Photoaffinity Labeling of Parathyroid Hormone Receptors: Comparison of Receptors across Species and Target Tissues and after Desensitization to Hormone†

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ABSTRACT: Cells derived from human giant cell tumors of bone and fibroblasts derived from human neonatal foreskin respond to parathyroid hormone (PTH) by increasing the intracellular and extracellular levels of adenosine cyclic 3′,5′-phosphate (cAMP). Using photoaffinity labeling methods, we examined these cells for the presence of a PTH receptor or a binding subunit of a receptor complex. A previously designed biologically active and photolabile radioligand analogue of PTH was reacted with these intact cells. After photolysis, the cells were extracted, and the proteins were denatured, reduced, and separated by electrophoresis on sodium dodecyl sulfate ($NaDodSO_4$)–polyacrylamide gels followed by autoradiography. A single membrane component, M_r 70 000, was labeled specifically in intact cells cultured from skeletal and dermal tissue. By mixing, in pairs, photolabeled proteins from (a) intact human cells derived from giant cell tumors of bone, (b) intact human fibroblasts, and (c) canine renal cortical membranes, the receptors (or their binding subunits) for PTH were compared directly and found to be identical in terms of molecular size (as determined by the migration position on $NaDodSO_4$–polyacrylamide gels) across species (dog and human) and target tissue (bone, skin, and kidney). Preincubation of cells cultured from giant cell tumors of bone with PTH resulted in loss of the PTH-induced cAMP response (desensitization). Preincubation with PTH was accompanied by a marked decrease in photoaffinity labeling of the PTH binding component and suggests that the loss of hormone response in cells preincubated with PTH was related to a decrease in the number or availability of PTH receptors. In contrast, the PTH-induced cAMP response and photoaffinity labeling of the PTH binding component were not altered by preincubating cells with a PTH inhibitor which binds to PTH receptors but fails to increase cAMP levels. These results suggest that simple occupancy of the hormone receptor is not sufficient to produce desensitization to PTH and that biological events that take place after the binding of PTH to the receptor are involved in the subsequent loss of hormone responsiveness.

The biological effects of peptide hormones, including parathyroid hormone (PTH),[1] are initiated through interaction of the hormone with specific receptors on the plasma membrane of the target tissue. To identify the PTH binding component in target tissues, we developed a biologically active and photolabile radioiodinated analogue of PTH. In a prior investigation, we used this photoaffinity-labeled radioligand to covalently label PTH binding elements in membranes derived from canine renal cortical tissue (Coltrera et al., 1981). These studies identified a single membrane component, M_r 70 000, that was labeled specifically and is presumed to represent the PTH receptor or a binding subunit of the receptor. Employing a different photolabile radioligand, Draper et al. (1982) identified a PTH binding element with similar characteristics in canine renal membranes.

Recent extensive studies with PTH and synthetic analogues of this hormone in several assay systems in vitro and in vivo (Rosenblatt, 1981) provided information regarding the structural requirements for receptor interaction and the subsequent biological response. When the properties of intact PTH were compared with several PTH fragments and analogues (Tregear et al., 1973, 1974; Parsons et al., 1975; Goldring et al., 1979; Coltrera et al., 1981; Rosenblatt et al., 1981), it was found that the biological properties of these compounds were consistent across the different assay systems used, suggesting that the functional binding requirements of the receptors may be conserved across species and target tissues.

To examine physicochemically the receptors for PTH and to compare the properties of the receptors in different target tissues and different species, we used our previously developed photoaffinity-labeled radioligand of PTH (Coltrera et al., 1981) to label the binding component in human tissues of skeletal and dermal origin and in canine renal tissue. Electrophoretic analyses of the labeled proteins permitted comparison of the PTH binding components in these different target tissues.

In prior studies, we showed that monolayer cell cultures derived from human giant cell tumors of bone respond to PTH by increasing the intracellular and extracellular levels of adenosine cyclic 3′,5′-phosphate (cAMP) (Goldring et al., 1978). Although skin is not conventionally regarded as a PTH target tissue, some monolayer cultures of fibroblasts from human foreskin and adult dermis are also responsive to PTH (Goldring et al., 1978, 1979, 1981). Monolayer cell cultures prepared from these human sources exhibit PTH structure–activity relations similar to those of the canine renal system (Goldring et al., 1979).

Preincubation of human fibroblasts or cells cultured from human giant cell tumors of bone with PTH results in subsequent loss of the cAMP response when cells are rechallenged with PTH; the term "desensitization" has been used to describe this phenomenon. Desensitization to PTH requires more than

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[1] Abbreviations: PTH, parathyroid hormone; cAMP, adenosine cyclic 3′,5′-phosphate; EDTA, ethylenediaminetetraacetate; bPTH, bovine parathyroid hormone; Tris, tris(hydroxymethyl)aminomethane; PBS, phosphate-buffered saline; $NaDodSO_4$, sodium dodecyl sulfate.

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simple occupancy of hormone receptors, since preincubation with a PTH analogue [which has been demonstrated to bind to PTH receptors (Segre et al., 1979) but which fails to stimulate a cAMP response (Rosenblatt et al., 1977; Goldring et al., 1979)] does not produce desensitization to PTH (Goldring et al., 1981). This result suggests that postbinding events, as postulated for other hormonal systems (Caro & Amatruda, 1980; Marshall & Olefsky, 1980; Su et al., 1980; King & Cuatrecasas, 1981), mediate hormone-specific desensitization.

Desensitization to PTH must result ultimately either from decreased receptor number or availability (termed "down regulation") or from uncoupling of the receptor from its intracellular effector system(s). Despite attempts in several laboratories, this issue could not be resolved because a PTH radioligand–receptor assay had not been developed for intact cell systems. The technique of photoaffinity labeling has provided a tool for demonstrating directly the availability of the specific hormone binding component in intact cells. In these studies, we have used the PTH photolabile radioligand as a probe of the PTH receptor binding capacity in intact cells and to directly investigate the role of receptor availability before and after desensitization to PTH.

Materials and Methods

Target Tissues. (*A*) *Canine Renal Membranes.* Purified canine renal cortical membranes were prepared in a fashion identical with that employed in an adenylate cyclase assay for PTH (Rosenblatt et al., 1977) and a radioligand–receptor binding assay for PTH (Segre et al., 1979) and were used previously in studies identifying PTH receptors by photoaffinity labeling (Coltrera et al., 1981; Draper et al., 1982).

(*B*) *Tissues from Giant Cell Tumor of Bone and Human Foreskin.* Sterile specimens of tumor tissue were obtained at the time of surgery from patients undergoing therapeutic resection of giant cell tumor and allograft transplantation (Goldring et al., 1978) and were kindly provided by Dr. Henry J. Mankin. The diagnosis of giant cell tumor of bone was established on the basis of clinical course, tumor site, and histological evaluation. Tissues from giant cell tumors were prepared for monolayer culture by dispersion with trypsin–EDTA and clostridial collagenase (clostridiopeptidase A, Worthington Biochemical CLS, 125–200 units/mg), and cells were cultured in plastic petri dishes, as previously described (Goldring et al., 1978). Samples of human neonatal foreskin were obtained at the time of circumcision of normal male infants, and the cells (fibroblasts) were grown by a standard explant technique. After primary culture and growth to confluence in plastic petri dishes (10 cm), cells from dermal and skeletal sources were subcultured, as previously described (Goldring et al., 1978, 1979, 1981). Forty-eight hours before incubation with hormone, or hormone analogues, cells were transferred to plastic trays containing 24 individual wells, each 1.6 cm in diameter (Costar), at a density of 5×10^4 cells/well.

(*C*) *PTH and PTH Analogues.* Solutions of PTH or its analogues were prepared by dissolving, in buffer medium, lyophilized samples of either highly purified bovine PTH-(1–84); an analogue of enhanced biopotency, [Nle^8,Nle^{18},Tyr^{34}]bPTH-(1–34)-amide; or an inhibitory analogue, [Nle^8,Nle^{18},Tyr^{34}]bPTH-(3–34)-amide. Concentrations were adjusted by serial dilution. Bovine PTH-(1–84) [3000 Medical Research Council (MRC) units/mg], a gift from Dr. Henry T. Keutmann, was prepared from the trichloroacetic acid precipitate of bovine parathyroid glands and purified by gel filtration, followed by ion-exchange chromatography on carboxymethylcellulose (Keutmann et al., 1971). Synthetic analogues of PTH were prepared by a modification (Rosenblatt et al., 1977a,b) of the Merrifield method of solid-phase peptide synthesis (Merrifield, 1962, 1969; Erickson & Merrifield, 1976). Details of the purification and chemical analysis have been reported previously (Rosenblatt et al., 1977a,b). The analogue [Nle^8,Nle^{18}Tyr^{34}]bPTH-(1–34)-amide has a potency approximately 200% that of bPTH-(1–34) in the renal adenylate cyclase assay (Rosenblatt & Potts, 1977). The PTH antagonist [Nle^8,Nle^{18}Tyr^{34}]bPTH-(3–34)-amide has been previously shown to be a pure competitive inhibitor of PTH action in vitro and lacks PTH-like agonist properties in vitro (Rosenblatt, 1981). The analogue can inhibit completely PTH-stimulated increases in cAMP in canine renal membranes (Rosenblatt et al., 1977), cells from human giant cell tumors, and human fibroblasts (Goldring et al., 1979). The analogue also has been shown to bind to PTH receptors with an avidity comparable to PTH (Segre et al., 1979) and to inhibit labeling of PTH receptors by a photolabile radiolabeled PTH analogue (Coltrera et al., 1981).

Preparation of a Photolabile Radiolabeled Analogue of PTH. [Nle^8,Nle^{18},Tyr^{34}]bPTH-(1–34)-amide was used for radiolabeling and reacted with photolabile groups as previously described (Coltrera et al., 1981). This analogue, previously synthesized, purified, and analyzed for chemical homogeneity and biological activity (Rosenblatt & Potts, 1977), was used as the radioligand for a PTH renal radioreceptor assay (Segre et al., 1979). [Nle^8,Nle^{18},Tyr^{34}]bPTH-(1–34)-amide (8 μg) was iodinated with 6 mCi of Na^{125}I (New England Nuclear) by a modification (Coltrera et al., 1981) of the method of Hunter & Greenwood (1962). The specific activity estimated in samples of the radiolabeled analogue precipitated with 10% trichloroacetic acid was 500 mCi/mg, which corresponds to an average iodine to peptide ratio of 1. Twenty-five microliters of a 380 μM solution of 4-fluoro-3-nitrophenyl azide (Pierce Chemical Co.) in dimethyl sulfoxide (Fisher Scientific Co.) was added to a 100-μL solution containing 2 μg of ^{125}I-labeled [Nle^8,Nle^{18},Tyr^{34}]bPTH-(1–34)-amide dissolved in 10 μM triethylamine in dimethyl sulfoxide. The solution was protected from light and agitated continuously for 5 h at room temperature. Fifteen micrograms of glycine in 20 μL of 0.2 M sodium phosphate buffer (pH 7.4) was added to terminate the reaction. These methods generate a fully biologically active analogue of PTH, as previously demonstrated in renal adenylate cyclase assays conducted in darkness (Coltrera et al., 1981).

Photoreaction of the PTH Analogue with Target Tissues. Aliquots of 100 μg of canine renal cortical membranes were suspended in 100 μL of reaction buffer consisting of 50 mM Tris-HCl, 9 mM theophylline, 4.2 mM $MgCl_2$, 26 mM KCl, and 0.12% (w/v) bovine serum albumin and placed in glass test tubes (12 × 75 mm). In the dark, 3.0×10^6 cpm of the photolabile radiolabeled PTH analogue in 40 μL of assay buffer was added to each test tube, and the contents were incubated at 15 °C. After 5 min, 100 μL of buffer with or without 10 μg of unlabeled [Nle^8,Nle^{18},Tyr^{34}]bPTH-(1–34)-amide was added, and the contents were incubated for an additional 15 min in the dark. The dose of "competing" unlabeled PTH was selected because it generates a maximal cAMP response in these cell-based systems and would be expected to saturate available binding sites. At the end of the incubation period, all the tubes (at 15 °C) were exposed to light from a high-pressure mercury vapor lamp at 30 cm for 15 min. After centrifugation of the tubes at 600*g* for 4 min at 4 °C, the supernatant was aspirated. The pellets were resuspended in a solution consisting of 75 μL of water, 25 μL

of 10% $NaDodSO_4$, and 25 μL of sample buffer. The sample buffer consisted of 5 mL of glycerol, 1 μg of $NaDodSO_4$, 2.5 mL of β-mercaptoethanol, 0.25 mL of 0.2% bromphenol blue in doubly distilled water, and 2.5 mL of a stock solution made by dissolving 3 g of Tris base in a total volume of 25 mL of water (pH 6.8). The suspensions were then boiled for 2 min and mounted immediately on $NaDodSO_4$–polyacrylamide gel slabs.

Monolayer cells from human giant cell tumors of bone and dermis were passaged into 10-cm plastic petri dishes 48 h before study at a density of 2×10^6 cells/plate. Immediately before photolabeling, the petri dishes were washed 4 times with Dulbecco's PBS supplemented with 0.9 mM calcium and 0.5 mM magnesium. After the addition of 3 mL of PBS, this solution served as the incubation buffer in the cell culture studies. The photolabeling was conducted as described above for canine renal membranes. Plates were then washed gently with 2 mL of PBS, and the cells were dissolved in 200 μL of a solution consisting of 75 μL of water, 25 μL of 10% $NaDodSO_4$, and 25 μL of sample buffer (see above). The dishes were scraped with a plastic policeman, and the contents were transferred to glass tubes. After the aliquots were boiled for 2 min, they were mounted immediately on $NaDodSO_4$–polyacrylamide gel slabs.

Electrophoresis and Autoradiography. (*A*) *Electrophoresis of $NaDodSO_4$–Polyacrylamide Gels.* $NaDodSO_4$–polyacrylamide (9–20%) gradient slab gels (10 × 15 × 0.15 cm) were prepared by the method of Laemmli (1970). Aliquots of the membrane suspensions (25 μL) were mounted in each lane. Marker proteins were run in separate lanes. The $NaDodSO_4$–polyacrylamide gels were put at constant voltage until the bromphenol blue stacking line reached the bottom. After electrophoresis, the gel was stained with Coomassie blue, destained, and then dried and mounted for autoradiography on Kodak X-ray film type SB-5.

(*B*) *Analysis of Mixed Preparations of Photolabile Target Tissues.* An aliquot containing 60 000 cpm of each photoreacted preparation of canine renal membranes and cells from human giant cell tumor of bone and human foreskin fibroblasts were combined in pairs (renal membranes + giant cell tumor cells, renal membranes + fibroblasts, giant cell tumor cells + fibroblasts), mounted on $NaDodSO_4$–polyacrylamide gel slabs as described above, and subjected to electrophoresis followed by autoradiography.

Desensitization Studies. Cells were preincubated for 48 h in the presence or absence of bPTH-(1–84) or the inhibitory analogue [Nle^8,Nle^{18},Tyr^{34}]bPTH-(3–34)-amide in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, NY) plus 10% fetal calf serum (Microbiological Associates, Bethesda, MD) and penicillin (100 units/mL) and streptomycin (100 μg/mL) (Grand Island Biological Co., Grand Island, NY). After preincubation, the cells in the dishes were reacted with the photolabile PTH radioligand, as described above. The cAMP response to PTH in the cells in multiwell trays was assessed by washing of the cells in 1 mL of PBS 4 times followed by incubation in the presence or absence of bPTH-(1–84) for 20 min at 37 °C in 0.2 mL of PBS supplemented with 0.25% bovine serum albumin (Pentex, Miles Laboratories, Kankakee, IL) and 0.1% glucose plus 1 mM 3-isobutyl-1-methylxanthine (Aldrich Chemical Co., Milwaukee, WI).

cAMP Assays. To assay for cAMP content in cells plus medium, trays containing the cultured human cells were floated in a boiling-water bath, and the contents were brought to dryness by evaporation. One milliliter of cAMP assay buffer was then added to the wells, the cells were freed by scraping, and the contents were transferred to tubes. After centrifugation for 10 min at 600*g*, the supernatant solution was assayed for cAMP content by radioimmunoassay with a cAMP kit (New England Nuclear, Boston, MA) (Goldring et al., 1979).

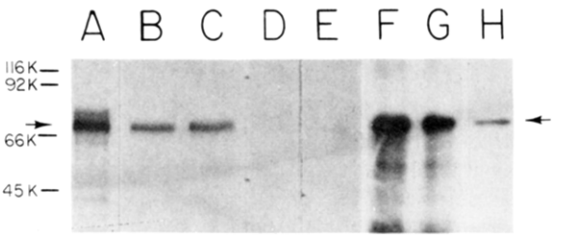

FIGURE 1: Identification and comparison of the PTH binding component in canine renal membranes and cells derived from human giant cell tumors of bone and human dermis. Renal cortical membranes and intact human cells were incubated with the photolabile PTH radioligand in the presence or absence of competing unlabeled hormone. Autoradiographs were prepared after solubilization, reduction, and $NaDodSO_4$–polyacrylamide gel electrophoresis of the photoreacted tissue or cells. The migration position of "marker" proteins of known molecular weight is indicated along the left margin. A component of comparable mobility (indicated by the arrow) can be identified in the renal tissue (lane A) and in the human cells of skeletal (lane B) and dermal (lane C) origin. Addition of excess unlabeled PTH before exposure to light results in almost complete disappearance of the labeled component in the human cells (lane D, skeletal cells; lane E, dermal cells). In addition, renal membranes or human cells, after reaction with the photolabile PTH radioligand, were solubilized and reduced, and mixed preparations in pairs were subjected to electrophoresis simultaneously on $NaDodSO_4$–polyacrylamide gel slabs. Lane F shows a mixture of photolabeled canine renal membranes and cells from human giant cell tumor of bone; lane G represents a mixture of canine renal membranes and human fibroblasts; lane H is a mixture of cells from human giant cell tumor of bone and human fibroblasts. A single labeled band of identical electrophoretic mobility is present in each of the mixed pairs in the autoradiograph.

Results

To identify the PTH binding component in human tissues and to compare it to the previously characterized PTH binding component in canine renal membranes, monolayer cells from human skeletal and dermal tissues were incubated with the photolabile PTH radioligand in the presence or absence of excess unlabeled PTH. After photolysis, reduction, and denaturation, the proteins were separated by electrophoresis. The labeling pattern shown by electrophoresis of $NaDodSO_4$–polyacrylamide gel slabs was consistent with the presence of a single binding component in intact cells derived from human giant cell tumors of bone and human foreskin fibroblasts (Figure 1, lanes B and C). Addition of excess unlabeled [Nle^8,Nle^{18},Tyr^{34}]bPTH-(1–34)-amide markedly diminished the radiolabeling of the binding component in monolayer cell cultures from both tissues (Figure 1, lanes D and E). Similar results were obtained when the radiolabeled photoreactive derivative and unlabeled analogue were added simultaneously, or when cells were incubated first with unlabeled hormone followed by photolabile derivative (data not shown), implying that equilibrium conditions are achieved by the time of photolabeling. It was estimated that the molecular weight of the labeled component was 70 000 by comparison with protein standards of known molecular weight run simultaneously in $NaDodSO_4$–polyacrylamide gels in adjacent lanes (Figure 1) and by comparison with the apparent molecular weight of the previously characterized PTH receptor in canine renal membranes (Figure 1, lane A).

To further establish that labeling of the PTH binding component in these cells was specific for PTH, cultured human cells that lacked a cAMP response to PTH were investigated.

Table I: Effect of Preincubation without PTH, with PTH, or with the PTH Inhibitor on the Subsequent PTH-Induced cAMP Response[a]

preincubation condition	cAMP levels (pmol/well) after test incubation	
	buffer	PTH
no hormone	0.47 ± 0.10	2.51 ± 0.23
PTH (1×10^{-7} M)	0.80 ± 0.06	0.80 ± 0.08
PTH inhibitor (3×10^{-7} M)	0.72 ± 0.16	2.91 ± 0.76

[a] After being plated in multiwell trays at 5.0×10^4 cells/well, cells from human giant cell tumors of bone were preincubated for 48 h without hormone, with PTH, or with the PTH inhibitor [Nle^8,Nle^{18},Tyr^{34}]bPTH-(3–34)-amide. After preincubation, cells were washed 4 times with PBS. The cAMP response to subsequent exposure to PTH was evaluated by incubation (test incubation) of the cells at 37 °C for 20 min in a buffer containing 1 mM isobutylmethylxanthine in the presence or absence of PTH (1.0×10^{-7} M). Values represent the mean ± SEM for three wells under each test condition. PTH-induced cAMP responses were significantly different from control (buffer) ($p < 0.1$) and PTH inhibitor incubations.

A human melanoma cell line grown from tumor tissue obtained from a patient with malignant melanoma was used. These cells failed to increase cAMP levels when acutely exposed to PTH. The presence of a PTH binding component could not be demonstrated by using the identical photoaffinity labeling approach described above (data not shown). In addition, cells from a giant cell tumor, which (after serial passage) had lost the capacity to increase cAMP levels when exposed to PTH, were also studied. Loss of the cAMP response to PTH in these "late-passage" cells was accompanied by failure to photolabel a PTH binding component. Lastly, when photolabile PTH radioligand is added to target tissue in the absence of photoactivation, a PTH binding component fails to label.

For direct comparison of the physicochemical properties of the photoaffinity-labeled binding components detected in the canine renal membranes and intact human cells, we mixed labeled preparations from membranes or cells in pairs for electrophoresis. Each combination of labeled material was analyzed simultaneously on adjacent lanes of a NaDodSO$_4$–polyacrylamide gel slab (Figure 1, lanes F–H). Components derived from each of the three systems appeared identical in terms of molecular size.

The photoreactive PTH radioligand was also used in studies in which cells cultured from human giant cell tumors of bone were preincubated with or without bPTH-(1–84) or with the PTH inhibitory analogue [Nle^8,Nle^{18},Tyr^{34}]bPTH-(3–34)-amide to examine the mechanism of tissue desensitization to PTH. After preincubation, the subsequent cAMP response to PTH was assessed by challenging cells acutely with PTH (test incubation). The PTH photoaffinity labeling pattern was evaluated under corresponding conditions. As shown in Table I, preincubation of the bone tumor cells with PTH resulted in almost complete loss of the PTH-induced cAMP response. In contrast, preincubation with the PTH inhibitor did not affect the subsequent PTH-induced cAMP response. The loss of the PTH-induced cAMP response in cells preincubated with this hormone was associated with a marked diminution in labeling of the PTH binding component (Figure 2, lane C). Preincubation of cells with the PTH inhibitor did not appreciably diminish subsequent photoaffinity labeling of the PTH binding component (Figure 2, lane D).

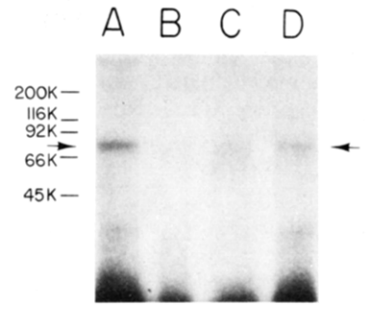

FIGURE 2: Effect of preincubation with PTH or with a PTH inhibitory analogue on labeling of the PTH binding component in cells cultured from human giant cell tumor of bone. Cells were preincubated without hormone or with PTH (1.0×10^{-7} M) or the PTH inhibitor [Nle^8,Nle^{18},Tyr^{34}]bPTH-(3–34)-amide (3.0×10^{-7} M). Cells were washed and then reacted with the photolabile PTH radioligand in the presence or absence of excess unlabeled PTH. After solubilization, reduction, and NaDodSO$_4$–polyacrylamide slab gel electrophoresis, the PTH binding components were examined by autoradiography. Lane A represents the labeling pattern in cells preincubated without hormone. The effect of addition of unlabeled PTH (acutely) before exposure to light is shown in lane B. The effect of preincubation with PTH is shown in lane C, and the effect of preincubation with the PTH inhibitor is shown in lane D.

Discussion

Employing the technique of photoaffinity labeling, we now have demonstrated directly the presence of PTH binding elements in PTH-responsive intact human cells derived from giant cell tumors of bone and from human foreskin fibroblasts (Figure 1). These membrane components are labeled specifically (Figure 1) and hence represent, presumably, the PTH receptor or a binding subunit of the PTH receptor. The molecular size of both of these receptors or receptor subunits is identical with that of the previously identified PTH receptor (or subunit) in canine renal membranes, as determined by electrophoresis with NaDodSO$_4$–polyacrylamide gel slabs (Figure 1). These findings, taken together with previously reported structure–activity studies (Tregear et al., 1973, 1974; Parsons et al., 1975; Goldring et al., 1978, 1979, 1981; Rosenblatt et al., 1981; Rosenblatt, 1981), demonstrate that the physicochemical properties of PTH receptors appear to be highly conserved across at least two species (dog and human) and the three "target" tissues (kidney, bone, and skin) examined.

The specificity of photoaffinity labeling of the intact human cells is further supported by the failure to demonstrate the presence of a PTH binding component in cultured human melanoma cells, which lack a PTH-induced cAMP response. Furthermore, demonstration that the loss (after serial passage) of the PTH binding component in cells cultured from human giant cell tumors of bone coincided with the loss of the PTH-induced cAMP response also provides evidence favoring specificity of labeling by photoaffinity techniques. Although the loss of the PTH binding component in these late-passaged cells could reflect overgrowth of a cell type not responsive to PTH, the absence of a change in cell morphology or growth characteristics makes the latter explanation unlikely.

We previously reported that some cell cultures prepared from dermal tissues are responsive to PTH (Goldring et al., 1979). Structure–activity studies with PTH fragments and analogues demonstrated similarities in the characteristics of the receptor for PTH in the dermal cells and cells derived from tissues regarded as conventional target tissues for PTH, i.e., bone and kidney (Goldring et al., 1979, 1981). Studies done with murine skeletal tissue have shown that osteoblasts or "osteoblast-like" fibroblasts exhibit a number of biologic responses to PTH, including stimulation of adenylate cyclase activity (Luben et al., 1976; Peck et al., 1977). Furthermore, we showed that, in culture, some human synovial cells, which also are of mesenchymal origin, respond to PTH with an

increase in cAMP levels (Goldring et al., 1980). Thus, it is not surprising that fibroblast-like cells in dermal tissues, which are presumably of mesenchymal origin, have receptors for PTH that are comparable with the receptors in skeletal tissue.

Finally, in these studies we examined directly the mechanism of desensitization to PTH. Other means of investigating the mechanism of desensitization, such as analysis by receptor binding studies, could not be used because radioreceptor assays for PTH have not been developed for these intact cells. Pretreatment of intact human cells of either skeletal or dermal origin with PTH results in a diminished cAMP response to subsequent reexposure to this hormone (Goldring et al., 1981). In previous reports, we indicated that desensitization does not result from receptor occupancy alone, inasmuch as the hormone inhibitor [Nle^{8},Nle^{18},Tyr^{34}]bPTH-(3–34)-amide (which binds to the PTH receptor and can competitively inhibit PTH action, but which does not itself elicit a cAMP response) does not produce the desensitization phenomenon (Goldring et al., 1981). Theoretically, several mechanisms could be responsible for hormone-specific desensitization. A decrease in receptor number by internalization, destruction, or an altered steric conformation or accessibility of the receptors after exposure to PTH could account for diminished responsiveness to the hormone (down regulation). Alternatively, receptor number, conformation, or accessibility could remain unchanged, but receptors could become uncoupled from their effector system (e.g., adenylate cyclase and the guanyl nucleotide regulatory protein), resulting in cellular refractoriness to hormonal stimulation. Our earlier studies could not distinguish between these possibilities. Use of photoaffinity labeling, however, provides direct evidence supporting a decrease in receptor number or receptor availability as the mechanism responsible for the desensitization of human cells to PTH.

Photoaffinity labeling has permitted direct characterization and comparison of PTH receptors in intact human cells from skeletal and dermal origin. The identification of a specific PTH binding component in intact human cells (which can be grown in monolayer culture) should now permit harvesting, further characterization, and perhaps, ultimately, isolation of the human receptor for PTH.

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Registry No. Parathyroid hormone, 9002-64-6; adenosine cyclic 3′,5′-phosphate, 60-92-4.

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