing 2 mM-Ca²⁺. The cells were identified as chondrocytes by morphology, by the presence of alkaline phosphatase activity and by their reaction with antibody against type II collagen [25]. The cells were used when confluent.

Assay of CK activity

After treatment for 24 h, cells were washed with PBS and sonicated, in three 3 s bursts, in homogenizing buffer containing 5 mM-magnesium acetate/0.4 mM-EDTA/2.5 mM-dithiothreitol/50 mM-Tris/HCl (pH 6.8)/250 mM-sucrose. In some cases, cells were disrupted by freezing and thawing three times. Supernatant extracts were obtained by centrifugation in an Eppendorf Microfuge at 12000 *g* for 5 min at 4 °C [12]. Assays were performed at 30 °C in a Gilford 250 automatic recording spectrophotometer using an assay, as described previously [17,18], in which the ATP formed was coupled, via the action of hexokinase and glucose-6-phosphate dehydrogenase, to the formation of NADH. Protein concentrations were determined by the Coomassie Brilliant Blue dye binding method of Bradford [26], with bovine serum albumin as the standard.

Determination of cyclic AMP production

Cyclic AMP was measured, after hormonal treatment of cells for 15 min, by the competitive binding assay of Gilman with a slight modification [27] using a cyclic [³H]AMP assay kit from Diagnostic Products Corporation (Los Angeles, CA, U.S.A.). Protein was assayed by the Bradford method [26].

[³H]Thymidine incorporation into DNA

The rate of DNA synthesis was measured by determination of [³H]thymidine incorporation into DNA by pulse labelling at 22–24 h after the start of the incubation. At 2 h before termination of the experiments, 5 μ Ci of [³H]thymidine (5 Ci/mmol: Amersham) was added to each culture dish. At the end of the incubation the medium was removed, and cells were collected and homogenized in 1 ml of deionized distilled water. The total uptake of [³H]thymidine and the incorporation into materials insoluble in 5% trichloroacetic acid were measured [27]. In all cases, the changes in uptake of [³H]thymidine into the cells were much too small to account for the changes in [³H]thymidine incorporation into DNA. DNA was determined by the diphenylamine method described by Burton [28].

RESULTS

Comparison of bPTH-(1–84) and hPTH-(28–48) in osteoblast-enriched rat calvaria cell cultures

Osteoblast-enriched calvaria cell cultures were incubated with full-length bPTH-(1–84), and cyclic AMP production (at 15 min) was compared with the mitotic indicators [³H]thymidine incorporation and stimulation of CK specific activity (Fig. 1).

Fig. 1. Dose-dependent stimulation by bPTH-(1–84) of osteoblast-enriched rat embryo calvaria cell cultures

Experimental details for the determination of cyclic AMP (□) (15 min after stimulation) and incorporation of [³H]thymidine into DNA (▲) and CK (●) specific activity (24 h after stimulation) are given in the Materials and methods section. The results are means $\pm$ S.E.M., $n \geq 5$. In several cases the S.E.M. was less than the size of the symbol. Control values were: cyclic AMP, 25 ± 5 pmol/mg of protein; DNA, 240 ± 30 c.p.m./ μ g of DNA; CK, 200 ± 20 nmol/min per mg of protein. Statistical significance of differences between treated and corresponding control groups by Student's *t* test: * $P < 0.05$; ** $P < 0.01$.

Fig. 2. Dose-dependent stimulation by hPTH-(28–48) of osteoblast-enriched rat embryo calvaria cell cultures

Experimental details and symbols are the same as for Fig. 1. Control values are: cyclic AMP, 32 ± 5 pmol/mg of protein; DNA, 630 ± 30 c.p.m./ μ g of DNA; CK, 225 ± 50 nmol/min per mg of protein.

There was a significant increase in cyclic AMP production at a concentration as low as 0.1 nM and a maximum increase at 10 nM. For stimulation of CK activity or DNA synthesis after 24 h of treatment a concentration of 1 nM-bPTH-(1–84) was needed to detect a significant increase, but a maximum effect was also reached at 10 nM.

By contrast, when cells were treated with hPTH-(28–48), a fragment from the middle portion of the molecule (Fig. 2), there was no significant increase in cyclic AMP production at any of the concentrations tested [from 10 nM, the optimum for bPTH-(1–84), to 1 μ M]. However, DNA synthesis and CK specific activity were stimulated by hPTH-(28–48) at all three concentrations tested. The stimulation of CK specific activity was maximal at 10 nM, whereas the increase in [³H]thymidine incorporation reached a peak at 100 nM.

Table 1. Inhibition by hPTH-(28-48) of cyclic AMP formation induced by bPTH-(1-84) in rat embryo calvaria cell cultures

bPTH-(1-84) was used at 10 nM and hPTH-(28-48) at 1 μ M. Cyclic AMP was determined 15 min after stimulation as described in the Materials and methods section. The results are means $\pm$ S.E.M., $n \geq 5$. Statistical significance of the difference between treated and control groups by Student's *t* test: **P* < 0.005.

PTH fragment	Cyclic AMP production (pmol/mg of protein)
None	16.4 $\pm$ 2.0
bPTH-(1-84)	55.6 $\pm$ 10.4*
hPTH-(28-48)	16.2 $\pm$ 2.4
bPTH-(1-84) + hPTH-(28-48)	18.0 $\pm$ 1.6

To investigate the influence of the mid-region fragment on the stimulation of cyclic AMP production by the complete molecule, the two molecules were tested in combination. When osteoblast-enriched calvaria cell cultures were treated with 10 nM-bPTH-(1-84) for 15 min there was a 3-fold increase in cyclic AMP formation (Table 1); hPTH-(28-48) at 1 μ M did not stimulate any cyclic AMP production. However, simultaneous treatment with 10 nM-bPTH-(1-84) and a 100-fold excess of hPTH-(28-48) prevented stimulation of cyclic AMP production by bPTH-(1-84) (Table 1).

Mitogenic activity of PTH fragments on osteoblast-enriched calvaria cell cultures

Rat calvaria cultures were treated with fragments of PTH derived from the central portion of the molecule. The concentration used in this comparison was based on the results obtained above (Figs. 1 and 2) and on previous experiments using chick sternum chondroblasts [16]. When treated for 15 min for stimulation of cyclic AMP production (Table 2), only the complete PTH containing the N-terminal part of the molecule [or

Table 2. Stimulation of cyclic AMP production, CK activity and DNA synthesis in rat embryo calvaria cell cultures by defined PTH fragments

Experimental details are described in the Materials and methods section. The results are means $\pm$ S.E.M., $n = 5-15$ for at least two sets of cultures. Control values were: cyclic AMP, 29 $\pm$ 2.9 pmol/mg of protein; DNA, 624 $\pm$ 73 c.p.m./ μ g of DNA; CK, 457 $\pm$ 46 nmol/min per mg of protein. Statistical significance of differences between treated and control groups by Student's *t* test: **P* < 0.05; ***P* < 0.01.

PTH fragment (100 nM)	Effect of fragment (experimental value/control value)		
	Cyclic AMP concentration	CK specific activity	[³ H]Thymidine incorporation
None	1.00 $\pm$ 0.10	1.00 $\pm$ 0.15	1.00 $\pm$ 0.11
bPTH-(1-84)	3.74 $\pm$ 0.30**	2.27 $\pm$ 0.09**	2.61 $\pm$ 0.11**
hPTH-(25-39)	0.87 $\pm$ 0.10	2.15 $\pm$ 0.24*	2.96 $\pm$ 0.18**
hPTH-(28-48)	1.22 $\pm$ 0.09	2.15 $\pm$ 0.15**	2.82 $\pm$ 0.18**
hPTH-(28-47)	0.52 $\pm$ 0.39	2.18 $\pm$ 0.12*	2.11 $\pm$ 0.09**
hPTH-(29-47)	0.57 $\pm$ 0.17	1.67 $\pm$ 0.11*	1.96 $\pm$ 0.14**
hPTH-(30-47)	1.00 $\pm$ 0.22	1.27 $\pm$ 0.03*	1.86 $\pm$ 0.18*
hPTH-(31-47)	0.78 $\pm$ 0.10	1.21 $\pm$ 0.18	1.82 $\pm$ 0.18*
hPTH-(32-47)	0.96 $\pm$ 0.10	1.09 $\pm$ 0.12	1.86 $\pm$ 0.18*
hPTH-(33-47)	1.13 $\pm$ 0.26	0.79 $\pm$ 0.09	1.61 $\pm$ 0.18*
hPTH-(34-47)	1.04 $\pm$ 0.09	0.70 $\pm$ 0.15	1.49 $\pm$ 0.23*

Table 3. Stimulation of CK specific activity and DNA synthesis in rat epiphyseal cartilage cell cultures by defined fragments of PTH

Experimental details are described in the Materials and methods section. No significant increase in the amount of DNA per plate was detectable by 24 h. The results are means $\pm$ S.E.M.; $n = 6$ for two sets of cultures. Statistical significance of differences between test and control groups by Student's *t* test: **P* < 0.05; ***P* < 0.01.

PTH fragment	CK specific activity (nmol/min per mg of protein)	[³ H]Thymidine incorporation into DNA (c.p.m./plate)
Control	225 $\pm$ 32	350 $\pm$ 25
bPTH-(1-84) (10 nM)	389 $\pm$ 39*	556 $\pm$ 50*
hPTH-(25-39) (100 nM)	268 $\pm$ 36	544 $\pm$ 44*
hPTH-(28-48) (100 nM)	346 $\pm$ 36*	731 $\pm$ 44**
hPTH-(34-47) (100 nM)	236 $\pm$ 32	356 $\pm$ 75

hPTH-(1-34); results not shown] was able to stimulate cyclic AMP formation; all other peptides tested were ineffective. When cells were treated for 24 h and CK activity was measured (Table 2), bPTH-(1-84) [as well as (hPTH-(1-34); results not shown], hPTH-(25-39), hPTH-(28-48) and hPTH-(28-47) were maximally active in increasing CK activity, whereas hPTH-(29-47) was stimulatory but to a lesser degree, and hPTH-(30-47) was slightly stimulatory. All other fragments used [hPTH-(31-47), hPTH-(32-47), hPTH-(33-47) and hPTH-(34-47)] were inactive.

When DNA synthesis was measured, the stimulation by the different fragments was found to be maximal with bPTH-(1-84) [as well as with hPTH-(1-34); results not shown], hPTH-(25-39) and hPTH-(28-48), and less with hPTH-(28-47) to hPTH-(34-47) (Table 2).

Mitogenic activity of PTH fragments on rat epiphyseal cartilage cell cultures

To compare the stimulations seen in osteoblast-like cells (Figs. 1 and 2) with PTH effects in a cartilage system, cultures of rat epiphyseal cartilage cells were incubated with full-length PTH and selected PTH fragments (Table 3). CK activity was stimulated by bPTH-(1-84) and hPTH-(28-48) but not by hPTH-(25-39) or hPTH-(34-47). However, DNA synthesis was stimulated by all molecules tested except hPTH-(34-47) (Table 3). The comparison between the complete molecule and the mid-regional fragment hPTH-(28-48) is similar to but not identical with the results obtained for calvaria cell cultures (Figs. 1 and 2) and in the chick sternum chondrocyte system [15].

Mitogenic activity of PTH fragments on ROS 17/2.8 osteosarcoma cell cultures

When cultures of the widely used model for osteoblasts [23], the osteoblast-like clone of rat osteosarcoma, ROS 17/2.8, were incubated with full-length PTH and PTH fragments (Table 4), cyclic AMP production was stimulated only by bPTH-(1-84) and hPTH-(1-34), whereas hPTH-(25-39), hPTH-(27-48), hPTH-(28-48) and hPTH-(34-47) were ineffective. Both CK activity and DNA synthesis were stimulated by all of the cell fragments tested except hPTH-(34-47) (Table 4). These results are also similar to the results obtained in calvaria cell cultures.

Inhibition of induction of CK activity by cycloheximide or actinomycin D in ROS 17/2.8 cells

The ability of inhibitors of RNA and protein synthesis to inhibit stimulation of CK activity by a mitogenic mid-region

Table 4. Stimulation of cyclic AMP production, CK specific activity and DNA synthesis in ROS 17/2.8 osteoblast-like cells by defined PTH fragments

Experimental details are given in the Materials and methods section. Concentrations were chosen on the basis of results obtained with chick sternum chondroblasts [16]. The results are means $\pm$ S.E.M.; $n \geq 5$. Control values were: cyclic AMP, 29 ± 5.2 pmol/mg of protein; DNA, 392 ± 58 c.p.m./ μ g of DNA; CK, 698 ± 70 nmol/min per mg of protein. Statistical significance of differences between treated and control groups of Student's *t* test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

PTH fragment	Effect of fragment (experimental value/control value)		
	Cyclic AMP	CK specific activity	[3 H]Thymidine incorporation
bPTH-(1-84) (10 nM)	$4.60 \pm 0.10^{***}$	$1.92 \pm 0.11^{**}$	$1.71 \pm 0.18^*$
hPTH-(1-34) (40 nM)	$4.67 \pm 0.09^{***}$	$1.83 \pm 0.09^{**}$	$2.06 \pm 0.09^{**}$
hPTH-(25-39) (200 nM)	1.05 ± 0.15	$1.71 \pm 0.08^*$	$1.36 \pm 0.08^*$
hPTH-(28-47) (400 nM)	1.03 ± 0.16	$1.66 \pm 0.11^*$	$1.44 \pm 0.11^*$
hPTH-(28-48) (400 nM)	1.18 ± 0.28	$1.56 \pm 0.08^*$	$1.56 \pm 0.12^*$
hPTH-(34-47) (500 nM)	0.88 ± 0.31	1.07 ± 0.10	1.05 ± 0.11

Table 5. Inhibition by actinomycin D and cycloheximide of PTH stimulation of CK activity in ROS 17/2.8 cells

Cells were grown to subconfluence as described in the Materials and methods section and treated for 24 h with 10 nM-bPTH-(1-84) or 100 nM-hPTH-(28-48), with or without actinomycin D (act. D) (5 μ g/ml) or cycloheximide (10 μ g/ml). CK activity was measured as described in the Materials and methods section. Results are means $\pm$ S.E.M.; $n = 5$, * $P < 0.05$.

Treatment	Creatine kinase specific activity (μ mol/min per mg of protein)
Control	2.23 ± 0.32
Act. D (5 μ g/ml)	2.36 ± 0.27
Cycloheximide (10 μ g/ml)	2.90 ± 0.38
bPTH-(1-84) (10 nM)	$4.49 \pm 0.65^*$
bPTH-(1-84) + act. D	3.19 ± 0.32
bPTH-(1-84) + cycloheximide	2.84 ± 0.38
hPTH-(28-48) (100 nM)	$4.00 \pm 0.27^*$
hPTH-(28-48) + act. D	2.43 ± 0.35
hPTH-(28-48) + cycloheximide	2.16 ± 0.16

fragment of PTH or by bPTH-(1-84) was tested. ROS 17/2.8 cells incubated with 10 nM-bPTH-(1-84) or 100 μ M-hPTH-(28-48) showed, at 24 h, the expected significant increase in CK activity (Table 5); treatment with either actinomycin D (5 μ g/ml) or cycloheximide (10 μ g/ml) completely inhibited this stimulation by both the complete hormone and the mitogenic fragment. This is similar to previous findings for the complete PTH molecule tested in calvaria cells [18].

DISCUSSION

The results presented in this paper extend our previous reports that bPTH-(1-84) stimulates DNA synthesis and CK activity in rat osteoblasts [12] and chondroblasts [16], in agreement with findings from other laboratories on such skeletal-derived cells [11,13]. As we reported previously [12,16], the stimulation by PTH of cell proliferation and the induction of CK synthesis in

these cells is independent of cyclic AMP formation. The only cell type in which PTH stimulation of DNA synthesis was shown to be mediated (and mimicked) by cyclic AMP was the chick epiphyseal growth plate chondroprogenitor cell [29]. Moreover, the fact that a mid-region fragment which does not stimulate cyclic AMP production inhibits bPTH-(1-84) stimulation of cyclic AMP production (Table 1) suggests an as yet undefined competitive interaction between the two molecules. These data, taken together with previous data [12,16], provide evidence for a mechanism by which PTH activates two distinct second messenger systems, one via the phosphoinositide pathway for the action of PTH on stimulation of CK and DNA synthesis (cell proliferation) and the other via adenylate cyclase to increase cyclic AMP formation (which leads to bone resorption [30,31]). The inhibition of cyclic AMP formation by fragments from the mid-region of PTH might indicate the use of these fragments *in vivo* in a dual role as inhibitors of bone resorption as well as stimulators of bone formation.

Until now, only N-terminal fragments were found to have effects similar to those of the complete PTH molecule [32] or of PTH-(1-34) [which has the same activity as PTH-(1-84)]. Only bPTH-(1-27) amide, in addition to the widely used hPTH-(1-34), was shown to increase cyclic AMP formation and ornithine decarboxylase activity and glycosaminoglycan synthesis in rabbit rib growth cartilage chondrocytes [33]. It was recently reported that the middle part of the molecule of hPTH (amino acids 28-48) activates cell proliferation in cartilage [16]. The fragments hPTH-(28-48) and hPTH-(25-39), which share the amino acid sequence 30-34 [16], were found to be effective stimulators of bone cell proliferation. Other fragments in this central region were also active in the osteoblast cell culture systems in the present study. We conclude that in bone, as well as in cartilage, the central region of the molecule, which is its most hydrophobic part, is active in the stimulation of proliferation, with slight differences in the responsiveness of the two tissues to specific mid-region fragments when comparing DNA synthesis and CK stimulation. This portion, of up to 39 residues, is conserved in human, cow and pig, emphasizing its potential physiological importance. The use of human PTH fragments in this study to stimulate rat cells provides an overall picture which may be slightly modified in completely homogeneous systems. Similarly, although the analysis of CK specific activity reveals the same differential stimulation as shown by thymidine incorporation, the extents of stimulation of these two parameters are not necessarily equal. It will be important to determine whether any mid-region fragments of PTH are present in the circulation under physiological or pathological conditions.

The mechanism by which PTH fragments and the complete molecule of PTH stimulate cell proliferation cannot be via cyclic AMP, since the active mid-region fragments do not stimulate adenylate cyclase activity (Tables 1 and 2), but probably activate phospholipase C and protein kinase C [12]. The direct effect of the fragments in activating phospholipase C and protein kinase C has yet to be demonstrated. The inhibition by actinomycin D and cycloheximide of stimulation of CK activity in ROS 17/2.8 cells by both bPTH-(1-84) and hPTH-(28-48), and the rapid (within 1 h) increase in the steady-state level of mRNA for CK and *c-fos* [34] in calvaria cell cultures after stimulation by bPTH-(1-84) are first results in the attempt to elucidate the signal transduction cascade from the membrane to the gene regulation level.

The findings in this paper have now been paralleled *in vivo* [35]; D. Sömjen, K. D. Schlüter, E. Wingender, H. Mayer & A. M. Kaye, unpublished work) in prepubertal Wistar-derived rats. We found that hPTH-(28-48) stimulated CK specific activity in a time- and dose-dependent manner, reaching a maximum at 4 h

after intraperitoneal injection of 1.25 mg/rat. A fragment closer to the C-terminal, hPTH-(34–47), was inactive compared with equimolar concentrations of hPTH-(28–48) or hPTH-(25–39), which stimulated both CK and DNA synthesis. Therefore, data obtained both *in vitro* and *in vivo* support the idea that different effects of PTH are exerted by different functional domains of the molecule and raise the possibility that, for therapeutic purposes, the overall balance of the anabolic and catabolic effects of PTH could be changed *in vivo* to favour bone-forming processes.

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REFERENCES

- Potts, J. T., Jr., Kronenberg, H. M. & Rosenblatt, M. (1982) *Adv. Protein Chem.* **35**, 323–396
- Rasmussen, H. & Tenenhouse, A. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **59**, 1364–1370
- Takigawa, M., Watanabe, R., Ishida, H., Asada, A. & Suzuki, F. (1979) *J. Biochem. (Tokyo)* **85**, 311–314
- Sömjen, D., Yariv, M., Kaye, A. M., Korenstein, R., Fischler, H. & Binderman, I. (1982) *Adv. Polyamine Res.* **4**, 713–718
- Borle, A. B. & Neumann, W. F. (1965) *J. Cell Biol.* **24**, 316–323
- Morgan, J. I., Hall, A. K. & Perris, A. D. (1975) *Biochem. Biophys. Res. Commun.* **66**, 188–194
- Whitfield, J. F., Rixon, R. H., Perris, A. D. & Yondale, T. (1969) *Exp. Cell Res.* **57**, 8–12
- Perris, A. D. & Whitfield, J. F. (1967) *Nature (London)* **214**, 302–303
- Perris, A. D. & Whitfield, J. F. (1967) *Nature (London)* **216**, 1350–1351
- Whitfield, J. F., Boynton, A. L., MacManus, P., Skorska, M. & Tsang, B. K. (1979) *Mol. Cell. Biochem.* **27**, 155–179
- van der Plas, A., Feyen, J. H. M. & Nijweide, P. J. (1985) *Biochem. Biophys. Res. Commun.* **129**, 918–925
- Sömjen, D., Zor, U., Kaye, A. M., Harell, A. & Binderman, I. (1987) *Biochim. Biophys. Acta* **931**, 215–223
- Lewinson, D. & Silbermann, M. (1986) *Calcif. Tissue Int.* **38**, 155–162
- Slovik, D. M., Rosenthal, D. I., Doppelt, S. H., Potts, J. T., Jr., Daly, A. M., Campbell, J. A. & Neer, R. M. (1986) *J. Bone Mineral Res.* **1**, 377–382
- Farndale, R. W., Sandy, J. R., Meghi, S. & Meikle, M. C. (1988) *Biochem. J.* **252**, 263–268
- Schlüter, K. D., Hellstern, H., Wingender, E. & Mayer, H. (1989) *J. Biol. Chem.* **264**, 11087–11092
- Kaye, A. M. (1983) *J. Steroid Biochem.* **19**, 33–40
- Binderman, I., Berger, E., Fine, N., Shimshoni, Z., Harell, A. & Sömjen, D. (1989) *Connect. Tissue Res.* **20**, 44–47
- Sömjen, D., Kaye, A. M., Rodan, G. A. & Binderman, I. (1985) *Calcif. Tissue Int.* **37**, 635–638
- Watts, D. S. (1973) *Enzymes* 3rd Ed. **8** part 2, 383–455
- Kaye, A. M., Reiss, N. A., Weisman, Y., Binderman, I. & Sömjen, D. (1986) in *Myocardial and Skeletal Muscle Bioenergetics* (Brautbar, N., ed.), pp. 83–102, Plenum, New York
- Binderman, I. & Sömjen, D. (1982) in *Current Advances in Skeletogenesis: Development, Biomineralization, Mediators and Metabolic Bone Diseases* (Silberman, M. & Slavkin, H. C., eds.), pp. 412–416, Excerpta Medica, Amsterdam
- Majeska, R. J., Rodan, S. B. & Rodan, G. A. (1980) *Endocrinology (Baltimore)* **107**, 1494–1503
- Kaye, A. M., Weisman, Y., Harell, A. & Sömjen, D. (1990) *J. Steroid Biochem. Mol. Biol.* **37** in the press
- Levy, J., Shimshoni, Z., Sömjen, D., Berger, E., Fine, N., Silberman, M. & Binderman, I. (1988) *In Vitro* **24**, 620–625
- Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254
- Sömjen, D., Binderman, I., Berger, G. & Harell, A. (1980) *Biochim. Biophys. Acta* **627**, 91–99
- Burton, K. (1956) *Biochem. J.* **62**, 315–323
- Pines, M. & Hurwitz, S. (1988) *Endocrinology (Baltimore)* **123**, 360–365
- Wong, G. L., Luben, R. A. & Cohn, D. V. (1977) *Science* **197**, 663–665
- Peck, W. A. & Klahr, S. (1979) *Adv. Cyclic Nucleotide Res.* **11**, 89–130
- Sakaguchi, K., Fukase, M., Kobayashi, I., Kimura, T., Sakakibara, S., Katsuragi, S., Morita, K., Noda, T. & Fujita, T. (1987) *J. Bone Mineral Res.* **2**, 83–90
- Takano, T., Takigawa, M., Shirai, E., Suzuki, F. & Rosenblatt, M. (1985) *Endocrinology (Baltimore)* **116**, 2536–2542
- Sömjen, D., Berger, E., Weisman, A., Binderman, I. & Kaye, A. M. (1989) *Israel Endocrine Soc. Annu. Meeting, Jerusalem*, 16–17 January 1989, p. 82 (abstract)
- Sömjen, D., Schlüter, K. D., Wingender, E., Mayer, H. & Kaye, A. M. (1990) 20th FEBS Meeting, Budapest, 19–24 August 1990, p. 261 (abstract)

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