Peptide ligands of opiate receptors can thus exert an inhibitory action on activity of the SAS. Considering data showing elevation of the opioid peptide level in the body in extremal situations [8], it can be postulated that, through depression of function of the SAS, they can limit the pathogenic action of stress developing under conditions of hyperergia. Further arguments in support of this hypothesis may be given by the existence of opiate receptors in the adrenals [10] and inhibition of NA release from sympathetic neurons under the influence of opioid neuropeptides [7].

Normalization of certain constants of homeostatis in rats with AMI following their injection with enkephalins may partially explain the weakening of the cardiotoxic action of an excess of adrenomimetics, which the writers demonstrated previously [3].

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STRUCTURAL CHANGES IN THE GASTRIC MUCOSA IN EXPERIMENTAL HYPERTHYROIDISM

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The secretory function of the stomach is known to depend on the functional state of the endocrine glands [1, 4, 11]. Data on the character of the digestive disorders arising in patients with thyrotoxicosis, obtained by different investigators, are highly contradictory. Some workers have observed reduced proteolytic activity of the gastric juice in patients with toxic goiter [10, 12], whereas others found increased activity [13].

The essentially contradictory nature of different items of information about disturbances of the structure of the gastric mucosa in thyrotoxicosis, which cannot yet be explained, has led to a search for objective criteria for evaluating the morphological changes in this disease.

The aim of the present investigation was accordingly to study the morphological substrate lying at the basis of the disturbed pepsin-forming function of the stomach in rats with experimental hyperthyroidism.

EXPERIMENTAL METHOD

Experiments were carried out on mature male Wistar rats weighing 180-200 g. Thyroxine (from Reanal, Hungary) was injected intraperitoneally into the animals in doses of 0.1 mg/kg (small) and 2.5 mg/kg (large)

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TABLE 1. Secretion of Gastric Juice in Rats Receiving Thyroxine in Different Doses

Experimental conditions	Parameter studied			
	proteolytic activity, mg% hydrolyzed per minute	P	pepsin output, mg/min	P
Control	3,29±0,10		12,54±0,52	
Thyroxine (0,1 mg/kg):				
10 days	5,68±0,17	0,001	29,07±0,68	0,001
20 days	3,51±0,21	0,1	11,55±0,37	0,1
30 days	2,50±0,29	0,05	7,25±0,24	0,001
Control	3,48±0,19		11,55±0,64	
Thyroxine (2,5 mg/kg):				
10 days	4,68±0,17	0,001	29,07±0,68	0,001
20 days	2,54±0,39	0,1	12,11±1,91	0,1
30 days	2,44±0,15	0,01	6,07±0,9	0,001

daily for 10, 20, and 30 days. At each stage of the experiment 24 animals were used. For each experimental group there were nine control animals, kept under identical conditions and receiving intraperitoneal injections of the corresponding volume of solvent (0.05 N KOH) at the same times. Each series was divided into two groups: the stomachs of one group underwent histochemical and electron-microscopic study, whereas in the second group the gastric juice, collected through a fistula from the rats 10 days after formation of the fistula, was studied. Proteolytic activity was determined by a microexpress method [3].

The following oxidoreductases were determined histochemically: succinate dehydrogenase (SDH), NADH dehydrogenase (NADH-DH), and lactate dehydrogenase (LDH) [6]. Paraffin sections 6 μ thick were stained with gallocyanin and chrome alum by Einarson's method to determine RNA. An integrating cytospectrophotometer (Institute of Morphology, Academy of Medical Sciences of the USSR) was used for quantitative analysis. Enzyme activity was expressed in relative optical density units. The significance of differences was determined by Student's test.

For electron microscopic investigation the gastric mucosa was fixed in 2% glutaraldehyde solution in phosphate buffer and then postfixed in 1% OsO_4 solution. Morphometric analysis of the chief cells of the rat stomach was carried out by the method in [15], using a multipurpose test system. The surface area of the membrane components of the cells per unit volume of cytoplasm was determined, and dimensionality (in cm^2/cm^3) was calculated from the number of intersections of membrane of the organelle studied with test lines [6]. The results were subjected to statistical analysis and coefficients of correlation were calculated. Of the total number of correlations, moderately strong (coefficient of correlation r = 0.5-0.69), strong (r = 0.70-0.89), and strongest, or "functional" (r = 0.90-1.0) were selected.

EXPERIMENTAL RESULTS

Injection of thyroxine in the small dose for 10 days led to an increase of 73% in the proteolytic activity of the gastric juice, whereas if the period of injection of the hormone was lengthened (30 days) activity fell by 24%. A similar time course was observed also when large doses of thyroxine were injected (Table 1).

Changes in activity of oxidoreductases in the chief glandulocytes of the stomach after administration of thyroxine were described previously [6]. Activity of the enzymes in the chief cells was shown to change insufficiently depending on the duration of injection of thyroxine. When thyroxine was given in a dose of 2.5 mg/kg for 10 days a significant increase of 48% ($P < 0.05$) in SDH activity and of 23% ($P < 0.01$) in NADH-DH activity was observed, but changes in cytochrome oxidase (CO), LDH, and glucose-6-phosphate dehydrogenase (G6PDH) activity were not statistically significant. The sharp increase in activity of these dehydrogenases may be connected with swelling of the intracellular organelles and increased permeability of their membranes, which the writers described previously [7] and regard as a compensatory response to injection of an excessive quantity of the hormone into the animal, and also with activation of one of the most energy-yielding pathways of glucose oxidation – the Krebs cycle. After injection of the hormone for 30 days SDH activity fell to 58% ($P < 0.01$) and

TABLE 2. Correlation between Parameters Studied

Parameter studied	Proteolytic activity	
	thyroxine (2.5 mg/kg)	
	10 days	30 days
SDH	0,51	0,94
NADH-DH	0,60	0,98
G6PDH	0,05	0,18
LDH	0,13	0,24
CO	0,20	0,15
RNA	0,95	0,85
Surface area of mitochondrial cristae	0,68	0,87
Area of membranes of rough cytoplasmic reticulum	0,98	0,95

NADH-DH activity to 53% ($P < 0.01$), which we regarded as increasing exhaustion of the enzyme systems as a result of the collapse of adaptation.

Correlation analysis of SDH and NADH-DH activity in the chief cells of the stomach and proteolytic activity of the gastric juice of the rats showed moderately strong correlation between these parameters ($r = 0.51$, $r = 0.61$) after injection of thyroxine in a dose of 2.5 mg/kg for 10 days, and the strongest, or "functional" correlation ($r = 0.90$, $r = 0.98$) after injection of the hormone for 30 days (Table 2). Meanwhile CO, LDH, and G6PDH activity did not correlate with proteolytic activity.

Comparison of the RNA content in the chief cells with proteolytic activity of the gastric juice of rats receiving thyroxine for 10 days revealed strong correlation ($r = 0.95$). An increase in the RNA content (by 40%) corresponded to an increase in proteolytic activity of the gastric juice (by 35%). After injection of thyroxine for 30 days, the reduced RNA content in the chief glandulocytes corresponded to reduced proteolytic activity of the gastric juice (strong correlation; $r = 0.87$).

Ultrastructural analysis showed that in the early stages of hyperthyroidization most of the chief cells were in an enhanced functional state. This was shown by their well developed rough cytoplasmic reticulum (its cisterns in the basal part of the cell were dilated by 2.8 times).

Strongest, or "functional" correlation was established between the area of the membranes of rough cytoplasmic reticulum (in the basal part) and proteolytic activity of the gastric juice at different times of thyroxine administration. For instance, in the early periods of hyperthyroidization, the increased area of the membranes of the rough cytoplasmic reticulum correlated with increased proteolytic activity of the gastric juice ($r = 0.98$), and in the late stages, the reduced area of the membranes of the rough cytoplasmic reticulum correlated with reduced proteolytic activity of the gastric juice ($r = 0.95$).

Moderately strong correlation ($r = 0.69$) was found between the surface area of the mitochondrial cristae and proteolytic activity of the gastric juice after injection of thyroxine for 10 days, and strong correlation ($r = 0.87$) after injection of the hormone for 30 days. Examination of the specific character of the action of thyroxine (in the early stage) on organelles of the chief cells indicates that the most important changes among cellular structures were those undergone by the protein-synthesizing apparatus.

Whereas the chief point of application of thyroxine in the parietal cells at the organelle level is the mitochondria [7], in the chief cells the most important changes are undergone by the protein-synthesizing apparatus of the cell, and changes in the mitochondria are less marked. This conclusion is confirmed by the moderately strong correlation observed between SDH and NADH-DH activity, the surface area of the mitochondrial cristae of the chief cells, and proteolytic activity of the gastric juice of rats receiving thyroxine for 10 days ($r = 0.51$, $r = 0.60$, and $r = +0.68$ respectively).

After injection of thyroxine for 30 days correlation analysis revealed strong correlation between enzyme activity and proteolytic activity. For instance, the coefficient of correlation between SDH and NADH-DH activity and the proteolytic activity of the gastric juice was 0.94 compared with 0.51 and 0.98 compared with 0.60 in the early stages of hyperthyroidization. Equally strong correlation was found between the surface area of the membranes of the rough cytoplasmic reticulum, the RNA content in the chief cells, and the proteolytic activity of gastric juice.

It can be concluded from these data that the increase in proteolytic activity of the gastric juice in the early stages of hyperthyroidization may be the result of an increase in the intensity of biosynthesis in the chief cells of the stomach. The reduction of proteolytic activity, on the other hand, during prolonged hyperthyroidization may be due to a decline equally in energy production and biosynthesis in the chief cells.

Considering all the facts described above, and also data published by the writers previously [8], showing that in the early stages of hormone injection protein biosynthesis in the gastric mucosa rose to 387% and the RNA concentration to 112%, it can be tentatively suggested that the dominant mechanism responsible for the increase in enzyme activity in the chief glandulocytes is evidently stimulation of enzyme protein synthesis by thyroxine. This hypothesis is supported by the results of biochemical investigations [5, 14], according to which, during short-term exposure to thyroxine, synthesis of messenger and ribosomal RNA is intensified, and aggregation of ribosomes into polysomes is increased in the liver tissues, leading to increased efficiency of the protein-synthesizing apparatus of the cell.

Contradictory data in the literature on disturbance of the enzyme activity of the gastric juice in patients with thyrotoxicosis may be explained on the grounds that many investigations frequently did not take into account the severity and duration of the disease.

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