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A parathyroid hormone antagonist stimulates epidermal proliferation and hair growth in mice

(parathyroid hormone-related peptide/skin)

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ABSTRACT **The biologic action of parathyroid hormone (PTH)-related peptide (PTHrP) in normal skin was investigated in cultured human keratinocytes and in SKH-1 hairless mice. The results indicate that the PTHrP agonists human PTHrP-(1–34) and PTH(1–34) are potent inhibitors of epidermal cell proliferation. [Nle^{8,18},Tyr^{34}]bovine PTH-(7–34)-amide, an antagonist of the PTH/PTHrP receptor, blocked the inhibitory effect of PTH-(1–34) in cultured keratinocytes. In the SKH-1 mice, PTH-(7–34) caused a 244% increase of [^{3}H]thymidine incorporation into isolated epidermal DNA and 246% and 180% increases in the number and length of hair shafts, respectively. Thus, PTH and PTHrP may play an important role in the normal physiology of skin, and their agonists and antagonists have potentially wide therapeutic applications in the treatment of hyperproliferative skin disorders and aging skin and could also be effective in stimulating and maintaining hair growth.**

Parathyroid hormone (PTH)-related peptide (PTHrP) has been identified as responsible for humoral hypercalcemia of malignancy, which occurs most commonly in squamous cell tumors of the lung and kidney (1, 2). The full-length cDNA clones for PTHrP have been shown to encode a 141-amino acid protein that shares 70% homology with PTH in its first 13 amino acids but diverges completely in its primary structure thereafter (3, 4). Studies using synthetic PTHrP amino-terminal fragments have demonstrated that these peptide fragments bind to the PTH receptor and cause biological effects on calcium and phosphorus metabolism similar to PTH in cultured bone and kidney cells (3, 4). Thus, it has been postulated that a single receptor species mediates many physiological functions of both PTH and PTHrP (5). A PTH/PTHrP receptor cDNA has been cloned (5). Using the cDNA probe for this PTH/PTHrP receptor, Urena *et al.* (6) found that the PTH/PTHrP receptor mRNAs were widely expressed in many tissues beside classic PTH target organs. In addition to being a product of tumors that are associated with humoral hypercalcemia of malignancy, PTHrP is also expressed by a variety of normal and neoplastic tissues, including the skin and hair follicles (7, 8). The presence of PTHrP bioactivity in nonmalignant cells was first demonstrated in conditioned medium harvested from confluent human keratinocyte cultures (9). However, the physiological role of this peptide in normal skin is still not clear. Preliminary data indicated that human PTHrP-(1–34)-peptide [hPTHrP-(1–34)] and human PTH-(1–34) [hPTH-(1–34)] inhibited the proliferation and induced terminal differentiation of cultured human keratinocytes (10, 11). The PTH antagonist [Nle^{8,18},Tyr^{34}]bovine PTH-(7–34)-amide [bPTH-(7–34)] restored the proliferative activity and inhibited the cornified envelope formation of the cultured keratinocytes exposed to either PTHrP-(1–34) or 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] in cultures (10). We reasoned that if we could inhibit the antiproliferative activity of endogenously synthesized PTHrP with the PTH/PTHrP receptor antagonist PTH-(7–34), then we could use the peptide to promote epidermal cell proliferation and hair growth *in vivo*. To further explore the potential biologic action of PTHrP in epidermis, we conducted a study to determine the effect of PTHrP agonists, hPTHrP-(1–34) and hPTH-(1–34), and an antagonist, bPTH-(7–34), on keratinocyte proliferation *in vitro* and on the incorporation of [^{3}H]thymidine into epidermal DNA as well as their effect on hair growth in SKH-1 hairless mice *in vivo*.

METHODS

SKH-1 hairless mice, 5–6 weeks old (20–25 g), from Charles River Breeding Laboratories, were fed normal mouse chow ad libitum and were handled in accordance with the Boston University School of Medicine Institutional Guidelines for laboratory animal care. hPTHrP-(1–34), hPTH-(1–34), and bPTH-(7–34) were purchased from Bachem and 1,25$(OH)_2D_3$ was kindly provided by M. Uskokovic of Hoffman–La Roche. [^{3}H]Thymidine was obtained from DuPont/New England Nuclear. All reagents used for DNA extraction were analytical grade. Groups of three to six mice all of which were similar in size and hair distribution received either hPTH-(1–34), bPTH-(7–34), 1,25$(OH)_2D_3$, or control vehicle intraperitoneally for 3 or 7 days. On day 3, the mice were administered 45 μCi (1 μCi = 37 kBq) of [^{3}H]thymidine intraperitoneally. Four hours later, mice were killed by cervical dislocation and skin samples were immediately removed and stored at −80°C until the epidermal layer (identified by light microscopy) was scraped from the skin for DNA extraction (12). On day 7 an analysis of hair growth was determined in a blinded fashion on the animal's back skin. The culture of human keratinocytes and the quantification of the number of basal cells in the presence of various PTH or PTHrP fragments were carried out as previously described (13). Statistical analyses were performed with Student's *t* test and analysis of variance.

RESULTS

hPTH-(1–34) and hPTHrP-(1–34) caused a dose-dependent decrease in the number of keratinocyte basal cells (Fig. 1). 1,25$(OH)_2D_3$, a potent inhibitor of keratinocyte proliferation (10, 13), at 10 nM caused a 42% inhibition in the number of basal cells, similar to hPTH-(1–34) and hPTHrP-(1–34) at 10 nM, and served as a positive control. bPTH-(7–34) by itself

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Abbreviations: PTH, parathyroid hormone; PTHrP, parathyroid hormone-related peptide; prefix h, human; bPTH-(7–34), [Nle^{8,18},Tyr^{34}]bovine PTH-(7–34)-amide; 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3.

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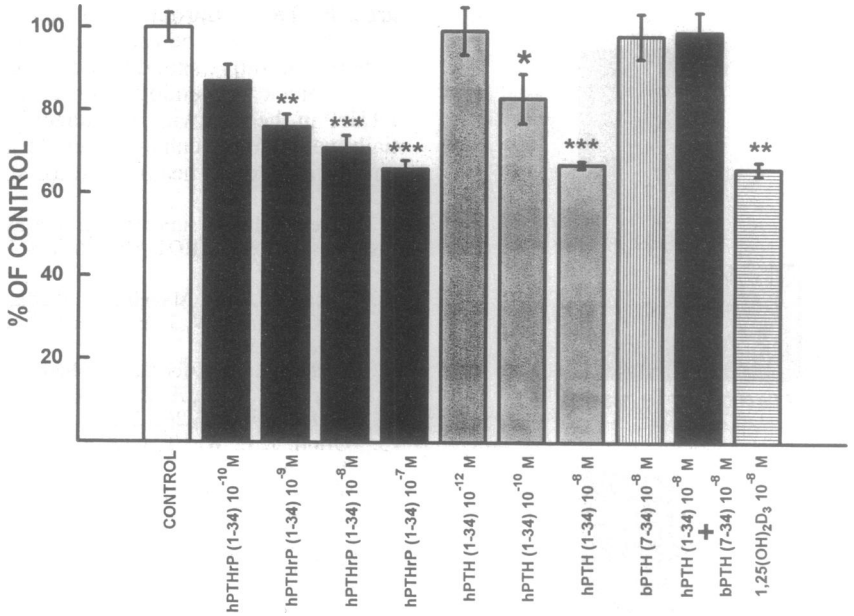

FIG. 1. Inhibition of keratinocyte proliferation in the presence of either 0.1–100 nM to hPTHrP-(1–34), 0.001–10 nM hPTH-(1–34), 10 nM bPTH-(7–34), 10 nM hPTH-(1–34) plus 10 nM bPTH-(7–34), or 10 nM $1,25(OH)_2D_3$. Keratinocytes were dosed three times with the indicated compound or compounds for 7 days as previously described (13). For the group which was treated with both hPTH-(1–34) and bPTH-(7–34), the two compounds were added simultaneously to keratinocytes. The results are presented as the percent of the number of basal cells in the control group. Each value is the mean ± SEM of three culture dishes. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$ compared with the control group by analysis of variance.

at 10 nM had no effect on keratinocyte proliferation, but, it completely reversed the hPTH-(1–34)-induced inhibitory effect (Fig. 1).

The group of mice which received 0.5 μg of hPTH-(1–34) daily for 3 days showed a 59% decline in the incorporation of [^{3}H]thymidine into epidermal DNA compared with the control group (Fig. 2). A similar degree of inhibition was observed with a daily dose of 0.1 μg of $1,25(OH)_2D_3$ for 3 days. bPTH-(7–34) caused a dose-dependent increase in the incorporation of [^{3}H]thymidine into epidermal DNA. At a dose of 10 μg per day for 3 days, bPTH-(7–34) caused a 244% increase in [^{3}H]thymidine incorporation into epidermal DNA when compared with the control group. In three additional experiments, bPTH-(7–34) at a daily dose of 10 μg for 3 days induced 196%, 300%, and 355% increases in [^{3}H]thymidine incorporation into epidermal DNA (data not shown).

After 7 days the mice which had received 10 μg of bPTH-(7–34) had noticeably more hair on their bodies when compared with the control group. Indeed, there was a 246% increase in the number of hair shafts (32 ± 1 vs. 13 ± 1 per cm^2; $P < 0.01$) and a 180% increase in the hair shaft length (4.5 ± 0.2 vs. 2.5 ± 0.2 mm; $P < 0.01$) when compared with the controls (Fig. 3). The mice that received hPTH-(1–34) for 7 days showed no effect on either hair shaft number (11 ± 1 vs. 13 ± 1 per cm^2) or hair shaft length (2.4 ± 0.1 vs. 2.5 ± 0.2 mm). The serum calcium concentrations were all within the normal range and there was no statistical difference among the groups at the end of the experiments.

DISCUSSION

PTHrP is widely distributed in normal tissues in substantial quantities (3, 4, 6, 7). It has been suggested that, in at least some of these tissues, PTHrP may function as an autocrine or paracrine factor (3, 4). PTHrP has been demonstrated to be a potential modulator of cell growth in several cell types besides keratinocytes (14–16). Cells in which endogenous PTHrP synthesis had been blocked by transfection of an antisense RNA for PTHrP into a human keratinocyte cell line had an increase in [^{3}H]thymidine incorporation and an accelerated growth, further supporting the concept that PTHrP is an endogenous inhibitor of cell growth (17). Increasing evidence has indicated differential biological activity exerted

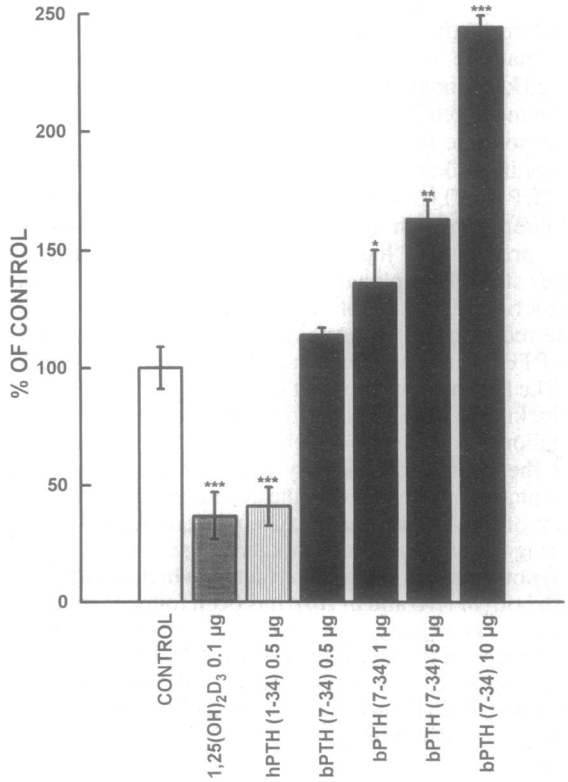

FIG. 2. *In vivo* effects of $1,25(OH)_2D_3$, hPTH-(1–34), and bPTH-(7–34) on incorporation of [^{3}H]thymidine into epidermal DNA. Data presented are means ± SEM of three to seven animals. No statistically significant difference ($P > 0.1$) was observed between the control and the group given 0.5 μg of bPTH-(7–34) daily. *, $P < 0.1$; **, $P < 0.01$; ***, $P < 0.005$ compared with the control.

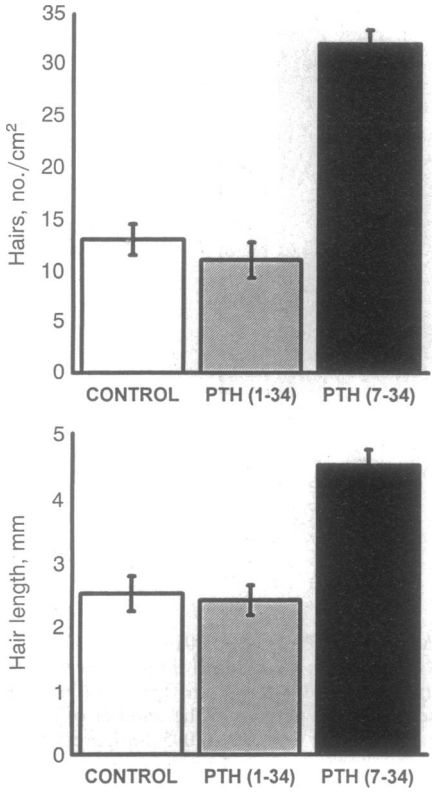

FIG. 3. Number of hairs per cm^2 and hair length in mice treated with vehicle control, hPTH-(1–34), or hPTH-(7–34).

by different PTHrP fragments (18–22) and suggests the presence of a tissue-specific PTHrP receptor(s) which may be distinct from the classical PTH/PTHrP receptor (5). It is likely that the actions of PTH and PTHrP fragments on cultured keratinocytes and mouse skin employed in our study may be mediated by the PTH/PTHrP receptor. However, it is also possible that there may be a separate PTHrP/PTH receptor in the keratinocytes that only recognizes a fragment of PTHrP or PTH which in turn results in the enhancement of proliferation such as that induced by bPTH-(7–34).

The presence of high concentrations of PTHrP in hair follicles strongly implicates its potential role in the paracrine or autocrine regulation of hair growth (7). This is supported by the recent observation that transgenic mice that overexpress PTHrP have a disturbance in hair follicle development (23). The finding that the onset of anagen can be induced with the plucking of resting hairs from telogen follicles has led to the chalone hypothesis, which states that the hair cycle is under the control of a locally active inhibitor synthesized during anagen phase and that the accumulation of a sufficient amount of this inhibitor will cause the termination of anagen and bring the hair cycle into catagen phase (24). In support of the hypothesis, an unknown factor which inhibited hair growth both *in vivo* and *in vitro* has been found in murine skin epidermis. This factor was present in telogen but not in anagen phase of the hair follicle cycle (25). Our data showing that hair growth was stimulated by bPTH-(7–34) strongly suggest that PTHrP, its fragments, or the glycosylated PTHrP (26) may be the inhibitory factor described in the chalone hypothesis.

In summary, the present results provide strong evidence that PTHrP is an endogenous antiproliferative factor that participates in the regulation of epidermal and hair follicle cell growth. The antiproliferative activity of PTH-(1–34) and PTHrP-(1–34) may be valuable for the clinical use of treating hyperproliferative skin disorders such as psoriasis. The ability to block the endogenous antiproliferative activity of PTHrP in the skin with its antagonist bPTH-(7–34) is potentially valuable for enhancing epidermal growth in aged skin and during wound healing and for stimulating hair growth.

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