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Serotonin Stimulates Adenosine 3',5'-Monophosphate Accumulation in Parathyroid Adenoma*

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ABSTRACT. The effect of serotonin on cAMP accumulation in parathyroid adenoma tissue from patients with primary hyperparathyroidism was studied *in vitro*. Incubation with 10^{-5} M serotonin elicited a marked increase (of 90–150%) in cAMP content in slices of parathyroid adenoma tissue. This stimulatory effect of serotonin was already apparent after 2 min of incubation; stimulation by serotonin was dose dependent, with the highest stimulation being achieved at 10^{-4} M serotonin. The serotonin antagonists, methylsergide and cinanserin, in concentrations equimolar to serotonin completely blocked the stimulatory effect of serotonin on cAMP increase. The serotonin content in surgically removed parathyroid adenoma tissue, as determined by fluorometric assay, was 6.4 ± 1.2 pmol/mg wet wt ($\sim 0.8 \times 10^{-5}$ M). The present observations demonstrate that parathyroid adenoma tissue has a high content of serotonin, and serotonin stimulates cAMP accumulation in this tissue. Since cAMP acts as a mediator of parathyroid hormone (PTH) release, our results suggest that serotonin could be one of the factors regulating PTH secretion and/or contributing to PTH hypersecretion in various forms of primary hyperparathyroidism. (*J Clin Endocrinol Metab* **51**: 1274, 1980)

SEROTONIN-producing neoplasms have been found in association with hyperparathyroidism in some patients with multiple endocrine neoplasia types 1 (1–11) and 2a (12). While it is not known whether there is any causal association between elevated blood serotonin levels in these patients (13, 14) and hypersecretion of parathyroid hormone (PTH), many observations *in vivo* and *in vitro* provide strong evidence that a variety of other biogenic amines, such as β-adrenergic agonists (15, 16), dopamine (17), and histamine (18), are potent stimuli of PTH secretion by parathyroid tissue. This effect of biogenic amines as well as of other stimuli of PTH secretion (15, 19, 20) appears to be mediated by increases in cAMP generation and accumulation of this cyclic nucleotide in parathyroid tissue (15, 19–21). That increased cAMP generation in this setting is due to activation of adenylate cyclase is supported by the observation that human parathyroid tissue contains adenylate cyclase which can be stimulated by β-adrenergic agonists (20, 22).

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Like various other biogenic amines, serotonin has been shown in several cell types and organ systems to stimulate cAMP formation (23, 24) and/or to activate adenylate cyclase (25, 26). In view of these considerations, we addressed ourselves to the question of whether serotonin can stimulate cAMP formation and accumulation in human hyperparathyroid tissue and, by extension, whether it may potentially serve as a PTH secretagogue. To further explore the possibility of a pathogenic role for serotonin in hyperparathyroidism, we also measured serotonin levels in parathyroid adenoma tissue. The results demonstrate that serotonin is a potent agonist of cAMP formation in parathyroid adenoma tissue and that this tissue itself contains a considerable amount of serotonin.

Materials and Methods

Tissue was obtained from parathyroid adenomata of patients who underwent partial parathyroidectomy for primary hyperparathyroidism diagnosed by clinical criteria. The average age of the patients (four males and two females) was approximately 40 yr. After surgical removal of the parathyroid tissue, part of the tissue specimen was immediately chilled in ice-cold 0.9% sodium chloride solution. All subsequent preparatory steps were performed at 0–2 C. After the tissue was trimmed of adipose and connective tissue, a portion was taken for histological examination. In some patients, another part of the tissue block was removed, immediately frozen on dry ice, and stored for determination of serotonin concentration. The rest of the

tissue was quickly transferred in cold buffer to the laboratory, and slices were prepared with a Stadie-Riggs tissue slicer. Slices of parathyroid tissue were extensively washed in ice-cold modified Krebs-Ringer solution, and the effect of serotonin on cAMP accumulation was determined using basically the design described in our previous studies of serotonin's effect on cAMP accumulation in renal tissue (24). Slices were divided into individual flasks (~5–15 mg wet wt/incubation) and incubated in 350 μl modified Krebs-Ringer buffer (KRB) (24) at 37 C for 20 min. After the preincubation period, 50 liters of 5 mM 1-methyl-3-isobutyl xanthine and of other agents dissolved in the same buffer (KRB) were added within 6–10 sec up to a final volume of 0.5 ml. After the addition of these agents, slices were incubated for an additional 5 min, unless specified otherwise in *Results*. The incubation was stopped by the addition of 0.5 ml 10% trichloroacetic acid (TCA) and homogenization of the tissue. Aliquots of the TCA homogenate were taken for determination of protein. After the addition of trace amounts of [^{3}H]cAMP to monitor recovery of the nucleotide (27), precipitated protein was removed by centrifugation, and TCA from the supernatant was removed by subsequent extraction with ethyl ether. cAMP content in the extract was determined by RIA after acetylation with acetic anhydride and triethanol amine using the procedure described in full detail in our previous studies (24, 27). Validation of the RIA used was described in detail elsewhere (27); neither serotonin nor its antagonists interferes with cAMP determination (24).

The effects of serotonin and other agents were tested in each experiment in such a way that control and experimental tissue slices from the same block of tissue were simultaneously incubated with and without added agents. The protein and cAMP contents were determined in experimental and control homogenates (with and without drugs, respectively) at the same time using the same standards. The content of cAMP in the incubation mixture was expressed in relation to tissue protein in each incubation as picomoles of cAMP per mg tissue protein.

With use of the present experimental design, cAMP which accumulated in the tissue and that which escaped into the medium were determined together. The determination of tissue protein, RIA for cAMP, and sources of agents and biochemicals were described in detail in our previous communications (24, 27, 28).

For determination of tissue levels of serotonin, the frozen tissue was homogenized and extracted as described by Snyder *et al.* (29), except that tissues were homogenized in 4 ml ice-cold 0.1 M HCl, as employed by Bogdanski *et al.* (30), rather than in 8 ml 0.4 N perchloric acid. Serotonin content was determined by fluorometric assay and expressed as picomoles of serotonin per mg tissue. To ensure validity of measurements, in preliminary experiments, serotonin content was measured with this assay in several rat tissues (intestine, spleen, and liver). The serotonin content found in these preliminary experiments agreed with values of rat tissue serotonin reported from other laboratories (31).

Serotonin (5-hydroxytryptamine creatine sulfate) was purchased from Sigma Chemical Co. (St. Louis, MO); 1-methyl-3-isobutyl-xanthine was obtained from Aldrich Chemical Co. (Milwaukee, WI). Methysergide maleate was a gift from Sandoz Pharmaceuticals (Hanover, NJ), and cinanserine hydrochloride was a gift from Squibb and Sons, Inc. (Princeton, NJ). All agents were dissolved before use in Krebs-Ringer phosphate buffer.

Results

Serotonin (10^{-5} M) consistently caused a marked increase in the cAMP level in incubated parathyroid adenoma tissue of examined patients (Table 1). The cAMP increase was dependent on the dose of serotonin; the highest stimulation was observed at a concentration of 10^{-4} M (Fig. 1). Serotonin caused a significant cAMP increase as early as 2 min after addition, and the effect persisted up to 30 min (Table 2). To ascertain that serotonin's action was specific for this biogenic amine, the effect of antiserotonin drugs was tested. Although methylsergide and cinanserin (10^{-5} M) alone had no effect on cAMP levels in parathyroid tissue (data not shown), these drugs completely blocked the stimulatory action of serotonin on cAMP levels (Table 3).

The average serotonin content (mean ± SE; n = 6) determined in the frozen tissue samples was 6.4 ± 1.2 pmol/mg tissue. Assuming that parathyroid adenoma tissue contains about 70–80% water, as tissues do in general (32), the actual concentration of serotonin in parathyroid tissue fluid was about 0.8×10^{-5} M. This concentration of serotonin is comparable to the serotonin content of human ileum, lower than that in human spleen, and higher than that in most central nervous system structures (except the hypothalamus and pineal gland), as determined by the same method (31).

Discussion

In the present study, serotonin was found to elicit marked and dose-dependent stimulation of cAMP accumulation in parathyroid adenoma tissue. Inhibition of this stimulatory effect by specific serotonin antagonists

TABLE 1. Effect of serotonin on cAMP levels in parathyroid tissue from hyperparathyroid patients

Case no.	cAMP (pmol/mg protein)		
	Basal (no additions)	Serotonin (10^{-5} M)	Δ%[a]
1	27.2 ± 3.4[b]	67.7 ± 4.9	+149
2	12.0 ± 0.8	28.8 ± 2.2	+140
3	20.8 ± 2.0	39.8 ± 10.3	+91
4	18.6 ± 1.0	35.6 ± 4.7	+94
5	25.8 ± 3.4	61.2 ± 11.0	+137
Mean ± SE	20.9 ± 2.7	46.6 ± 7.6[c]	122.2 ± 12[c]

[a] Percent increase from basal.

[b] Each value denotes the mean ± SE of triplicates.

[c] Significantly different from basal and/or Δ% is significant ($P < 0.005$, by paired t test).

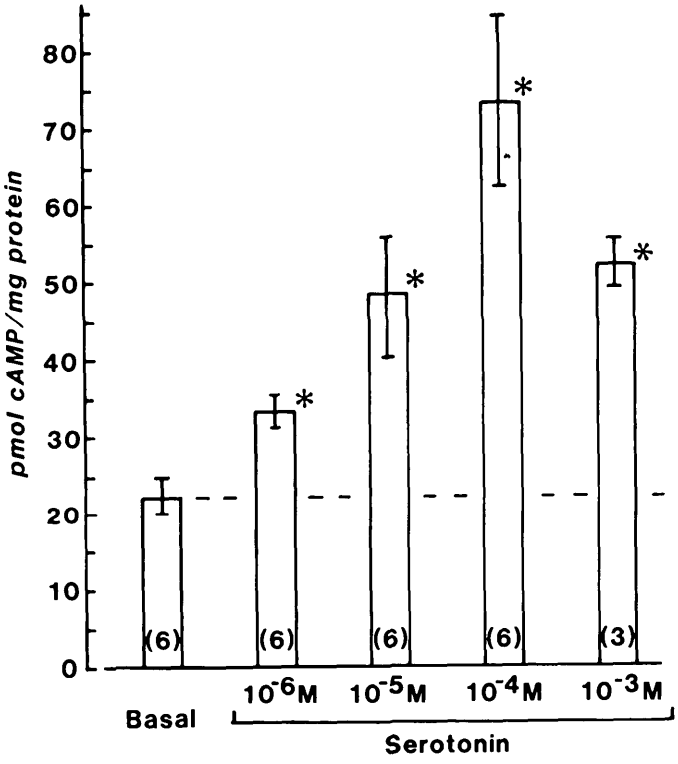

FIG. 1. Effect of various concentrations of serotonin on cAMP accumulation in parathyroid adenoma tissue. For experimental details, see *Materials and Methods*. Each *bar* denotes the mean ± SE of several incubations; the number of incubations (n) is indicated at the bottom of the bars. *, Values significantly ($P < 0.05$ or higher level of significance by *t* test) higher than basal value (no serotonin added).

TABLE 2. Effect of serotonin on parathyroid tissue levels of cAMP at different time intervals. (For details see Methods)

Time	cAMP (pmol/mg protein)			Δ%[a]	*P*[b]
	Basal (no addition)	n	Serotonin (10^{-5} M)		
2 (5)[c]	13.7 ± 1.4[d]	(5)	26.9 ± 4.1	+96	<0.01
5 (5)	17.1 ± 3.4	(5)	44.4 ± 9.7	+126	<0.05
15 (5)	22.6 ± 5.3	(5)	60.0 ± 14.0	+131	<0.05
30 (5)	20.3 ± 4.3	(5)	44.1 ± 3.8	+137	<0.05

[a] Percent increase from basal.

[b] For significance of difference of serotonin-stimulated values from the corresponding basal values during the same time interval (by *t* test).

[c] The number of samples (incubations) is indicated in parentheses. For details see *Materials and Methods*.

[d] Mean ± SE; picomoles of cAMP per mg protein.

indicates that serotonin most likely increased cAMP levels by acting on serotonin receptors in a manner analogous to serotonin's actions on some other tissues (23-26). Since cAMP appears to be an intracellular mediator of PTH secretion (15, 19, 20), and since physiological and pharmacological stimuli which increase cAMP accumulation in parathyroid tissue invariably stimulate PTH secretion (15, 19, 20), it is reasonable to expect that

TABLE 3. Effect of methylsergide and cinanserin on stimulatory action of serotonin in parathyroid tissue

Condition	n	cAMP (pmol/mg protein)
Basal (no additions)	6	16.4 ± 2.2[a]
Serotonin (10^{-5} M)	6	34.3 ± 5.3[b]
Serotonin (10^{-5} M) + methylsergide (10^{-5} M)	6	15.9 ± 2.2
Serotonin (10^{-5} M) + cinanserine (10^{-5} M)	5	13.3 ± 4.9

[a] Values given are the mean ± SE. Number of observations (incubations).

[b] Value significantly ($P < 0.02$, by *t* test) different from the basal value or values with methylsergide and cinanserin.

serotonin-stimulated cAMP accumulation will also result in PTH release from parathyroid adenoma tissue.

The present observations, therefore, point to the possibility that serotonin may play a role in the physiological regulation of PTH secretion or at least in pathological PTH hypersecretion in hyperparathyroid states. Since PTH secretion may, in part, be under neural control (33-35), it is conceivable that serotonin may serve as one of the neurotransmitters in this neural regulatory pathway (36). Under normal circumstances, circulating serum levels of serotonin were reported to be approximately 0.8×10^{-6} M (14), which is somewhat lower than the lowest concentration shown in our experiments to stimulate cAMP accumulation in parathyroid tissue. However, in the carcinoid syndrome, serum levels of serotonin may reach levels in the range of about 10^{-5} M (14), a concentration in which serotonin directly stimulates cAMP formation in parathyroid glands, as suggested in the present experiments. Thus, it should be considered a possibility that the pathologically high blood serotonin levels encountered in some cases of carcinoid syndrome may contribute to PTH hypersecretion and subsequent hyperparathyroidism which have been described in several instances (1-12).

Parathyroid adenoma tissue contains serotonin in a concentration within the range capable of stimulating cAMP accumulation. Whether these relatively high levels of serotonin result from a high rate of serotonin synthesis and storage in parathyroid tissue or, alternatively, are due to uptake of serotonin from the blood perfusing the parathyroid glands remains undetermined. It is of interest that parathyroid adenomata contain numerous argentaffin cells (37, 38), a major site of serotonin storage in various tissues (39), while normal parathyroid tissue is devoid of these cells (37, 38). It should be realized that serotonin in parathyroid adenoma tissue might be sequestered in certain cell populations or cell compartments distinct from the serotonin receptor-adenylate cyclase system in plasma membranes. It is possible that serotonin should be released from such putative

stores in response to various (including neural) stimuli before it acts on the serotonin receptor and stimulates adenylate cyclase. Alternatively, serotonin synthesized *de novo* in parathyroid adenoma tissue may have direct access to serotonin-sensitive cAMP system. However, regardless of the serotonin localization in parathyroid adenoma tissue, its presence in the range of concentrations capable of stimulating cAMP accumulation (and, by extension, PTH secretion) suggests that serotonin-stimulated elevation of cAMP may contribute to anomalously high PTH secretion in hyperparathyroid states, albeit the details of such mechanism remain to be clarified.

In conclusion, although many points remain to be clarified, the present results provide evidence that human parathyroid adenoma tissue contains a cAMP system sensitive to stimulation by serotonin, and that the tissue itself contains considerable amounts of this biogenic amine. If serotonin indeed contributes, as postulated, by increasing cAMP levels in parathyroid tissue to control PTH secretion and, especially, to produce hypersecretion in pathological situations, blockade of serotonin's action on parathyroid tissue by specific antagonists (40) may be considered as a potential pharmacological tool to inhibit PTH secretion in clinical and experimental situations.

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