TSH-Induced Release of 5-Hydroxytryptamine and Histamine from Rat Thyroid Mast Cells

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ABSTRACT. The effect of TSH on rat thyroid mast cells was investigated by a combination of chemical, histochemical, electron microscopical, and bio-assay procedures. In T_4-pretreated rats, a single iv injection of TSH reduced the concentration of 5-hydroxytryptamine (5-HT) and histamine in thyroid mast cells, whereas mast cells outside the thyroid seemed unaffected. In rats kept on propylthiouracil and iodine-poor diet for 4 weeks, plasma TSH levels were raised ten-fold. At the same time, follicle cells and mast cells in the thyroid increased in number, but the concentration of 5-HT and histamine in individual thyroid mast cells was reduced. Ultrastructurally, there were no overt signs of mast cell degranulation, whether upon short-term or long-term TSH stimulation. It is concluded that, in the rat, TSH induces a release of 5-HT and histamine from intrathyroidal mast cells, which seems to occur without any concomitant degranulation. This release may be involved in the process of thyroid hormone secretion. (*Endocrinology* **90**: 795, 1972)

5-HYDROXYTRYPTAMINE (5-HT) and certain catecholamines have been shown to stimulate release and synthesis of thyroid hormone by a direct action on the thyroid follicle cell (1–4). The presence of such amines in the thyroid may therefore have physiological significance (1–7). Amine-containing mast cells occur in varying numbers in the thyroid of different species (5,6,8,9), and recent observations imply that the amount of thyroid mast cells is related to the level of plasma TSH (7, *cf* 10). The number of mast cells in the rat thyroid is large, and they contain both 5-HT and histamine (5,6,10–14). TSH is known to induce a rapid release of 5-HT from these cells, and it has been postulated that this explains the increased thyroid blood flow which follows TSH stimulation (5,6). No rapid effect of TSH on the histamine level of the rat thyroid has been recorded (5,6); upon prolonged stimulation with TSH, however, thyroid histamine is reduced (13).

Recent studies have shown that in mice, normally having low plasma TSH levels and few thyroid mast cells, elevation of plasma TSH induces the appearance of 5-HT- and histamine-containing mast cells in the thyroid (7). At the same time, TSH seems to reduce their content of both 5-HT and histamine, and of metachromatic material as well. The present investigation aims at a further understanding of the relation between plasma TSH and thyroid mast cells in the rat, where these cells are frequent. For this purpose, a combination of chemical, histochemical, electron microscopical, and bio-assay procedures has been used. The results show that TSH induces release of both 5-HT and histamine from thyroid mast cells, that the release of amines is associated with a reduction in their metachromasia, and that these effects are not accompanied by overt ultrastructural signs of degranulation.

Materials and Methods

Animals. A total of 140 Sprague-Dawley rats (Anticimex, Stockholm, Sweden) of either sex, weighing about 50–100 g, and 200 female NMRI mice (Lab. Animal Breeding and Research Centre, Ry, Denmark), weighing about 20 g, were used. Unless otherwise specified, the animals were kept on a standard pellet diet (Ewos, Södertälje, Sweden) and tap water *ad lib*. The rats were killed by a blow on the head or by dislocation of the neck.

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Drugs and hormone preparations. PTU: Propylthiouracil (Tiotil; Pharmacia, Uppsala, Sweden). T_4: L-thyroxine sodium (British Drug House, Poole, England); TSH: ovine thyrotropin (NIH-TSH-S6) and bovine thyrotropin (NIH-TSH-B5) were gifts from the Endocrinology Study Section, National Institutes of Health, Bethesda, Md.

Chemical determinations of 5-HT and histamine. The thyroid was dissected out, freed from surrounding tissue, and weighed. In some experiments thyroids were paired. The thyroids were homogenized in 2 ml 0.4 N perchloric acid. Precipitated proteins were spun down. For determination of 5-HT, 1.5 ml of the supernatant was extracted with *n*-butanol as described by Kuntzman *et al.* (15). 5-HT was then determined fluorometrically by the method of Maickel and Miller (16). For determination of histamine, 0.5 ml of the supernatant was extracted with a mixture of *n*-butanol and chloroform as described by Burkhalter (17). Histamine was then measured fluorometrically by the method of Shore, Burkhalter and Cohn (18). Amine concentrations were expressed in μg/g thyroid tissue.

Histochemistry of 5-HT and histamine. The thyroid with the adjacent part of the trachea, oesophagus, and surrounding connective tissue was immediately dissected out. The two thyroid lobes with surrounding tissue were separated, one being used for histochemical demonstration of 5-HT, and the other for that of histamine. Both preparations were frozen in a propane-propylene mixture to the temperature of liquid nitrogen. *Demonstration of 5-HT.* After freeze-drying, the preparation was treated with formaldehyde gas (1 hr, 80 C), embedded in paraffin, sectioned at 6 μ, and mounted for flourescence microscopy. For technical details see Falck and Owman (19). *Demonstration of histamine.* The frozen specimen was sectioned at 20 μ 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 gentle hydration for 5 sec; they were then mounted in xylene for fluorescence microscopy (20).

Toluidine blue staining for demonstration of mast cells. Sections obtained from the freeze-dried formaldehyde-treated material were deparaffinized and stained in an ethanol solution of toluidine blue (1%) for the light microscopic demonstration of mast cells (21). In some cases, also sections from glutaraldehyde-fixed material (see below) were stained with toluidine blue.

Electron microscopy. Twenty rats, anesthetized with ether, were perfused via the ascending aorta with a glutaraldehyde fixative (3% glutaraldehyde in 0.075 M sodium cacodylate, pH 7.2). Thyroid specimens were excised and kept in the glutaraldehyde fixative for 1–2 hr. They were postfixed for 2 hr in 1% osmium tetroxide in Veronal acetate buffer, pH 7.2. After embedding in Epon, sections were cut on an LKB Ultrotome, stained with uranyl acetate and lead citrate, and examined at 80 kV in a Siemens Elmiskop I or a Philips 300 electron microscope.

Plasma sampling. Blood samples (1.1–1.2 ml/animal) were taken from unanesthetized rats by orbital puncture with a heparinized constriction pipette. Plasma (500 μl/animal) was obtained by centrifugation in a Beckman-Spinco Microfuge. Plasma samples were pooled (5 × 500 μl) and immediately assayed for TSH as described below.

TSH bioassay was carried out by the McKenzie (22) method, as modified by Rerup and Melander (23). In short, mice were kept on an iodine-poor diet (Ewos, Södertälje, Sweden) for 3–7 days prior to radioiodine (^{131}I) injection. They received 8 μCi Na ^{131}I ip once, and 20 μg T_4 sc twice. Blood was sampled (100 μl) by orbital puncture before and 2 hr after the iv injection of rat plasma (0.2–0.4 ml into a tail vein). Two standards of TSH, 0.05 and 0.20 mU, were given in parallel. Blood ^{131}I activity was measured in a gas flow counter (Nuclear-Chicago) and expressed as the ^{10}log percentage cpm ratio of the 2 hr to 0 hr value (22,23). At least 6 mice were used per dose.

Experimental. Acute effects of TSH: In order to achieve a high sensitivity to exogenous TSH, endogenous TSH was suppressed by sc T_4 injections to 70 rats, 20–40 μg/100 g body weight on days 0, 3, and 6 (*cf* 1,2,5,6). The effect of exogenous TSH was studied on day 7, by one

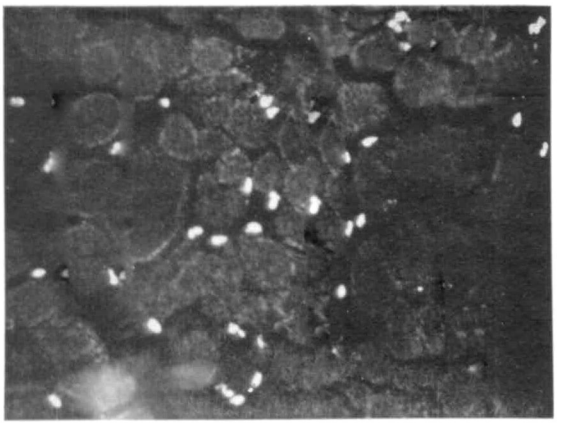


FIG. 1. Fluorescence photomicrograph of thyroid from T_4-pretreated rat, showing *o*-phthaldialdehyde-induced histamine-fluorescence in several cells distributed among the follicles.

iv injection (tail vein, no anesthesia). The thyroids were removed as described above, 15 min or 2 hr after the TSH injection. Control animals received 0.9% saline iv. Chronic effects of TSH: 25 rats were given PTU (0.05% in the drinking water) and an iodine-poor diet (Ewos, Södertälje, Sweden) for 4 weeks, and 25 rats were kept as controls. Plasma was then sampled and assayed for TSH as described above, and the thyroids were removed for the various analyses.

Results

Acute effects of TSH on thyroid mast cells. Numerous mast cells, containing both histamine (Fig. 1) and 5-HT (Fig. 2) were seen in the rat thyroid. A single dose of TSH reduced the concentration of thyroid 5-HT and histamine concentrations within 15 min (Fig. 3). After 2 hr 5-HT was further reduced, whereas the histamine level was back to normal (Fig. 3). Thyroid mast cells exhibited decreased fluorescence reactions for histamine at 15 min and for 5-HT at both 15 min and 2 hr (Fig. 2c). A reduction of their metachromasia was seen at the 15-min but not at the two-hr interval. Mast cells outside the thyroid (in the trachea, the oe-

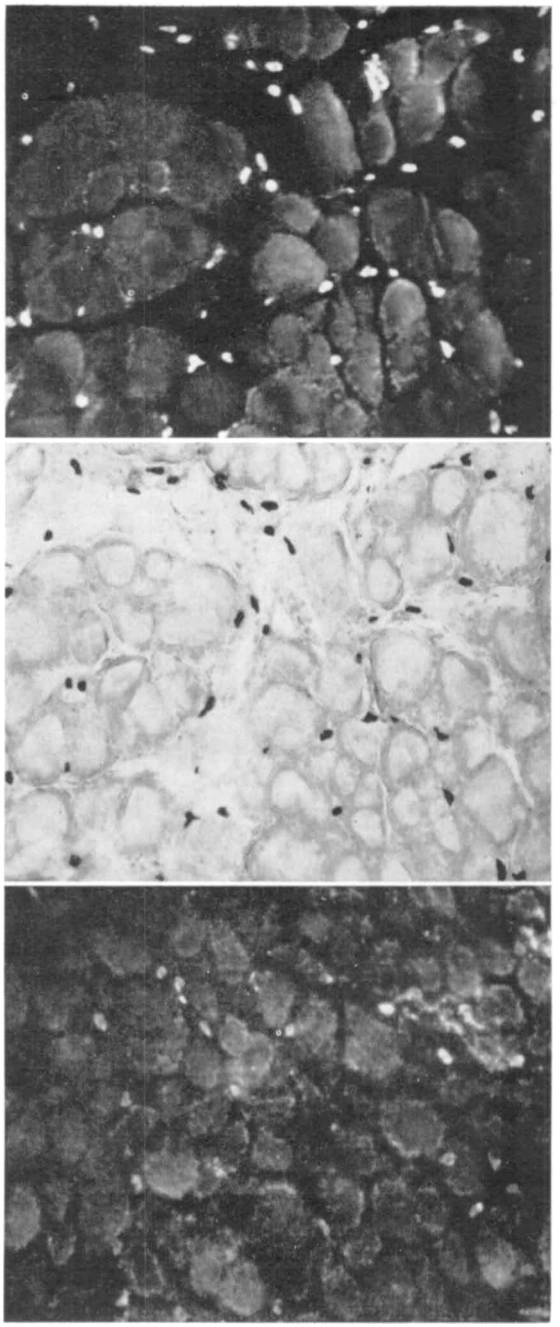


FIG. 2. Thyroid from T_4-pretreated rat. a) and b) control; c) 2 hr after TSH injection (500 mU/100 g iv).

a (top) Fluorescence photomicrograph showing formaldehyde-induced 5-HT-fluorescence in several cells among the follicles.

b (center) Subsequent toluidine-blue staining revealed that these cells were metachromatic, and thus identified as mast cells.

c (bottom) 2 hr after TSH injection the 5-HT-fluorescence intensity of the mast cells was reduced.

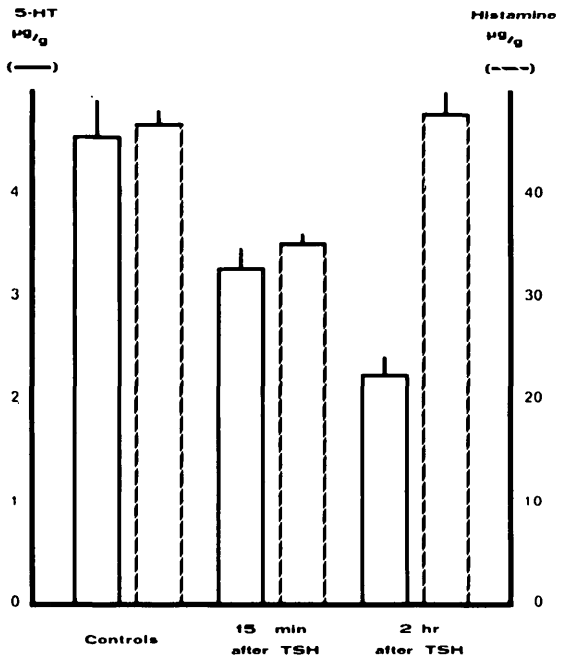


FIG. 3. Effect of a single TSH injection (500 mU/100 g iv) on the 5-HT and histamine concentrations in thyroids (5 per group) from T_4-pretreated rats. Means and SEM indicated. Significance of differences: 5-HT: 15 min vs. controls $p < 0.05$; 2 hr vs. controls $p < 0.001$; 2 hr vs. 15 min $p < 0.01$. Histamine: 15 min vs. controls $p < 0.001$; 2 hr vs. controls not significant.

sophagus, and the thyroid capsule) appeared unaffected by TSH treatment, as judged by both light and fluorescence microscopy.

Ultrastructurally, TSH evoked signs of endocytosis and formation of colloid droplets in the follicle cells. After 15 min microvilli and pseudopods were frequent, and colloid droplets were found in the apical regions (Fig. 4c). After 2 hr, colloid droplets had appeared also in the basal parts. Thyroid mast cells, however, seemed to be unaffected by TSH, whether located within the parenchyma or in the capsule (Fig. 4a–c); they all contained numerous electron-dense granules, and there were no signs of granule extrusion.

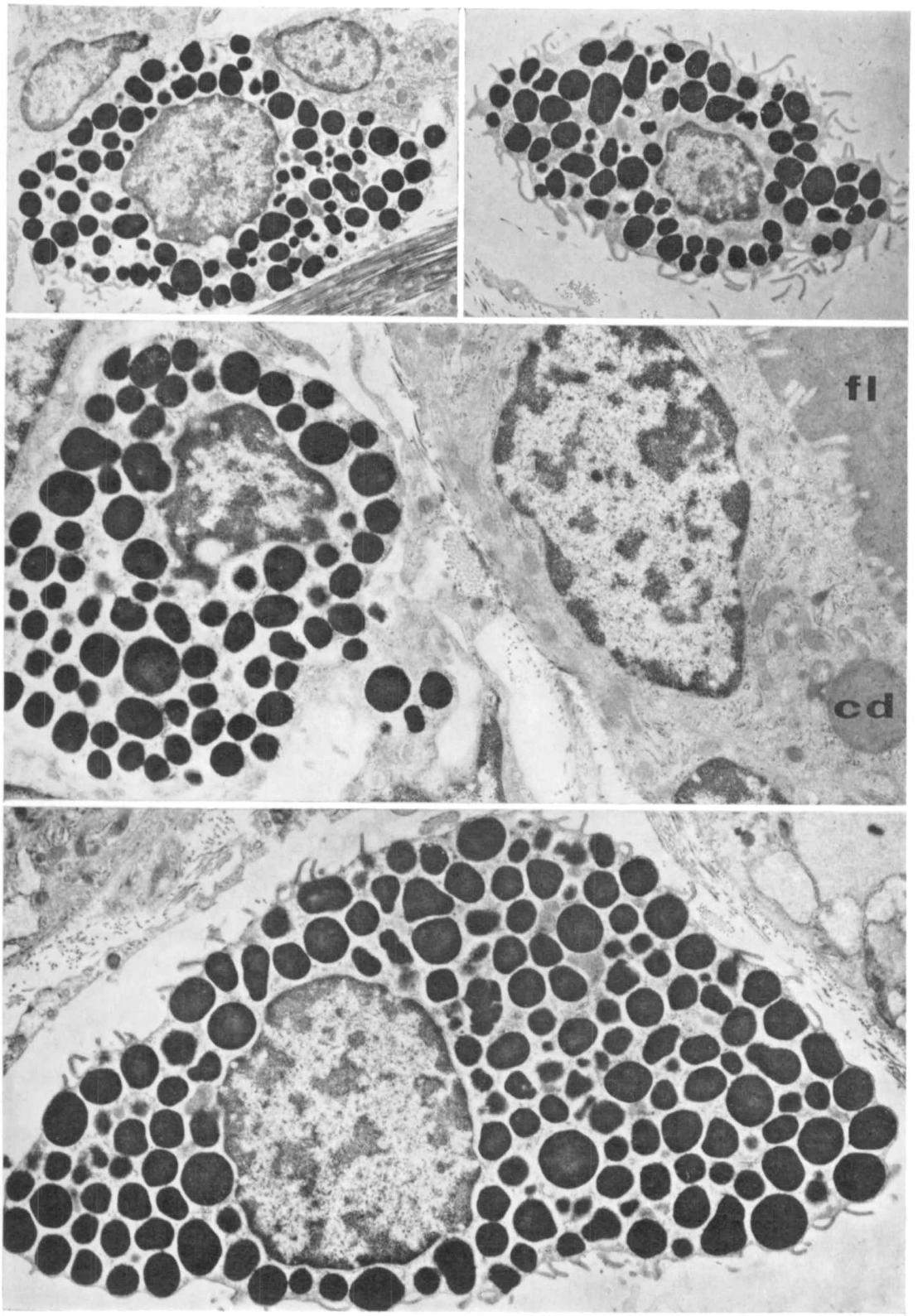
Chronic effects of TSH on thyroid mast cells. In rats kept on PTU and iodine-poor diet for four weeks, plasma TSH had increased almost ten-fold (Fig. 5). Their thyroids had an average weight of 54.3 ± 4.2 mg (mean ± SEM; n = 8), as compared to 13.7 ± 0.4 mg in controls ($p < 0.001$). As judged by light and fluorescence microscopy, the number of follicle cells and mast cells per visual field showed no gross change and their relative proportions were not overtly altered; *i.e.*, the total number of both cell types was increased. The total thyroid content of 5-HT and histamine was also increased ($p < 0.01$ and 0.02, respectively); however, the amine concentrations were reduced (Fig. 5). These reductions reflected a reduced amine content in individual thyroid mast cells, as judged by fluorescence microscopy. There were no histochemically visible alterations in mast cells outside the thyroid.

Ultrastructurally, no changes were observed in thyroid mast cells (Fig. 4d), whereas signs of activation, such as an increase in cell size, endoplasmic reticulum and number of mitochondria, were evident in the follicle cells.

Discussion

A single injection of TSH reduced thyroid 5-HT and histamine concentrations in T_4-pretreated rats. The level of 5-HT was reduced for a longer period than was that of histamine, which returned to normal within 2 hr. The rapid changes indicate a release of the amines, and fluorescence histochemistry revealed that this release occurred from in-

FIG. 4. Electron micrographs of rat thyroids. *a-c*: T_4-pretreated animals; *d*: treatment with PTU. *a*) (top *left*, ×5,000): Control interfollicular mast cell with typical dense granules. *b*) (*top right*, ×5,000): Mast cell located in the thyroid capsule 15 min after iv injection of TSH (500 mU/100 g). Same morphology as in interfollicular mast cells (*a* and *c*). *c*) (*center*, ×11,000): 15 min after TSH (500 mU/100 g iv). Interfollicular mast cell without signs of granule extrusion. Follicular cell with an apical colloid droplet (cd). Follicular lumen (fl) to the right. *d*) (*bottom*, ×6,000): Four weeks on PTU. Interfollicular mast cell with a morphology similar to that of mast cells in the other groups.



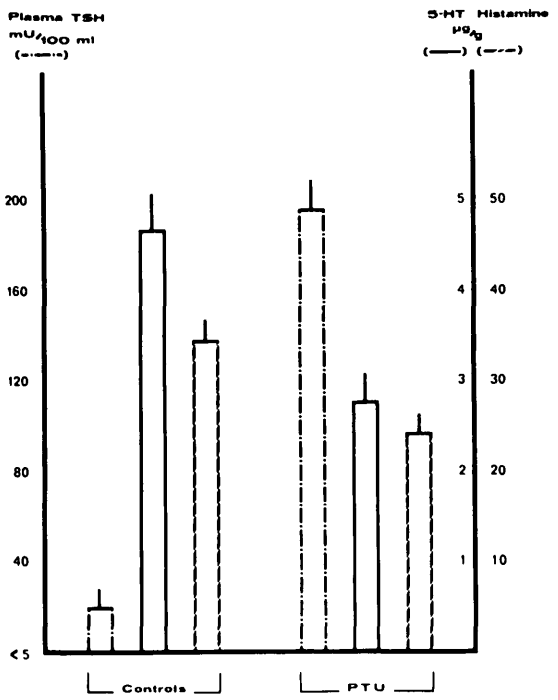


FIG. 5. Relation of plasma TSH levels (mU/100 ml) and thyroid concentrations of 5-HT and histamine (μg/g thyroid tissue) in rats. Eight control animals, eight kept on PTU and iodine-poor diet for 4 weeks. Significance of differences between controls and PTU-treated animals: plasma TSH $p < 0.001$, 5-HT $p < 0.001$, histamine $p < 0.01$.

trathyroidal mast cells. This was accompanied by a reduction in the metachromasia of these cells, implying that heparin was also released (24). During treatment with PTU and iodine-poor diet, plasma TSH levels were raised about ten-fold, and hyperplasia of both follicular cells and mast cells in the thyroid developed. At the same time the amounts of 5-HT and histamine in individual thyroid mast cells were reduced. In extrathyroidal mast cells, including those in the thyroid capsule, no changes were recorded whether upon acute or chronic exposure to TSH. As judged by electron microscopy, the TSH-induced release of 5-HT and histamine was not associated with any extrusion of granules; nor was any other ultrastructural change noted in the mast cells. The follicle cells, on the other hand, were activated, as evidenced by the development of pseudopods and intracellular colloid droplets. On the basis of these findings, it is concluded that, in the rat thyroid, TSH stimulates not only the formation of follicle cells but also the appearance of mast cells (*cf* 10), and induces both release of hormone from follicle cells and release of 5-HT, histamine and, possibly, heparin from mast cells. This amine release, which is restricted to mast cells within the thyroid, seems to take place without any concomitant degranulation. In mice, hypersecretion of TSH induces the appearance of intrathyroidal mast cells and also stimulates the release of amines from the mast cells (7). It follows that activation of thyroid mast cells constitutes part of the thyroid's reaction to TSH. Conceivably, thyroid mast cells are involved in the regulation of thyroid function.

Clayton and co-workers have previously shown that exogenous TSH rapidly mobilizes 5-HT from intrathyroidal mast cells (5,6). They postulated that 5-HT thus released is responsible for the increase in thyroid blood flow which is part of the glandular response to TSH (5,6). Hoffman and Levey (13) suggested that histamine might be the cause of the TSH-induced thyroid hyperemia since PTU treatment resulted in a marked reduction of thyroid histamine. From the present study, showing a TSH-induced release of 5-HT, histamine and, possibly, heparin, it follows that the nature of the mediator(s) of the TSH-induced increase in thyroid blood flow is still an open question.

The functional significance of thyroid mast cells need not be restricted to actions on thyroid circulation (*cf* 5,6). Depletion of thyroid mast cells by compound 48/80 is accompanied first by secretion of thyroid hormone, and subsequently by impairment of the thyroid secretory response to TSH (25). 5-HT, but not histamine, stimulates release (1,2) and synthesis (3,4, and unpublished) of thyroid hormone by a direct effect on the follicle cells. Moreover, thyroid mast cells are rich in an alkaline protease

which can hydrolyze thyroglobulin (14). Thus, thyroid mast cells may be involved in the regulation of thyroid function by multiple actions.

Acknowledgments

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Regulation of the Adipose Tissue Mass (Proceedings to be published by Excerpta Medica Foundation)

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