Biochemical Pharmacology, Vol. 32, No. 2, pp. 355–360, 1983.
Printed in Great Britain.

0006-2952/83/020355-06 $03.00/0
Pergamon Press Ltd.

INDUCTION OF MAST CELL SECRETION BY PARATHORMONE*

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(*Received* 3 *February* 1982; *accepted* 23 *June* 1982)

Abstract—The biologically active fragment of human parathormone (PTH) and intact bovine PTH were found to induce secretion of both serotonin and histamine from rat peritoneal mast cells *in vitro*. Release of serotonin and histamine was demonstrated with 25 units/ml PTH or higher. This level is within the higher limits of the elevated PTH levels found in advanced uremia. Mast cell secretion by PTH was dose, time and energy dependent and was not cytotoxic. Although mast cell activation was independent of extracellular calcium, it required intracellular calcium, thus resembling the action of certain other peptide secretagogues. Intradermal injection of PTH induced immediate increases in vascular permeability suggesting that PTH could induce mast cell secretion *in vivo*. Light and electron microscopic observations confirmed mast cell degranulation by exocytosis. These results demonstrate that elevated levels of PTH can induce mast cell secretion *in vitro* and *in vivo* and suggest a possible role for mast cells in the pathophysiology of non-allergic disease states.

Mast cells are ubiquitous in the body but, aside from their involvement in immune responses such as immediate and delayed hypersensitivity [1, 2], their role in normal physiology remains speculative [3, 4]. The recent demonstration that certain biologically important peptides are secreted [5], or can induce secretion [6], from mast cells has prompted speculations that mast cells may be involved in cellular interactions that are regulated by peptide hormones [7]. We report here that parathormone (PTH) can induce release of serotonin and histamine from mast cells and that this secretion is dependent on calcium and metabolic energy. PTH-induced secretion by mast cells may be involved in the pathophysiology of some conditions involving PTH, such as chronic renal failure where elevations in blood levels of PTH [8–10] and mast cell proliferation have been noted [11, 12].

METHODS AND RESULTS

The secretory granules of rat peritoneal mast cells were labeled with [^{3}H]serotonin by preincubation for 1 hr at 37°. After washing, the cells were incubated for 30 min at 37° with various doses of the biologically active fragment of human PTH (hPTH 1-34) or intact bovine PTH (bPTH 1-84). Both [^{3}H]serotonin and endogenous histamine were secreted. Secretion of about 5% serotonin at 25 units/ml (about 5 μg/ml) and of about 15% at 100 units/ml PTH was accompanied by about 3 and 12% histamine respectively (Fig. 1a). Similar results were obtained with mast cells enriched to over 90% purity [13] with about 10% of both serotonin and histamine having been released at 100 units/ml PTH (Fig. 1b). This secretion was time dependent, became maximal at 30 min, and was non-cytotoxic since cell viability was consistently higher than 95% (Fig. 1c). Fragments of hPTH without parathormone biologic activity (hPTH 44-68 and hPTH 53-84) were without any effect on mast cell secretion (Fig. 1d).

The potential *in vivo* secretion of mast cells in response to PTH was also investigated. Intradermal injection of PTH into the skin of guinea pigs that were injected intravenously a few minutes earlier with 1% Evans blue dye resulted in immediate extravasation of dye. This result indicated an immediate increase in local vascular permeability consistent with secretion of vasoactive mediators by local mast cells (Fig. 2). Light and electron microscopy at skin sites of rats that received intradermal injections of PTH showed degranulation of mast cells (Fig. 3).

To further characterize the secretory response to PTH, mast cells either were placed in a calcium-free medium or were deprived of calcium ions by chelation with 2×10^{-3} M EDTA for 2 hr at 37°. In either instance, the secretory response to PTH persisted (Table 1). However, marked inhibition of the secretory response was produced (Table 1) if the cells were depleted of *intracellular* calcium. This was accomplished by preincubation with small amounts of the divalent cation ionophore A23187 (0.1 μg/ml) in the presence of 0.1 mM EDTA, followed by washing and resuspension in calcium-free Locke's solution. This technique has been shown previously to effectively deplete mast cells of intracellular calcium

* Supported in part by grants from the United States Public Health Service, National Institutes of Health, AI-12211, AI-11077, and AI-10497.

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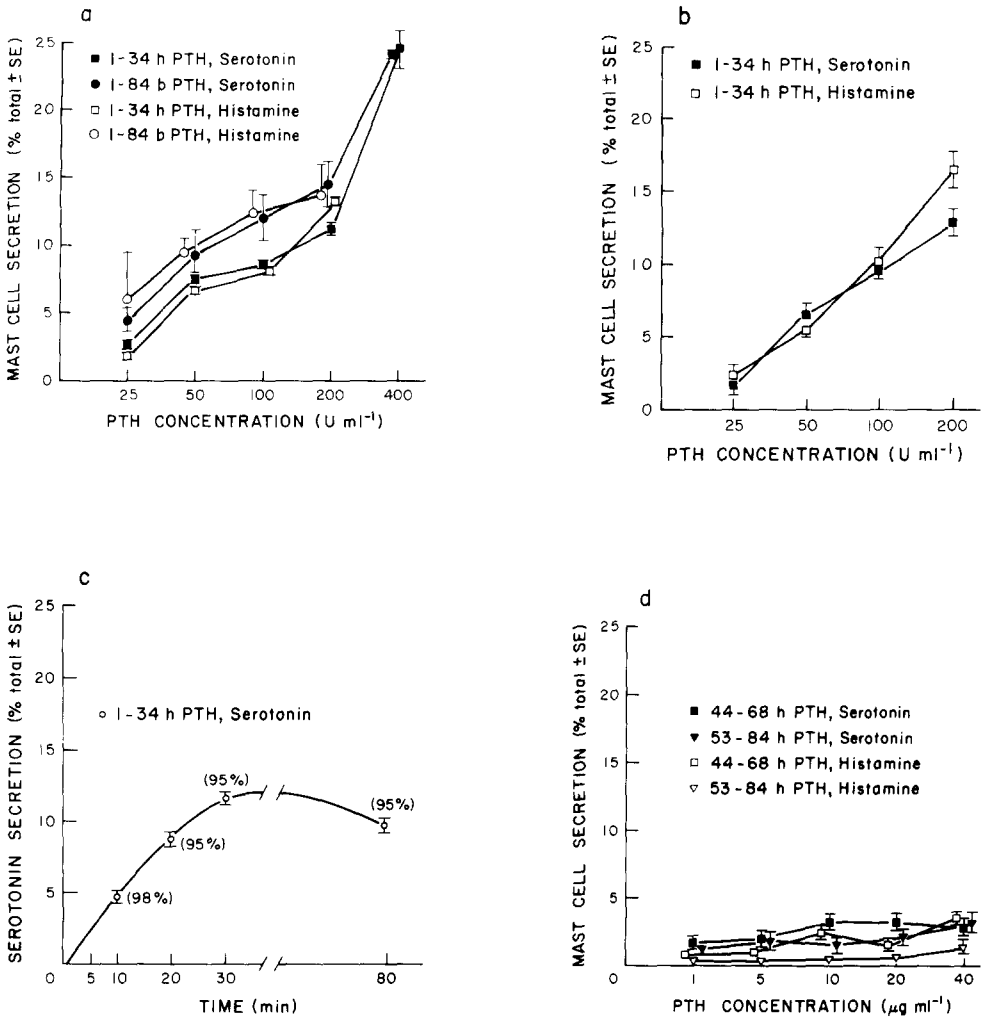
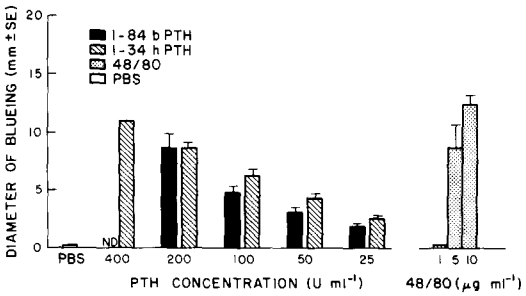


Fig. 1. Effect of PTH on mast cell secretion. Peritoneal cell suspensions containing mast cells from Sprague–Dawley male rats (400 g) were collected and resuspended in Locke's solution buffered with 5 mM 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (Hepes), pH 7.0 [6]. Peritoneal cells were preinicubated with [^{3}H]serotonin (10 μCi/10^6 cells) for 1 hr, were then washed by centrifugation at 180 *g* for 5 min at 24°, resuspended in 5 ml of Locke's solution, followed by centrifugation again, and, finally, resuspended in Locke's solution. Mast cell secretion was quantitated by scintillation counting of [^{3}H]serotonin, expressed as the amount measured in the supernatant fraction divided by that present in the supernatant fraction and pellet combined. Release of endogenous histamine was also measured using the *o*-phthalaldehyde fluorometric method [14], and the results are expressed in the same way as serotonin. Some slight variability in the ability to induce mast cell secretion was noticed with hPTH 1-34 preparations from different suppliers, the Beckman material showing somewhat more activity than that of Armour; storage and shipping conditions may account for this effect. Mast cell secretion was identical using hPTH 1-34 diluted in Locke's solution containing 0.1% bovine serum albumin (BSA), 1.0% BSA, or 1.0% gelatin. Panel a: Mast cell-containing suspensions (0.2 ml) were incubated for 30 min with various concentrations of biologically active PTH: human synthetic PTH 1-34 (hPTH 1-34, Beckman, Palo Alto, CA, or Armour/Revlon, Tuckahoe, NY), or native bovine PTH (bPTH 1-84, a gift of Dr. James W. Hamilton, Kansas City Veterans Hospital, Kansas City, MO). The results indicate the net mean secretion ± S.E. of five experiments in the case of serotonin and two in the case of histamine performed in duplicate. Net secretion is reported throughout and represents evoked minus basal secretion. Panel b: Mast cells were purified (better than 90%) over metrizamide as previously described [13] and were then incubated with various concentrations of hPTH 1-34 as shown. The results represent the net means ± S.E. of two experiments performed in duplicate. Panel c: Mast cells were added to tubes containing hPTH 1-34 (100 units/ml Beckman) and were incubated for various periods of time as shown, with numbers in parentheses indicating percent viability at each time point as measured with Trypan blue dye exclusion. The results represent the net means ± S.E. of three experiments performed in duplicate. Panel d: Mast cells were incubated for 30 min at 37° with various concentrations of biologically inactive hPTH 44-68 and hPTH 53-84 (Bachem, Torrance, CA) as indicated. The results represent the net means ± S.E. of five experiments (performed in duplicate) for serotonin and three for histamine.



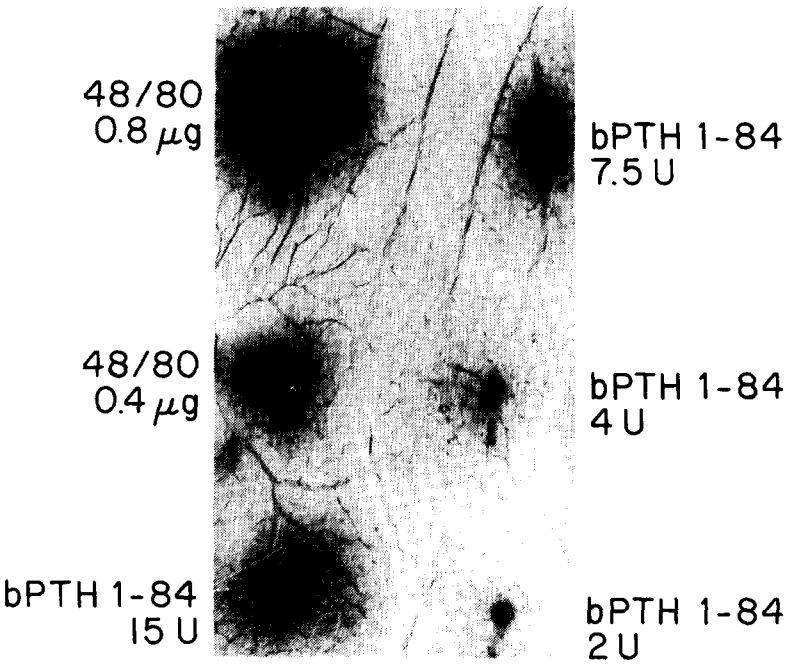


Fig. 2. Immediate increase in vascular permeability induced in skin by intradermal injection of PTH. In two separate experiments, two to three male guinea pigs (250 g) were shaved and, following intravenous injection of 1% Evans blue dye, various flank skin sites were injected intradermally with 75 μl of Locke's solution that contained various concentrations of bPTH 1-84, hPTH 1-34, the classic mast cell secretagogue compound 48/80, hPTH 44-68, and hPTH 53-84. Twenty minutes later the animals were killed, skin was reflected, and the diameter of dye extravasation was measured in two perpendicular directions. Panel a: A bar graph of the mean diameter ± S.E. of blueing induced by various doses of PTH, compound 48/80 or phosphate-buffered saline (PBS) (hPTH 44-68 and hPTH 53-84 did not induce blueing, data not shown). Panel b: A photograph showing the extravasation of dye in the underside of the flank skin of a guinea pig 20 min after challenge with the indicated agents.

ion stores without an effect on metabolic requirements [6, 18]. Deprivation of mast cell energy stores by pretreatment with antimycin A (0.2 μM) and 2-deoxyglucose (5×10^{-4} M) for 30 min resulted in complete inhibition of mediator release induced by 100 units/ml PTH (Table 1). Mast cell secretion induced by PTH was, therefore, dependent on intracellular calcium stores and metabolic energy and, thus, was similar to that induced by certain other mast cell secretagogues, such as compound 48/80 (Table 1).

Preincubation of mast cells for 30 min with suboptimal concentrations of PTH (10 units/ml) had no effect on secretion by mast cells preincubated with a 1:40,000 dilution of anti-2,4-dinitrophenol IgE hybridoma antibody [19] followed, after washing, by addition of 1:40 rabbit anti-mouse IgE [19]. Furthermore, incubation with calcitonin, another peptide hormone, had no effect on mast cell secretion over a wide range of concentrations (results not shown). It, therefore, appeared that the effect of PTH was neither mediated non-specifically through an effect on IgE-dependent secretion, nor was it a general non-specific peptide effect. Phosphatidyl

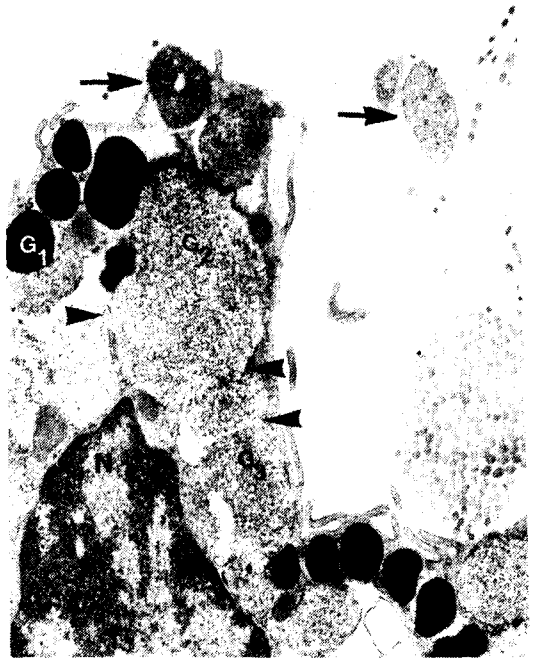


Fig. 3. *In vivo* degranulation of mast cells by PTH. Pairs of rats were injected intradermally with 75 μl of Locke's solution either with or without hPTH 1-34 (100 units/ml, 7.5 units/site). Twenty minutes later the sites of injection were removed into Karnofsky's fixative [15] and processed into 1 μm Spurr-embedded [16], stained [17] sections. Mast cells in multiple sections from each site were evaluated for degranulation as judged by variability in the staining of cytoplasmic granules and vacuolization of the cytoplasm. Examination of between 50 and 100 mast cells per animal showed that PTH induced degranulation in 28% of mast cells as compared to only 3% degranulation in control sites. The figure shows an electron micrograph of a dermal mast cell (N, nucleus) typical of those found at sites of PTH injection. There was degranulation by compound exocytosis. Some normally dense cytoplasmic granules (G_1) were swollen (G_2 and G_3) leading to alteration of the granule matrix and fusion of granule membranes with each other and with the cell membrane, causing swollen granules to lie within common channels in the cytoplasm (arrow heads) and to be extruded from the cell (arrows).

Table 1. Effect of energy and calcium deprivation on PTH-induced secretion of [^{3}H]serotonin by mast cells

	Serotonin release (% total ± S.E.)				
			Calcium deprivation procedure		
Stimulus added (final conc)	Normal Locke's	No energy	Ca^{2+}-free Locke's	Ca^{2+}-free Locke's + 2 mM EDTA	Ca^{2+}-free Locke's + 0.1 μg/ml A23187 + 1 mM EDTA
hPTH 1-34 (100 units/ml)	8.4 ± 0.4	0.6 ± 0.1	8.9 ± 3.5	8.6 ± 0.2	0.7 ± 0.3
bPTH 1-84 (100 units/ml)	12.1 ± 6.1	1.3 ± 0.6	ND†	12.1 ± 0.9	0.8 ± 0.7
Compound 48/80 (1 μg/ml)	58.2 ± 4.6	0.7 ± 0.5	30.5 ± 0.8	22.1 ± 1.0	3.2 ± 1.8

* The reported values represent the net mean secretions ± S.E.
† Not determined.

serine was required to demonstrate IgE-dependent secretion but was not necessary for PTH-induced secretion.

DISCUSSION

We have shown that the complete bovine PTH molecule (bPTH 1-84), and the synthetic fragment of human PTH (hPTH 1-34) that is biologically active [20], can induce mast cell secretion. A similar *in vitro* action of hPTH 1-34 was recently described independently and was shown not to require any extracellular calcium [21]. Our experiments, however, demonstrate, that this action of PTH is dependent on *intracellular* calcium and thus resembles the action of another peptide secretagogue, the tetradecapeptide somatostatin [6, 18, 22].

Although it has been shown previously that several other peptides induce mast cell secretion [23, 24], only recently has the possible mechanism of action of biologically important peptide mast cell secretagogues been investigated [25, 26]. From recent findings [18, 24–26], it appears that a high number of cationic charges on a peptide is not sufficient to induce mast cell secretion. Instead, an appropriate conformation of the molecule seems to be required. In this view, it is interesting that two PTH *fragments* (hPTH 44-68 and 53-84) were devoid of ability to induce mast cell secretion. Both hPTH 1-34 and bPTH 1-84 carry a net cationic charge of six [27]; it is, therefore, of interest that they had *comparable* secretory activity although their cationic charges per number of amino acids is about 1 per 6 and 1 per 14 respectively. Furthermore, hPTH 44-68 has a net cationic charge of 3, or 1 per 8 amino acids, but had *no* secretory activity. It, therefore, appears that the action of PTH on mast cell secretion was not due to cationic charges alone. The fact that another peptide, neurotensin, has been shown to induce mast cell secretion [28] and to bind to mast cell receptors [29, 30] suggests that PTH may also bind to some kind of a receptor on the surface of mast cells. If PTH binding to mast cell receptors occurs *in vivo*, it may actually reduce the amount of PTH available for measurements in various clinical states where PTH is involved. Furthermore, the high *in vitro* levels of PTH required in this and other mast cell studies may be partly due to alterations of a possible receptor, especially in enriched mast cell preparations [13]. In view of the similarities in mast cell secretion induced by PTH and somatostatin, it may be of interest that somatostatin has been shown to inhibit PTH receptor binding and subsequent action [31].

Blood PTH levels are elevated (1–100 units/ml) in advanced uremia [8–10], and uremic patients show tissue mast cell proliferation [11, 12], a finding reproduced experimentally in bone marrow of rats that receive injections of PTH [32]. PTH levels in uremic patients include the levels (about 30 units/ml) that we have found capable of stimulating mast cell secretion *in vitro*. These levels are within the range (10–30 units/ml) recently shown to have an inhibitory effect on erythropoiesis and thus suggested as a cause for anaemia in patients with uremia [33]. However, the immunoassayable PTH that accumulates in patients with uremia is predominantly the C-terminal fragment which does not induce mast cell secretion. Nonetheless, since uremic patients show mast cell proliferation [11, 12] and some clinical signs of possible mast cell involvement such as generalized pruritus [9, 11], and since mast cell degranulation results in a subsequent local increase in mast cell numbers [34], it is possible that some association may exist in uremia between elevated blood PTH levels, mast cell secretion (and proliferation) and elevated plasma histamine levels. In fact, our preliminary results show that histamine levels are elevated in the blood of uremic patients, but much more work is needed before this can be connected with PTH abnormalities. It is also of interest that hyperparathyroidism has been associated recently with some cases of chronic urticaria [35], which is generally due to activation of mast cells in the skin.

In summary, our results show that PTH can activate mast cells to secrete mediators both *in vitro* and *in vivo*. This finding may broaden the possible role of mast cells as it further implicates them in the pathophysiology of non-immunologic disease states.

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