TSH-Induced Appearance and Stimulation of Amine-Containing Mast Cells in the Mouse Thyroid

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ABSTRACT. Intra- and extrathyroidal mast cells in mice were located by their metachromasia and histochemically demonstrable 5-hydroxytryptamine (5-HT) and histamine content, and plasma TSH levels were determined by bioassay. Untreated mice had no detectable plasma TSH (<0.1 mU/ml), and no or very few intrathyroidal mast cells, whereas numerous mast cells were found in adjacent tissues. During propylthiouracil (PTU) treatment, plasma TSH rose progressively (to 4.0 mU/ml at 4 weeks) and several amine-containing mast cells appeared in the thyroid. Concomitant administration of thyroid hormone prevented these effects, and withdrawal of PTU was followed by decreased plasma TSH levels and a disappearance of the thyroid mast cells. However, the content of amines and metachromatic material in thyroid mast cells seemed inversely related to the plasma TSH level. The extrathyroidal mast cells were never affected by changes in TSH. The findings indicate that, in mice, TSH induces the appearance of 5-HT- and histamine-containing thyroid mast cells, and promotes a release from these cells of the amines and metachromatic material. This may be one of the mechanisms by which certain amines are directly involved in the function of the thyroid follicle cell. (*Endocrinology* **89:** 528, 1971)

RECENT INVESTIGATIONS on mice have shown that 5-hydroxytryptamine (5-HT) and certain catecholamines can, like TSH, induce endocytosis of thyroglobulin and release of thyroid hormone by a direct *in vivo* action on the thyroid (1–3). 5-HT and epinephrine also stimulate several facets of *in vitro* thyroid activity (4–6), among them the synthesis of thyroid hormone (5, 6). The presence of different amine-storing cell systems in this gland—adrenergic nerve terminals, parafollicular cells and mast cells (cf 7–9)—suggests a physiological significance of these findings (*cf* 1–3). Amine-containing mast cells are found in the thyroid of various species (7, 8, 10, 11), and changes in the number and metachromatic reaction of thyroid mast cells have been reported to follow changes in thyroid activity in guinea pig and rat (12, 13). In the rat thyroid, 5-HT and histamine are present in considerable amounts (14–18), and it has been shown in this species that TSH induces an acute mobilization of 5-HT from intrathyroidal mast cells (11, 14), and that chronic stimulation is followed by a depletion of thyroidal histamine (17).

In view of the assumption that 5-HT may have a physiological role in the secretion process of the thyroid (1–3, 11, 14), the relation between thyroid mast cells and plasma TSH levels was studied in mice with different degrees of thyroid activity. The present report will show that, whereas no or very few thyroid mast cells are seen in the normal mouse, TSH induces the appearance in the gland of mast cells containing 5-HT and histamine, that their number is correlated to the level of circulating TSH, and that TSH promotes a release of the amines from these cells.

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Materials and Methods

Animals. Female mice of the NMRI strain (Laboratory Animal Breeding and Research Centre, Ry, Denmark), weighing about 20 g, were used. When not otherwise specified (see *TSH bioassay* and *Experimental*) they were kept on a standard pellet diet (SAN-Bolagen, Malmö, Sweden) and tap water *ad lib.*

Drugs and hormone preparations. PTU: propylthiouracil (Tiotil, Pharmacia, Uppsala, Sweden); 48/80: polymer of N-methylhomoanisylamine and formaldehyde (Leo, Helsingborg, Sweden); TH: thyroid hormone as thyroid

powder (USP); T_4: L-thyroxine sodium (British Drug House, Poole, England); TSH: ovine thyrotropin 2.47 U/mg (NIH-TSH-S6), a gift from the Endocrinology Study Section, National Institutes of Health, Bethesda, Md.

Plasma sampling. Plasma samples from experimental mice (see *Experimental*) were prepared as follows: Blood was sampled (250 μl/mouse) by orbital puncture with a heparinized constriction pipette, and plasma was obtained by centrifugation in a Beckman-Spinco Microfuge. Plasma samples (100 μl/250 μl blood) from identically treated mice were pooled (8 ×100 μl/pool) and frozen until assayed for TSH.

TSH bioassay. About 300 mice were used for TSH bioassay. This was carried out by the McKenzie (19) method, as modified by Rerup and Melander (20). In short, mice were kept on a diet without fish meal and no extra salt added (SAN-Bolagen, Malmö, Sweden) 7–14 days prior to the radioiodine (^{131}I) injection. They received 8 μCi carrier-free [^{131}I]NaIip once, and 20 μg T_4 sc twice. Blood was sampled (100 μl) by orbital puncture before and 2 hr after injection of the test material. Blood ^{131}I activity was measured in a gas flow counter with end window (Nuclear-Chicago) and expressed as the $\log_{10}$ percentage cpm ratio of the 2-hr to the 0-hr value (20). TSH standards and plasma samples were injected iv (tail vein) in a volume of 0.1–0.2 ml; necessary dilutions were made with 0.9% saline. At least 5 test mice were used per dose.

Fluorescence microscopy. Experimental mice (see *Experimental*) were killed by neck traction, and the thyroid with the adjacent part of the trachea, oesophagus and surrounding connective tissue was immediately dissected out. The whole preparation was frozen in a propane-propylene mixture to the temperature of liquid nitrogen. After freeze-drying the preparations were treated in formaldehyde gas (1 hr, 80 C) for the histochemical demonstration of 5-HT (21–23), embedded in paraffin, sectioned at 6 μ, and mounted for fluorescence microscopy. For further technical details see Falck and Owman (24).

For the histochemical demonstration of histamine (25–27), similar preparations were frozen as above and sectioned at 20 μ thickness in a cryostat (−30 C). After freeze-drying over P_2O_5 overnight in a desiccator kept in the cryostat, the sections were exposed to gaseous *o*-phthaldialdehyde for 90 sec followed by hydration for 5 sec, and mounted in xylene for fluorescence microscopy.

Light microscopy. Some of the sections (6 μ thickness) obtained from the freeze-dried formaldehyde-treated material were deparaffinized and stained in an ethanol solution of toluidine blue for the light microscopic demonstration of metachromatic cells (28).

Counting of mast cells. The number of mast cells in the thyroid was estimated in sections from formaldehyde-treated tissue by counting fluorescent cells (10 ×objective, 12.5 ×ocular) in 10 randomly chosen sections from each thyroid lobe from 3 animals in the different experimental groups.

Experimental. The effects of the following treatments were tested:

I. *PTU*: One hundred animals, kept on the standard diet, received 0.1% PTU in the drinking water. Twenty of these were analyzed after 2 weeks. Eighty animals received this treatment for 4 weeks, whereafter 20 of them were returned to tap water for 2 weeks, while 20 continued on PTU. Ten animals, on PTU for 4 weeks, received a single injection of 48/80 ip (200 μg/animal) and another 10 a single control injection of 0.9% saline.

II. *TH*: Twenty animals, on tap water, received thyroid hormone in a pellet diet containing 0.066% thyroid powder, for 4 weeks.

III. *PTU+TH*: Forty animals received a combination of PTU (see I) and TH (see II) for 4 weeks.

IV. *PTU* and *T_4*: Thirty-five animals on PTU for 4 weeks were given T_4 (20 μg sc) on day 28 and again on day 30.

V. *PTU and T_4 and TSH*: Fifteen animals on PTU for 4 weeks were given T_4 as under IV, and, in addition, 100 mU TSH iv on day 31.

VI. *Controls*: Control animals were kept on the standard diet and tap water during the entire experimental time.

Results

Controls. In untreated mice no plasma TSH was detected (Fig. 1). Mast cells were characterized by their metachromasia after toluidine blue staining, by their formaldehyde-induced yellow flourescence due to 5-HT, and by their *o*-phthaldialdehyde-induced yellow flourescence due to histamine. No, or very few, mast cells were found within the thyroid (Fig. 3a), whereas they occurred frequently in adjacent tis-

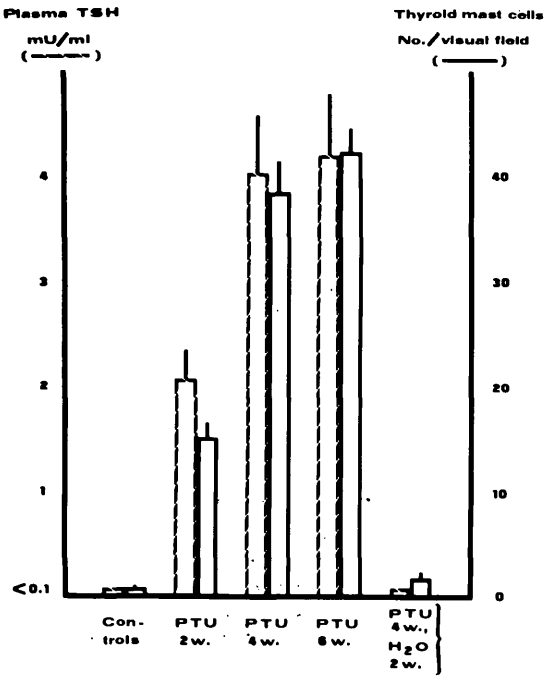

FIG. 1. Correlation of plasma TSH level and number of 5-HT-containing thyroid mast cells in PTU-treated mice. Plasma TSH values are derived from 8 mice followed for 6 weeks (w.), and from 8 mice in which PTU was withdrawn after 4 weeks. Mast cell counting was performed on thyroids from 3 mice in each group. SEM indicated.

sues, including the trachea and esophagus (Fig. 3c).

PTU. Within two weeks of PTU treatment, plasma TSH had increased to 2.1 ± 0.27 (mean $\pm$ SEM) mU/ml (Fig. 1). Light and flourescence microscopy of the thyroid now revealed the presence of several 5-HT- and histamine-containing metachromatic mast cells, diffusely distributed between the follicles (Fig. 1, 2). The gland showed signs of increased activity, such as an increase in height and number of the follicle cells and a decrease in luminal colloid, as compared to the picture obtained from control animals. After four weeks on PTU, when the plasma TSH level had reached 4.0 ± 0.56 mU/ml (Fig. 1) and the signs of follicular activation were further marked, even more mast cells were seen in the thyroid (Fig. 1, 3b), while the mast cells in surrounding tissues (Fig. 3d) seemed unchanged in number. Moreover, these still exhibited a prominent metachromatic reaction, and an intense flourescence, whereas the thyroid mast cells appearing during PTU treatment has a distinctly low degree of metachromasia and fluorescence intensity.

Both the intra- and extrathyroidal mast cells responded to 48/80 treatment with a diminished metachromasia and a disappearance of the fluorescence.

Continuation of PTU treatment for another two weeks did not produce any marked deviations from the findings at four weeks (Fig. 1), but replacement of PTU with tap water for two weeks resulted in a return of plasma TSH to undetectable levels, signs of diminished follicular activity, and a disappearance of the thyroid mast cells (Fig. 1). The extrathyroidal mast cells seemed to remain unchanged whether PTU was continued or not.

PTU+TH. In mice given a combination of PTU and TH for four weeks the results resembled those obtained in control animals. Thus, no or very few mast cells were seen in the thyroid, and plasma TSH was below detection. The follicles appeared inactive. Similar findings were made after treatment with TH only for four weeks.

PTU and T_4. Three days after the start of T_4 treatment of mice on PTU, plasma TSH had fallen to undectable levels. After this short-time suppression of TSH, no overt decrease was seen in the number of intrathyroidal mast cells; in fact, they now showed a more intense fluorescence and a distinctly stronger metachromatic reaction than in mice given PTU only.

PTU and T_4 and TSH. Five min after an iv injection of TSH (100 mU) to mice pretreated with PTU and T_4 as above, there was again a low degree of fluorescence intensity and metachromasia in the thyroid mast cells, *i.e.*, the picture was similar to that from mice given PTU only.

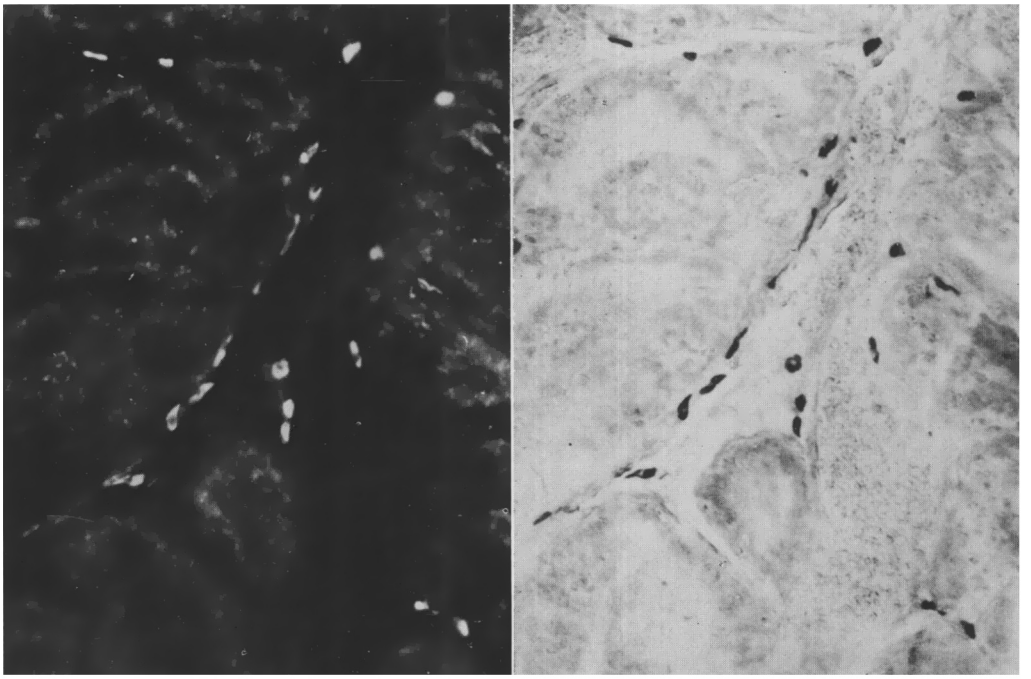

FIG. 2. Thyroid from animal after 2 weeks of PTU treatment. a (*left*). Fluorescence photomicrograph showing formaldehyde-induced yellow 5-HT-fluorescence in several cells distributed among the follicles. b (*right*). Subsequent toluidine blue staining of the section reveals that the 5-HT-containing cells are metachromatic. (×340.)

Discussion

The present investigation has shown that, whereas no or very few thyroid mast cells could be detected in the untreated mouse, numerous metachromatic cells, containing histochemically demonstrable 5-HT and histamine, appeared in the thyroids of mice treated with PTU. These cells were classified as mast cells (7, 10, 11), which was further justified by the finding that they, like mast cells in other tissues, were depleted of their metachromatic material and fluorogenic material when the animals were treated with compound 48/80 (*cf* 10, 18). In agreement with previous observations (20, 29), it was also found that plasma TSH levels increased progressively upon PTU treatment, and returned to normal upon withdrawal of the drug. The number of thyroid mast cells showed a concomitant increase and decrease, whereas in mice given PTU and TH (or TH alone) no plasma TSH was detected and virtually no mast cells appeared in the gland. Hence it is probable that TSH, rather than PTU, is responsible for the appearance of thyroid mast cells; moreover, their number can be correlated with the level of circulating TSH. Further, this effect of TSH seems to be restricted to the thyroid in the sense that mast cells in other tissues studied did not show any obvious alteration in number, metachromasia, or amine content upon the various treatments used. It follows that TSH stimulates not only the follicle cells but at least one other cell system within the thyroid, and this in turn implies that the target for TSH is the gland itself rather than a single specific cell system within it.

In the rat, numerous mast cells are already seen in the thyroid under normal conditions (10, 11, 18). It is notable that this animal has a high plasma TSH level (20, 29) and, since the number of thyroid mast cells also seems to vary with the thyroid activity in this species (11), a correlation between

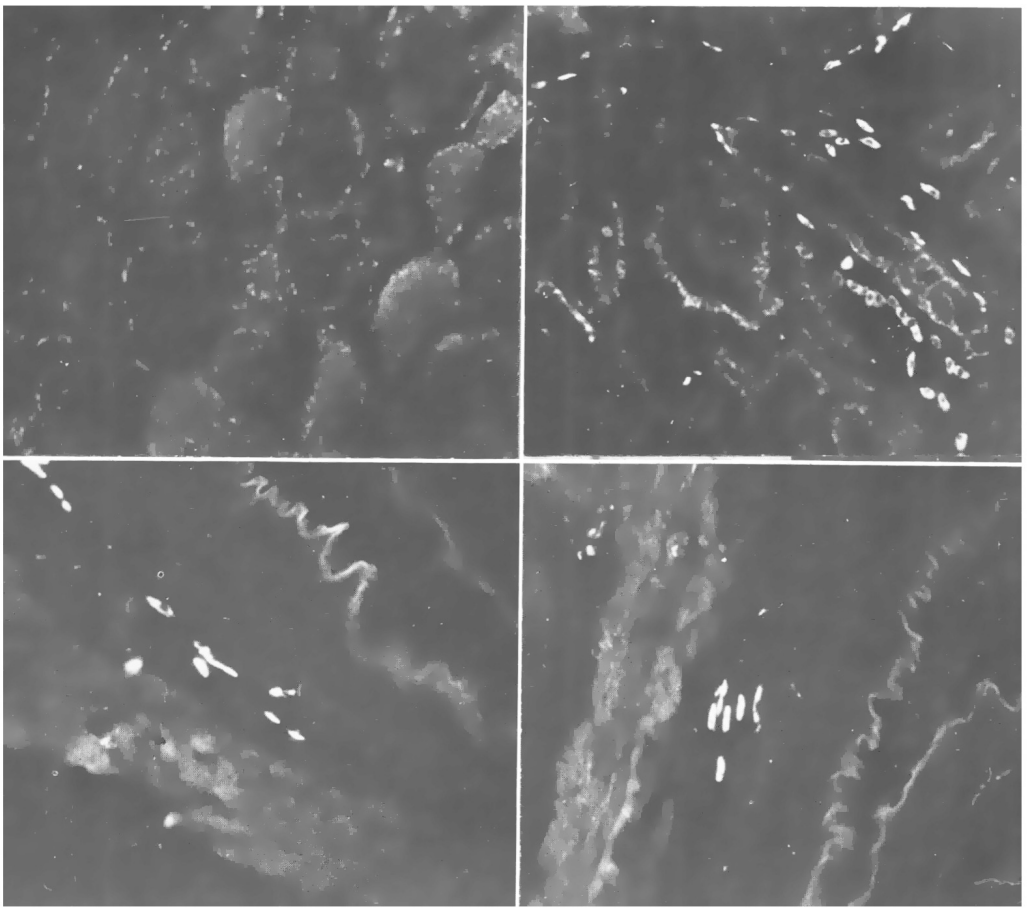

FIG. 3. Fluorescence photomicrographs from formaldehyde-treated specimens to demonstrate 5-HT-containing mast cells. (×260.) a (*top, left*). Thyroid from control animal. No fluorescent mast cells are seen. Small autofluorescent granules in follicle cells. b (*top, right*). Thyroid after 4 weeks of PTU treatment. Numerous 5-HT-containing mast cells have appeared. c (*bottom, left*). Esophageal tissue from control animal containing several fluorescent mast cells. d (*bottom, right*). Esophageal tissue after 4 weeks of PTU treatment. Mast cells as in control animals.

plasma TSH level and thyroid mast cell number may be a general feature.

The TSH-induced thyroid mast cells had a low content of 5-HT and histamine, and a low degree of metachromasia, as compared to extrathyroidal mast cells. However, during a short-time T_4-blockade of TSH secretion, when the thyroid mast cells were not overtly reduced in number, their content of amines and metachromatic material were increased. These findings indicate that TSH not only induces an appearance of amine-containing mast cells in the thyroid but also promotes a release of amines—and of metachromatic material as well—from these cells. In accordance with this, a low content of amines and metachromatic material was again seen, when a high plasma TSH level was re-established by TSH treatment of PTU mice acutely suppressed with T_4. Conversely, when TSH levels are reduced, there is an initial reduction in the release of 5-HT and histamine and an ultimate reduction in the number of mast cells, as was observed after withdrawal of PTU.

Clayton and co-workers (11, 14) have demonstrated that in the T_4-suppressed rat TSH promotes a rapid mobilization of 5-HT from intrathyroidal, perivascular mast

cells. This occurs concomitantly with an increase in thyroidal blood flow (11, 14), and hence it was suggested that the physiological action of TSH includes a release of 5-HT (and possibly of histamine, *cf* 11) from perivascular mast cells in the thyroid which enhances the glandular blood flow (11, 14). It seems most probable that mast cell amines released can and do affect the intrathyroidal blood flow in both rat and mouse; however, this does not preclude other intrathyroidal effects of 5-HT, or histamine (*cf* 11). 5-HT stimulates *in vivo* endocytosis of thyroglobulin (3) and release of thyroid hormone (1–3, 30, 31) in a way that cannot be entirely explained by an effect on the thyroid vascular system (2, 30, 31). Furthermore, 5-HT stimulates iodine metabolism in isolated thyroid cells (5).

In conclusion, TSH induces an appearance, and also stimulates the activity, of amine-containing mast cells in the thyroid and this may be one of the mechanisms by which certain amines are directly implicated in the function of the thyroid follicle cell.

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