Potentiation of Antigen-Induced Mast Cell Activation by 1-34 Bovine Parathyroid Hormone (43222)

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Abstract. **Peptides such as parathyroid hormone (PTH), somatostatin, and gastrin have been reported to stimulate mast cell mediator release. Preincubation of rat serosal mast cells with synthetic 1-34 bovine parathyroid hormone (1-34bPTH) significantly enhanced antigen-induced 5-hydroxytryptamine (5-HT) release. Enhancement of 5-HT release by 1-34bPTH was dose dependent between 5 and 2000 n*M*. In the absence of antigen, mean net 5-HT release was less than 1% when naive or passively sensitized mast cells were incubated with 1000 n*M* 1-34bPTH for time intervals up to 90 min. These findings indicate that 1-34bPTH, at relatively low concentrations, potentiates antigen-induced 5-HT release from mast cells.** [P.S.E.B.M. 1991, Vol 197]

Parathyroid hormone (PTH) (1, 2), an important regulator of bone turnover, and other peptide hormones such as gastrin (3) and somatostatin (4) have been reported to stimulate mast cell mediator release. Mast cells are laden with a broad spectrum of potent bioactive compounds which are released into the microenvironment upon activation of the cells (5, 6). Participation of the mast cell in inflammatory responses is well established. The mast cell has also been implicated as a potential contributor to the regulation of bone and cartilage metabolism. Systemic mastocytosis with bone involvement is associated with both osteosclerosis and osteoporosis (7–10). Elevated mast cell numbers have been observed in the bone marrows of patients with postmenopausal osteoporosis (11, 12) and renal osteodystrophy (13, 14). Increased mast cell numbers have also been recognized in both juvenile (15, 16) and adult (17, 18) rheumatoid synovium and, in some patients, at the sites of cartilage erosion (19). There is a positive correlation between mast cell numbers and histologic inflammatory index in rheumatoid synovium (20). In rats, local mastocytosis has been seen in areas of experimentally induced bone resorption (21) and postfracture healing (22). The precise role of the mast cell in bone and joint pathology (23, 24) has not been defined.

The present study was performed in order to further characterize the responsiveness of mast cells to PTH. The results demonstrate modulation of antigen-induced mast cell activation by the biologically active peptide fragment of PTH.

Materials and Methods

Male Sprague-Dawley rats (250–300 g) were purchased from Sasco, Omaha, NE. Care and use of laboratory animals was in accordance with the Guide for the Care and Use of Laboratory Animals, NIH Publication 85-23. Monoclonal IgE antibody reactive against dinitrophenyl bovine serum albumin (DNP-BSA) was produced by TIB-141, clone no. IGELL-C4, American Type Culture Collection and was generously supplied by Drs. Gary Wood and Joan Hunt of the Department of Pathology and Oncology, University of Kansas Medical Center, Kansas City, KS. DNP-BSA was prepared as described (25). ^{14}C-labeled 5-hydroxytryptamine (5-HT), 57 mCi/mmol, was obtained from ICN Radiochemicals, Irvine, CA. Metrizamide (Grade I), porcine intestinal heparin, compound 48/80, calcium ionophore A23187, and deoxyribonuclease (type I) were purchased from Sigma Chemical Co., St. Louis, MO. Synthetic N-terminal 1-34bPTH (1-34bPTH) with a specific activity of approximately 7 units/μg was purchased from Bachem, Torrance, CA. Purified native intact 1-84 bovine PTH (1-84bPTH) with a specific

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activity of approximately 5 units/μg was obtained from Drs. Robert Jilka and James Hamilton of the Calcium Research Laboratory, Veterans Administration Medical Center, Kansas City, MO.

Mast Cell Collection and Preparation. Rat serosal cells were collected by lavage of the peritoneal and thoracic cavities of each rat with 15 ml of Tyrode's buffer (26) containing 0.1% gelatin and 50 μg of heparin/ml (27). The cells were sedimented at 400*g* for 15 min, pooled, washed twice with Tyrode's buffer (pH 7.5) containing 0.1% gelatin and 30 μg of deoxyribonuclease (Tyrode's gelatin deoxyribonuclease buffer, TGD), and resuspended in 1 ml of TGD. An aliquot of the cell suspension was diluted 1/1 with 0.1% toluidine blue in 50% ethanol adjusted to pH 3.0 (28), and the cells were quantitated by counting on a standard hemacytometer. The cell suspension was diluted to contain 15×10^6 mast cells/ml and then labeled with 0.1 μCi of ^{14}C-labeled 5-HT per 10^6 mast cells in a 37°C shaking water bath for 60 min (29). For those experiments requiring passively sensitized mast cells, the cell suspension was preincubated with monoclonal IgE reactive against DNP-BSA at 37°C for 30 min before the addition of ^{14}C-labeled 5-HT. Two-milliliter volumes of cell suspension containing approximately 4×10^6 mast cells were layered over 4-ml cushions of 22.5% metrizamide (density 1.125 g/ml) and centrifuged at 200*g* for 15 min (27). Mast cell pellets were combined, washed three times, and resuspended to the desired concentration in TGD. Isolated in this manner, mast cell suspensions were 90–95% pure by toluidine blue staining with 99–100% viability by trypan blue exclusion.

Incubation with PTH, Antigen Challenge, and 5-HT Release. All experiments were performed in triplicate in a final reaction volume of 0.5 ml. Aliquots (0.3 ml) of a cell suspension containing 1×10^5 sensitized mast cells were preincubated with either 0.1 ml of 1-34bPTH dilution in TGD or 0.1 ml of TGD in a 37°C water bath with gentle shaking for periods ranging from 0 to 60 min. At the end of the preincubation time, either 0.1 ml of TGD containing 100 ng of DNP-BSA or 0.1 ml of TGD was added and the incubation continued for 30 min. In this manner, each PTH concentration or exposure time tested included triplicate controls for any direct effect of the PTH on unchallenged mast cells.

For experiments specifically examining the direct effect of 1-34bPTH on mast cells, passively sensitized or unsensitized mast cells were incubated with various concentrations of 1-34bPTH for varying time periods without antigen challenge. Each experiment included a control for mast cell activation, either DNP-BSA (200 ng/ml), compound 48/80 (2 μg/ml), or calcium ionophore A23187 (1 μg/ml).

Reactions were terminated by chilling on ice followed by centrifugation at 400*g* for 10 min. Supernatants were collected and the released ^{14}C-labeled 5-HT was quantitated by liquid scintillation spectrometry. Release of ^{14}C-labeled 5-HT was expressed as the percentage of total cellular content.

Statistical Methods. Statistical analysis was carried out by using Student's *t* test, and *P* values of less than 0.05 were considered significant. Correlation coefficients were calculated by linear regression analysis.

Results

PTH Modulation of Mast Cell Function. Passively sensitized mast cells were preincubated for 1 min with synthetic 1-34bPTH at concentrations ranging from 0.5 to 2000 n*M* before challenge with DNP-BSA. Mean data from three separate experiments are shown in Figure 1. Incubation with 1-34bPTH before antigen challenge enhanced antigen-induced release of 5-HT in a dose-dependent manner. The correlation coefficient of the best-fitting curve obtained by linear regression analysis of the mean data was 0.96. Combining data from three separate experiments, mean enhancement of 5-HT release was significant with 1-34bPTH concen-

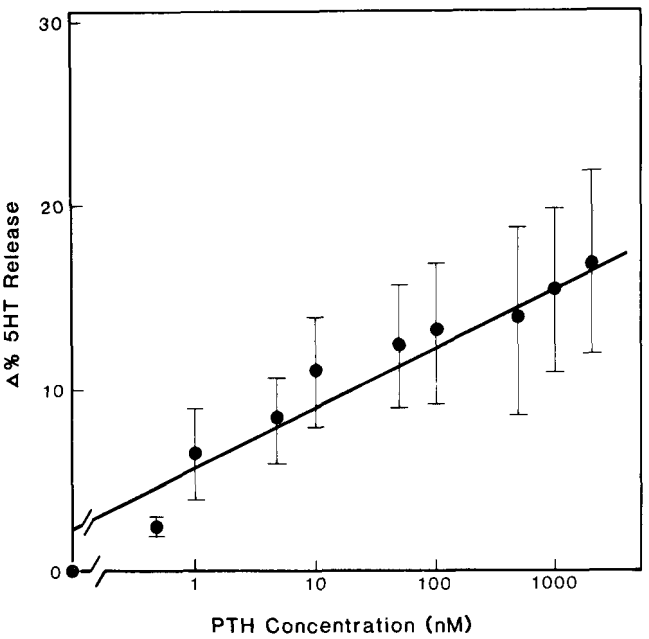

Figure 1. Enhancement by 1-34bPTH of antigen-induced 5-HT release from mast cells. IgE-sensitized mast cells were preincubated with 0.5–2000 n*M* 1-34bPTH or TGD buffer control for 1 min before challenge with DNP-BSA. For each PTH concentration, Δ% 5-HT release is the net percentage of 5-HT release from PTH-treated cells minus the net percentage of 5-HT release from non-PTH-treated cells. Controls for percentage of spontaneous 5-HT release from unchallenged mast cells (less than 8% in all experiments) and for any additional direct effect of each PTH concentration on unchallenged mast cells (range, 0–1.35% net 5-HT release) were subtracted to yield the net percentage of 5-HT release from antigen-challenged mast cells. Each data point is the mean ± SEM of three experiments, each performed in triplicate. Antigen-induced net 5-HT release from non-PTH-treated controls ranged from 11 to 29% in these experiments.

trations of 5 nM (.165 units/ml) and greater ($P < 0.05$). Within each of the three individual experiments, enhancement was significant with 1-34bPTH concentrations of 1 nM (0.33 units/ml) and greater ($P < 0.01$). Correlation coefficients for the three individual experiments were 0.96, 0.96, and 0.82. The mean intraexperiment coefficients of variation of triplicate determinations for the three experiments were 0.02, 0.03, and 0.05.

Effect of Duration of PTH Exposure on Mast Cell Response to Antigen Challenge. Passively sensitized mast cells were preincubated with either 100 nM 1-34bPTH or TGD buffer for varying periods of time before challenge with DNP-BSA (Fig. 2). PTH enhancement of antigen-induced mast cell activation was maximal with a 1-min prechallenge incubation, and this enhancement was significant ($P < 0.01$) compared to the non-PTH-treated buffer control. Complete loss of the PTH effect was observed by 30 min.

Direct Effect of PTH on Mast Cell Mediator Release. At the concentrations and incubation times tested in the above experiments, 1-34bPTH directly caused only up to 1.4% net 5-HT release from mast cells in the absence of antigen challenge. Incubation of mast cells with 1000 nM 1-34bPTH for as long as 90 min did not cause any further increase in direct 5-HT release (Fig. 3). In Figure 3, data were combined from eight experiments using either sensitized or unsensitized isolated serosal mast cells incubated with 1000 nM 1-34bPTH for time periods ranging from 15 to 90 min with no antigen challenge. The mean net percentage of 5-HT release, after subtracting non-PTH-treated controls, was less than 1% at all time intervals tested. In experiments (data not shown) using unfractionated rat serosal cells containing 10% mast cells incubated for 30 min with 1-34bPTH at concentrations up to 1000 nM, net 5-HT release ranged from 0.5 to 2.9%. Similarly, experiments using native intact 1-84bPTH at concentrations up to 1000 nM and exposures up to 40 min resulted in a maximum of 3% net 5-HT release from isolated, unchallenged mast cells (data not shown). In all of these experiments, mast cell activation using either DNP-BSA, compound 48/80, or calcium ionophore A23187 ranged from 11 to 57% net 5-HT release (mean 28.5%).

Discussion

The synthetic, biologically active, N-terminal fragment of bovine PTH (1-34bPTH) significantly en-

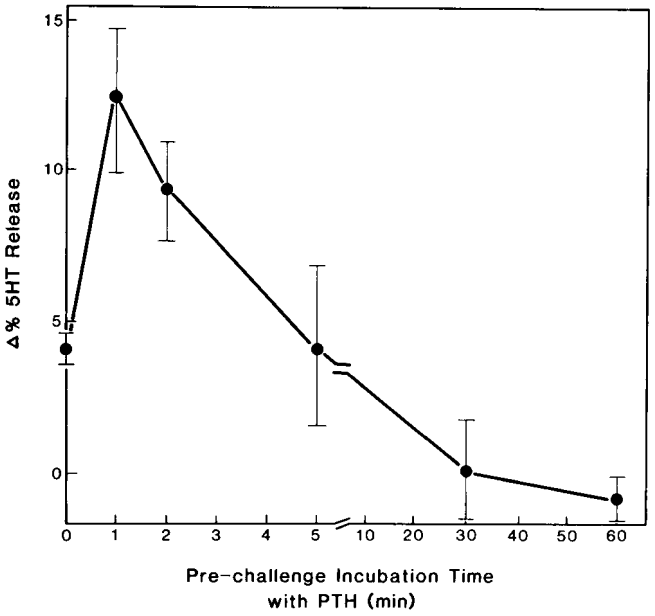

Figure 2. Effect of prechallenge incubation time with 1-34bPTH on potentiation of antigen-induced 5-HT release from mast cells. IgE-sensitized mast cells were incubated with 100 nM 1-34bPTH or TGD buffer control for 0 to 60 min before DNP-BSA challenge. Zero minutes represents the simultaneous addition of PTH or TGD and DNP-BSA. For each prechallenge incubation time, Δ% 5-HT release is the net percentage of 5-HT release from PTH-treated cells minus the net percentage of 5-HT release from non-PTH-treated control cells. Net percentage of 5-HT release was obtained by subtracting the percentage of spontaneous 5-HT release from unchallenged mast cells for each incubation time (range, 5–12%). At these incubation times, PTH induced less than 0.7% net 5-HT release from unchallenged mast cells. Each data point is the mean ± SD of triplicate samples within a representative experiment. Mean net antigen-induced 5-HT release from non-PTH-treated cells at all incubation times was 31.4%.

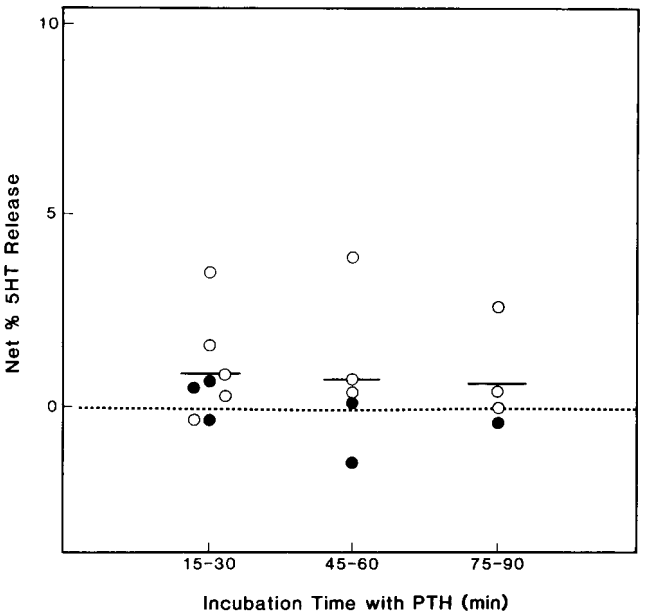

Figure 3. Direct effect of 1000 nM 1-34bPTH on 5-HT release from unchallenged isolated mast cells. Unsensitized (●) or IgE-sensitized (○) mast cells were incubated with 1-34bPTH for time periods ranging from 15 to 90 min without antigen challenge. Data from eight separate experiments yielding 17 data points are grouped into three incubation time ranges. Each data point is the mean of triplicate samples. Net percentage of 5-HT release is the percentage of 5-HT release from PTH-treated cells minus the spontaneous percentage of 5-HT release from non-PTH-treated cells. For each time range, the mean net percentage of 5-HT release ± SEM is as follows: 0.86 ± 0.44 for 15 to 30 min ($n = 8$); 0.77 ± 0.87 for 45 to 60 min ($n = 5$); 0.70 ± 0.68 for 75 to 90 min ($n = 4$). In these eight experiments, mean net percentage of 5-HT release with DNP-BSA, compound 48/80, or calcium ionophore A23187 was 28.9%.

hanced IgE-mediated 5-HT release from isolated rat serosal mast cells in a dose-dependent manner. Enhancement occurred at PTH concentrations considerably lower than those (10 n*M*) used for *in vitro* rodent bone resorption assays (30) and much lower than those (0.5 to 100 units/ml) which have been associated with biologically important effects in other *in vitro* animal and human cell systems (31–34). The PTH effect was rapid and transient in nature. Enhancement was maximal when mast cells were preincubated with the 1-34bPTH for 1 min before antigen challenge and was not evident with prechallenge incubation times of 30 min or more.

The concentrations of 1-34bPTH which enhanced antigen-induced 5-HT release induced less than 1.5% net 5-HT release directly from unchallenged isolated serosal mast cells. This was true for either passively sensitized or unsensitized mast cells with incubation times up to 90 min using 1000 n*M* 1-34bPTH. By using unfractionated serosal cell populations, maximum net 5-HT release was 2.9% with 1-34bPTH concentrations up to 1000 n*M*. At higher concentrations, synthetic N-terminal PTH has been shown to directly induce mediator release from unchallenged rat serosal mast cells (1, 2). Since phosphatidylserine was present in the incubation mixture used by Wilhelms *et al.* (2), the present data cannot be compared. Our results using 1000 n*M* 1-34bPTH (33 units/ml) are similar to those observed by Tsakalos *et al.* (1) at the lower end of their concentration curve in which 25 units/ml of synthetic 1-34 human PTH directly induced 2 to 3% net mediator release from mixed or isolated rat peritoneal mast cells. We observed up to 3% net 5-HT release with native intact 1-84bPTH up to 1000 n*M* (50 units/ml) using isolated serosal mast cells. This is similar to the 10% net mediator release observed by Tsakalos *et al.* (1) using native 1-84bPTH at 50 units/ml and a mixed peritoneal cell population.

Several limitations to interpretation of the present observation must be considered. Rat serosal mast cells are readily obtainable in the numbers required for *in vitro* studies; however, the results obtained cannot necessarily be extrapolated to the mast cells of other tissues or to human mast cells. PTH modulation of newly generated mast cell mediator release was not evaluated. The biochemical mechanism for the observed PTH effect cannot be determined on the basis of the present experiments. Furthermore, PTH potentiation of antigen-induced mast cell activation *in vitro* does not necessarily imply that the same effect occurs *in vivo* or that it is of physiologic significance. In view of the transient nature of the observed PTH effect, one would have to postulate fluctuations in the level of biologically active and available PTH within the immediate environment of the mast cell *in vivo*. Whether or not such microenvironmental fluctuations occur *in vivo* has not been reported. Nevertheless, wide fluctuations in serum PTH levels with time (35) and diurnal variations (36) in humans have been observed.

The present studies extend previous observations of PTH-mast cell interaction to include potentiation by 1-34bPTH of antigen-induced rat serosal mast cell preformed mediator release. Direct stimulation of mediator release from the mast cells by 1-34bPTH was not observed at the concentrations tested. Although the results suggest a potential role for PTH in the modulation of mast cell activation, the pathophysiologic significance of such an interaction *in vivo* is speculative.

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