Effects of Biogenic Amines on the Formation of Adenosine 3', 5'-Monophosphate in Human Thyroid Slices

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Synopsis

The effects of various concentrations of biogenic amines on the formation of adenosine-3', 5'-monophosphate (cyclic AMP) and their interactions with other thyroid stimulators were investigated in human thyroid slices from normal and Graves' disease. Most of biogenic amines were found to have the stimulatory effects to some extent. Among the biogenic amines tested, histamine was the most potent thyroid stimulator, norepinephrine and serotonin, the intermediate in terms of cyclic AMP formation. The effect of histamine was almost as potent as TSH in thyroid slices from Graves' disease. This stimulatory effect of histamine was blocked by metiamide, a histamine H_2 -receptor antagonist, but not by chlorpheniramine, a histamine H_1 -receptor antagonist. The effect of norepinephrine was completely inhibited by propranolol, but not by phentolamine. Polyphloretin phosphate did not inhibit norepinephrine- or histamine-induced cyclic AMP formation, while it significantly depressed cyclic AMP formation induced by prostaglandin E_2 . The maximal effect of histamine was additive to that of TSH. It is suggested that biogenic amines, histamine and norepinephrine, in particular, have the thyroid receptors different from that of TSH or prostaglandin E_2 and could play an important role in thyroid physiology.

The resemblance of sympathetic over-activity to hyperthyroidism and the similarities between the physiologic effects of hormonal materials isolated from the thyroid and the adrenal medulla early pointed to relation between thyroid hormones and the catecholamines. Although extensive evidence of an interrelationship between these hormones has been accumulated (Waldstein, 1966; Maayan *et al.*, 1973; Spaulding and Burrow, 1975; Jacoby *et al.*, 1975; Onaya and Hashizume, 1976), the nature and mechanism of their interaction remain to be elucidated. It has been recently proposed that biogenic amines are involved in thyroid hormone secretion; norepinephrine released from sympathetic nerve terminals and his-

tamine from mast cells within the thyroid (Melander *et al.*, 1971; Ericson *et al.*, 1972; Melander and Sundler, 1973; Melander *et al.*, 1974a; 1974b). Melander *et al.* (1974a; 1974b) have reported that sympathetic adrenergic nerve fibers were numerous in the human and mouse thyroids in relation to arterioles and in interfollicular spaces, suggesting that the sympathetic innervation of the thyroid serves as means for effecting prompt, short-term alterations in the rate of thyroid hormone secretion. Furthermore, they have demonstrated several lines of evidence that the mast cells within the thyroid might also be involved in thyroid hormone secretion induced by thyroid stimulators such as TSH or the long-acting thyroid stimulator (LATS) (Melander *et al.*, 1971; Ericson *et al.*, 1972; Melander and Sundler, 1972; 1973). Ade-

nosine cyclic 3', 5'-monophosphate (cyclic AMP), which have been known to be a mediator of many hormonal actions in various endocrine organs, has also been suggested to play an important role in the thyroid. These considerations led us to test the effects of biogenic amines and other agents on the formation of cyclic AMP in human thyroid tissues *in vitro*.

Materials and Methods

Human thyroid tissues were obtained at surgery from patients with treated Graves' disease or, when normal thyroid tissues were used, from patients with encapsulated thyroid adenoma or cyst. The patients with Graves' disease usually received methimazole (30–40 mg/day) for several weeks and Lugol's solution (10 drops/day) for 7 to 10 days prior to surgery. The variations of the basal levels of cyclic AMP in human thyroid tissues are probably due to the fact that thyroid activities of these patients were quite different from each other even after the similar treatment. For the cyclic AMP study, human thyroid slices, 0.5 mm thick and weighing 10–20 mg, were made with a Stadie-Riggs microtome. Thyroid slices were distributed into 10 ml Erlenmeyer flasks containing 2 ml of Krebs-Ringer bicarbonate buffer (pH 7.4), 50 mg/100 ml of glucose and 10^{-2} M theophylline. The flasks were gassed with 95% O₂–5% CO₂, capped and incubated at 37°C for 30 min in a Dubnoff metabolic shaker before addition of test substances. The flasks were further incubated for 10 min. The incubation was terminated by freezing the thyroid tissues in test tubes immersed in dry ice-acetone. Before freezing, thyroid tissues were lightly blotted on filter paper. Frozen slices were weighed and dropped into boiling cyclic AMP-assay buffer on a boiling-water bath. The tubes were covered to prevent evaporation and boiled for 10 min (Cooper *et al.*, 1972). Thyroid tissues were then homogenized and denatured protein was removed by centrifugation at 4°C for 20 min. All supernatants were stored at –20°C until the cyclic AMP concentrations were measured. Cyclic AMP was measured by the direct binding method of Gilman (1970) as described previously (Sato *et al.*, 1974). The neurohormones and other substances, dissolved in saline buffered with 0.01 M sodium phosphate (pH 7.4) (PBS), were added in small volumes to give the desired final concentrations. The following hormones and compounds were used: dopamine hydrochloride, L-norepinephrine bitartrate, L-epinephrine bitartrate, histamine dihydrochloride, serotonin creatinine sulfate, L-isoproterenol, DL-propra-

nolol. Polyphloretin phosphate (PPP) was a gift of Dr. Seiya Sato, Kitazato Biochemical Laboratories, Japan. Prostaglandin E₂ (PGE₂) was kindly supplied by Ono Pharmaceutical Co., Ltd. Metiamide was a gift of Smith Kline & French Laboratories, Ltd., Japan. ³H-Labeled cyclic AMP was supplied by the Radiochemical Centre (RCC). Statistical analysis of the significance of difference between groups was done by means of Student's *t* test. A *p* value less than 0.05 was considered statistically significant.

Results

Effects of biogenic amines and other substances on the formation of cyclic AMP in thyroid slices from patients with Graves' disease

The effects of various concentrations of biogenic amines on the formation of cyclic AMP in thyroid slices from Graves' disease are shown in Table 1. The concentration of cyclic AMP slightly but significantly increased with increasing doses of dopamine incubated with thyroid slices (Exp. 1; Table 1). A slight but significant increase of cyclic AMP was also observed in thyroid slices when 10^{-5} M to 10^{-4} M epinephrine were used. In contrast, TSH markedly stimulated cyclic AMP formation in thyroid slices in comparison to dopamine or epinephrine. In Exp. 2 of Table 1, norepinephrine induced a significant increase of cyclic AMP at the concentrations from 10^{-5} M to 10^{-4} M. L-Isoproterenol caused a significant increase of cyclic AMP only with a concentration of 10^{-6} M. Serotonin also elevated cyclic AMP levels, but significantly only with a concentration of 10^{-4} M (Exp. 3; Table 1). Histamine at concentrations from 10^{-5} M to 10^{-4} M markedly stimulated the formation of cyclic AMP even as compared to the effect of TSH (Exp. 3; Table 1).

Table 1. Effects of biogenic amines and other substances on the formation of cyclic AMP in thyroid slices from patients with Graves' disease

Test substances (M)	No. of determinations	Cyclic AMP (picomoles/mg wet weight tissue)
Exp. 1. Dopamine		
0	6	0.12±0.01*
10 ⁻⁶	5	0.18±0.01**
10 ⁻⁵	5	0.25±0.05**
10 ⁻⁴	5	0.33±0.08**
Epinephrine		
10 ⁻⁶	5	0.22±0.06
10 ⁻⁵	5	0.30±0.04**
10 ⁻⁴	5	0.27±0.05**
TSH (100 mU/ml)	5	2.40±0.34**
Exp. 2. Norepinephrine		
0	5	0.55±0.04
10 ⁻⁶	4	0.54±0.09
10 ⁻⁵	4	0.84±0.05**
10 ⁻⁴	4	0.93±0.08**
L-isoproterenol		
10 ⁻⁶	4	0.76±0.06**
10 ⁻⁵	4	0.59±0.02
10 ⁻⁴	4	0.69±0.11
Exp. 3. Serotonin		
0	5	0.86±0.20
10 ⁻⁶	5	1.83±0.38
10 ⁻⁵	5	1.38±0.29
10 ⁻⁴	5	1.83±0.07**
Histamine		
10 ⁻⁶	5	1.39±0.52
10 ⁻⁵	5	3.14±0.52**
10 ⁻⁴	5	3.44±0.56**
TSH (100 mU/ml)	5	2.86±0.41**

* Mean±S.E. of the mean.

** Statistically significant from its control group (p<0.05).

Effects of phentolamine and propranolol on norepinephrine-induced cyclic AMP formation in thyroid slices of Graves' disease

Phentolamine, an alpha-adrenergic blocking agent, and propranolol, a beta-adrenergic blocking agent, were tested for their ability to block norepinephrine-induced cyclic AMP formation in thyroid slices of Graves' disease. Norepinephrine at a concentration of 10⁻⁴ M significantly stimulated the formation of cyclic AMP as shown in Table 2 (group B). Phentolamine alone did neither affect the basal level of cyclic AMP significantly, nor inhibit norepinephrine-induced cyclic AMP formation at all (groups C and D). Propranolol, on the other hand, completely inhibited norepinephrine-stimulated formation of cyclic AMP, while it alone did not alter the basal level of cyclic AMP (groups E and F).

Effects of chlorpheniramine and metiamide on histamine- and TSH-induced cyclic AMP formation in normal or Graves' disease thyroid slices

In order to investigate histamine receptors in human thyroid glands, chlorpheniramine, an H₁-receptor antagonist, and metiamide an H₂-receptor antagonist, were tested for TSH or histamine stimulation of cyclic AMP formation in human thyroid slices (Table 3). TSH (20 mU/ml) markedly

Table 2. Effects of phentolamine or propranolol on norepinephrine-induced cyclic AMP formation in thyroid slices of Graves' disease

Groups	Addition to incubation medium	No. of determinations	Cyclic AMP (picomoles/mg wet weight)	p values
A	PBS	5	0.27±0.11*	
B	Norepinephrine (10 ⁻⁴ M)	5	0.67±0.06	A-B, p<0.05
C	Phentolamine (10 ⁻⁴ M)	4	0.51±0.12	A-C, N.S.
D	Phentolamine+Norepinephrine	5	0.87±0.17	B-D, N.S.
E	Propranolol (10 ⁻⁴ M)	5	0.33±0.03	
F	Propranolol+Norepinephrine	5	0.31±0.03	B-F, p<0.01

* Mean±S.E. of the mean.

Thyroid slices were preincubated for 30 min in the presence or absence of phentolamine or propranolol, respectively.

stimulated cyclic AMP formation in normal human thyroid slices (Exp. 1; group D). This stimulatory effect of TSH was not inhibited either by chlorpheniramine or by metiamide. In contrast to the effect of TSH, histamine stimulation of cyclic AMP formation was significantly inhibited by 10^{-4} M metiamide, whereas chlorpheniramine had no effect on histamine stimulation (groups H and I). Furthermore, 10^{-4} M chlorpheniramine alone significantly increased cyclic AMP concentration, while metiamide alone did not affect the basal level of cyclic AMP. A similar experiment was designed using thyroid tissues of Graves' disease (Exp. 2; Table 3). Similarly with normal human thyroid tissues, an increase by histamine of cyclic AMP formation was significantly inhibited by metiamide, but not by chlorpheniramine. Chlorpheniramine, however, exhibited no effect on the basal level of cyclic AMP in this case (Exp. 2; group B).

Effects of polyphloretin phosphate (PPP) on the formation of cyclic AMP induced by TSH, norepinephrine, histamine and PGE_2 in thyroid slices of Graves' disease

All of these stimulators tested significantly elevated cyclic AMP levels in thyroid slices of Graves' disease (Exp. 1 and 2; Table 4). PPP alone did not affect the basal levels of cyclic AMP (Exp. 1 and 2). PPP significantly inhibited PGE_2 -induced cyclic AMP formation (Exp. 1; group F), but did not affect TSH-, histamine- and norepinephrine-induced cyclic AMP formation (Exp. 1 and 2). Furthermore, the maximal effect of histamine was significantly additive to that of TSH (Exp. 1; groups G and H).

Discussion

The interrelation of the thyroid gland and the sympathetic nervous system has

intrigued thyroid researchers since many years ago. Recently, however, considerable attention has been paid to the effect of the biogenic amines upon thyroid hormone secretion in several species (Ericson *et al.*, 1972; Melander *et al.*, 1974a). It has been reported that not only TSH but also the long-acting thyroid stimulator (LATS) share the effects with norepinephrine released from sympathetic nerve terminals and with histamine released from mast cells within the thyroid (Melander *et al.*, 1971; 1974a; 1974b; Ericson *et al.*, 1972; Melander and Sundler, 1973). It seems logical, therefore, to investigate, if any, the possible role of these amines in the pathological activation of the human thyroid which causes the symptoms of thyrotoxicosis. In the present study, biogenic amines were tested for their ability to stimulate thyroid tissues of Graves' disease in terms of cyclic AMP formation *in vitro*. Some investigators have failed to demonstrate the stimulation by biogenic amines of thyroidal adenylate cyclase activity (Pastan and Katzen, 1967; Kendall-Taylor, 1972). Recently, however, thyroidal adenylate cyclase has been shown to be stimulated by dopamine (Maayan *et al.*, 1973). More recently, Spaulding and Burrow (1975) have reported the beta-adrenergic stimulation of cyclic AMP and protein kinase activity in the thyroid. Here, we have demonstrated that, among the biogenic amines tested, histamine was a quite potent thyroid stimulator for Graves' disease thyroid; norepinephrine and serotonin, the intermediate. Histamine was almost as potent as a high concentration of TSH in thyroid slices from Graves' disease. Histamine is known to stimulate the secretion of acid by the stomach (Loew and Chickering, 1941), increase the heart rate (Trendelenburg, 1960) and inhibit contractions in the rat uterus (Dews and Graham, 1946). These actions cannot be antagonized by typical anti-histaminic drugs such as mepyramine (a histamine H_1 -receptor antagonist) and have

Table 3. Effects of chlorpheniramine or metiamide on histamine or TSH-induced cyclic AMP formation in human thyroid slices

Groups	Addition to incubation medium	No. of determinations	Cyclic AMP (picomoles/mg wet weight)	p values
Exp. 1. (Normal thyroid)				
A	PBS	4	0.42±0.07*	
B	Chlorpheniramine (10 ⁻⁴ M)	5	0.70±0.04	A-B, p<0.01
C	Metiamide (10 ⁻⁴ M)	4	0.63±0.07	A-C, N.S.
D	TSH (20 mU/ml)	5	6.30±0.44	A-D, p<0.01
E	Chlorpheniramine+TSH	5	7.36±0.23	D-E, N.S.
F	Metiamide+TSH	5	5.34±0.46	D-F, N.S.
G	Histamine (10 ⁻⁴ M)	5	1.51±0.08	A-G, p<0.01
H	Chlorpheniramine+Histamine	5	1.35±0.09	G-H, N.S.
I	Metiamide+Histamine	5	1.03±0.10	G-I, p<0.01
Exp. 2. (Graves' disease)				
A	PBS	5	1.12±0.06	
B	Chlorpheniramine (10 ⁻⁴ M)	5	1.20±0.11	
C	Metiamide (10 ⁻⁴ M)	5	1.01±0.12	
D	Histamine (10 ⁻⁴ M)	5	1.94±0.11	A-D, p<0.01
E	Chlorpheniramine+Histamine	5	1.79±0.23	D-E, N.S.
F	Metiamide+Histamine	5	0.70±0.07	D-F, p<0.01

* Mean±S.E. of the mean.

Thyroid slices were preincubated for 30 min in the presence or absence of chlorpheniramine or metiamide, respectively.

Table 4. Effects of polyphloreitin phosphate (PPP) on the formation of cyclic AMP induced by TSH, norepinephrine, histamine and PGE₂ in thyroid slices from Graves' disease

Groups	Test substances	No. of determinations	Cyclic AMP (picomoles/mg wet weight)	p values
Exp. 1.				
A	PBS	5	0.57±0.06*	
B	PPP+PBS	5	0.58±0.04	
C	TSH (100 mU/ml)	5	0.99±0.16	A-C, p<0.05
D	PPP+TSH	5	1.23±0.16	C-D, N.S.
E	PGE ₂ (5×10 ⁻⁹ M)	5	2.06±0.25	A-E, p<0.05
F	PPP+PGE ₂	5	1.10±0.30	E-F, p<0.05
G	Histamine (10 ⁻⁴ M)	5	0.88±0.08	A-G, p<0.05
H	Histamine+TSH	5	1.42±0.18	G-H, p<0.05
Exp. 2.				
A	PBS	5	0.81±0.11	
B	PPP+PBS	5	0.91±0.08	
C	TSH (100 mU/ml)	5	2.00±0.30	A-C, p<0.01
D	PPP+TSH	5	1.53±0.45	C-D, N.S.
E	Histamine (10 ⁻⁴ M)	5	1.89±0.18	A-E, p<0.01
F	PPP+Histamine	5	2.12±0.13	
G	Norepinephrine (10 ⁻⁴ M)	5	2.11±0.50	A-G, p<0.05
H	PPP+Norepinephrine	5	2.25±0.20	

* Mean±S.E. of the mean.

Thyroid slices were preincubated for 30 min in the presence or absence of PPP at a concentration of 100 µg/ml.

been recently ascribed to mediation by histamine H₂-receptors (Black *et al.*, 1972). In our study, stimulation by histamine of cyclic AMP formation in Graves' disease thyroid was significantly inhibited by metiamide, a histamine H₂-receptor antagonist, but not by chlorpheniramine, a histamine H₁-receptor antagonist at all, giving the first evidence for the existence of a histamine H₂-receptor in the thyroid. The effect of norepinephrine was completely inhibited by propranolol, a beta-adrenergic blocking agent, but not by phentolamine, an alpha-adrenergic blocking agent. On the other hand, TSH stimulation of cyclic AMP formation in Graves' disease thyroid was affected by none of these agents. Levey *et al.* (1969) also failed to observe the inhibitory effect of propranolol (10⁻⁴ M) on TSH-induced adenylate cyclase activity in the canine thyroid *in vitro*. It was further demonstrated that PPP significantly inhibited only PGE₂-stimulated cyclic AMP formation in our hands, although Burke (1974) reported that PPP inhibited induction of cyclic AMP formation by TSH in mouse thyroid lobes. These observations could suggest that in Graves' disease thyroid there exist an H₂-receptor for histamine and a beta-adrenergic receptor for norepinephrine in mediating thyroid activation although they might not be pathologically specific for Graves' disease. In addition, from the present data, these receptors for the biogenic amines seem to be different from those of TSH and PGE₂. If histamine, which could be released from the mast cells by homocytotropic antibody (IgE), were a potent thyroid stimulator especially for Graves' disease, the deposition of IgE in the stroma and follicular basement membranes of the Graves' disease thyroid would be of great interest (Werner *et al.*, 1972). The present findings seem to shed a light to the assumption that local release of vasoactive amines within the thyroid might play some role in the pathogenesis of Graves' disease in addition to the other

thyroid stimulators such as LATS (Adams, 1965), LATS-protector (Adams and Kennedy, 1967; Shishiba *et al.*, 1973; Adams *et al.*, 1974) and the human thyroid stimulator (HTS) (Onaya *et al.*, 1973). Needless to say, much work must be done to prove this hypothesis. Recent report by Melander *et al.* (1975) on the influence of histamine-containing thyroid mast cells on thyroid blood flow and permeability must be also considered.

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