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CLINICAL INVESTIGATION

Effect of parathyroid hormone on human T cell activation

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Effect of parathyroid hormone on human T cell activation. Lymphocytes have receptors for PTH and patients with chronic renal failure have high blood levels of PTH and impaired lymphocyte function. It is possible, therefore, that PTH affects lymphocyte function. We studied the interaction between PTH and proliferation of human lymphocytes in vitro and examined potential mechanisms for such an interaction. 1-84 PTH stimulated in a dose dependent manner PHA-induced proliferation of T cells but had no effect on PWM-induced proliferation. The hormone did not alter CD4/CD8 ratio. Inactivation of PTH abolished its stimulatory effect. PTH augmented IL-2 production by PHA-activated T cells but did not increase expression of IL-2R. 1-34 PTH also stimulated PHA-induced T cell proliferation. TPA augmented PHA-induced T cell proliferation but the addition of PTH to the culture stimulated by PHA and TPA did not augment further the proliferation of T cells. Staurosporin reversed the stimulation by PTH of the PHA-induced lymphocyte proliferation. Both 1-34 and 1-84 PTH stimulated cyclic AMP production by lymphocytes. Forskolin did not affect PHA-induced T cell proliferation although it stimulated cyclic AMP generation. The results show that: 1) PTH acts on T cells; 2) acute exposure to PTH augments PHA-induced T cell proliferation and IL-2 production; 3) this action of PTH is related to its biological activity and is most likely due to the ability of PTH to enhance entry of calcium into cells and/or stimulation of protein kinase C but is independent of cyclic AMP generation.

End-stage renal failure is associated with derangements in cellular immunity [1–6]. The mechanisms of these abnormalities are not clear. It has been postulated that factor(s) in the uremic serum may participate in the genesis of the impaired cellular response [7, 8].

Patients with chronic renal failure have secondary hyperparathyroidism and elevated blood levels of parathyroid hormone (PTH) [9–11]. Available data indicate that the excess PTH affects the function of many organs in patients with uremia [12]. It is theoretically possible that PTH may also affect lymphocyte function and play, at least in part, a role in the genesis of the abnormalities of cellular immunity in uremia.

Several pieces of information lend a potential support for this proposition. First, preliminary data of Hoette et al [13] suggested that PTH may inhibit blastogenic response of lymphocytes to PHA. Also Shasha et al [14] also reported that both human and bovine PTH significantly inhibited lectin-induced lymphocytes transformation. Second, several investigators reported data consistent with the presence of PTH receptors in human and bovine lymphocytes [15, 16]. Third, interleukin (IL)2 which is produced by the T cells is important in the proliferation and differentiation of the T and B lymphocytes [17]. The production and/or secretion of IL-2 by T cells is calcium dependent [18], and PTH through its ability to enhance calcium entry into cells [19–23] may affect IL-2 production.

The present study was undertaken to examine the interaction between intact PTH (1-84 PTH) and its aminoterminal fragment (1-34 PTH) and lymphocytes from normal subjects, and to investigate the mechanisms underlying such an interaction.

Methods

Normal healthy volunteers were studied. There were 11 females and 23 males. Their ages ranged between 23 to 39 (30 ± 0.7) years. None were taking medications known to affect the immune system or lymphocyte function.

Preparation of PBMC

Peripheral venous blood was drawn under sterile conditions into vacutainers containing preservative-free heparin (GIBCO Labs, Grand Island, New York, USA), 20 U per each 1.0 ml blood. The blood was diluted with an equal volume of HBSS, (Flow Laboratories, McLean, Virginia, USA) containing penicillin (100 U/ml) and streptomycin (100 μg/ml; GIBCO). Twenty milliliters of diluted blood were layered on top of 15 ml of Ficoll-Hypaque solution (Isolymph, Gallard-Schlesinger, New York, USA), density 1.077 g/ml, in sterile 50 ml conical tubes (Corning, New York, New York, USA). The samples were centrifuged 30 minutes at 400 g at room temperature. PBMC (87 ± 3% lymphocytes) were recovered from the plasma-isolymph interface, washed twice with 50 ml of HBSS, and resuspended in RPMI-1640 (GIBCO), supplemented with 10% heat-inactivated fetal calf serum (FCS; Irvine Scientific, Irvine, California, USA), penicillin 100 U/ml, streptomycin 100 μg/ml, and 2 mM glutamine (GIBCO). Cell suspensions were adjusted to obtain a final concentration of 2×10^6 PBMC/ml.

Proliferation assay

Except where noted otherwise, lymphocytes were cultured for five days at 37°C in a humidified, water-jacketed incubator (Forma Scientific, Marietta, Ohio, USA) in 5% CO_2. The cells were cultured at a density of 2×10^5/well in standard flat bottom, 96 well microplates (Dynatech, Alexandria, Virginia,

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USA). Each well received 0.1 ml of cells in medium, either 0.1 ml of phytohemagglutinin (PHA), (reagent grade Wellcome Diagnostics, Dartford, UK), or 0.1 of pokeweed mitogen (PWM; GIBCO) and 0.05 ml of desired dilution of PTH in medium. Mitogens were dissolved in culture medium and used at a final concentration, of 1:450 vol/vol of the stock PHA and 1:250 vol/vol of the stock PWM. In cultures without PTH or mitogen, desired amount of medium was added to reach the final volume of 250 μl.

The choice of the dilution of 1/450 vol/vol of the PHA stock, the dose of 4×10^{-7} M 1-84 PTH and the duration of culture of five days was based on preliminary studies. We evaluated the PBMC response to varying dilutions of PHA (1/225 to 1/3600 vol/vol) both in the presence and absence of 1-84 PTH (1×10^{-7} to 1×10^{-6} M). These preliminary studies revealed that the dilution of 1/450 vol/vol PHA is suboptimal, that PTH stimulated PHA-induced lymphocyte proliferation and the maximal effect was noted at a concentration of 4×10^{-7} M. This concentration of PTH exerted its stimulatory effect at dilutions of PHA of 1/225, 1/450 and 1/900 vol/vol; however, the maximal effect of PTH was noted at the suboptimal dilution of PHA which was 1/450 vol/vol. We also found that this effect of PTH on PHA-induced lymphocyte proliferation was variable during the second and third days of culture. In these days PTH modestly inhibited, had no effect or modestly stimulated lymphocyte proliferation. However, the stimulatory effect of PTH was more consistent on day five of the culture.

After five days, the cultures were pulsed for four hours with 0.5 μCi of ^{3}H-thymidine (Methyl-^{3}H thymidine, specific activity 6.7 Ci/mmol, ICN Radiochemicals, Irvine, California, USA). The cells were then harvested onto glass fiber filter paper using an automated cell harvester (Cambridge Technology Inc., Cambridge, Massachusetts, USA). The filters were dried overnight, quenched in Betafluor (National Diagnostics, Manville, New Jersey, USA) and counted in a liquid scintillation counter (Model LS7000, Beckman Instruments, Palo Alto, California, USA). Each determination was performed in four to six replicates. The proliferation of lymphocytes is expressed as ^{3}H-thymidine uptake (counts per minute, CPM).

Preparations of PTH

Synthetic 1-34 bovine PTH was obtained from Bachem (Torrance, California, USA). Bovine intact hormone (1-84 PTH) was purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). In some experiments inactivated PTH was used. The inactivation of the hormone was done as follows: the PTH was dissolved in 0.2 ml of 0.1 mM acetic acid adjusted to the desired volume with 30% H_2O_2 and incubated at 37°C for four hours; the reaction was stopped by rapid freezing at −70°C and the product was recovered by lyophylization.

Production of IL-2 by cultured cells

Cultures were set as described earlier for the proliferation assay except that 5% FCS was used, and 20 mM HEPES was also added to the media. Cells were cultured with 1-84 PTH (4×10^{-7} M) and PHA (1/450 vol/vol dilution) and with PHA alone with triplicate for each group. At 48 hours of culture, supernatants from the replicates were collected, pooled into round bottom microplates, and stored at −20°C until assayed. IL-2 was determined using a proliferative assay with an IL-2 dependent murine CTLL cell line and recombinant human IL-2 as a reference standard as previously described [24].

Expression of interleukin 2 receptors (IL-2R)

To determine the expression of IL-2R by lymphocytes, PBMC were cultured in sterile tubes (Falcon, Division of Becton Dickinson, Lincoln Park, New Jersey, USA) in RPMI-1640 supplemented as previously described except that HEPES (10 mM) was added. Cells were cultured at a density of 10^6 cells per tube in a total volume of 1.25 ml. At 24, 48, and 72 hours, cells were harvested, stained with a Phyco-Erythrin monoclonal antibody to IL-2R (PE-IL2R, Becton Dickinson, Mountain View, California, USA) and analyzed by flow cytometry using a fluorescence activated cell sorter (Ortho System tp, Orthodiagnostic, Inc. Raritan, New Jersey, USA). Results are expressed as the percent of cells positive for the IL-2R.

IL-2 bound to lymphocytes under high affinity conditions was evaluated ($N = 3$) as described by Robb, Greene and Rusk [25]. PBMC, 3×10^5, were incubated with 20 pM to 200 pM ^{125}I-IL-2 (NEN Products, Massachusetts, USA) for 10 minutes at 37°C. Cell bound IL-2 was separated from excess lymphokine by centrifugation at 10000 RPM through 200 μl paraffin oil, and both fractions were counted with a gamma counter. Control cell aliquots were preincubated with 200 units of unlabeled recombinant IL-2 (Cetus Corp.). The percent bound ^{125}I-IL-2 was corrected for nonsaturable binding.

Effect of PTH on CD4/CD8 ratio

The CD4/CD8 ratios were determined on PBMC of normal subjects before culture and after five days of unstimulated and PHA-induced proliferation of lymphocytes with and without 4×10^{-7} M 1-84 PTH. For the determination of CD4/CD8 ratio, two FITC conjugated monoclonal antibodies to T cell surface antigens (Becton Dickinson) were used. These were anti-Leu 3 antibody, which reacts with the CD4 subpopulation and anti-Leu 2 which reacts with the CD8 subpopulation. Pellets of PMBC were incubated with 5 μl monoclonal antibody, at 4°C for 30 minutes. After washing the pellets, they were resuspended in 5% FCS complete RPMI medium and read on an Ortho System 50 Cytoflow graph.

Effects of 12-0-tetradecanoyl phorbol 13-acetate (TPA) or forskolin

These experiments were designed to examine the effects of TPA alone and in the presence of PHA, 4×10^{-7} M 1-84 PTH and PHA plus PTH on lymphocyte proliferation. TPA was dissolved in DMSO and added to culture at a final TPA concentration of 62 ng/ml. DMSO alone had no effect on PHA-induced lymphocyte proliferation (PHA alone $36.1 \times 10^3 \pm 2.9 \times 10^3$ CPM vs. PHA plus DMSO $36.4 \times 10^3 \pm 4.8 \times 10^3$ CPM). The effects of forskolin in the presence and absence of PHA on lymphocyte proliferation were also examined. Thirty microliters of forskolin solution (5 mg forskolin/1.0 ml of ethanol) were added to 270 μl of culture media, giving a final concentration of 20 μg forskolin/ml. The studies with TPA and forskolin were performed simultaneously.

Effect of Staurosporin

The effect of 10^{-8} M Staurosporin, an inhibitor of protein kinase C on the stimulatory effect of 1-84 PTH, on the PHA-induced proliferation of lymphocytes was examined. Staurosporin was dissolved in DMSO to prepare a stock solution of 10^{-3} M; culture media was then added to obtain a final concentration of 10^{-8} M.

Effect of PTH on cyclic AMP production of PBMC

PBMC were isolated as described above. Two $\times$ 10^6 cells were suspended in 1.0 ml Krebs-Ringer solution with 10 mM HEPES, 2.8 mM D-glucose, 0.5 mg/ml BSA (pH 7.4). Twenty-five microliters of either Krebs-Ringer, 1-34 PTH, 1-84 PTH or forskolin solutions were added to 0.5 ml of cell suspension giving a final concentration of 4×10^{-7} M of 1-34 and 1-84 PTH and 20 μg/ml of forskolin. The cells were incubated in a water bath at 37°C for 30 minutes. The reaction was terminated by the addition of 240 μl of 10% perchloric acid. The mixture was then neutralized with 180 μl of 2 N potassium carbonate, diluted with 1.0 ml of distilled water and centrifuged at 3000 g for eight minutes. The supernate was saved, the pellet was washed and centrifuged twice and the supernate was saved after each centrifugation. The pooled supernate was dried in an oven (80°C) for 18 hours and then dissolved in 2 ml of the cyclic AMP assay buffer. Cyclic AMP was measured utilizing the cyclic AMP (^{125}I) assay kit (Amersham Co., Arling Heights, Illinois, USA).

Statistical analysis

All experiments were performed with paired controls. Student's *t*-test was used for statistical analysis. *P* values below 0.05 were considered significant. Data are expressed as mean ± 1 SE.

Results

Effects of various PTH preparations on lymphocyte proliferation

We first examined the effect of 1-84 PTH on unstimulated lymphocyte cultures. The hormone produced a significant ($P < 0.01$) increase in lymphocyte proliferation as depicted by ^{3}H-thymidine uptake of $4.6 \times 10^3 \pm 0.5 \times 10^3$ CPM compared to that noted in unstimulated cultures without PTH ($2.0 \times 10^3 \pm 0.2 \times 10^3$ CPM).

We then examined the effect of PTH on PHA-induced lymphocyte proliferation. Figure 1 depicts the effects of increasing doses of 1-84 PTH on PHA-induced lymphocyte proliferation ($N = 8$). The hormone augmented the PHA-stimulated ^{3}H-thymidine uptake by PBMC in a dose dependent fashion. Inactivation of the hormone ($N = 8$) abolished its enhancing effect on PHA-induced lymphocyte proliferation (Fig. 2). The aminoterminal fragment of the hormone (1-34 PTH) also augmented PHA-induced lymphocyte proliferation, but only at the higher peptide concentration of 4×10^{-7} M (Fig. 3), $P < 0.01$ ($N = 8$). Doubling the concentration of 1-34 PTH to 8×10^{-7} M did not produce further stimulation. Moreover, the increase in the PHA-induced lymphocyte proliferation by the 1-34 PTH (+28 ± 5%) was significantly ($P < 0.01$) less than that produced by equimolar concentration (4×10^{-7} M) of 1-84 PTH (+51 ± 9%).

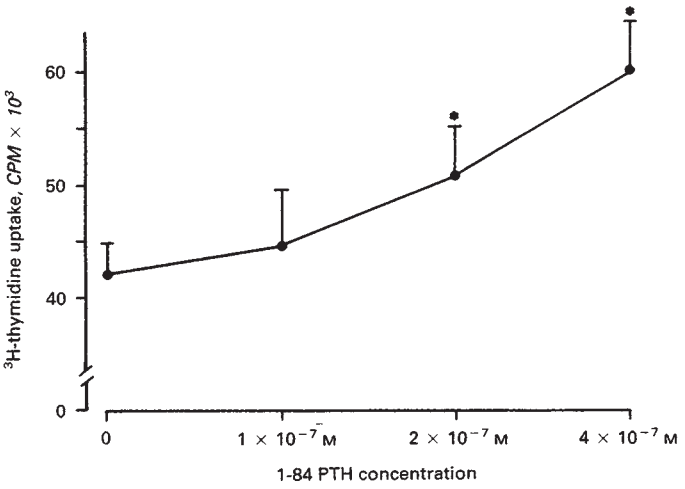

Fig. 1. *Effects of increasing concentrations of 1-84 PTH on PHA-induced proliferation of human T cells.* Each data point represents mean value of 8 studies and the brackets denote 1 SE. *$P < 0.01$ from data without PTH.

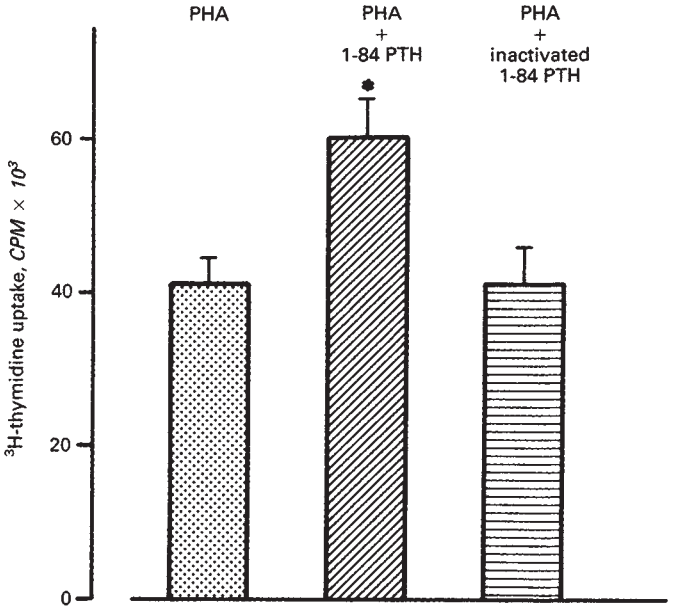

Fig. 2. *Effect of inactivated 1-84 PTH on PHA-induced human T cell proliferation.* Each column represents the mean value of 8 studies and brackets denote 1 SE. *$P < 0.01$ versus PHA alone or PHA + inactivated 1-84 PTH.

PTH had no effect on proliferation of lymphocytes stimulated with PWM (Fig. 4).

Effect of PTH on IL-2 production

Since IL-2 is required for the sustainment of mitogen-induced lymphocyte proliferation, we asked whether the PTH effect was mediated by this lymphokine. Figure 5 depicts the effects of 4×10^{-7} M 1-84 PTH on PHA-induced IL-2 production determined after 48 hours of culture ($N = 5$). The mean IL-2 concentration in the supernatants of cells cultured with PHA alone was 5.0 ± 2.0 U/ml. The presence of PTH in culture media was associated

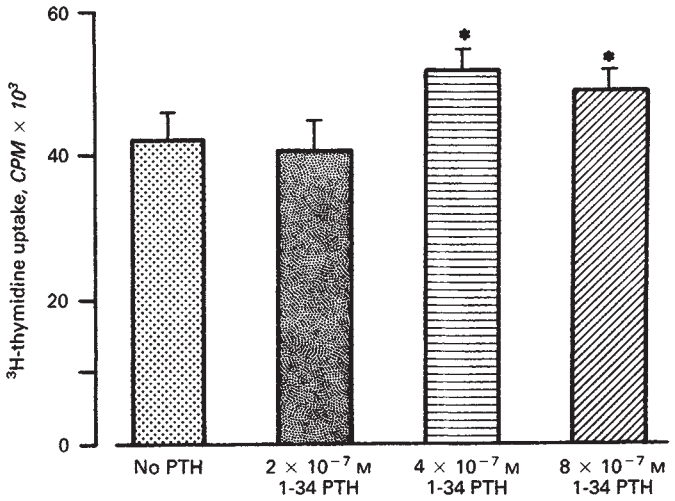

Fig. 3. *Effects of increasing concentrations of 1-34 PTH on PHA-induced human T cell proliferation.* Each column represents mean value of 8 studies and brackets denote 1 SE. *$P < 0.01$ versus no PTH or 2×10^{-7} M 1-34 PTH.

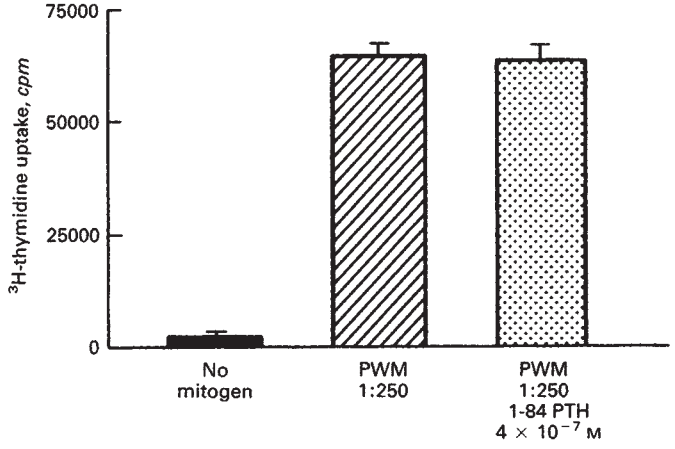

Fig. 4. *Effect of 1-84 PTH on PWM-induced T cell proliferation.* Each column represents mean value of 8 studies and brackets denote 1 SE.

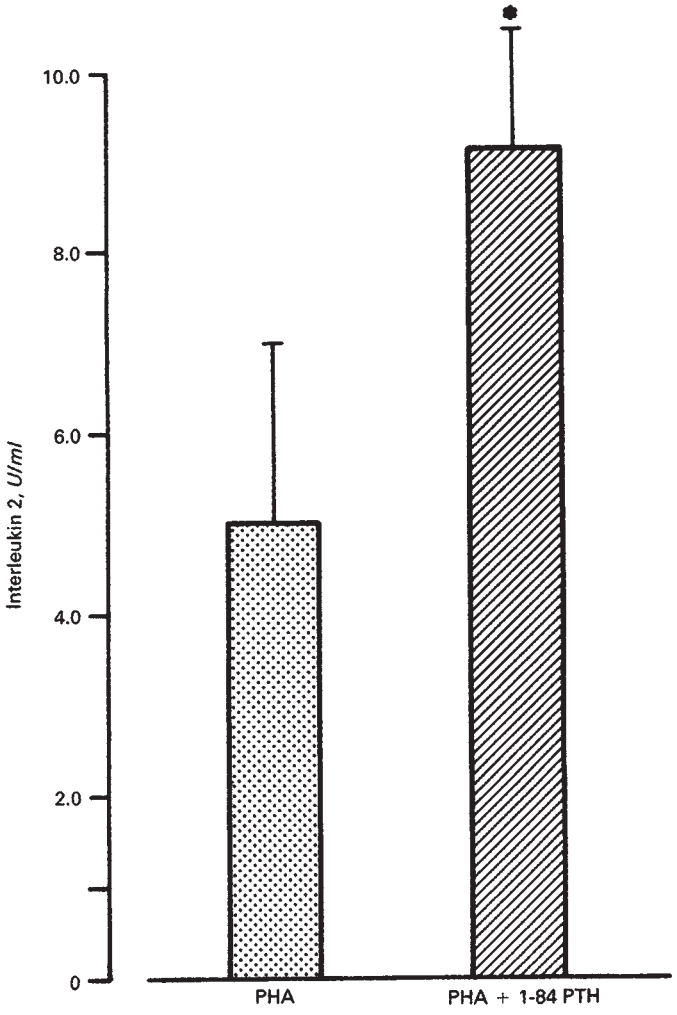

Fig. 5. *Effect of 1-84 PTH (4×10^{-7} M) on PHA-induced IL-2 production by human T cells after 48 hours of culture.* Each column represents mean value of 5 studies and brackets denote 1 SE. *$P < 0.01$.

with a significant ($P < 0.01$) increase in IL-2 concentration to 9.1 ± 1.8 U/ml. Thus, 1-84 PTH had a significant stimulatory effect on PHA-induced IL-2 production by PBMC. However, it should be mentioned that 1-84 PTH did not cause a detectable increase in IL-2 production by the unstimulated lymphocytes.

Effect of PTH on expression of IL-2R

Since mitogen-induced proliferation of lymphocytes requires the expression of IL-2R on lymphocytes and since PTH augmented PHA-induced lymphocyte proliferation, we examined the effect of the hormone on expression of IL-2R. The IL-2R were determined at 24, 48 and 72 hours in spontaneously proliferating cells, in those stimulated with PHA alone, in cells cultured with 4×10^{-7} M 1-84 PTH alone, and in cells cultured with both PHA and PTH ($N = 4$). The hormone alone did not augment IL-2R by the unstimulated cells and did not alter their expression on cells stimulated by PHA (Fig. 6). Table 1 depicts the result of a representative experiment evaluating the expression of high affinity IL-2R on lymphocytes. PTH did not augment the expression of these receptors.

Effect of TPA and forskolin on lymphocyte proliferation

Figure 7 depicts the effects of TPA and forskolin on lymphocyte proliferation ($N = 10$). TPA stimulated lymphocyte proliferation to a degree not different from PHA (TPA: $37.8 \times 10^3 \pm 6.2 \times 10^3$ CPM vs. PHA: $36.5 \times 10^3 \pm 3.3 \times 10^3$ CPM). TPA significantly augmented the PHA-induced lymphocyte proliferation (PHA + TPA: $56.3 \times 10^3 \pm 5.7 \times 10^3$ CPM, $P < 0.01$). The addition of 1-84 PTH to the cultures stimulated with PHA and TPA did not augment further the lymphocyte proliferation (PHA + TPA: $56.3 \times 10^3 \pm 5.7 \times 10^3$ CPM vs. PHA + TPA + PTH: $57.2 \times 10^3 \pm 6.1 \times 10^3$ CPM). Forskolin did not augment PHA-induced lymphocyte proliferation (PHA: $36.5 \times 10^3 \pm 3.3 \times 10^3$ CPM versus PHA + forskolin $41.5 \times 10^3 \pm 4.3 \times 10^3$ CPM).

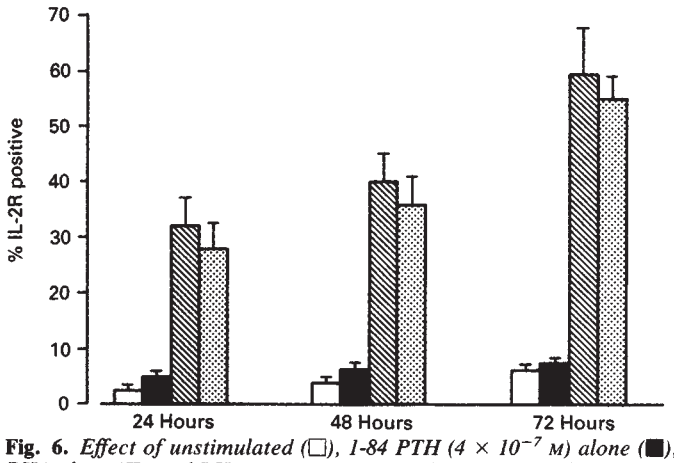

Fig. 6. *Effect of unstimulated (□), 1-84 PTH (4 × 10^{-7} M) alone (■), PHA alone (▧) and PHA + PTH (▨) on IL-2R expression by human T cells at various times of culture.* Each column represents mean value of 4 studies and brackets denote 1 SE.

Table 1. Expression of high affinity IL-2R on lymphocytes

	Specifically bound ^{125}I-IL-2%			
Hours	Unstimulated	PHA	PTH	PHA + PTH
24	0.8 ± 0.4	0.4 ± 0.3	0.9 ± 0.4	1.1 ± 0.5
48	0.9 ± 0.4	2.6 ± 0.5	0.9 ± 0.4	2.3 ± 0.2
72	1.6 ± 1.0	6.8 ± 1.7	1.7 ± 0.7	6.3 ± 0.8

Values indicated mean % ±SEM ^{125}I-IL-2 specifically bound by 3 × 10^5 lymphocytes after 24, 48, 72 hours of culture.

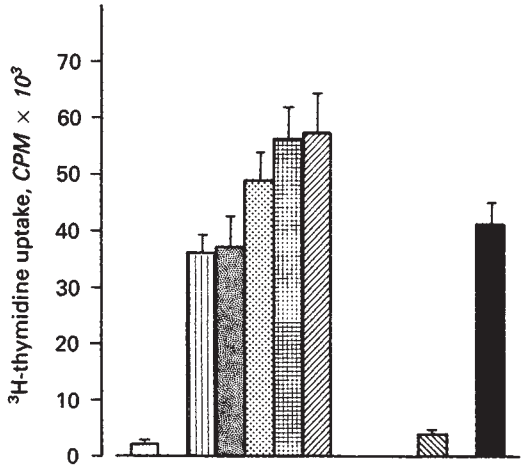

Fig. 7. *Effects of TPA (62 ng/ml) and forskolin (20 μg/ml) on PHA or PHA + 1-84 PTH (4 × 10^{-7} M)-induced human T cell proliferation.* Symbols are: (□) unstimulated; (▥) PHA; (▥) TPA; (▥) PHA + PTH; (■) PHA + TPA; (▨) PHA + TPA + PTH; (▧) forskolin; (■) forskolin + PHA. Each column represents mean value of 10 studies and brackets denote 1 SE.

Effect of PTH on CD4/CD8 cells ratio

Table 2 reveals the data obtained in studies examining the effect of 1-84 PTH on CD4/CD8 ratios. At time 0 of culture the value of this ratio was 1.49 ± 0.22, which is not different from that reported by others [26]. In the unstimulated culture, we observed proliferation of the CD4 but not CD8 cells resulting in a relative increase in CD4/CD8 ratio to 1.72 ± 0.10. In the presence of PTH alone, a similar effect was obtained, resulting in an increase in CD4/CD8 ratio (1.99 ± 0.11) which was modestly but significantly ($P < 0.02$) higher than that observed with unstimulated cultures. PHA induced proliferation of both CD4 and CD8 cells with a greater effect on CD8 resulting in a significant decrease in CD4/CD8 ratio as compared to unstimulated cultures. 1-84 PTH did not alter the effect of PHA on CD4/CD8 ratio.

Table 2. Effect of parathyroid hormone on percent CD4 and CD8 cells after unstimulated or PHA-stimulated lymphocyte culture

	Preculture	Unstimulated	PTH alone	PHA alone	PTH + PHA
CD4%	35.7 ±3.2	47.1 ±4.0	47.3 ±3.4	51.7 ±3.2	54.0 ±3.5
CD8%	25.5 ±3.5	27.6 ±2.2	24.1[a] ±2.2	43.9[b] ±3.0	42.5[a] ±4.2
CD4/CD8	1.49 ±0.22	1.72 ±0.10	1.99[a] ±0.11	1.21[c] ±0.14	1.35 ±0.21

Value shown are means ± SEM.
[a] $P < 0.02$ versus corresponding value during unstimulated culture
[b] $P < 0.01$ versus corresponding value during unstimulated culture
[c] $P < 0.05$ versus corresponding value during unstimulated culture

Effect of Staurosporin

The data showed that this inhibitor of protein kinase C reversed the stimulation by PTH of the PHA-induced lymphocyte proliferation (Table 3).

Effect of PTH on cyclic AMP

Both 1-34 and 1-84 PTH significantly stimulated cyclic AMP production by PBMC (Fig. 8). The basal cyclic AMP level was 11.2 ± 0.46 pmol/10^6 cells and increased to 19.1 ± 3.2 pmol/10^6 ($P < 0.05$) with 1-34 PTH and to 26.3 ± 5.2 pmol/10^6 cells ($P < 0.025$) with 1-84 PTH. Similarly, forskolin significantly ($P < 0.01$) increased cyclic AMP generation by these cells (37.3 ± 1.3 pmol/10^6 cells).

Discussion

The results of the present study demonstrate that intact 1-84 PTH augmented PHA-induced lymphocyte proliferation in a dose dependent manner. This effect of 1-84 PTH is related to its biological activity and not to a contaminant of the hormone preparation since inactivation of the hormone abolished its effect. Further, the synthetic, pure, aminoterminal fragment, (1-34 PTH) also augmented the PHA-induced lymphocyte proliferation. In contrast PTH did not affect lymphocyte proliferation induced by PWM.

It is of interest that the 1-84 PTH was more potent than its aminoterminal fragment in augmenting the PHA-induced lymphocyte proliferation. This is not surprising since previous studies showed similar observations on the effect of these two moieties of the hormone on isolated heart cells [27], on myocardial bioenergetics [23] and on fatty acids oxidation by skeletal muscle [28]. These observations suggest that other parts of the PTH molecule besides the aminoterminal fragment may have biological activity or that the intact molecule of the hormone attaches more tightly to its receptor than does the aminoterminal fragment.

Table 3. Effect of Staurosporin on lymphocyte proliferation

	No mitogen	Staurosporin	PHA	PHA+PTH	PHA+PTH+Staurosporin
	^{3}H-thymidine uptake (CPM × 10^3)				
Study 1	2.4 ± 0.5	5.4 ± 1.5	42.5 ± 3.9	65.6 ± 9.7[a]	42.2 ± 6.5
Study 2	3.0 ± 0.2	3.5 ± 1.0	46.3 ± 0.8	71.7 ± 0.2[b]	41.2 ± 2.5

Data are presented as mean ± SE. Each study is the mean of three measurements.
[a] $P < 0.05$ versus PHA, and PHA+PTH+Staurosporin
[b] $P < 0.01$ versus PHA, and PHA+PTH+Staurosporin

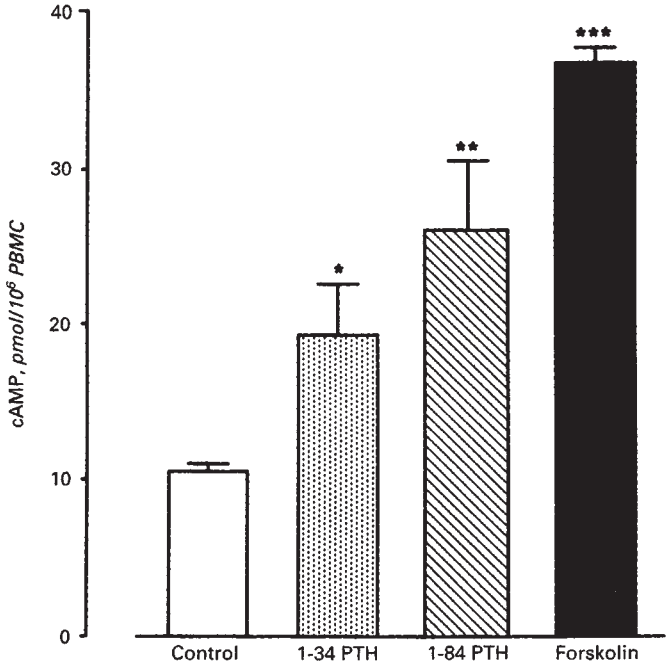

Fig. 8. *Effect of vehicle, 4 × 10^{-7} M 1-34 and 1-84 PTH and of 20 μg/ml of forskolin on cyclic AMP production by lymphocytes.* Each column represents the mean of 3 studies (each measurement done in duplicate) and the brackets denote 1 SE. *$P < 0.05$, **$P < 0.025$, ***$P < 0.01$ versus control.

PHA is a T cell mitogen [17, 29, 30]. Since PTH augmented PHA-induced lymphocyte proliferation and since the percents of CD4 and CD8 cells noted during PHA and PTH stimulation were not different from that with PHA alone, one can suggest that the stimulatory effect of the hormone on lymphocyte proliferation is due to an action on both CD4 and CD8 cells. Available data indicate that both murine [31, 32], and human [33] CD4 cells can be divided into at least two distinct subsets; one type (Th1) responds to PHA to produce IL2 and the other type (Th2) responds to PWM to produce IL4 [34]. Our finding that PTH stimulated PHA and not PWM-induced cell proliferation and enhanced IL2 production, suggest that the hormone may stimulate proliferation of Th1 subset of the CD4 cells.

Data suggesting an action of PTH on lymphocytes are limited. Whitfield, Mac Manns and Rixon [35] reported in 1970 that PTH stimulated thymocyte (T cell) DNA synthesis. Perry [36] found that 1-34 PTH activated mononuclear leukocytes, most likely T cells, and caused them to produce a substance(s) that enhances bone resorption. Others reported data consistent with the presence of PTH receptors on lymphocytes [15, 16]. These observations are in agreement with our results. Recently, Atkinson et al [37] reported that 1-34 PTH and 1-38 PTH stimulate proliferation of rat thymic lymphocytes. Thus, the available data and our results demonstrate that T cells are a target for PTH, and the hormone stimulates their proliferation. It should be mentioned, however, that two studies reported that PTH may inhibit the PHA-induced lymphocyte proliferation [13, 14]. The data of Hoette et al [13] were preliminary; they used Eli Lilly PTH extracts which contain a great deal of impurities, the conditions of their culture (2% heated pooled normal serum) were suboptimal and the duration of the culture (2 days) was short. The studies of Shasha et al [14] used 1 × 10^5 cells per well and a three day culture compared to 2 × 10^5 cells per well and five day culture in our study. These may provide partial explanation for the differences between the two studies since we also found that the effect of PTH on PHA-induced lymphocyte proliferation is variable after two or three days of culture. In addition, it is difficult to compare the amount of hormone in our studies with those in the other two, since we do not know how to convert mIU/ml concentration of their PTH into molar concentrations of the hormone as used in our study.

The mechanisms underlying the effect of PTH on PHA-induced proliferation may be complex. The proliferation of T cells requires the presence of monocytes and their production of IL-1, production of IL-2 by T cells and the action of IL-2 on its surface receptors on the lymphocytes [38–40]. It has been shown that an increase in cytosolic calcium of T cells by a calcium ionophore is associated with an increase in IL-2 production and augmentation of lymphocytes proliferation induced by suboptimal doses of PHA [17, 18]. Since PTH is known to enhance calcium entry into many cells [19–23], it is reasonable to suggest that the action of the hormone on T cell proliferation is related to its ability to augment movement of calcium into these cells and the consequent production of IL-2. Indeed, the finding that PTH causes a significant increase in IL-2 production in the presence of PHA is consistent with such a sequence of events. Further support to this notion is found in the data of Atkinson et al [37] showing that the action of PTH on proliferation of rat thymic lymphocytes depends on the presence of calcium in the culture media and that the hormone causes a rise in cytosolic calcium of these cells.

It is interesting that 1-84 PTH did not cause expression of IL-2R in the unstimulated lymphocyte nor did it affect expression of IL-2R in PHA-treated cells. It appears, therefore, that a potential PTH-induced movement of calcium into the T cells is not associated with expression of IL-2R. This is not surprising since it has been shown that a change in cytosolic calcium is not required for induction of IL-2R [41]. One may speculate that adequate number of expressed IL-2R induced by suboptimal

doses of PHA were available and permitted the enhancing effect of PTH on T cell proliferation.

A PTH-induced calcium movement into T cells may also stimulate lymphocyte proliferation independent of IL-2 production. The hormone produced a modest but significant increase in ^{3}H-thymidine uptake in the unstimulated lymphocyte culture without a detectable increase in IL-2 production. This observation is in agreement with data by Koretzky et al [42] who demonstrated that A23187 calcium ionophore can induce lymphocyte proliferation independent of IL-1 and IL-2.

Several investigators have provided data consistent with the presence of PTH receptors in the lymphocytes. Therefore, PTH-PTH-receptor interactions may provide, at least, two other pathways for the action of the hormone on the T cells. First, PTH may stimulate an adenylate cyclase system in the lymphocyte and generate cAMP which, in turn, may mediate the effect of the hormone. Indeed, our data show that both 1-34 and 1-84 PTH stimulated cyclic AMP generation by PBMC. However, this effect of PTH does not seem to be responsible for its stimulation of PHA-induced T cell proliferation. First, forskolin, an agent that stimulates adenylate cyclase activity [43] did not augment PHA-induced lymphocyte proliferation in our studies despite significant increase in cyclic AMP generation. Furthermore, others have found that forskolin, while significantly increases cyclic AMP, inhibited mitogen-induced lymphocyte proliferation [44]. Also Atkinson et al [37] found that PTH-induced proliferation of rat thymic lymphocytes is independent of cAMP.

Second, PTH- PTH-receptor interaction has been shown to affect phospholipid turnover in many tissues [45–47]. Such an effect would generate diacylglycerol which is a potent stimulus of protein kinase C. The increased activity of this enzyme may augment the PHA-induced lymphocyte proliferation. Indeed, our studies as well as those of others [44, 48] have demonstrated that TPA, an agent that stimulates protein kinase C [49, 50] augments PHA-induced lymphocyte proliferation. These observations and the finding in our study that PTH does not produce further augmentation in PHA-induced lymphocytes proliferation in cultures with PHA and TPA is consistent with the notion that action of PTH is mediated, at least in part, by an effect on the activity of protein kinase C. Further support for this postulate is provided by the reversal of the stimulatory effect of PTH by staurosporin, an inhibitor of protein kinase C.

The clinical implications of the effects of PTH on T cell function in the genesis of the abnormalities in cell immunity in uremia need further investigation. We must emphasize that our studies deal with the effect of acute exposure to PTH. However, in patients with advanced chronic renal failure, there is chronic exposure to markedly elevated blood levels of PTH. Such sustained exposure may result in a calcium overload of the lymphocytes, down regulation of their PTH receptors and altered phospholipid metabolism of their cell membrane. Either or any combination of such events may have adverse effects on lymphocyte function. Indeed, chronic exposure to PTH in renal failure was associated with increased calcium content of many organs [51–57] and down regulation of its receptors in bone [58]. Furthermore, many studies have demonstrated that the acute effect of PTH may be different from its chronic action. For example, excess amounts of PTH acutely stimulate the chronotropic [27] and inotropic [59] properties of the heart cells and enhances random motility of polymorphonuclear leukocytes [60], but chronic exposure to excess PTH decreases or stops the beating of the heart cells [27], impairs the metabolism and function of the heart [23] and reduces random motility of the leukocytes [60].

In this context, Chatenoud et al [61] reported the presence of preactivated T cells in hemodialysis patients. This observation could be, at least in part, due to the chronic exposure of the T cells to high levels of PTH in these patients. Also Shasha et al [62] found that the lectin-stimulated lymphocyte transformation was significantly reduced in three patients with primary hyperparathyroidism, and this abnormality was corrected one month after the removal of the parathyroid adenoma. These data further support the notion that chronic exposure of the T cells to excess PTH may affect their function.

We would like to mention that the PTH dose in our in vitro studies demonstrating a direct effect of the hormone on the T cells is high and even exceeds the high blood levels of PTH in patients with chronic renal failure. This may raise the question about the physiological significance of this action of the hormone. However, we would like to emphasize that the in vitro dose of PTH required to elicit most of the non-traditional effects of PTH such as those on the heart cells [27], hematopoietic cells [63], red blood cells [22], white blood cells [60, 64], pancreatic islets [65] and vascular smooth muscle [66] are also high. Several possibilities should be considered to explain this phenomenon as discussed in detail by Potts [67]. First, these non-traditional actions of PTH could be non-specific and do not represent hormone-receptor interaction. However, in many of these studies including the present one, PTH stimulated cyclic AMP production [27, 65], suggesting the presence of receptors responsive to PTH in many of these cells. Second, the non-traditional actions of PTH are mediated by stimulation of cell receptors that are responsive to other agonists. This implies that PTH acts as a "surrogate for the normally active agonist" [67]. If this postulate is correct, one might expect that different regions of the PTH molecule other than those involved in mediating the mineral action of the hormone would be responsible for the non-traditional effects. Indeed, in many studies, 1-34 PTH, considered to be the biologically active fragment for mediating the mineral actions, does not elicit the non-traditional effects [60, 63, 64]. If PTH mediates these latter actions as a surrogate for other agonist, one would expect that higher doses of PTH are needed. Third, it is also possible that the non-traditional actions of PTH are mediated by activation of specific receptors that may or may not be related to the PTH receptors responsible for the mineral actions of the hormone. Such potential PTH receptors responsible for its non-traditional actions may be present at low concentration and may require higher doses of the hormone for their activation. Finally, the in vitro cell preparation may contain cell components, such as proteases, which may degrade the hormone, and hence higher quantities are required to elicit its action.

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