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. [Nle8,O18,Tyr34]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 [3H]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 aminoterminal 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 [Nle8,18,Tyr34]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 D3 [1,25(OH)2D3] 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 [3H]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)2D3 was kindly provided by M. Uskokovic of Hoffman-La Roche. [3H]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)2D3, or control vehicle intraperitoneally for 3 or 7 days. On day 3, the mice were administered 45 μCi (1 μCi = 37 kBq) of [3H]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)2D3, 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

Abbreviations: PTH, parathyroid hormone; PTHrP, parathyroid hormone-related peptide; prefix h, human; bPTH-(7–34), [Nle8,18,Tyr34]bovine PTH-(7–34)-amide; 1,25(OH)2D3, 1,25-dihydroxyvitamin D3.

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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 [3H]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)2D3 for 3 days. bPTH-(7-34) caused a dose-dependent increase in the incorporation of [3H]thymidine into epidermal DNA. At a dose of 10 μg per day for 3 days, bPTH-(7-34) caused a 244% increase in [3H]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 [3H]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 cm²; 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 cm²) 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 [3H]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...
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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