HISTOCHEMICAL BASIS OF THE CORRELATION BETWEEN RNP CONTENT AND ACTICITY OF SOME OXIDOREDUCTASES IN THE ALBINO RAT LIVER DURING EXPERIMENTAL THYROID DYSFUNCTION

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UDC 616.441-092.9-07:616.36-008.9-074

Changes in the RNP content and in the activity of mitochondrial 1-glycerol-3-phosphate: (menadione)-tetrazolium oxidoreductase (1.1.99.5) and decarboxylating 1-malate: NADP-oxidoreductase (1.1.1.40) in the liver cells depending on the level of thyroid hormones in the body were studied by histochemical methods in albino rats. A statistically significant correlation was found between the RNP content and the activity of these enzymes.

Biochemical investigations have demonstrated the effect of many hormones on the activity of enzyme systems, on protein synthesis, and on the synthesis of ribonucleoproteins (RNP) in corresponding target tissues [16, 18]. Some hormones have the properties of DNA depressors, possibly by forming a complex with histones [3], or of inducers of enzyme systems, by activating the corresponding genes or inactivating their blocking agents [7]. Administration of exogenous hormones, especially thyroid hormones, gives rise to appreciable changes in the activity of enzyme systems [8, 11, 12, 17], but these have so far received little study by morphological methods.

The object of the present investigation was to study by histochemical methods the relationship between the activity of some oxidoreductases and the RNP content in the liver cell of albino rats with experimental disturbances of the thyroid hormone level.

EXPERIMENTAL METHOD

Experiments were carried on 84 sexually mature male albino rats weighing initially 150-200 g. The animals were divided into seven groups with 12 rats in each group: 1) intact animals; 2) animals undergoing mock thyroidectomy (all stages of the operation without actual removal of the thyroid gland); 3) intact animals receiving thyroxine (reanal) in a dose of 200 μg/kg body weight parenterally daily for 16 days; 4) animals receiving thyroxine parenterally like the rats of group 3, and also receiving 6-methylthiouracil (6-MTU) in a dose of 200 mg and aminophylline in a dose of 300 mg/kg body weight with their food daily from the 5th day until the end of the experiment; 5) animals undergoing thyroidectomy and sacrificed 48-60 days after the operation; 6) thyroidectomized animals receiving thyroxine parenterally in a dose of 250 μg/kg body weight 32-44 days after the operation for a period of 16 days; 7) thyroidectomized animals receiving thyroxine like the animals of group 6, and also 6-MTU and aminophylline, like those of group 4 32-44 days after the operation. All the animals were kept under identical conditions and received a balanced diet.

The rats were killed by decapitation. Pieces of liver were taken simultaneously from the animals of all groups, mounted in the same block on the stage of the microtome, and frozen with solid CO_2. Serial sections were cut in a cryostate at $-20°C$ using a very cold knife, mounted on dry slides, and dried in air.

Morphological Laboratory, Chervonograd Medical Department, L'vov Region, Ukrainian SSR. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 10, pp. 116-119, October, 1972. Original article submitted June 24, 1971.

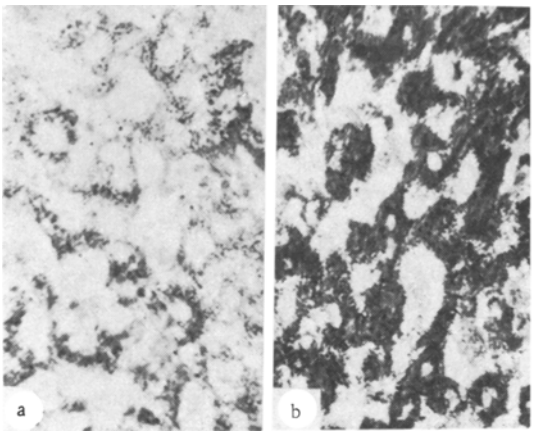

Fig. 1

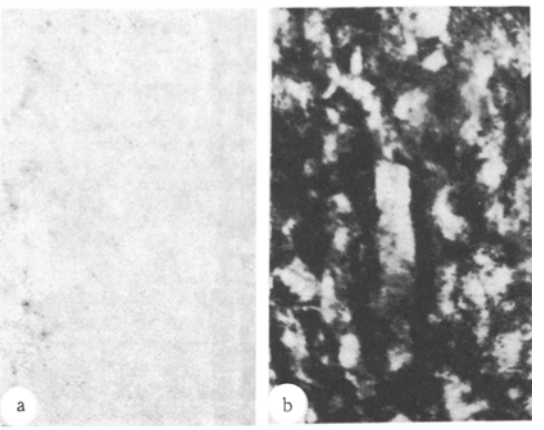

Fig. 2

Fig. 1. Histochemical reaction for RNP by Brachet's method in the liver of the control (a) and hyperthyroid (b) rats. Here and in Figs. 2 and 3: objective 20, ocular, homal 5.

Fig. 2. Histochemical reaction for G3POR by the method of Hess and Pearse in liver of control (a) and hyperthyroid (b) rats.

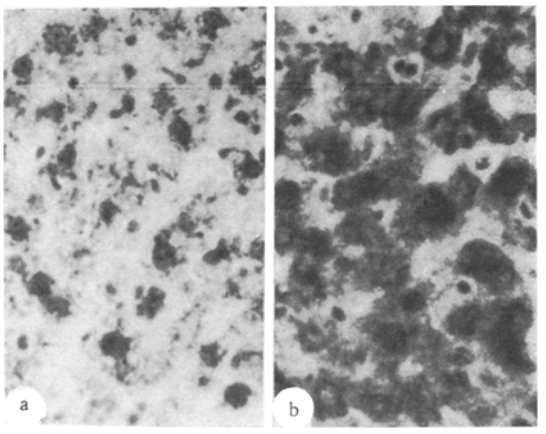

Fig. 3. Histochemical reaction for D1MOR by Krasnov's method in liver of control (a) and hyperthyroid (b) rats.

Some serial sections from each block were fixed in Gendre's fluid at −60°C, and in Carnoy's fluid. RNP was detected by Brachet's method with methyl green and pyronine G. Mitochondrial l-glycerol-3-phosphate: (menadione)-tetrazolium oxidoreductase (G3POR) (1.1.99.5) was detected by the method of Hess and Pearse [5] and decarboxylating l-malate: NADP-oxidoreductase (D1MOR) (1.1.1.40) by the method of Krasnov [2] in the modification used in V. V. Birov's laboratory. Histological survey sections were stained with gallocyanin and chrome alum by the method of De Boer and Arnaker [10].

The control and experimental material was studied by the method of qualitative comparative assessment of the intensity of staining of RNP or the intensity of deposition of tetrazolium salts reduced to formazan. A double control (the animals of groups 1 and 2) was used for the initial level. The results were subjected to statistical analysis as described by Kendall [6], and the degree of correlation between the RNP content and the activity of these two enzymes was assessed.

EXPERIMENTAL RESULTS

Administration of an excess of thyroid hormones to the intact animals led to a marked increase in the RNP content in the cytoplasm of the liver cells. Their localization also was changed: RNP was detected in the cytoplasm of the liver cells of intact animals as pink masses forming a peripheral ring, while in animals with exogenous hyperthyroidism (group 3) the dye was distributed more diffusely as fine granules; the zone of demonstration of RNA in the cell was widened (Fig. 1). There was always a noticable decrease in the intensity of RNP staining in the liver cells of the thyroidectomized rats. Combined administration of L-thyroxine, 6-MTU, and aminophylline (group 4) led to a decrease in the intensity of staining of RNP compared with that observed in exogenous hyperthyroidism, but the normal intensity of staining was not restored. In the other groups of animals, a study of RNP localization revealed a varied picture: in some cases staining for RNP resembled that in the control group, in others there was a marked increase in the intensity of staining in the liver cells (group 6).

Activity of G3POR in the liver cells of the control animals was fairly low. Formazan granules were arranged diffusely throughout the cytoplasm and were comparatively few in number. In the group of rats

undergoing thyroidectomy, activity of the enzyme in the liver cells could not be detected histochemically. After administration of an excess of thyroid hormones (animals of groups 3 and 6) a sharp increase was observed in the activity of the enzyme (Fig. 2), and administration of 6-MTU together with aminophylline to the rats with exogenous thyrotoxicosis (group 4) restored its normal level. In the animals of group 7, the normal activity of the enzyme in the liver cells was not restored.

A marked increase in the activity of D1MOR was found in the liver cells of the animals with exogenous hyperthyroidism (Fig. 3). Activity of this enzyme in the liver of the rats of group 4 was reduced by comparison with its level in exogenous hyperthyroidism, but was not fully returned to normal. Activity of the enzyme was always reduced in the liver cells of the thyroidectomized animals, while in the rats of groups 6 and 7 no definite rule could be established for the changes in activity of the enzyme.

Comparison of the RNP level and activity of the above enzymes showed a marked parallel between the intensity of staining for RNP and the deposition of reduced tetrazolium (formazan) in material from the same individual animals. A high correlation was found between the RNP level and activity of G3POR ($r = 0.8$; $P < 0.001$) and an average level of correlation between the RNP content and activity of D1MOR ($r = 0.6$; $P < 0.01$).

The increase in the RNP content in the liver cells of laboratory animals in experimental hyperthyroidism is confirmed by the results of biochemical tests. Such an increase has been found in nuclear structures [1] and in the cytoplasm of the cell [4, 19]. A similar increase in the RNP content in the liver cells has also been found by histochemical methods [13]. An increase in the activity of mitochondrial oxidoreductases has been observed by many workers, and by as much as 8-10 times [17]. The induction of these enzymes takes place through and is connected with protein synthesis. It has been postulated that protein synthesis is preceded by the synthesis of messenger RNA, a process maintained by thyroid hormones [9, 14, 15]. The results obtained by histochemical tests, showing correlation between the RNP content and the activity of these two enzymes of the glycerophosphate and malate cycles are in agreement with the results of the biochemical tests. They demonstrate the complex mechanism of action of thyroid hormone on the body, involving the stimulation of RNP synthesis and effected through the activation of various enzyme systems.

In experimental thyroid dysfunction the RNP content and the activities of mitochondrial G3POR and D1MOR in the liver cells of albino rats undergo changes. There is a statistically significant correlation between the RNP content and the activity of the two enzymes. These results accord with the concept that thyroid hormones stimulate the synthesis of messenger RNA and protein, and since they can be studied by histochemical methods this considerably simplifies the technique.

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