PROTEIN BIOSYNTHESIS IN THE RAT LIVER DURING NATURAL FLUCTUATIONS OF THE STEROID BACKGROUND

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The results of an investigation of protein and RNA removal and of induction of glucose-6-phosphatase activity in the rat liver during natural (seasonal) fluctuation of the steroid background are described. It was shown that seasonal adrenal activity increases the uptake of labeled precursors (leucine-1-^{14}C and orotic acid-1-^{14}C) into total protein and RNA of the liver, and also increases glucose-6-phosphatase activity in that organ. It is concluded that changes in the endogenous corticosteroid level in the body play a regulatory role for protein biosynthesis in the rat liver.

KEY WORDS: protein biosynthesis; liver corticosteroids.

Prolonged administration of large doses of glucocorticosteroids increases the protein content in the hepatocytes and the weight of the liver [8]. It has been shown that under these circumstances different stages of protein biosynthesis are stimulated in the liver and certain enzymes are induced in the liver cells [14]. However, the effects obtained as a result of the use of above-physiological quantities of exogenous hormones must often be reproduced subsequently in experiments which take into account the functional possibilities of the hormone-forming systems in the adrenals.

It was accordingly decided to study protein renewal, RNA synthesis, and enzyme induction in the liver during natural fluctuations of the steroid background in rats.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. The level of hormone formation and the degree of protein metabolism were compared in animals of the same population in March and April. In the course of the experiments particular attention was directed to maximal standardization of the diet, the conditions of keeping of the animals, and the time of sacrifice. Protein biosynthesis was judged from the incorporation of the specific protein precursor (leucine-^{14}C) into total proteins of liver homogenate. Leucine-1-^{14}C (specific radioactivity 24.7 mCi/mmole) was injected intraperitoneally over a period of 5 min in a dose of 6 μCi/g body weight. The protein precipitate obtained after treatment of the homogenate with 10% TCA was concentrated on Synpor AUFS (Czechoslovakia) membrane filters. The radioactivity of the precipitate was measured by means of a Mark II Nuclear Chicago liquid scintillation counter, using universal toluene scintillator. The counting efficiency was 86%.

To study total RNA biosynthesis, the radioactivity of samples from liver homogenate obtained 40 min after intraperitoneal injection of orotic acid-1-^{14}C (specific radioactivity 15 mCi/mmole) in a dose of 0.2 μCi/g body weight, was determined. Incorporation of label into protein and RNA (specific radioactivity) was calculated relative to the total protein and RNA content [9] in the liver. Glucose-6-phosphatase activity in the liver [11] and the adrenocorticosteroid level in the blood [15] also were investigated.

EXPERIMENTAL RESULTS

The secretory function of the adrenals is known to fluctuate regularly, with a frequency that is synchronized with the rhythm of changes taking place in the environment. Diurnal and seasonal changes of the hormonal background have been studied the most. It was shown previously that in rats there is a rise of adrenal

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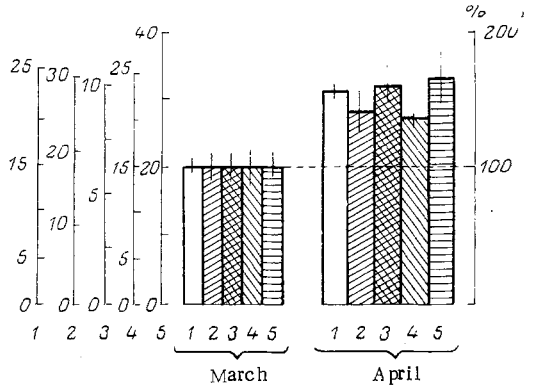

Fig. 1. Effect of seasonal changes in blood corticosteroid level (in μg %) on protein content (in mg/100 mg tissue), glucose-6-phosphatase activity (in μmoles P_i/30 min/100 mg tissue), and incorporation of leucine into total protein (in thousands of cpm/mg protein) and orotic acid into RNA (in thousands of cpm/min/mg RNA) in rat liver. 1) Corticosteroid level; 2) protein content; 3) glucose-6-phosphatase activity; 4, 5) incorporation of leucine into total protein and of orotic acid into RNA respectively.

functional activity in the spring, which lasts all summer, and ends in the fall [1, 2]. The beginning of this rise, i.e., the switch from a lower to a higher level of secretion, is regarded as a convenient model with which to study the effect of endogenous hormones on protein biosynthesis. As Fig. 1 shows, the corticosteroid concentration in the blood and adrenal tissues of the rats was much higher in April than in March. The total protein in the liver was correspondingly higher also. The increase in uptake of labeled leucine into total protein could indicate stimulation of protein metabolism under these conditions. An increase in weight of the liver and in its protein and nucleic acid content has been observed in animals after awakening from hibernation, which is accompanied by marked activation of hormone formation in the adrenals [3]. The stimulating action of corticosteroids on protein biosynthesis is usually associated with their effect on ribosomes or on the cell genome. It has recently been shown that an increase in protein synthesis takes place in the liver ribosomes during acute stress induced by injection of a celite suspension into rats [13]. Experiments with puromycin-^{3}H showed that under these circumstances the rate of initiation of the peptide chain is increased. Celite had no effect on adrenalectomized animals. The authors cited suggest that corticosteroids activate or increase the concentration of a special factor in the ribosomes, which regulates protein synthesis [13]. Meanwhile corticosteroids can increase the number of ribosomes themselves in hormonally sensitive tissues [7]. Under the influence of adrenocortical hormones not only structural proteins [3], which provide for the increase in weight of the organ, but also certain specific enzyme proteins [14] are synthesized in the liver. A unique marker of the effect of corticosteroids on protein biosynthesis is the glucose-6-phosphatase activity of the tissues, which is directly determined by the quantity of enzyme proteins specifically hydrolyzing glucose-6-phosphate [14]. For example, we know that activity of the enzyme in the liver in intact mice is maximal in darkness and minimal during daylight, i.e., at a time when adrenal function has correspondingly maximal and minimal values [5]. Since the increase in the blood corticosteroid level under these conditions corresponded quantitatively to the degree of activation of the liver glucose-6-phosphatase (Fig. 1), it can be assumed that this correlation is maintained by hormonal induction of enzyme synthesis.

It is considered that an important role in the mechanism of stimulation of the protein-synthesizing system of the cells by corticosteroids is played by their direct influence on the transcribing apparatus of the genome [12].

The possibility thus cannot be ruled out that changes found in the protein content, incorporation of labeled precursor into protein, and the glucose-6-phosphatase activity in the liver during natural fluctuations of the corticosteroid background are the result of hormonally determined changes in RNA synthesis.

As Fig. 1 shows, elevation of the blood corticosteroid level is accompanied by stimulation of incorporation of the injected labeled precursor (orotic acid) into total liver RNA. It is considered [6] that adrenocortical hormones somehow influence DNA so as to stimulate the formation of messenger RNA, which is then transported from the nucleus into the cytoplasm, where it participates in protein synthesis. According to Kenney et al. [10], the kinetics of increased production of protein molecules in the liver under the influence of corticosteroids corresponds in time with increased synthesis of at least three types of RNA: messenger, transfer, and ribosomal.

From the quantitative aspect, the final level of hormonal stimulation of protein biosynthesis can evidently be determined by modulation of polypeptide synthesis on ribosomes itself – specifically of the stages of its synthesis such as initiation and chain elongation [4, 13]. However, the stage limiting the velocity of the whole process of induction of protein synthesis by steroids can now be confidently regarded as template RNA formation [4].

Analysis of data in the literature [1-3, 5, 7, 13, 14] and of our own results thus suggests that the operation of the steroid-dependent mechanism of regulation of protein synthesis in the rat liver does not contradict the capacity of the hormone-forming functions of the adrenals, for characteristic changes in the level of protein metabolism are brought about even in response to seasonal [2] and diurnal [5] fluctuations in the steroid background. Consequently, the experimental results can be explained in terms of the generally accepted notions of the mechanism of action of exogenous corticosteroids on the protein-synthesizing system of the cells.

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