Cytological Effects of TSH on the Thyroid of Hypophysectomized Rats with and without Previous Administration of Actinomycin D. An Electron Microscope Study

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The present paper is concerned mainly with two problems: the fine structural changes of the thyroid gland after hypophysectomy and the inhibition of the endocytotic effect of TSH by actinomycin D.

It has been well known that the thyroid gland is stimulated by the TSH secreted from the adenohypophysis. Numerous papers have been published dealing with the electron microscopy of the thyroid gland following the injection of TSH (Wissig 1963, Fujita 1963, 1968, Sheldon et al. 1964, Stein and Gross 1964, Wetzel et al. 1965, Seljelid 1967 a, b, c, etc.). However, as to the fine structure of the thyroid after hypophysectomy, information is scarce except for the papers of Dempsey and Peterson (1955), Kurosumi (1964, personal communication), Wetzel et al. (1965), Seljelid (1967), and Schwarz (1967); finer pictures and more detailed descriptions seem needed.

Numerous authors have reported that large droplets appear in the apical part of the follicular cell from 5 minutes to several hours after injection of TSH. These large droplets have recently been regarded as an endocytotic reabsorbed colloid by autoradiographic and microinjection methods (Sheldon et al. 1964, Stein and Gross 1964, Bauer and Meyer 1964, Ekholm and Smeds 1966, Seljelid 1967 a, b, Fujita 1968). The present authors who have studied the effect of TSH and actinomycin D on the thyroid gland of the hypophysectomized rat, noticed by chance that the endocytotic action of TSH is inhibited by actinomycin D.

Materials and methods

Thirty six male rats weighing about 150–200 g were used for this study. Three normal animals served as control. Twenty animals were hypophysectomized by the transauditory method (Koyama 1962). Nine animals were separated into three groups and each group was sacrificed 10 days, 20–21 days and 30 days after hypophysectomy.

Twelve of the hypophysectomized animals 20–21 days after the operation were given a single injection of 300–600 µ of actinomycin D (Lederle, 3008 A–30 B) intraperitoneally 4–10 hrs before sacrifice. Eleven hypophysectomized animals treated with actinomycin D and eleven hypophysectomized animals free of actinomycin D
were singly injected with 5 mg (about 15 USP units) of TSH (NIH-TSH-B3) intraperitoneally. The animals were sacrificed 30 min., 1, 2 or 4 hrs after the TSH injection. In addition, two hypophysectomized animals actinomycin D-treated and actinomycin D-free, received double injections of 5 mg (about 17 USP units) and 2 mg (about 7 USP units) of TSH (NIH-bovine-B2), 4 hrs and 2 hrs before sacrifice. The thyroid glands removed were fixed for 2 hrs in a 2% osmium tetroxide solution buffered with s-Collidine to pH 7.4, or in a mixture of 1 part of 4% osmium tetroxide solution, 2 parts of 0.2M cacodylate buffer of pH 7.4 and 1 part of 10% glutaraldehyde solution. After fixation the tissues were dehydrated with graded concentrations of alcohol, and embedded in Epon epoxy resin. Sections, cut on a Porter-Blum ultramicrotome and stained with Millonig's lead, were examined in a Hitachi HS-7 type electron microscope.

**Observations**

1. **Thyroid of normal and hypophysectomized rats**

The fine structure of the normal mammalian thyroid has been reported by numerous authors (Wissig 1960, 1964, Heimann 1966, Lupulescu and Petrovici 1968, etc.) and will not be described in detail in the present paper. The thyroid cell of a normal rat is characterized by a well developed rough endoplasmic reticulum with its relatively enlarged cisternae (Fig. 1). Mitochondria are distributed throughout the cytoplasm, especially in the ergastoplasmic region. The Golgi apparatus is usually located at the supranuclear region. Several kinds of granules, large pale or dense, small dense, and small low-dense, are located in the apical part of the cytoplasm, as described in previous papers. At the basal part of the follicle cells or among the follicle tissues, parafollicular cells are located singly or in small groups. A few small dense granules about 200-500 m\(\mu\) in diameter are scattered in the cytoplasm.

Ten to thirty days after hypophysectomy, most follicle lumens became larger and all the thyroid follicular cells were markedly attenuated, especially at the peripheral cytoplasmic region (Fig. 2, 3). The height of the cell was about 0.5-2 \(\mu\) at the peripheral cytoplasmic region and 2-5 \(\mu\) at the perinuclear region, while that of the normal cell about 5-20 \(\mu\) and almost similar at both regions. At the basis of the remarkably thin cytoplasm, there was a large bay, where sometimes a blood capillary or connective tissue cell was located (Fig. 2). The nucleus became flattened and the cytoplasm appeared compact with a marked decrease of the cytomembrane system. In most cells, elements of the rough endoplasmic reticulum were almost entirely lost and the remainder became smaller and flattened (Fig. 2, 3). The mitochondria were fairly well preserved in the cytoplasm, though they seemed to be somewhat smaller and round or oval as compared with the longer ones of the normal thyroid cell. The Golgi apparatus located in the supranuclear region was also markedly reduced in size. The Golgi vacuoles disappeared and only a few small lamellar structures and vesicles remained in this area. The cytoplasmic matrix looked relatively dark, and numerous free ribosomes were distributed. Most cytoplasmic granules and droplets disappeared, and only a few small lysosome-like dense bodies were sometimes seen (Table 1). The microvilli were relatively well preserved at
Fig. 1. A part of a normal thyroid follicular cell of a rat. Well developed rough endoplasmic reticulum and a large droplet (D) are seen. N nucleus, L follicle lumen, G Golgi apparatus. ×16,000
Fig. 2. Part of thyroid follicular cells (F), endothelial cells (E), and interfollicular connective tissue (S) in the 20 day-hypophysectomized rat. The cytomembranes are markedly reduced in number and size, and the free ribosomes are distributed throughout the scanty cytoplasm of the attenuated cell. L follicular lumen. ×17,000
Fig. 3. Part of three flattened follicular cells in the 20 day-hypophysectomized rat. *F* follicular cell, *L* follicle lumen, *S* connective tissue space, *E* capillary endothelium. ×17,000
the free surface, though their population seemed somewhat lower than that in the normal cell. No conspicuous fine structural changes were observed in the parafollicular cell as reported by Matsuzawa (1966) and Schwarz (1967).

2. Effect of TSH on hypophysectomized rats (Fig. 4-6)

Several large (0.5-4 μ) low-dense droplets appeared in most thyroid cells of the hypophysectomized rats, 30 min., 1, 2 and 4 hrs after the injection of TSH.

Fig. 4. Part of thyroid follicular cells in the 20 day-hypophysectomized rat 4 hrs after an injection of 5 mg (about 15 USP units) of TSH (NIH-bovine-B3). Notice large droplets (D) in the pseudopod and cytoplasm. L follicle lumen, C capillary lumen. ×16,000
Sometimes large pseudopods containing large pale droplets suggesting the endocytosis of the follicle colloid were noticed (Fig. 4). These droplets were very few in the animals 30 min—1 hr after the injection of TSH and tended to increase in number for several hrs. The reaction to TSH seemed to occur somewhat later in the hypophysectomized rat as compared with that in the normal animals. Numerous large droplet appeared especially in the rats given double injections of TSH, 4 and 2 hrs before sacrifice (Fig. 6). Small lysosome like dense bodies were also noticed near the large droplets. Elements of the rough endoplasmic reticulum of most cells, which were very poor in the hypophysectomized rat, showed somewhat enlarged cisternal structures 2–4 hrs after the injection of TSH. However, the well developed reticular structures with numerous cisternal elements which are usually seen in normal rats, were not recognized in these experimental animals. The Golgi apparatus also became relatively large and the smooth-surfaced vacuolar elements

**Fig. 5.** Part of thyroid follicular cells in the 20 day-hypophysectomized rat treated with 5 mg (about 15 USP units) of TSH 4 hrs before sacrifice. Notice low-dense and moderately dense large colloid droplets (D) in the cytoplasm. *L* follicular lumen, *C* capillary lumen. ×11,000
Fig. 6. A part of a thyroid follicular cell in the 21 day-hypophysectomized rat given two injections of 5 mg (about 17.5 USP units) and 2 mg (about 7 units) of TSH (NIH-bovine B2) 4 and 2 hrs before sacrifice. Two large and several small droplets are seen in the cytoplasm. $L$ follicle lumen, $D$ large droplet. $\times 21,000$
Fig. 7. Part of thyroid follicular cells and a blood capillary (C) in the 20 day-hypophysectomized rat treated with 600 μg of actinomycin D 8 hrs before sacrifice and with 5 mg (about 15 USP units) of TSH 4 hrs prior to fixation. Notice the flattened cytoplasm with poorly developed cytomembranes and a few granules. No large droplets are seen. L follicle lumen. ×14,000
Fig. 8. Part of thyroid follicular cells and interfollicular connective tissue in the same experimental animal as Figure 7. Rough endoplasmic reticulum is poorly developed and no large droplets are seen. Notice the small Golgi apparatus (G), and lysosome-like dense bodies (B). L follicle lumen. ×14,000
appeared 2–4 hrs after the injection of TSH. Small vesicles 0.1–0.3 μ in diameter were markedly increased and were located near the Golgi field and in the subapical region.

3. Effect of actinomycin D and TSH on the thyroid of hypophysectomized rats (Fig. 7, 8).

The large droplets which appeared in the thyroid follicular cell of the TSH-treated animals were hardly recognized in the actinomycin D-treated animals, 30 min, 1, 2 and 4 hrs after TSH injection.

Table 1. The number of cytoplasmic droplets and subapical vesicles (except for Golgi vesicles) in the thyroid follicular cell of experimental animals (Number per 100 cell sections cut through the plane showing the large nucleus)

<table>
<thead>
<tr>
<th></th>
<th>Round or oval colloid droplets and small low-dense vesicles (Long diameter) (μ)</th>
<th>Large irregularly sized droplets</th>
<th>Lysosome-like small dense bodies</th>
<th>Endocytotic figures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1–0.3/0.3–1.0/1.0–2.0/2.0–3.0/3.0–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypophysectomized*</td>
<td>9/0/0/0/0/0/136/0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypophysectomized +TSH**</td>
<td>839/103/85/31/5/90/13/</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypophysectomized +act. D+TSH***</td>
<td>478/19/2/1/0/3/238/0</td>
<td></td>
<td></td>
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</tr>
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</table>

In each experimental animal, about 100 cell sections were examined.

* Rat 20 days after hypophysectomy
** Hypophysectomized rat treated with 5 mg (about 15 USP units) of TSH (NIH-bovine-B3) 4 hrs before sacrifice.
*** Hypophysectomized rat treated with 600 γ of actinomycin D 8 hrs before sacrifice and with 5 mg of TSH 4 hrs before sacrifice.

Table 1 shows as a sample the number of the cytoplasmic droplets, granules and vesicles per 100 follicular cells in normal and experimental animals. From these data, it is clear that the large droplets, which are considered to be endocytosed from the follicular colloid, are conspicuously decreased in number in the experimental group treated with actinomycin D. Pseudopods suggesting the endocytosis of the follicle colloid were also hardly seen. Small subapical vesicles 0.1–0.3 μ in diameter were also reduced in number in the follicular cell of actinomycin D- and TSH-treated rats, as compared with that of TSH-treated animals.

The endoplasmic reticulum was flattened and was not as well developed in the actinomycin D-treated, hypophysectomized animals after TSH injection, as those of hypophysectomized animals without any treatment. The Golgi apparatus was also small in size and lacking in large vacuoles. Free ribosomes were distributed throughout the cytoplasmic matrix.

Discussion

It has been generally reported by numerous biochemical papers, that TSH stimulates the follicular cell in accelerating the uptake and organization of iodine,
reabsorption and hydrolysis of colloid droplet, and synthesis of thyroglobulin. Hence, it is easily understood that the follicular cell is attenuated and its cytomembrane becomes very poor in the thyroid follicular cell of the hypophysectomized rat. KUROSUMI (1964) and SCHWARZ (1967) also observed a decrease in number of the cytoplasmic granules and droplets in the apical region of the cell and reduction of the Golgi apparatus in size. However, SCHWARZ (1967) who observed the thyroid gland of the rat 6–10 days after hypophysectomy recognized a considerable number of large droplets in the basal region of the cell. This fact suggests that it takes a fairly long time to hydrolyse completely the reabsorbed colloid in the hypophysectomized rat. However, we could not find any colloid droplets in the follicular cell 10–30 days after hypophysectomy. The reduction in size or disappearance of the rough endoplasmic reticulum and of the Golgi apparatus suggests the cessation of the thyroglobulin synthesis in the follicular cell. Recently, it has been believed that the large droplet is not a secretory substance but a reabsorbed colloid as described in the introduction. The disappearance of the large droplets in the apical cytoplasm should mean the stop of the reabsorption of the follicle colloid. From this viewpoint, it is considered that the thyroid gland ceases its function almost entirely in the hypophysectomized rat.

SELJELID (1967 a) reported, in the thyroid suppressed for a few days by thyroxine-treatment or hypophysectomy, that TSH injections lead to the reappearance of colloid droplets in 5 min, and the recovery of the follicular cell to its normal appearance in 60–120 min. However, in the long term-hypophysectomized rat in the present study the thyroid did not react so markedly. This fact suggested that the sensitivity to TSH becomes lower in the long term suppressed thyroid.

As actinomycin D has been considered to act on the nuclear DNA and inhibit the messenger RNA formation (REICH et al. 1962, GOLDBERG and RALINOWITZ 1962, HURWITZ et al. 1962, REICH et al. 1962, et al.), it is easy to expect the thyroglobulin synthesis to be blocked by the treatment of this agent. The decrease in number of small subapical vesicles in the actinomycin D- and TSH-treated rats may suggest the block of synthesis of thyroglobulin, since the small subapical vesicles have been regarded as secretory substances containing thyroglobulin by NADLER et al. (1964). In addition, in the actinomycin D- and TSH-treated rat, large droplets are hardly recognized in the thyroid follicular cell. It is considered that actinomycin D inhibits the action of TSH in reabsorption of the colloid by endocytosis. Several possibilities are speculated as to the mechanism of this phenomenon as follows.

1. GREENSPAN and HARGADINE (1965) reported using the antibody fluorescence method that TSH administered intravascularly or applied directly on tissues in vitro are demonstrated in a high concentration within the nucleus of the thyroid cell of the dog and guinea pig. TSH may possibly stimulate the nucleus to synthesize messenger RNA, which might act on the cytoplasm to produce enzymes necessary for reabsorption of the colloid. Actinomycin D, then, by inhibiting the synthesis of that messenger RNA, could block the endocytosis in the thyroid follicular cell.

2. TSH might stimulate the nucleus to synthesize the messenger RNA. The messenger RNA might act on the cytoplasm to produce the cell membrane necessary for endocytosis. Then, actinomycin D, by inhibiting the synthesis of that messenger RNA, might block the endocytosis.
3. Messenger RNA is necessary for the formation of the cytoplasmic structural protein of the follicular cell cytoplasm which was reduced in quantity by hypophysectomy. Actinomycin D, by blocking the synthesis of this messenger RNA, would suppress the formation of the structural protein and the vital activity of the thyroid follicular cell. Thus, the endocytic activity induced by the TSH-treatment should also be inhibited. If so, the inhibition of the endocytosis is not a specific reaction caused by actinomycin D.

4. The actinomycin D might inhibit directly the endocytosis without passing through the nucleus.

The possibility of (4) is considered to be very low from general biochemical knowledge on actinomycin D. It is a problem to be solved in the future as to how actinomycin D inhibits the effect of TSH on the reabsorption of the colloid.

Summary

Electron microscopic studies were made of thyroid glands from 10–30 day-hypophysectomized rats, 20–21 day-hypophysectomized rats treated with TSH 30 min–4 hrs before sacrifice, and 20–21 day-hypophysectomized rats treated with actinomycin D 4–10 hrs before sacrifice and with TSH 30 min–4 hrs prior to fixation.

1. In the hypophysectomized rats the thyroid follicular cells were markedly attenuated and conspicuously reduced in size of their rough endoplasmic reticulum and Golgi apparatus. Most cytoplasmic small vesicles and colloid droplets had disappeared except for a few lysosome-like dense bodies. No fine structural changes were recognized in the parafollicular cell after hypophysectomy.

2. Some elements of the rough endoplasmic reticulum showed simple (but not reticular) enlarged cisternal structures and the small vesicles were increased markedly 2–4 hours after injection of TSH in the hypophysectomized rats, while in the hypophysectomized animals treated with actinomycin D the rough endoplasmic reticulum remains flattened and small vesicles were smaller in number after TSH injection.

3. Cytoplasmic large colloid droplets appeared in the hypophysectomized thyroid cell 30 min after injection of TSH, and were increased in number with time. However, these droplets were hardly recognized in the rat treated with actinomycin D and TSH. Actinomycin D is considered to inhibit the endocytic activity of the follicular cell induced by TSH-injection; the possible mechanisms of this effect were discussed.

Addendum

Since this paper was submitted for publication, the present authors have read an interesting paper by Drs. Ehkholm and Elovqvist, dealing with the inhibition of endocytosis in the thyroid follicle cells by actinomycin D in the Experimental cell Research 48: 640–643 (1967). We would like to express our agreement with Drs. Ehkholm and Elovqvist.
アクチノマイシン D 投与および非投与下の、下垂体摘出ラットに対する TSH の影響（内容自抄）

1. 下垂体摘出後のラットの甲状腺機能上皮細胞は極端に扁平になり、粗面小胞体とグリル装置ははなはだしく減少し、細胞内のコロイド滴（droplet）や電子密度の低い小顆粒（small vesicles）はほとんど消失する。下垂体摘出ラットの甲状腺の腺泡細胞の微細構造は、正常の場合と大差がなかった。

2. TSH 投与後 2 〜 4 時間の下垂体摘出ラットの甲状腺機能上皮細胞の粗面小胞体はやや拡張し、電子密度の低い小顆粒（small vesicles）が出現するが、アクチノマイシン D で処理の後に TSH を投与した群では、粗面小胞体は扁平で、小顆粒の数は少ない。

3. TSH 注射後30分で下垂体摘出ラットの甲状腺機能上皮細胞内に大きなコロイド滴が出現し、時間と共に数を増す。しかしアクチノマイシン D の処理後 TSH を与えたものでは、大きなコロイド滴はほとんど出現しない。このことから、アクチノマイシン D によって起こされるコロイドの取込み（endocytic activity）を阻害すると考えられる。

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