RESPONSE OF THE PROTEIN-SYNTHESIZING APPARATUS OF THE CHIEF CELLS OF THE RAT STOMACH TO THYROXINE

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Thyroid hormones have a marked effect on various forms of protein biosynthesis [2-4, 11]. There have been reports of the effect of thyroid hormones on protein synthesis in the liver, myocardium, and muscles [1, 2, 5]. According to data in the literature [1-5], the tissues of these organs are hormonally sensitive, whereas the lungs, brain and spleen are insensitive. The response of the stomach to thyroid hormones was not studied by the workers cited above.

Our previous investigations [7, 8] showed that the main point of application of thyroxine in the parietal cells at the organelle level is the mitochondria. The aim of this investigation was to study the response of the protein-synthesizing apparatus of the chief cells of the stomach.

EXPERIMENTAL METHOD

Experiments were carried out on 46 male Wistar rats weighing 180-200 g. The control group consisted of 16 rats; the 16 rats of group 1 received thyroxine by intraperitoneal injection in a dose of 2.5 mg/kg daily for 10 days, and the 14 animals of group 2 received the hormone by the scheme mentioned above for 30 days. This scheme of production of thyrotoxicosis caused the animals to lose weight (from 17 to 24%) and gave rise to marked tachycardia, with an average increase in heart rate by 150 beats/min; particular attention was paid to maximal standardization of the diet, management of the animals, and the time of sacrifice. The stomach was fixed in Carnoy's fluid. Paraffin sections 6 μ thick were stained with gallocyanin and chrome alum by Einarson's method for RNA determination, followed by cytophotometry. Electron-microscopic analysis and subsequent morphometry were carried out by the method in [8]. Protein biosynthesis was judged from incorporation of the specific protein precursor ^{14}C-leucine into total proteins of the homogenate of the gastric mucosa (specific radioactivity 24.7 mCi/mmole), which was injected intraperitoneally at the 5th minute in a dose of 6 μCi/g. The protein residue obtained after treatment of the homogenate with 10% TCA was concentrated on membrane filters (Aufs, Synpor, Czechoslovakia). Radioactivity of the residue was measured on a Mark II liquid scintillation counter (Nuclear Chicago, USA), using a universal toluene scintillator. The counting effectiveness 86%.

To study protein biosynthesis and the total RNA content the radioactivity of samples of homogenate of gastric mucosa obtained 40 min after intraperitoneal injection of 0.2 μCi/g of ^{14}C-orotic acid (specific radioactivity 15 mCi/mmole) was measured. Incorporation of label into protein and RNA (specific radioactivity) was calculated relative to the total protein and RNA content [9] in the gastric mucosa.

EXPERIMENTAL RESULTS

Changes in ultrastructure of the chief cells differed depending on the duration of administration of the hormone. In the early stages of hyperthyroidism, most of the cells were in an enhanced functional state. This was confirmed by the fact that the area of the membranes of the rough endoplasmic reticulum in the basal part had increased almost threefold (Table 1). In the nuclei the volume of condensed chromatin was reduced, evidence of

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TABLE 1. Results of Morphometric Analysis of Chief Cells of Gastric Mucosa after Administration of Thyroxine to Rats

Parameter studied	Control	Administration of thyroxine for	
		10 days	30 days
S_{vi}^{rer}	6,21±0,19	17,94±0,12*	3,92±0,12*
N_{vi}^{br}	105,7±6,15	131,4±4,83*	44,5±3,71*
N_{vi}^{fr}	29,8±1,18	48,4±1,12*	18,4±1,19*

Legend. S_{vi}^{rer}) Surface area membranes of RER, N_{vi}^{br}) number of bound ribosomes (in $\mu^2/3$), N_{vi}^{fr}) Number of free ribosomes and polysomes (in $\mu^2/3$). *$P < 0.05$ compared with control.

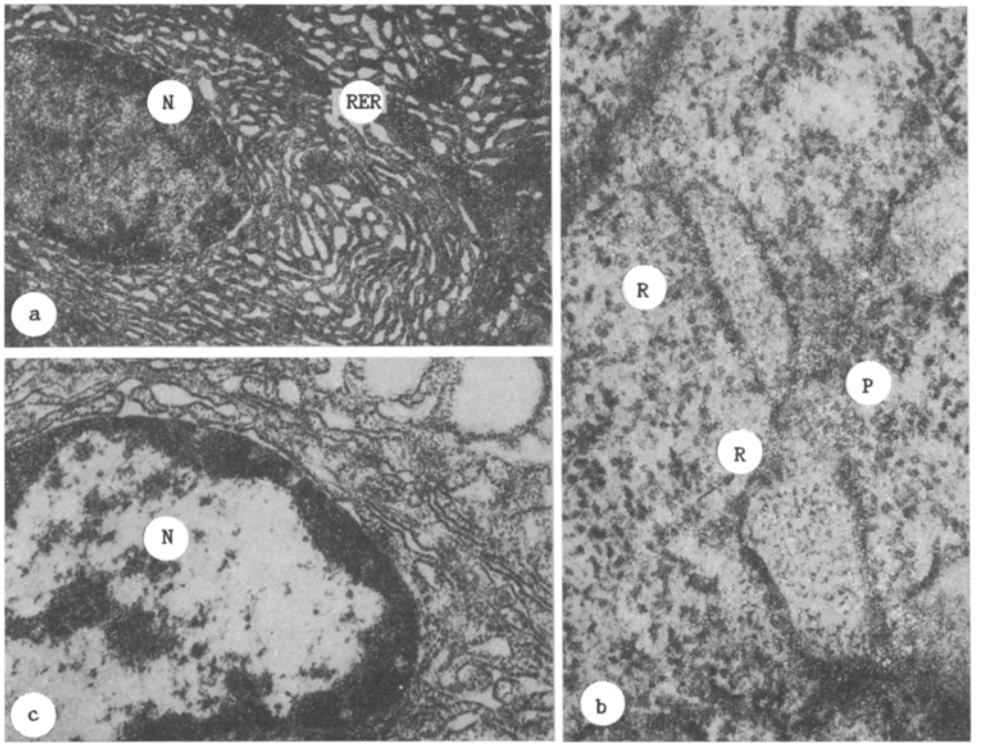

Fig. 1. Ultrastructure of chief cell in rat stomach: a) administration of thyroxine for 10 days (18,000×): membranes of RER well developed, nucleonemal part of nucleoli is clearly defined, fibrillary zones are small; b) administration of thyroxine for 10 days (45,000×): number of bound and free ribosomes (R) and polysomes (P) is increased; c) administration of thyroxine for 30 days (15,700×): heterochromatin of nucleus (N) arranged in clumps both at the periphery and at the center of the nucleus.

activation of transcription of the genome. Evidently as a result of this the number of free and attached ribosomes was increased (Table 1, Fig. 1). The RNA content in the chief cells, as shown by cytophotometry, was increased by 50% (Fig. 2). After administration of thyroxine for 10 days incorporation of labeled orotic acid into total RNA of the gastric mucosa and of labeled leucine into total protein were increased, which may indicate stimulation of its metabolism. It is considered [1, 6] that thyroid hormones interact with nuclear chromatin and activate definite operons, thereby increasing the rate of formation of mRNA, which is then transported from the nucleus into the cytoplasm, where it takes part in protein

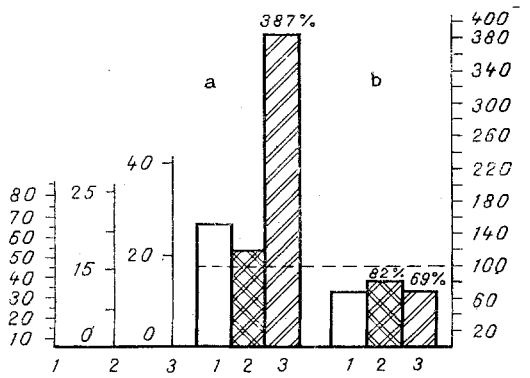

Fig. 2. Effect of thyroxine administered for 10 days (a) and 30 days (b) on RNA content in gastric chief cells (in optical density units), and incorporation of leucine into total protein (in cpm × 10^3/mg protein) and of orotic acid into total RNA (in cmp × 10^3/mg RNA) of gastric mucosa of rats: 1) RNA content in chief cells of stomach; 2, 3) incorporation of leucine into total protein and of orotic acid into RNA respectively.

synthesis. Comparison of the data of ultrastructural and biochemical analysis revealed definite correlation between the structure of the nucleoli and the biosynthetic activity of the secretory cells. Nucleoli of the chief cells, where intensive RNA synthesis takes place, had the typical structure: their nucleonemal part was well defined, they were rich in granules, and the fibrillary zones were small and could not be distinguished as separate regions. The nuclei in such cells were homogeneous (Fig. 1). After administration of thyroxine for 20 days synthetic processes were distinctly depressed in the chief cells of the stomach. The surface area of the membranes of the rough endoplasmic reticulum (RER) in the basal part of the chief cells was reduced by 1.6 times. The number of bound (by half) and free (by 1.6 times) ribosomes was reduced (Table 1) and the RNA content, as shown by cytophotometry, was reduced to 68% (Fig. 2).

According to the results of the biochemical investigations prolonged thyroxine administration was accompanied by a reduction in the incorporation both of leucine into total protein and of orotic acid into total RNA (Fig. 2). Nuclei of the chief cells of the stomach were completely different in structure after administration of thyroxine for 30 days. The chromatin in them was distributed in the form of clumps both at the periphery and in the center of the nucleus, and in individual cells heterochromatin was present in the form of large clumps with vacuoles, which occupied nearly the whole volume of the nucleus. As a result the nucleus came to resemble an osmiophilic mass of high electron density. The strongly osmiophilic nucleoli did not possess a nucleonemal structure. They were dense, RNA-containing formations (Fig. 1c), consisting entirely of closely arranged thin fibrils. This transition of the nucleoli from the normal to the compressed, fibrillary type, according to data in the literature [10], is observed when rRNA synthesis declines.

Thyroxine (early periods of administration), which induces synthesis of specific mRNA and rRNA, and facilitates their translation, by acting on many stages in the chain of protein biosynthesis can not only regulate the quantity of proteins but also, as the writers stated previously [7, 9], it can modify enzyme activity in the chief cells of the stomach through a change in the cAMP concentration, which is accompanied by increased proteolytic activity of the gastric juice. Enhanced function of the chief cells, uncompensated by a corresponidng intensification of bioenergetic and biosynthetic processes, undoubtedly causes damage to these cells with the development of destructive changes, and leads ultimately to reduction of the proteolytic activity of the gastric juice.

Specific features of the action of thyroxine on organelles of gastric secretory cells examined above led to the conclusion that, whereas the point of application of the hormone is the parietal cells at the organelle level in the mitochondria [8], in the chief cells the most important changes take place in the protein-synthesizing apparatus itself of the cell.

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