HORMONAL REGULATION OF SERUM ALBUMIN PRODUCTION BY PRE- AND POSTNATAL RAT HEPATOCYTES IN CULTURE

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One of the principal functions of the liver cells is synthesis and secretion of virtually all the blood plasma proteins except immunoglobulins. This function of the liver has been sufficiently fully characterized, but problems concerning its hormonal regulation are still far from their final solution. Serum albumin (SA) is the dominant protein, accounting for at least 10% of all proteins synthesized by the adult rat liver, and about 30% of the serum proteins synthesized by mature hepatocytes [10]. Attempts to investigate the role of certain hormones in regulating the production of this protein have not led to consistent conclusions. For instance, even though the role of glucocorticoids in the regulation and maintenance of specific functions of the hepatocytes is no longer disputed, only a few investigators have been able to demonstrate clearly the stimulating effect of these hormones on SA production by liver cells in vitro [6, 10]. The study of the role of different hormones in the regulation of this specific function of the hepatocytes in the perinatal and early postnatal periods of development of the liver is certainly no less interesting. However, these problems have not yet attracted due attention of research workers.

The aim of this investigation was to study the role of certain hormones in the regulation of SA production at different stages of ontogeny in rats, using primary cultures of liver cells.

EXPERIMENTAL METHOD

The following hormones and reagents were used: bovine insulin obtained from the Kaunas Endocrine Preparations Factory, human STH, obtained from the "Biotekhnologiya" Research and Production Combine, Ministry of the Medical and Biological Industry of the USSR, L-tri-iodothyronine, BSA (from "Serva," West Germany), cortisol, and type IV collagenase (from "Sigma," USA).

Fetal liver cells were obtained on the 21st-22nd day of pregnancy after anesthesia with pentobarbital (60 mg/kg), by a modification of the method in [14]. Cells suspended in medium 199, containing 10 mM HEPES, 2 mM glutamine, and 5% embryonic calf serum ("Serva," West Germany) were seeded on 24-well culture plates ("Flow Laboratories," England) at the rate of $2.0 \cdot 10^6$ cells/ml, using 0.75 ml of suspension per well. The wells in the plates were first coated with a thin layer of collagen, isolated from rat tail tendons. The cells were cultured for 5 days in an atmosphere of 5% CO_2 + 95% air. Hematopoietic cells were removed 20 h after seeding at the first change of medium [14].

Liver cells from 3-week-old male Wistar rats were obtained by the method in [1] with minor modifications. The cell suspension was seeded in growth medium in a concentration of $1.0 \cdot 10^6$ cells/ml and in a volume of 0.6 ml per well. The cells were incubated with the hormones on the 3rd day for 20 h.

The albumin concentration in the incubation medium was determined by our modification of the homologous radioimmunoassay method developed previously in our laboratory [2]. After incubation of samples of culture medium or of the standard albumin preparation with antibodies to SA and with ^{125}I-labelled albumin for 20 h, a staphylococcal reagent containing protein A (produced by the Pasteur Institute of Epidemiology and Microbiology, Leningrad), was added in the course of 20 h in a dilution of 1:50, after which the samples were centrifuged at 10,000*g* for 20 min. Radioactivity of the samples was measured on

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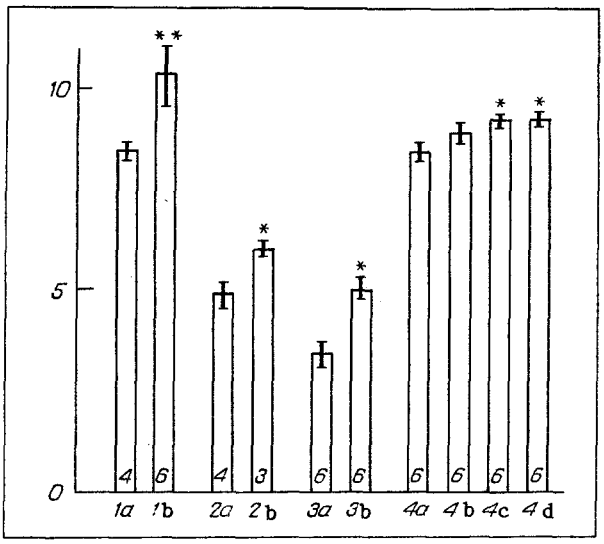

Fig. 1. Effects of hormones on albumin production ($M \pm m$) by liver cells from 3-week-old rats in culture. Abscissa: 1a-4a) controls in four different experiments, 1b) cortisol (10^{-6} M), 2b) insulin (5 μg/ml), 3b) STH (4 μg/ml), 4b-4d) T_3 in concentrations of 10^{-9}, 10^{-8}, and 10^{-7} M. Here and in Fig. 2: ordinate, level of albumin production (in μg/mg protein/20 h); number of observations shown inside columns; $*p < 0.05$, $**p < 0.01$ compared with corresponding control.

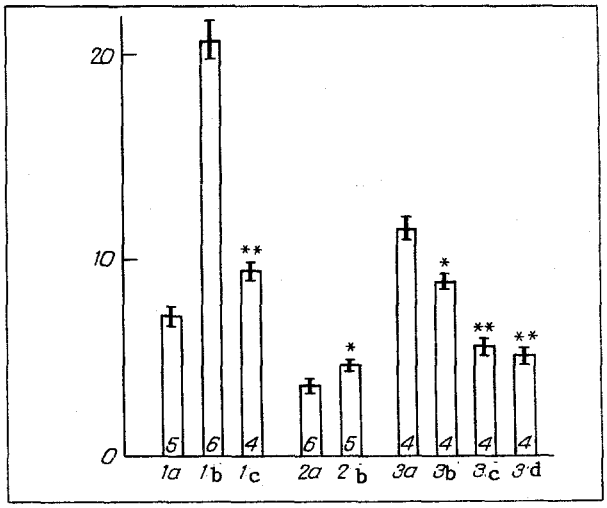

Fig. 2. Effect of hormones on albumin production ($M \pm m$) by fetal rat liver cells in primary cultures. Abscissa: 1a-3a) controls in three different experiments, 1b) cortisol, 1c) STH, 2b) insulin, 3b-3d) T_3 in increasing doses. Hormone concentrations as in Fig. 1.

a gamma-counter ("Trakor"). The albumin concentration in the medium reflected the level of its production as an integral parameter, representing both synthesis and secretion, and also, possibly, the degradation of this protein also.

Biosynthesis of total RNA and protein was estimated as incorporation of ^{3}H-uridine and ^{14}C-L-leucine, added 2 h before the end of incubation, in cell material insoluble in TCA [13]. The results were subjected to statistical analysis by Student's t-test.

TABLE 1. Effect of T_3 (10^{-7} M) Protein in Primary Cultures of Liver Cells from Fetal and Young Rats ($M \pm m$)

Test object	Incorporation into TCA-insoluble cell material (CPM/mg protein)	
	^{3}H-uridine	^{14}C-L-leucine
Fetuses		
Control	18 939±687 (4)	5681±606 (4)
T_3	27 200±1223 (4)	17 014±288 (4)
Young rats		
Control	7718±502 (6)	16 475±915 (6)
T_3	11 140±918 (6)	21 218±994 (6)

Legend. Number (n) in group given in parentheses; in all cases $p < 0.01$ compared with corresponding control.

EXPERIMENTAL RESULTS

After incubation of the hepatocytes from 3-week-old rats in culture with the hormones (Fig. 1) it was found that cortisol, insulin, and STH exhibited a moderately strong stimulating action on SA production (between 23 and 48%), whereas tri-iodothyronine (T_3) caused only a small increase (+9%). In experiments on primary cultures of fetal rat liver cells (Fig. 2) cortisol had the strongest stimulating action on SA production (+200%). In the same experiments STH and insulin increased SA production by 32 and 25% respectively; consequently, the efficacy of the action of the hormones on this process was comparable with that in primary cultures of liver cells from rat fetuses and 3-week-old rats. After the action of T_3 (10^{-9}-10^{-7} M) for 20 h on fetal liver cells it induced a dose-dependent decrease (by 1.4-2.4 times) in the SA concentration in the incubation medium. Characteristically, T_3 had a parallel stimulating action on incorporation of labeled precursors into total RNA and protein of liver cells of rat fetuses and 3-week-old rats in culture (Table 1).

These results relating to the effect of insulin, cortisol, and STH on liver cells from fetuses and young rats are on the whole in agreement with data in the literature relating to the hormonal sensitivity of hepatocytes of adult animals with respect to SA production and the level of albumin mRNA [2-4, 6, 10]. So far as thyroid hormones are concerned, their effect on SA production has not been adequately studied. In the accessible literature we found only one publication which demonstrated the inhibitory effect of T_3 on SA production by fetal rat liver cells in culture [9]. In experiments on chick embryonic hepatocytes in culture T_3 had an inhibitory action on the albumin mRNA level [11]. These last data and also results obtained on adult rat hepatocytes with respect to effects of insulin and glucocorticoids [4, 10] suggest that the observed effects of hormones on SA production by hepatocytes are mainly realized at the albumin gene transcription level.

The question of the physiological role of the hormonal effects on fetal rat hepatocytes observed in the present experiments may legitimately be asked. There is evidence in the literature that glucocorticoid hormones play a leading role in the early stages of development of the liver. At the end of pregnancy, cells of the fetal liver become capable of responding to glucocorticoids, and during this period there is a marked increase in the number of receptors for these hormones [12]. It has also been shown that the plasma corticosteroid level of the fetus peaks at the 19th day of pregnancy in rats [15]. These data may partly explain the increased sensitivity of fetal liver cells to cortisol which we observed. As regards STH, it has been shown that significant differences in the plasma level of this hormone exist at different stages of ontogeny. In fetal rats, for instance, the plasma STH level rises appreciably at the end of pregnancy and then falls in the neonatal period [8]. On the other hand, no significant differences have been found in binding of ^{125}I-labeled human and bovine STH in the liver of newborn and 3-week-old rats [7]. The number of insulin receptors in the fetal rat liver is significantly greater than their number in the liver of adult rats, but the sensitivity of the liver cells to insulin is much lower in fetuses than in adult animals [4]. Our experiments did not reveal any higher sensitivity of the hepatocytes of young rats to the two protein hormones. It will be clear from the facts stated above that the effects of cortisol, insulin, and STH on albumin production by fetal liver cells which we observed evidently reflect the readiness of these cells to take part in hormonally-regulated homeostasis immediately after birth. Meanwhile the opposite direction of the regulatory effect of T_3 on SA production by liver cells at different stages of ontogeny certainly deserves attention, although the physiological meaning of this change of regulation in the course of development is not yet clear.

The investigation thus showed that insulin, cortisol, and STH, during long-term incubation (20 h), can stimulate SA production in the cells of fetal rats and of 3-week-old rats in culture. Of all the hormones studied it is cortisol which has the strongest stimulating action on albumin production by fetal rat hepatocytes. In a culture of liver cells from young rats T_3 stimulated the production of this protein to a weak degree, whereas in fetal liver cells it had a marked and dose-dependent inhibitory effect. Comparison of these results with data in the literature shows that on the whole the direction of reactions to insulin, cortisol, and STH as regards SA production by fetal and young rat hepatocytes coincides with that in the case of adult rat hepatocytes. As regards T_3, separate studies are needed to elucidate the mechanisms of the opposite action of this hormone on liver cells of animals at different stages of development.

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