The Central Part of Parathyroid Hormone Stimulates Thymidine Incorporation of Chondrocytes*

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The stimulation of DNA synthesis in primary cell cultures of chicken chondrocytes by parathyroid hormone was studied by assaying [3H]thymidine incorporation into DNA. Optimal assay conditions were determined by varying cell age, plating density, and incubation time. Under these conditions DNA synthesis was significantly stimulated by parathyroid hormone (PTH) and some of its fragments: cells treated with human (h)PTH(1-84), bovine (b)PTH(1-34) and [Nle8,18,Tyr34]bPTH(3-34)amide and hPTH(13-34) displayed 2.6-fold enhanced [3H]thymidine incorporation in a dose-dependent manner. The fragment hPTH(28-48) led to a similar stimulation, whereas [Tyr62,Asp76]hPTH(52-84) had no effect. Using a series of synthetic hPTH peptides covering the central region of the hormone molecule (residues 25-47), we could delimitate further this putative mitogenic functional domain to a core region between amino acid residues 30 and 34. The effect of PTH on [3H]thymidine incorporation could not be mimicked by forskolin, indicating that the corresponding signal is not mediated by CAMP. It is, however, inhibited by EGTA and cannot be provoked in the absence of calcium ions in the medium. Therefore, the results presented indicate a hitherto unidentified functional domain of PTH in the central part of the molecule which exerts its mitogenic effect on chondrocytes in a CAMP-independent manner but seems to involve calcium ions for signal transduction.

Parathyroid hormone (PTH) is a peptide hormone of 84 amino acid residues which regulates calcium homeostasis by acting on renal cortex, cells of the skeletal system, and other target tissues. It stimulates the activity of adenylate cyclase (see Ref. 1 for review) or the induction of ornithine decarboxylase (2). Furthermore, PTH has been shown to exert effects on a variety of cells (3-7). In particular, it stimulates the DNA synthesis in secondary cultures of chicken osteoblasts (8), it enhances the growth of chicken embryonic cartilage in organ culture (9-11), and the bone apposition rate in vivo in a dose-dependent manner (12).

In contrast to the well characterized effect of native PTH, hormone variants, and fragments on the renal receptor which is linked to adenylate cyclase (reviewed in Ref. 1), the mechanism through which it acts on cell proliferation remains to be elucidated. Therefore, we attempted to define the functional domain of the PTH molecule which is necessary for the mitogenic response. For this purpose we established a primary cell culture system of chondrocytes, which were isolated from 16-day-old chicken embryos by separating the sternum as described (13). PTH and PTH-derived peptides were assayed for their capability to enhance incorporation of [3H]thymidine by these cells as a criterion for increase of DNA synthesis (9, 14, 15).

MATERIALS AND METHODS

Chemicals and Supplies—hPTH(1-84), bPTH(1-34), [Nle8,18,Tyr34]bPTH(1-34)amide, [Nle8,18,Tyr34]hPTH(3-34)amide, hPTH(13-34), [Tyr62,Asp76]hPTH(52-84) were obtained from Sigma, hPTH(25-48) from Bachem (U. S. A.). Tissue culture supplies were purchased from Nunc (Denmark), [methyl-3H]Thymidine (40 Ci/mmol) was obtained from Amer sham (United Kingdom), fetal calf serum (FCS) from Boehring (Mannheim, Federal Republic of Germany), and gentamycin from Biochrom (Berlin, Federal Republic of Germany) and collagenase type II, 143 units/mg ( Worthington). Forskolin was purchased from Sigma. All other chemicals were p.a. grade and obtained from Merck (Darmstadt, Federal Republic of Germany). Reagents and solvents for peptide synthesis were of the highest quality commercially available and were used without further purification.

Peptide Synthesis and Purification—All syntheses were performed in the automated mode in a modified, computer-controlled version of the Biosearch 9600 Peptide Synthesizer using fluorescamine-nitrocellulose-bonded strategy (16) and a p-benzoxycarbonyl alcohol resin. For peptide bond formation Castro’s reagent (17) was used in the activation step. Protected peptide resins were cleaved in trifluoroacetic acid/thio-phenol/amisol 8:1:1 for 2 h at 40 °C. The free peptides were precipi-tated afterwards by addition of ether. All crude peptides were routinely purified by gel filtration on Sephadex G10 and preparative reversed phase high performance liquid chromatography (column YMC ODS-5, 20 × 259 mm). The purified peptides were characterized by amino acid analysis (Biotronic LC 5001) and fast atom bombardment mass spectrometry (Kratos MS 50).

Isolation and Culture of Embryonic Chick Chondrocytes—Chondrocytes were isolated from sternum of 16-day-old embryonic chicks according to Yasu (13) with some modifications. Collagenase was dissolved in Hank’s balanced salt solution (0.4 mM NaHPO4, 140 mM NaCl, 5.5 mM KCl, 0.4 mM MgSO4, 1.3 mM CaCl2, 0.4 mM KH2PO4, 0.5 mM MgCl2, 5 mM glucose, 0.05% gentamycin) at a concentration of 286 units/ml. Sterna were treated for 30 min with collagenase solution at 37°C. Cells first released by this procedure were discarded because of their very poor responsiveness towards PTH. The collagenase treatment was subsequently repeated leading to a highly homogenous cell population of chondrocytes. The cells obtained by a second collagenase treatment were collected by centrifugation (1000 rpm, 10 min), washed twice with medium (Dulbecco’s modified Eagle’s medium, Gibco, supplemented with NaHCO3 (45 mM), glutamine (2 mM), penicillin G/streptomycin (65 mug/l and 100
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mg/L, respectively), Hepes (10 mM, pH 7.2), and FCS (10% v/v) and counted. The highly homogenous cell population isolated by this procedure revealed polyphenylalanine morphology which is typical for chondrocytes (13, 18). Their ornithine decarboxylase activity was inducible by PTH(1–34). They were devoid of alkaline phosphatase activity and de-differentiated upon senescence or secondary culture (data not shown). These observations strongly indicate the identity of chondrocytes and distinguish them from fibroblasts (ornithine decarboxylase (19)) or osteoblasts (alkaline phosphatase (20, 21)).

Assay for Thymidine Incorporation—The DNA synthesis rate was assayed in monolayer culture by the incorporation of [3H]thymidine into parathyroid acid-precipitable material. After variation of the essential parameters, the following conditions were found to be optimal to assay the stimulation of thymidine incorporation by PTH. Cells were seeded into microtiter plates with 96 wells (6-mm diameter; 14,000 cells/cm²) and a 200-μl volume of medium containing 10% FCS and were incubated at 37°C in an atmosphere of 5% CO₂. After 17 h, the medium was replaced by 200 μl of serum-free medium. After 4 days, chondrocytes were incubated for 4 h with the appropriate effector and 1 μCi of [3H]thymidine. Subsequently, medium was removed and the cells were washed twice with 200 μl of phosphate-buffered saline (PBS: 157 mM NaCl, 2.7 mM KCl, 1.5 mM KH₂PO₄, 0.8 mM Na₂HPO₄, pH 7.0). Then cells were lysed by 100 μl of 2% (v/v) Nonidet P-40 and 2% (w/v) sodium dodecyl sulfate treatment and the perchloric acid-insoluble material was precipitated by adding an equal volume of ice-cold 2% (w/v) perchloric acid in the presence of 1% herring sperm DNA as carrier. After storage for 10 min at -20°C, the precipitated material of each well was transferred into glass fiber filters with a Scatron-As semiautomatic cell harvester. Filters were dried at 80°C for 20 min, transferred into scintillation vials, and 2 ml of scintillation mixture (scintillator 299; United Technology Packard) were added. Radioactivity of the samples was determined with a β counter (Minimax; United Technology Packard). Each test contained 5 up to 10 identically treated wells and was repeated at least 3 times with freshly prepared chondrocytes. In separate experiments, we confirmed the correlation between enhancement of [3H]thymidine incorporation after 4 h and subsequent increase in cell number after a 24-h incubation period.

Assay for cAMP Determination—Chicken chondrocytes were seeded into 24-well plates with 500 μl of medium and 10% FCS (8000 cells/cm²). Cells were cultivated as described above. After 4 days, chondrocytes were incubated for 20 min with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine followed by an additional 10-min incubation with the corresponding PTH fragment. The induction was stopped by removing the medium and washing the cells once with ice-cold PBS. Subsequently, the cells were incubated in PBS at 85°C for 10 min. After replacement of the buffer by ice-cold PBS, the chondrocytes were harvested with a rubber policeman and sonicated in ice with three strokes for 5 s each. The cell homogenate was centrifuged (1800 × g, 15 s). The CAMP content of the supernatant was determined by radioimmunoassay (Amersham, Braunbach, West Germany).

**Protein Determination**—The protein concentration was determined using the Bio-Rad assay with bovine serum albumin as standard (22). 50 μl of cell extract were diluted to 800 μl and mixed with 200 μl of concentrated reagent; after vigorous mixing, absorption was measured at 595 nm.

**RESULTS**

**Effect of PTH Fragments on [3H]Thymidine Incorporation**—In a first approach we tested whether parathyroid hormone exhibits a stimulating influence on [3H]thymidine incorporation into acid-precipitable material by chicken chondrocytes. Extensive optimization of the assay system with respect to cell age, plating density, and induction period has been performed using bPTH(1–34) since this fragment exerts

**TABLE I**

| Stimulating agent | Concentration (μM) | [3H]Thymidine incorporation (E/C) |
|-------------------|-------------------|----------------------------------|
| None              |                   | 339 ± 28 (1.00)                  |
| FCS               | <1%               | 726 ± 65 (2.14)                  |
| hPTH(1–84)        | 5 × 10^{-10}      | 367 ± 79 (1.08)                  |
|                  | 5 × 10^{-9}       | 507 ± 59 (1.40)                  |
|                  | 5 × 10^{-8}       | 736 ± 52 (2.17)                  |
| bPTH(1–34)        | 5 × 10^{-10}      | 327 ± 63 (0.96)                  |
|                  | 5 × 10^{-9}       | 596 ± 55 (1.76)                  |
|                  | 5 × 10^{-8}       | 711 ± 50 (2.10)                  |
|                  | 5 × 10^{-7}       | 862 ± 196 (2.64)                 |
| bPTH(3–34)        | 5 × 10^{-10}      | 311 ± 29 (0.92)                  |
|                  | 5 × 10^{-9}       | 465 ± 68 (1.37)                  |
|                  | 5 × 10^{-8}       | 775 ± 89 (2.29)                  |
|                  | 5 × 10^{-7}       | 884 ± 203 (2.61)                 |
| hPTH(13–34)       | 5 × 10^{-10}      | 343 ± 76 (1.01)                  |
|                  | 5 × 10^{-9}       | 454 ± 29 (1.34)                  |
|                  | 5 × 10^{-8}       | 929 ± 108 (2.74)                 |
|                  | 5 × 10^{-7}       | 550 ± 65 (1.62)                  |
| bPTH(26–48)       | 5 × 10^{-10}      | 394 ± 94 (1.16)                  |
|                  | 5 × 10^{-9}       | 466 ± 73 (1.37)                  |
|                  | 5 × 10^{-8}       | 747 ± 87 (2.20)                  |
|                  | 5 × 10^{-7}       | 866 ± 143 (2.53)                 |
| bPTH(43–68)       | 5 × 10^{-10}      | 389 ± 91 (1.15)                  |
|                  | 5 × 10^{-9}       | 412 ± 69 (1.22)                  |
|                  | 5 × 10^{-8}       | 366 ± 35 (1.08)                  |
|                  | 5 × 10^{-7}       | 367 ± 79 (1.08)                  |
| bPTH(52–84)       | 5 × 10^{-10}      | 321 ± 11 (0.95)                  |
|                  | 5 × 10^{-9}       | 416 ± 57 (1.23)                  |
|                  | 5 × 10^{-8}       | 365 ± 80 (1.08)                  |
|                  | 5 × 10^{-7}       | 458 ± 88 (1.35)                  |

* p < 0.001.
* p < 0.01.
* p < 0.02.
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**FIG. 2. Summary of the results shown in Tables I and II.** On the top, the position numbers of the hPTH sequence (which is shown at the bottom and compared to the porcine and bovine PTH) are given. The hatched bars indicate the maximal stimulation of DNA synthesis we observed for the corresponding peptide. These values were related to that of the complete hormone, hPTH(1-84), whose activity is indicated by the dashed line. Below the position scale, the activities of the peptides covering the central region of the PTH molecule in stimulating [3H]thymidine incorporation are qualitatively given.

all the known biological effects of this hormone (e.g. binding to the renal PTH receptor and stimulation of adenylate cyclase) (1). Under optimal conditions (low plating density, serum-free medium, and 4 h of hormonal treatment) we found a distinct dose-response relationship for bPTH(1-34) in the concentration range between $5 \times 10^{-9}$ M and $5 \times 10^{-7}$ M resulting in a maximal stimulation of 2.6-fold (Fig. 1). This bPTH(1-34)-mediated stimulation of [3H]thymidine incorporation of chondrocytes corresponds to that obtained with serum factors (FCS) under the conditions applied (see below, Table I). FCS, however, exerts the maximal effect after 15-18 h; in contrast, no stimulation of DNA synthesis was observed after PTH treatment for $\geq 15$ h (data not shown).

Addressing the question, which part of the hormone molecule exhibits the [3H]thymidine incorporation enhancing activity, we studied the capability of several additional hormone fragments to stimulate DNA synthesis of chondrocytes. For comparison we simultaneously assayed, in addition to bPTH(1-34), the peptides hPTH(1-84), [Nle$^{38}$, Tyr$^{39}$] bPTH(3-34)amide, hPTH(13-34), hPTH(28-48), [Tyr$^{4}$] hPTH(43-68), and [Tyr$^{4}$, Asp$^{6}$]hPTH(52-84). All except the two peptides from the C-terminal half of the hormone molecule (covering the regions 43-68 and 52-84, respectively) displayed significant stimulation of [3H]thymidine incorporation in a dose-dependent manner and to an extent similar to fetal calf serum as a positive control (Table I). Above a critical value, the stimulation declined for any hitherto unknown reason (Fig. 1; cf. also Refs. 23 and 24).

Native hPTH(1-84) and the fragment bPTH(1-34) led to similar enhancement of [3H]thymidine incorporation (approximately 2.1- to 2.5-fold). Interestingly, [Nle$^{38}$, Tyr$^{39}$] bPTH(3-34)amide and hPTH(13-34) revealed nearly the same activity as bPTH(1-34). This indicates that the first 2 amino acid residues which are required for the stimulation of adenylate cyclase at the renal receptor (1) are dispensable for the enhancement of DNA synthesis by bPTH(1-34).

Even more surprisingly, hPTH(28-48) was observed to enhance [3H]thymidine incorporation comparably to that of the complete hormone (Table I). These results, which are summarized in Fig. 2, might indicate that the functional domain which is responsible for the [3H]thymidine incorporation enhancement by PTH resides in the overlapping sequence of the active fragments hPTH(13-34) and hPTH(28-48), i.e. in the region of amino acid residues 28-34.

The stimulation of DNA synthesis, e.g. by hPTH(28-48) appropriately measured after 4 h, is followed by an increase in cell number after 24 h of hormonal stimulation by 80% versus 17% in unstimulated control cells.

Mapping of the Amino-terminal Border of the Putative Mitogenic Functional Domain—For a more precise delineation of the functional PTH domain stimulating [3H]thymidine incorporation, we synthesized a series of peptides with variable amino termini; to avoid racemization of the first amino acid coupled to the resin (25), we started our synthesis with the glycine residue in position 47 and proceeded down to position 34. Subsequently, an aliquot of the peptide-loaded resin was removed after each condensation cycle. Thus, we obtained the hPTH fragments from amino acid residue 47 down to either position 34, 33, 32, 31, 30, 29, or 28, respectively. These peptides were applied to the [3H]thymidine incorporation assay described above. The fragments down to a length of 18 amino acid residues (i.e. hPTH(30-47)) were able to stimulate DNA synthesis, whereas those of 16 and less amino acids (corresponding to hPTH(32-47)) were inactive in the concentration range studied (Table II). The peptides hPTH(31-47) and hPTH(33-47) exhibited a slight stimulus on [3H]thymidine incorporation in some of our experiments (Table II), but this effect could not be reproduced in a statistically significant manner and also was not reproducible between experiments with different cell charges. Similar to the peptides 28/30-47, the fragment hPTH(28-39) was slightly active (Table II) indicating that the region beyond position 39 is not essential for stimulating [3H]thymidine incorporation. However, the shortest fragment assayed which still covers the presumptive functional domain, hPTH(28-39), was inactive (Table II). Most likely, this peptide comprising 12 amino acids is too short to adopt the structure which is required for binding to the corresponding receptor.

The Effect of PTH on DNA Synthesis Is cAMP-independent—As has been described for the PTH action via cAMP, a genuine amino terminus is required to stimulate the receptor-associated adenylate cyclase. As the stimulation of [3H]thymidine incorporation is exerted by a completely distinct region of the hormone molecule, we suggest that the corresponding signal is not mediated by cAMP. To substantiate this hypothesis, we directly measured the cAMP content of chondrocytes after stimulation with bPTH(1-34), [Nle$^{38}$, Tyr$^{39}$] bPTH(3-34), hPTH(28-48), and [Tyr$^{4}$, Asp$^{6}$] hPTH(52-84) at concentrations ensuring stimulation of [3H]thymidine incorporation in the appropriate assay and compared it to nonstimulated control cells (Table III). Only the fragment which has an intact amino terminus, bPTH(1-34), was able
enhancing the intracellular cAMP level with forskolin, which is known to be CAMP-mediated (29), by applying it in a concentration range between $10^{-10}$ and $10^{-7}$ M. The values given are mean ± S.E. for n parallel cultures. E/C ratios indicating a significant stimulation are indicated by Footnotes a–c.

TABLE II
Stimulation of [3H]thymidine incorporation in chondrocytes by midregional PTH peptides

| Stimulating agent | Concentration | n | [3H]Thymidine incorporation | E/C |
|------------------|--------------|---|---------------------------|-----|
| None             | mol/l        | 30 | 139 ± 6                   | (1.00) |
| hPTH(28–47)      | $4.5 \times 10^{-8}$ | 10 | 196 ± 25                   | 1.41* |
|                  | $4.5 \times 10^{-7}$ | 10 | 205 ± 22                   | 1.47* |
|                  | $4.5 \times 10^{-6}$ | 10 | 202 ± 17                   | 1.45* |
|                  | $4.5 \times 10^{-5}$ | 10 | 276 ± 36                   | 1.99* |
| hPTH(29–47)      | $4.8 \times 10^{-8}$ | 10 | 138 ± 15                   | 0.99* |
|                  | $4.8 \times 10^{-7}$ | 10 | 273 ± 33                   | 1.96* |
|                  | $4.8 \times 10^{-6}$ | 10 | 255 ± 20                   | 1.62* |
|                  | $4.8 \times 10^{-5}$ | 10 | 273 ± 30                   | 1.96* |
| hPTH(30–47)      | $5.1 \times 10^{-8}$ | 10 | 169 ± 23                   | 1.22 |
|                  | $5.1 \times 10^{-7}$ | 10 | 222 ± 19                   | 1.60* |
|                  | $5.1 \times 10^{-6}$ | 10 | 193 ± 20                   | 1.39 |
|                  | $5.1 \times 10^{-5}$ | 10 | 248 ± 31                   | 1.78* |
| hPTH(31–47)      | $5.3 \times 10^{-8}$ | 10 | 165 ± 20                   | 1.19 |
|                  | $5.3 \times 10^{-7}$ | 10 | 178 ± 24                   | 1.28 |
|                  | $5.3 \times 10^{-6}$ | 10 | 178 ± 35                   | 1.28 |
|                  | $5.3 \times 10^{-5}$ | 10 | 176 ± 8                    | 1.27* |
| hPTH(32–47)      | $5.7 \times 10^{-8}$ | 5  | 177 ± 29                   | 1.27 |
|                  | $5.7 \times 10^{-7}$ | 5  | 172 ± 12                   | 1.24 |
|                  | $5.7 \times 10^{-6}$ | 5  | 127 ± 22                   | 0.91 |
|                  | $5.7 \times 10^{-5}$ | 5  | 157 ± 33                   | 1.13 |
| hPTH(33–47)      | $6.1 \times 10^{-8}$ | 5  | 103 ± 38                   | 0.74 |
|                  | $6.1 \times 10^{-7}$ | 5  | 89 ± 21                    | 0.64 |
|                  | $6.1 \times 10^{-6}$ | 5  | 252 ± 82                   | 1.81* |
|                  | $6.1 \times 10^{-5}$ | 5  | 119 ± 36                   | 0.86 |
| hPTH(34–47)      | $6.5 \times 10^{-8}$ | 5  | 88 ± 27                    | 0.63 |
|                  | $6.5 \times 10^{-7}$ | 5  | 106 ± 18                   | 0.76 |
|                  | $6.5 \times 10^{-6}$ | 5  | 120 ± 23                   | 0.86 |
|                  | $6.5 \times 10^{-5}$ | 5  | 188 ± 70                   | 1.35 |
| None             | 10            | 324 ± 18                   | (1.00) |
| hPTH(25–39)      | $6.1 \times 10^{-8}$ | 5  | 452 ± 56                   | 1.40* |
|                  | $6.1 \times 10^{-7}$ | 5  | 418 ± 41                   | 1.29* |
|                  | $6.1 \times 10^{-6}$ | 5  | 517 ± 65                   | 1.60* |
|                  | $6.1 \times 10^{-5}$ | 5  | 655 ± 147                  | 2.02* |
|                  | $6.1 \times 10^{-4}$ | 5  | 542 ± 71                   | 1.67 |
| hPTH(28–39)      | $7.6 \times 10^{-8}$ | 5  | 319 ± 39                   | 0.97 |
|                  | $7.6 \times 10^{-7}$ | 5  | 473 ± 74                   | 1.46 |
|                  | $7.6 \times 10^{-6}$ | 5  | 324 ± 45                   | 1.00 |
|                  | $7.6 \times 10^{-5}$ | 5  | 399 ± 47                   | 1.23 |
|                  | $7.6 \times 10^{-4}$ | 5  | 387 ± 41                   | 1.19 |

* p < 0.01.
^* p < 0.001.
$^p p < 0.02.

to enhance the cellular cAMP content significantly, whereas the sequence 3–34 revealed only very weak activity, and the midregional and carboxyl-terminal regions of PTH were completely inactive.

Furthermore, we attempted to mimic the PTH effect by enhancing the intracellular cAMP level with forskolin, which is known to stimulate adenylate cyclase. This agent has been applied in a concentration range between $10^{-9}$ and $10^{-6}$ M. We were able to detect a distinct stimulation of the activity of ornithine decarboxylase in chondrocytes, which is known to be cAMP-mediated (29), by $10^{-7}$ M forskolin: and within the same time period of 4 h. However, this agent did not provoke any enhancement of DNA synthesis (see Fig. 3). Accordingly, attempts to provoke stimulation of DNA synthesis by administration of dibutyryl-cAMP failed also (data not shown).

It has been reported that the influx of calcium ions can be stimulated by PTH by a cAMP-dependent mechanism (26). To test if the PTH signal transduction leading to enhanced DNA synthesis is influenced by influx of calcium ions, we simultaneously added PTH and the calcium-chelating agent EGTA to chondrocyte cultures. Increasing concentrations of the chelator led to a progressive loss of the hormonal stimulation capability (Fig. 4, inset). From this finding we conclude that chelation of (presumably) calcium ions selectively abolishes the PTH-induced stimulation of DNA synthesis in chondrocytes rather than damages the cells unspecifically. Moreover, no enhancement of [3H]thymidine incorporation by hPTH(28–48) was observed when repeating the experiment in calcium-free medium. After supplementation with calcium ions, however, this activity was restored (Fig. 4, inset).

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The results presented above have shown that PTH stimulates \[^{3}H\]thymidine incorporation of chondrocytes. Additional experiments have also shown that the increase in DNA synthesis we measured after 4 h is followed by an increase in cell number observed after 24 h.

These results agree with those obtained for cartilage organ cultures (9–11), although other investigators described that PTH had little or no effect on DNA synthesis in chondrocytes (27, 28). The discrepancy is most likely due to the different assay conditions applied; the reports cited investigated the PTH response of chondrocytes at confluence after hormone treatment for at least 16 h in the presence of 10% FCS (27, 28). We found that high plating density (>56,000 cells/cm²), prolonged hormone treatment (>6 h), or the presence of calf serum (FCS > 1.5%) likewise impedes the PTH stimulus on the DNA synthesis of chondrocytes. Finally, it has been shown that in an organ culture system, the \[^{3}H\]thymidine incorporation of chondrocytes after PTH stimulation depends on their stage of differentiation (9). This could conceivably account for the differences discussed here.

Under the conditions applied in this study, chicken chondrocytes respond to PTH and appropriate hormone fragments by an approximately 2.6-fold increase in the rate of \[^{3}H\] thymidine incorporation. This corresponds to the effect PTH exerts on DNA synthesis of rat osteoblasts (29). Sömen et al. (29) could also show that the increase in DNA synthesis coincides with an induction of creatine kinase in these cells.

Up to now, only very few in vitro or in vivo agonist effects could be detected for amino-terminally truncated PTH fragments. [Nle\(^{8,18}\), Tyr\(^{24}\)]hPTH(28–48)amide has weak agonist properties (30), and this fragment as well as [Tyr\(^{24}\)]bPTH(7–34)amide and [Tyr\(^{24}\)]hPTH(13–34) are still able to stimulate glucose-6-phosphate dehydrogenase activity in the distal renal tubule (31). No function has hitherto been ascribed to the midregional fragment hPTH(28–48).

Moreover, Takano et al. (32) have shown that even the region bPTH(1–27) is sufficient to enhance the cAMP level as well as ornithine decarboxylase activity, although carboxyl-terminally adjacent sequences support its effects.

However, in the \[^{3}H\]thymidine incorporation assay optimized for induction with bPTH(1–34), the peptide hPTH(28–48) revealed a distinct stimulating activity. Hormone fragments covering very different regions of the PTH molecule such as residues 13–34 or 30–47 were also active (Fig. 2). This might either indicate (i) that there exist two independent mitogenic regions within these peptides or (ii) that a "core" of the mitogenic functional domain of hPTH resides in the overlapping region, i.e., residues 30–34. We presently favor the second hypothesis, since the central region, which is the most hydrophobic part of the PTH molecule, is highly conserved up to position 39 between different species such as human, bovine, and porcine PTH (Fig. 2) suggesting some physiological significance.

The sequences flanking the "core domain" residues 30–34 seem to stabilize some minimal structural requirement for receptor binding, since a peptide as short as residues 28–39 is inactive, although it contains the core region (Table II). As has been reported by Born et al. (33), comparison of the antagonist activities of [Nle\(^{8,18}\), Tyr\(^{24}\)]hPTH(3–34)amide and recombinant hPTH(3–34) also revealed distinct influences of the sequences carboxy-terminally from position 34. This region possibly strengthens receptor binding by inter- or intramolecular interactions (33).

Up to now, most PTH effects were studied either directly by investigating the stimulation of adenylate cyclase (27, 28, 32, 33) or indirectly by examination of cAMP-mediated effects, e.g., ornithine decarboxylase induction (27, 32). However, two lines of evidence strongly indicate that PTH enhances \[^{3}H\]thymidine incorporation of chondrocytes by a cAMP-independent mechanism: (i) amino-terminally truncated PTH fragments, which are known not to stimulate adenylate cyclase, are active in the \[^{3}H\]thymidine incorporation assay. This has been confirmed for hPTH(28–48) (Table III). (ii) The adenylate cyclase stimulating agent forskolin as well as dibutyryl-cAMP did not affect DNA synthesis, although both agents were able to induce ornithine decarboxylase in chondrocytes (data not shown), and the enhancement of the cAMP level was confirmed experimentally.

It has recently been shown that calcium channels of osteoblast-like cells are influenced by PTH stimuli in a cAMP-independent manner (26). On the other hand, experiments of other laboratories (35, 36) as well as preliminary results of our own group (data not shown) have shown that administration of low doses of a phorbol ester derivative increased the DNA synthesis of chondrocytes. This suggests the involvement of protein kinase C in this signal transfer process (37).

It is known that inositol trisphosphate after phosphorylation to tetrakisphosphate influences the influx of calcium ions into the cells (38), and it has been suggested that calcium might play an important role in the stimulation of cell proliferation by several growth factors (39). The results reported here also indicate that the mitogenic activity of PTH is dependent on external calcium ions (Fig. 4).

To achieve a more profound understanding of the mechanisms of PTH effects on its target cells, additional cell types (e.g., osteoblast-like and kidney-derived cells) have to be employed. Furthermore, a much broader spectrum of PTH peptides obtained from chemical peptide synthesis or variants of the complete hormone synthesized in Escherichia coli (40) will provide valuable information about the functional domains of the hormone with respect to its different activities. These experiments are in progress.

The finding that different hormonal effects are exerted by
different functional domains of the PTH molecule might lead to the attractive speculation that its apparently counteracting activities in vivo might be separable or that the overall balance of anabolic and catabolic effects might be changed in favor of bone forming processes for therapeutic purposes.

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