EFFECT OF ANDROGENS AND GROWTH HORMONE ON EXPRESSION OF THE UNUSUAL ESTROGEN-BINDING PROTEIN LEVEL IN HEPATOCYTES

T. G. Vishnyakova, O. V. Smirnova,
A. V. Bocharov, and V. B. Rozen

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Sexual differntiation of various aspects of liver metabolism is now well known [3]. It has been shown that sexual dependence of liver functions is formed on the basis of the fundamental genetic program, under the irreversible determinant action of androgens (AD) in the early period of postnatal ontogeny of males. An important role in the realization of the effects of sex hormones on the liver is played by the pituitary gland; the action of sex and pituitary hormones of the growth hormone (GH) family is closely interconnected [5, 7].

Some workers have suggested that liver functions are determined at the level of the hypothalamo-hypophyseal system [5, 8]. However, there is evidence of a possible direct action of sex hormones on certain functions of hepatocytes [1, 2, 6].

The aim of this investigation was to study the possibility of direct androgenic determination of the level of the unusual estrogen-binding protein (UEBP) of rat liver. It was shown previously that an essential role in sexual differentiation of the level of this protein, which is highly predominant in the liver of male rats, is played by androgenic programming [4]. In this connection we have studied the role of AD and GH, and also interaction between these hormones, in primary induction of the level of expression of UEBP.

EXPERIMENTAL METHOD

To study the direct effects of the hormones we used a primary culture of hepatocytes of ovariectomized adult females. The hepatocytes were isolated and cultured in accordance with the method described previously [1] and the following reagents and culture media were used: collagenase (569 U/mg), dexamethasone, and insulin were from "Sigma" (USA), Williams-E medium and HEPES solution from "Flow Laboratories" (England), and Hanks' solution without Ca^{2+} and Mg^{2+} from "Gibco" (Austria).

In different versions of the experiments, the duration of incubation of the culture with testosterone (TS, from "Serva," West Germany) and human GH (from Kaunas Endocrine Preparations Factory), or with both hormones, varied from 1 to 3 days, with concentrations of TS of 10^{-9}-10^{-5} M and of GH of 4.5-180 nM. In the experiments in vivo the following groups of a mixed population of female rats were used: 2-3 weeks after covariectomy and 1-14 days after hypophysectomy preceded (2-3 weeks beforehand) by removal of the gonads. Completeness of removal of the pituitary was tested as described previously [2].

Testosterone propionate (TP, from "Serva") was injected intramuscularly in 0.4 ml of propylene-glycol into ovariectomized females with an intact pituitary gland of 1 day after hypophysectomy in doses of 3 or 8 mg daily for 3 or 6 days, respectively.

The effect of GH was studied after subcutaneous injection of 60 or 120 μg of human GH (Kaunas Endocrine Preparations Factory) in 0.2 ml physiological saline twice a day for 3 or 6 days, respectively, to ovariectomized females 1 day after hypophysectomy. In one series of experiments to study the mechanism of interaction of AD and GH the hormones were injected simultaneously. The times of testing the UEBP level after the end of administration of the hormones are indicated in Table 1.

The concentration of E_2-binding sites of UEBP in liver cytosol (N_{UEBP}) was determined from binding of the minimal addition of 2,4,6,7,16,17-^{3}H-E_2 with specific radioactivity

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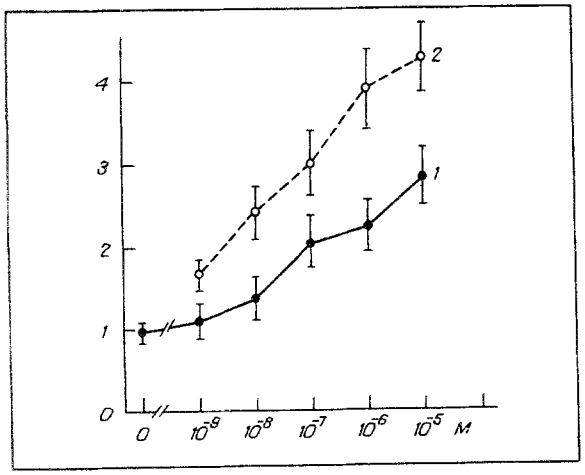

Fig. 1

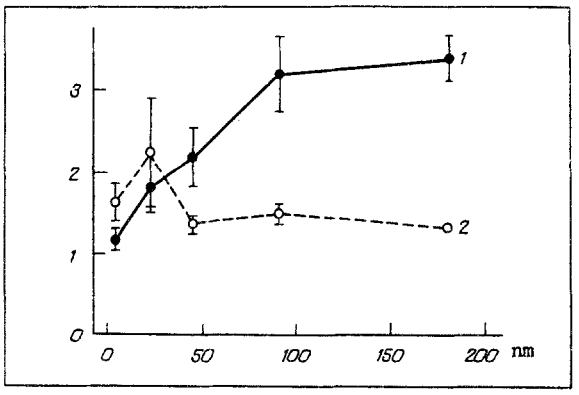

Fig. 2

Fig. 1. Action of TS on UEBP level in primary hepatocyte culture from ovariectomized females in the absence (1) and presence (2) of GH. Here and in Fig. 2: abscissa, TS concentration. Ordinate, N_{UEBP} (in pmoles/mg protein).

Fig. 2. Action of GH on UEBP level in primary hepatocyte culture from ovariectomized females in the absence (1) and presence (2) of TS.

TABLE 1. Role of AD and GH in Primary Induction of UEBP in Vivo (M ± m)

Serial No.	Group of ovariectomized, hypophysectomized females	Time of testing UEBP after last injection of hormones	N_{UEBP}, pmoles/mg protein	p
1	Without additional procedures	—	0,16±0,04 (31)	
2	After injection of TP			
	3 mg, 3 days	1 day	0,12±0,03 (5)	(1—2)>0,1
3	8 mg, 6 days	The same	0,61±0,15 (11)	(1—3)<0,05
4	8 mg, 6 days	10 days	0,07±0,03 (4)	(1—4)>0,1
5	After injection of TP			
	60 µg, twice a day, 3 days	1 day	0,14±0,016 (10)	(1—5)>0,1
6	120 µg, twice a day, 6 days	The same	0,59±0,08 (13)	(1—6)<0,05
7	120 µg, twice a day, 6 days	10 days	0,14±0,018 (5)	(1—7)>0,1
8	After combined injection of TP (3 mg 3 times a day) and GH (60 µg twice a day)	1 day	1,02±0,17 (19)	(1—8)<0,05
9	The same	10 days	1,82±0,088 (8)	(1—9)<0,05

Note. Number of determinations for N_{UEBP} and comparison between groups for p given in parentheses.

of 140 Ci/mmole ("Amersham," England) [4].

The remaining procedures were carried out as described previously [1].

EXPERIMENTAL RESULTS

Data showing the effect of AD (Fig. 1, curve 1) and GH (Fig. 2, curve 1) on the UEBP level in a hepatocyte culture from ovariectomized female rats, after incubation for 3 days with the hormones, are given in Figs. 1 and 2. The results showed that AD and GH separately, in above physiological concentrations, can cause an increase in UEBP expression. However, it is impossible to judge the stability of these effects because of the limited culture time, and incubation of a culture for 1 day with these hormones did not change the original UEBP level (Table 2). Similar results were obtained in vivo: injection of high doses of TP or GH raised the UEBP level in the experimental animals, but 10 days after discontinuing administration of the two hormones, the UEBP level fell (Table 1). The results are evidence that AD and GH can realize their action directly in hepatocytes, but their final effect is exhibited when large doses of the hormones are used and is unstable. Meanwhile, it was shown previously that in ovariectomized females with an intact pituitary gland AD causes stable induction of UEBP expression, which is maintained steadily for 10-40 days [4].

To study the conditions required for stable expression of UEBP and the relations between AD and GH, a hepatocyte culture was incubated in the presence of these two hormones. It will be clear from Fig. 1 (curve 2) that low concentrations of GH, while not acting alone, reduced

TABLE 2. Effect of AD and GH on Induction of UEBP in Hepatocyte Culture from Ovariectomized Females (M ± m)

Experimental material	Duration of incubation with hormone	N_{UEBP}, pmoles/mg protein	p
Hepatocytes of overiectomized females (control)	0 (a)	1,34±0,13 (5)	
The same	0 (b)	1,29±0,13 (6)	
The same + TS (10^{-6} M)	24	1,17±0,26 (6)	(1—3)>0,1
The same	72	2,58±0,4 (5)	(2—4)<0,05
The same + GH (90 mM)	24	1,45±0,21 (3)	(1—5)>0,1
The same	72	2,48±0,5 (4)	(2—6)<0,05
The same + GH (90 nM) + TS (10^{-6} M)	24	2,42±0,47 (4)	(1—7)<0,05
The same	72	3,77±0,66 (6)	(2—8)<0,05

Note. Incubation with hormone carried out 24 h after beginning of culture. Hepatocytes after 48 (a) and 96 (b) h of culture used in control. Remainder of legend as to Table 1.

the level of sensitivity to the action of AD by 1 or 2 orders of magnitude, and the dose-dependence curve of TS is shifted toward lower concentrations, corresponding to the physiological level. Moreover, GH increases the efficacy of the test concentrations of TS. Table 2 gives data on combined administration of TP and GH to ovariectomized and hypophysectomized females. Injection of small doses of GH, as may be seen, also increases their sensitivity to the action of AD: an ineffective does of TP induced UEBP in the experimental animals. Thus, as a result of the combined action of the hormones, the induced level of UEBP persisted throughout the period of culture, and at least for 10 days in the animals (Tables 1 and 2). It can be concluded that the stable expression of this trait requires the inducing action of AD accompanied by the permissive effect of low GH concentrations.

The effect of below-threshold concentrations of AD on development of the positive effect of GH during its action in hepatocyte culture is shown in Fig. 2 (curve 2). It will be seen that AD not only does not stabilize the rise of UEBP level under the influence of GH but, on the contrary, it has an inhibitory action on the effects of GH.

It can thus be concluded from experiments in vivo and direct experiments on hepatocyte culture from ovariectomized female rats that a leading role in determination of the liver for UEBP is played by AD. GH is evidently not a determinant, and in high concentrations it can exert only a regulatory (reversible) effect, the degree of which is under negative control by physiological concentrations of AD.

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