EFFECT OF THYROID HORMONES AND IGL in vivo ON PROTEIN SYNTHESIS IN THE MITOCHONDRIA OF THYROIDECTOMIZED ANIMALS

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The effect of tri-iodothyronine (T_3) and IGL on the intensity of incorporation of L-[^{14}C]tyrosine and on the rate of protein synthesis in the liver mitochondria of thyroidectomized rats and on the radioactivity of the amino-acid pool in the liver was investigated. The intensity of incorporation of L-[^{14}C]tyrosine into proteins in the liver mitochondria of thyroidectomized animals and the rate of protein synthesis in them were found to be only half of their values in animals undergoing mock operations. Administration of T_3 or IGL to thyroidectomized rats restored protein synthesis in the liver mitochondria to normal. IGL had a similar effect to T_3 on all biochemical indices studied. The absence of thyroid hormones circulating in thyroidectomized animals or administration of T_3 or IGL to them did not change the radioactivity of the free tyrosine pool in the liver tissue. KEY WORDS: thyroid hormones; thyroidectomy; protein synthesis in mitochondria; amino-acid pool; IGL.

The metabolically active form of the molecule of thyroid hormones is not yet known. According to one hypothesis of the mechanism of action of thyroid hormones it is suggested that these hormones probably exert their action after deiodination by means of iodine ions [1, 4, 5]. This hypothesis is confirmed by experimental data showing that in some cases iodine ions exhibit effects characteristic of thyroid hormones [2, 6, 7].

In the investigation described below the effect of tri-iodothyronine (T_3) and IGL on incorporation of L-[^{14}C]tyrosine into protein, changes in the intracellular pool of free tyrosine, and the rate of protein synthesis in the mitochondria of thyroidectomized rats was studied.

EXPERIMENTAL MATERIAL AND METHOD

Male albino rats 25 days old were thyroidectomized surgically (T_0). Control animals underwent a mock operation. Experiments were carried out 10 days after the operation. An intraperitoneal injection of T_3 (25 μg/100 g body weight) of IGL in the same dose was given to the animals 24 h before the experiment. L-[^{14}C]-tyrosine (20 μCi/100 g body weight, specific activity 125 μCi/mmole) was injected intraperitoneally 5, 15, 30, or 60 min before decapitation of the animals. The liver of the control and experimental rats was homogenized in medium consisting of 0.25 M sucrose, 0.05 M KCl, 0.01 M $MgCl_2$, and 0.005 M Tris-HCl (pH 7.6). The nuclei were isolated from the homogenate by centrifugation for 10 min at 600 g. The mitochondria were isolated at 8500 g by centrifugation for 10 min and washed three times in the isolation medium.

The radioactivity of the mitochondrial protein was determined by the method of Mans and Novelli [8] and protein was determined by the method of Lowry et al. [9]. The radioactivity of the samples was measured on a Packard Tricarb Spectrometer Model 3320 scintillation counter, using a scintillation fluid containing PPO (2,5-diphenyloxazole), POPOP (1,4-di-5-phenyloxazolyl)-benzene, dioxan, and naphthalene.

The tyrosine pool was analyzed by a combined method: to begin with the radioactivity of free L-[^{14}C]-tyrosine contained in the liver tissue was determined, after which the total quantity of free tyrosine in the liver was estimated. The analyses were carried out as follows: 1 g liver was homogenized in 1 ml water. The homogenate was treated with 1 ml of 10% $HClO_4$ and centrifuged at 10,000 g for 10 min. The precipitate was extracted twice with 5% $HClO_4$. All the cell extracts were pooled and then used for analysis. The total volume of the samples was made up to 4 ml. The precipitate was used to determine the protein content by the

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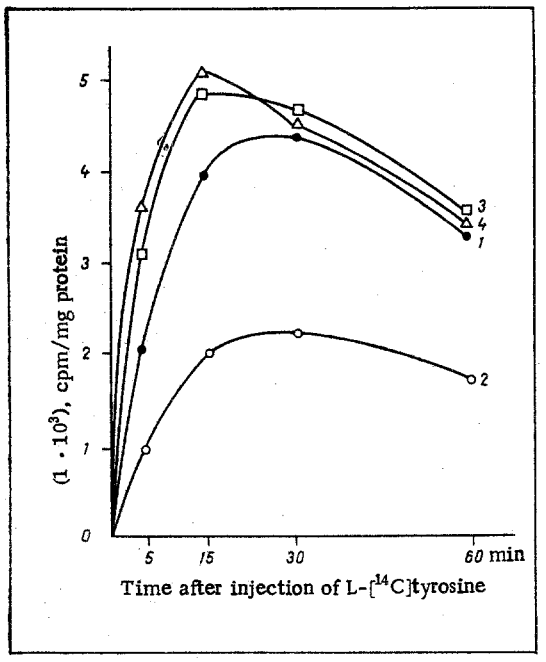

Fig. 1

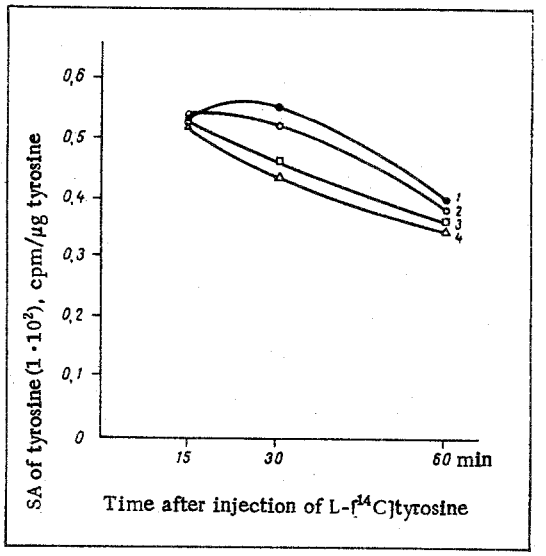

Fig. 2

Fig. 1. Kinetics of incorporation of L-[^{14}C]tyrosine into mitochondrial proteins of rat liver in vivo. Here and in Figs. 2 and 3: 1) animals undergoing mock operation (control); 2) thyroidectomized (T_0) animals; 3) $T_0 + T_3$; 4) T_0 + IGL.

Fig. 2. Changes in radioactivity of free tyrosine pool in rat liver.

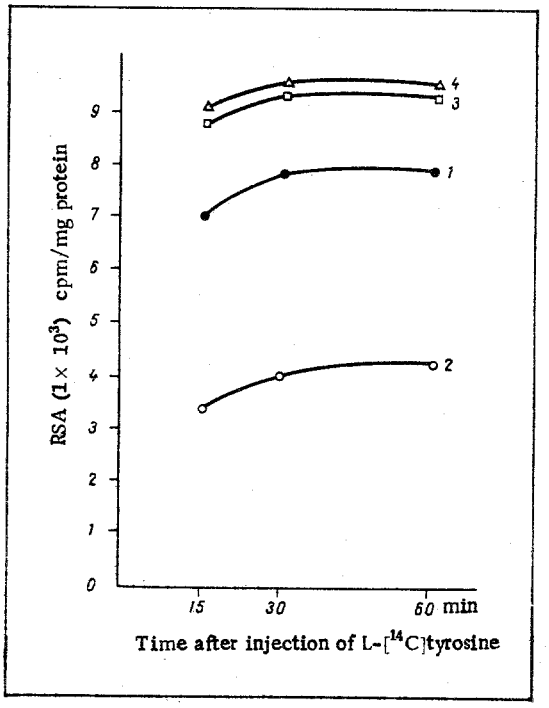

Fig. 3. Kinetics of protein synthesis in vivo in liver mitochondria of rats of different experimental groups.

method of Lowry et al. [9]. The radioactivity of free L-[^{14}C]tyrosine was measured by the method of Mans and Novelli [8] and calculated in cpm/mg protein of total liver tissue. The total quantity of tyrosine (labeled and nonradioactive) in the tissue was determined chromatographically [3] and expressed in μg/mg protein. The

radioactivity of the free tyrosine pool was characterized by the specific activity (SA) of this amino acid and expressed in cpm/μg free tyrosine present in the tissue. All values were expressed relative to 1 mg liver protein.

The rate of protein synthesis was estimated from the relative specific radioactivity (RSA), calculated from the ratio between SA of the protein after incorporation of L-[^{14}C]tyrosine (in cpm/mg protein) and the radioactivity of the free tyrosine pool (SA of tyrosine):

$$\text{RSA} = \frac{\text{SA of protein} \times 100}{\text{SA of tyrosine}}.$$

The results reflecting the kinetics of incorporation of L-[^{14}C]tyrosine after 5, 15, 30, and 60 min into mitochondrial proteins of the control and thyroidectomized animals (untreated, or after receiving injections of T_3 or IGL) are given in Fig. 1. Kinetic curves of incorporation of the precursor into mitochondrial proteins in all experimental groups were parabolic in character. The radioactivity of proteins in the liver mitochondria of animals undergoing the mock operation and of the thyroidectomized rats reached a maximum 30 min after injection of L-[^{14}C]tyrosine. In thyroidectomized animals treated with T_3 or IGL incorporation reached a maximum after only 15 min. The rate of incorporation of the precursor into liver mitochondrial proteins of the thyroidectomized rats was reduced by almost half compared with the rate in animals undergoing the mock operation. Administration of T_3 or IGL to the thyroidectomized animals restored the normal rate of incorporation of L-[^{14}C]tyrosine in these animals.

Action of T_3 and IGL on Amino-Acid Pool in Liver Cells. Graphs reflecting the effect of thyroidectomy and injection of T_3 or IGL on the radioactivity of the tyrosine pool in the liver are illustrated in Fig. 2. The results show that SA of free tyrosine in the liver of animals undergoing the mock operation and thyroidectomized rats reached a maximum 30 min after injection of labeled tyrosine. Meanwhile, in the animals receiving T_3 or IGL, SA of tyrosine reached a maximum at the first time of investigation, namely 15 min after injection of the precursor, and its value was a little lower after 30 min.

The maximum of incorporation of L-[^{14}C]tyrosine into mitochondrial proteins of the liver and the maximum of radioactivity of the free tyrosine pool of the liver thus coincided (compare Figs. 1 and 2). These correlations were almost unchanged either by thyroidectomy or by administration of T_3 or IGL to the thyroidectomized animals. Consequently, the difference in the rate of incorporation of the radioactive precursor into protein was due not to changes in the radioactivity of the free tyrosine pool, but to disturbances in the activity of the protein-synthesizing system of the cell.

Effect of T_3 and IGL on Protein Synthesis. After determination of SA of the mitochondrial proteins of the liver during incorporation of L-[^{14}C]tyrosine and also the radioactivity of the free tyrosine pool in the liver cells, it was then possible to calculate the rate of protein synthesis in the subcellular particles of the liver of the thyroidectomized animals and the effects of thyroid hormones and iodine ions on this process. The results reflecting the kinetics of protein synthesis in the liver mitochondria of the rats are given in Fig. 3. The graphs show that the rate of the protein synthesis in the mitochondria of the thyroidectomized rats was about half that in the subcellular particles of the liver of animals undergoing the mock operation. Administration of T_3, like that of IGL, stimulated protein synthesis in the liver mitochondria of the thyroidectomized rats. It is interesting to note that, as reflected in all the biochemical indices studied, the mechanism of action of iodine ions was analogous to that of tri-iodothyronine. The mechanism of the thyroxine-like effect of iodine ions is not clear. Nor is the molecular mechanism of action of T_3 on intracellular protein synthesis clear.

Iodination of aromatic amino acids assumes particular importance in the light of data in the literature showing that peptides rich in tyrosine and other aromatic amino acids can interact actively with bases in the DNA molecule, intruding between them and stabilizing the structure of the DNA [10-12]. Interaction between tyrosine-rich peptides and DNA and their stabilizing effect are perhaps due to conjugation of the π-electron system and the presence of atoms acting as electron donors. The introduction of an electron-acceptor atom such as iodine into this system, and iodination of tyrosyl residues completely abolish the stabilizing effect of these peptides [12]. It can thus be tentatively suggested that the iodination of residues of aromatic amino acids in the proteins bound with DNA may lead to events directly influencing its transcription activity.

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BIOSYNTHESIS OF RESERPINE-LINE SUBSTANCES IN THE MYOCARDIUM AND OTHER TISSUES OF MAN AND ANIMALS

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Reserpine-like substances were found to be synthesized in homogenates, microsomes, and cytosol from various human and animal tissues with the utilization of acetate, methyl groups of S-adenosylmethionine, tryptophan, reserpine, etc., as precursors. It is suggested that endogenous reserpine-like substances participate in the autoregulation of the levels of free and bound biogenic amines. KEY WORDS: endogenous reserpine-like substances; biosynthesis; biogenic amines.

Certain pharmacological agents are known to be able to reduce the reserves of biogenic amines in neuron terminals. These substances include, for example, guanethidine, 6-hydroxydopamine, and reserpine [3]. The last of the three has been well studied and is an alkaloid of indole type produced by certain species of tropical plants. Reserpine induces exhaustion of catecholamine depots as a result of blockade of the Mg-ATP-activated system responsible for their uptake at the level of adrenergic reserve granules [4, 6]. Exogenous reserpine, administered to an animal, has been shown [5] to bind with specific receptors on the membranes of catecholamine-containing granules in nerve endings and in the adrenals.

The writers showed previously that the human and animal myocardium contains endogenous substances similar to but not identical with reserpine [1].

The object of this investigation was to continue the study of the pathways of biosynthesis of respine-like substances of animal origin, to which the name "enderpines" or "RP-fraction" was given.

EXPERIMENTAL METHOD

The substances to be studied were isolated from animal tissues by the method described previously [1]. The intensity of fluorescence of the enderpines was measured on the Aminco–Bowman (USA) instruments. Tissue homogenates (ground with quartz sand for 5 min) and also freshly isolated cytosol and microsomes the rat liver were used as the enzyme systems. For the cytosol and microsomes the incubation system [7] suggested previously was used; its composition was as follows (2 ml): 6 μM ATP, 0.4 μM coenzyme A, 4 μM $MgCl_2$, 2 μM NaF, 0.4 μM dithiothreitol in 0.2 M Tris-HCl buffer, pH 7.2, protein concentration 0.5 mg/ml. The controlled enzyme reaction was started by the addition of 200 μl of the given labeled substrates and in-

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