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# THE UNUSUAL ESTROGEN-BINDING PROTEIN (UEBP) OF RAT LIVER: THE ROLE OF SEX STEROIDS AND HYPOPHYSIS IN ITS REGULATION

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**Summary**—The number of estradiol($E_2$) binding sites of rat liver unusual estrogen-binding protein ($N_{UEBP}$) was measured, using a novel modification of the quantitative method of specific UEBP determination. In liver cytosol of mature male and female rats, $N_{UEBP}$ amounted to $6.83 \pm 0.49$ and $< 0.05$ pmol/mg protein, respectively. Neonatal administration of testosterone-propionate (TP) and TP injections at later periods of ontogenesis increased $N_{UEBP}$ in female rat liver in a similar fashion. The elevated $N_{UEBP}$ was found in the liver of mature ovariectomized females 30 days after cessation of TP injections. Hypophysectomy (but not adrenalectomy or thyroidectomy) prevented TP induction of elevated $N_{UEBP}$ in pubertal females.

$E_2$ injections reversibly decreased $N_{UEBP}$ in the liver of all animals under study except of hypophysectomized males.

A stimulating regulatory effect of TP on $N_{UEBP}$ in male rat liver was observed only in the case of endogenous androgen deficiency and low $N_{UEBP}$. TP prevented the $E_2$-dependent decrease of $N_{UEBP}$ upon their simultaneous injections and increased the $E_2$-reduced $N_{UEBP}$ when injected after $E_2$.

Hypophysectomy led to a decrease of $N_{UEBP}$ in pubertal males but only slightly affected that in castrated animals. After TP injections to hypophysectomized males, $N_{UEBP}$ returned to a level next to the initial one.

It was concluded that estrogen–androgen regulation of the UEBP level led to the maintenance of sex differences in the UEBP content.

## INTRODUCTION

The unusual estrogen-binding protein (UEBP) is a constituent component of the system of intracellular proteins which specifically bind sex steroids. The main characteristic features of UEBP making it different from estrogen receptors and other estrogen-binding proteins are as follows: moderate affinity for the hormone ($K_a \sim 10^8\ M^{-1}$) and relatively high binding capacity ($\sim 10^{-11}$ mol per mg of protein); lability of steroid–protein complexes; ability to specifically bind estrogens and, in a lesser degree, androgens; lack of ability of UEBP–estrogen complexes to be translocated into the nuclei in a cell-free system [1–4]. It has been assumed that the special role of UEBP consists in the regulation of intracellular distribution of sex steroids between their receptors and the enzymes of their metabolism [4, 5].

At the same time, UEBP belongs to the group of sex-dependent hepatic proteins. This protein is abundant only in male rat liver [6–8]. The high content of UEBP in the liver of pubertal male rats is said to be due to neonatal imprinting by androgens [5, 9]. After administration of androgens, the UEBP level in female rat liver also increases [2, 7]. Therefore, it seemed essential to investigate the peculiarities of primary androgen induction of an increased UEBP content in female rat liver and to compare it with the androgen-dependent imprinting of UEBP in males.

The level of UEBP seems to be highly sensitive to the subsequent regulatory control by sex steroids [2, 7, 10]. For this reason we studied the role of androgens and estrogens also in the regulation of the already induced level of UEBP with the emphasis on the interaction of these hormones during the regulation of UEBP content.

There is some evidence of a significant role of hypophysis in the formation of sex differences in the metabolic activity of some liver systems [11–14]. With this in mind, we also investigated the direct participation of hypophysis in the regulation of UEBP content as well as in the realization of determining and regulatory effects of androgens and estrogens.

The present work seems to be important for the elucidation of some peculiarities of sex steroid determination and regulation of UEBP level as one of sex-dependent hepatic proteins as well as for better understanding of possible physiological functions of UEBP.

## EXPERIMENTAL

The experiments were carried out on rats of mixed population: immature (30–40 g), prepubertal (80–90 g) and pubertal (150–200 g) males and females; ovariectomized (ov/ect) 1–30 days prior to experiments, adrenalectomized (adr/ect), thyroid-

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ectomized (thr/ect) 10–14 days prior to experiments or hypophysectomized (hp/ect) 20–25 days prior to experiments mature females; castrated 14–50 days prior to experiments or hp/ect 20–25 days prior to experiments with intact or removed testes pubertal males. Hypophysectomy was performed transauricularly [15]. Criteria of the effectiveness of hypophysectomy were the reduction of body weight and the decrease of the corticosterone content in the blood, measured by the Murphy method [16] in our modification ($2.5 \pm 0.4$ μg% in hp/ect, and $8.9 \pm 0.7$ and $13.3 \pm 1.2$ μg% in sham operated males and females, respectively).

*Hormone administration*

Neonatal androgenization of female rats was performed on the 1st–3rd postnatal day(s) by a single subcutaneous injection of testosterone propionate (TP) (1.25 mg). In other cases, the hormones were injected intramuscularly in 0.4 ml of propylene glycol in the following doses: TP—3 mg, estradiol ($E_2$)–0.4–10.0 μg, hexestrol (Hex)—10.0 μg. The dosage, duration and injection patterns of hormone administration as well as the time between the cessation of injections and determination of the UEBP level are indicated in legends to Figures and Tables.

To induce the UEBP level in the liver of ov/ect females, TP was injected daily for 3 days in a dose of 3.0 mg or as a single dose (3.0 mg), reaching the same level of UEBP on the 3rd day. $E_2$ was administered to these females 3–5 days after cessation of TP injections.

*Preparation of liver cytosol*

Liver cytosol was prepared as described previously [4]. All procedures were performed at 0–4°C. The liver was homogenized in a Teflon-glass homogenizer in 10 mM Tris–HCl buffer containing 1.5 mM EDTA, 10 mM KCl and 6 mM dithiothreitol pH 7.5. The tissue to buffer ratio was 1.5:1. Total protein content in the cytosol (10–20 mg/ml) was determined according to Lowry *et al.* [17].

*Determination of the number of $E_2$-binding sites of UEBP ($N_{UEBP}$)*

For determination of $N_{UEBP}$, 10–50 μl of cytosol (4 parallel measurements) was incubated for 15 min at 0–4°C with 100 pg of [2,4,6,7-$^3$H] estradiol ([$^3$H]$E_2$, specific radioactivity 92–98 Ci/mmol, "Amersham", England) in the absence (total binding) or presence (nonspecific binding) of 200 ng of unlabeled $E_2$. To prevent the interaction of [$^3$H]$E_2$ with liver $E_2$ receptors, all the samples contained 100 ng hexestrol (Hex) incapable of binding to UEBP. The final volume of the samples was 150 μl. The free and protein-bound hormone was separated by a short time exposure of the samples to 25 μl of a charcoal suspension (Norit A, 2%) coated with dextran 80 (0.4%) for 1 min at 0–4°C. After 5 min centrifugation at 3.000 *g*, aliquots of supernatants were collected for radioactivity measurements on a liquid scintillation spectrometer (Nuclear Chicago Mark II).

$N_{UEBP}$ was calculated from equation I: $N = B_s/U \cdot 1/K_a$, where $B_s$ is the specifically bound hormone, U is the unbound hormone, $K_a$ is the apparent equilibrium association constant. The $B_s$ values were estimated according to [4]. The $K_a$ value was calculated from the Scatchard plots [18] for each lot of [$^3$H]$E_2$. The $K_a$ values determined under the given experimental conditions varied from 0.21 to $0.33 \cdot 10^8$ $M^{-1}$.

The application of equation I for $N$ value determination was based on the following principles: with $B_s \ll N$, the law of mass action for the hormone–protein interaction is transformed into the equation: $B_s/U = K_a \cdot N$, or $N = B_s/U \cdot 1/K_a$. Thus, at relatively low ("minimal") additions of the hormone, the $B_s/U$ ratio should correlate with $N$. Therefore in the present study "minimal" addition of [$^3$H]$E_2$ for the measurement of $N_{UEBP}$ was employed instead of the routinely used saturating concentration of the hormone. This allowed to achieve a high level of specific binding as compared to the nonspecific one and thus to increase the sensitivity and reliability of the method. The method used seems to be a convenient tool for determining the hormone-binding sites of the proteins possessing a moderate affinity for the hormone. Our experiments demonstrated that at $B_s/U \leq 2.0$, this ratio does correlate with the amount of UEBP added and is independent of the total protein content in the sample up to 2 mg (Fig. 1). The

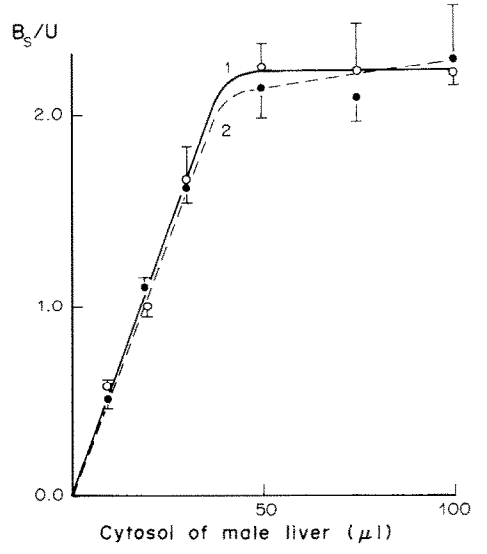


Fig. 1. Dependence of the level of specifically bound [$^3$H]$E_2$ after "minimal" addition of the hormone on the UEBP level and total protein content (M ± m). Concentration of UEBP was varied by adding to the samples different amounts of pubertal male rat liver cytosol. 10–100 μl of cytosol (total protein content 20 mg/ml) were adjusted to the constant volume of 100 μl by addition of a buffer (curve 1) or, in order to maintain the constant total protein level, by addition of pubertal female rat liver cytosol containing trace amounts of UEBP (curve 2) [total protein content 19.5 mg/ml]. The concentration of bound ($B_s$) and unbound (U) [$^3$H]$E_2$ was measured as described in Experimental.

$N$ values for UEBP in mature male rat liver determined by the above-described method were consistent with the results obtained earlier by the use of the equilibrium method of ion-exchange adsorption [19]. The removal of endogenous (free and UEBP-bound) steroids was achieved by incubating the samples with dextran-coated charcoal (15 min, 0°C) (effectiveness of procedure was >99%) and did not significantly influence the $N_{UEBP}$ values for intact animals as well as for animals injected with $E_2$ or TP in indicated doses. The absence of any noticeable effects of endogenous steroids on $N_{UEBP}$ is probably due to the relatively high capacity of the protein and to the lability of its complexes with the hormone.

## RESULTS

### *Some peculiarities of androgen determination of UEBP level in female rat liver*

*Androgen induction of elevated content of UEBP in female rat liver at different stages of ontogenesis.* As shown in Table 1 in untreated females of each age, the UEBP content was so low that it could not be determined quantitatively by the method described above. The low, but determinable level of UEBP was revealed in pubertal females only after ovariectomy. In males, the UEBP level reached the values exceeding those in females 10–20-fold (Table 1). The elevated content of UEBP in male rat liver is a result of natural neonatal imprinting by androgens [5, 9].

Neonatal androgenization of females led to the determination in the liver of immature animals of UEBP in amounts close to those in the males of the same age ($P > 0.1$, Table 1). In the liver of neonatally androgenized pubertal females, the imprinting by androgens of the UEBP level was unobserved (Table 1). The elevated content of UEBP in this group of animals can be revealed only after ovariectomy [5].

The data presented in Table 1 demonstrate that at later stages of ontogenesis androgens retain their ability to evoke primary stimulation of UEBP synthesis in female rat liver. After TP administration to immature females, the UEBP content in the liver of these animals approximated that in nonpubertal males and neonatally androgenized nonpubertal females ($P > 0.1$). In the liver of prepubertal females, the amounts of UEBP after TP injections was comparable to that in intact prepubertal males ($P > 0.1$) and markedly exceeded that in immature animals ($P < 0.001$). Androgen administration caused primary induction of an increased UEBP level also in pubertal females, both intact and ov/ect (Table 1).

These results suggest that androgens are capable of determining the increased level of UEBP in female rat liver, both when injected neonatally and upon primary induction at later stages of ontogenesis.

*Duration of elevated UEBP content in rat liver after cessation of androgen action.* The UEBP content, exceeding by about one order of magnitude that in ov/ect females, was observed for a long time (40–50 days) after castration of pubertal males (Table 2). Although the UEBP content in the liver of castrated males is lower than that in intact animals, the effect of natural neonatal imprinting by androgens was manifested in this case as the maintenance of a relatively high and constant level of UEBP in androgen-deficient males.

In the liver of pubertal ov/ect females, the high level of UEBP similar to that measured immediately after cessation of TP injections was observed for at least 1 month after the androgenization was stopped (Table 2). The presence of ovaries was a hindrance to the persistence of the high UEBP level after withdrawal of TP injections in the liver of pubertal females. Ovariectomy performed immediately after the cessation of TP administration prevented the disappearance of androgen effects (Table 2).

Thus, the duration of the androgen effect on the UEBP level after cessation of hormonal stimulation takes place both in the case of natural neonatal androgen imprinting of UEBP content in males and under postpubertal primary TP induction of the UEBP level in females. These findings suggest that in both cases androgens play a similar determining role.

Table 1. Androgen induction of $E_2$-binding sites of UEBP in female rat liver at different stages of ontogenesis in comparison with the UEBP content in male rats of various age

| Sex | Experimental group | $N_{UEBP}$, pmol/mg protein (M ± m) | | | |
|---|---|---|---|---|---|
| | | | | Pubertal | |
| | | Nonpubertal | Prepubertal | Intact | Gonadectomized |
| Females | Intact | <0.05 (6) | <0.05 (5) | <0.05 (7) | 0.39 ± 0.29 (23) |
| | Neonatally androgenized* | 0.59 ± 0.09 (11) | — | <0.05 (3)† | — |
| | Androgenized prior to experiment‡ | 0.46 ± 0.07 (10) | 1.30 ± 0.18 (8) | 0.39 ± 0.10 (10) | 1.92 ± 0.49 (24) |
| Males | Intact | 0.57 ± 0.05 (10) | 1.46 ± 0.23 (10) | 6.83 ± 0.49 (48) | 3.74 ± 0.69 (30) |

Here and further on the figures in brackets designate the number of determinations.

*TP (1.25 mg) was injected subcutaneously in a single dose on the 1st–3rd postnatal day.

†The TP-induced level of UEBP was revealed after ovariectomy of pubertal neonatally androgenized females [5].

‡TP (3.0 mg) was injected for 3 days: the number of $E_2$-binding sites of UEBP was determined 1 day after the last injection.

Table 2. Duration of revealing of the elevated UEBP content in female rat liver after cessation of TP injections in comparison with the dynamics of the UEBP level after castration of males

| Sex | Experimental group | $N_{UEBP}$, pmol/mg protein (M ± m) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Initial level | Days after castration of males or after cessation of TP injections to females | | | | | | |
| | | | 1 | 5 | 8–10 | 14–16 | 25–30 | 40–50 |
| Males | Pubertal | 6.83 ± 0.49 (48) | — | — | — | 3.14 ± 1.11 (12) | 3.74 ± 0.69 (30) | 3.30 ± 1.15 (5) |
| Females | Pubertal, ov/ect 30 days prior to TP injections* | 0.39 ± 0.23 (29) | 1.92 ± 0.49 (24) | 2.16 ± 0.41 (14) | 1.43 ± 0.05 (7) | 1.37 ± 0.35 (7) | 1.56 ± 0.58 (11) | —† |
| | Pubertal | < 0.05 (7) | 0.39 ± 0.10 (10) | < 0.05 (8) | — | — | — | — |
| | Pubertal, ov/ect immediately after cessation of TP injections* | < 0.05 | 0.99 ± 0.36 (6) | 0.67 ± 0.21 (5) | — | — | — | — |

*TP was injected as indicated in footnote ‡ to Table 1.
†UEBP content in ov/ect females remained at a high level 37–43 days after cessation of TP injections as well [7].

*Hormonal factors influencing the effectiveness of primary androgen induction of UEBP level in female rat liver*

*The role of adrenals and thyroid gland.* As can be seen from Table 3, adrenalectomy or thyroidectomy did not significantly influence either the level of TP-dependent induction of UEBP or the initial content of the protein in female rat liver.

*The role of hypophysis.* Hypophysectomy, on the contrary, prevented the TP induction of UEBP level in pubertal females (Table 3) without causing any appreciable influence on the initial level of UEBP. Presumably, the significant role of hypophysis in androgen induction of the high UEBP level in female rat liver is not due to the effects of adrenal, thyroid or ovarian hormones (Table 3) or their corresponding tropic pituitary hormones, but is conditioned by the existence of some other hypophyseal factor(s) which permit or mediate the androgen induction of the UEBP level.

*The role of ovaries and estrogen administration.* Ovarian hormones can diminish the degree of androgen induction of UEBP level in female rat liver as well as the initial level of UEBP (Table 3).

Estrogenization performed prior to TP injections did not significantly affect the TP-induced level of UEBP, whereas $E_2$ injected simultaneously with TP decreased the level of the TP-induced protein (Table 4). Thus, estrogens may influence only the degree of androgen induction of UEBP level in female rats. On the other hand, $E_2$ is incapable of exerting an androgen-like action. Injections of $E_2$ alone to ov/ect females resulted even in a slight decrease of the UEBP content (Table 4).

*Some peculiarities of regulation of UEBP level in rat liver*

*Regulatory effects of estrogens.* Administration of $E_2$ led to significant reduction of the initial UEBP content in all experimental groups, with the exception of hp/ect males. A similar effect was exerted by Hex, a synthetic estrogen incapable of binding to UEBP (Table 5).

Figure 2 shows the dynamics of changes in the UEBP level in male rat liver depending on the dose and duration of $E_2$ administration. In case of multiple injections of $E_2$, the rate of $N_{UEBP}$ decline increased with an increase in $E_2$ dose (Fig. 2B, D). Taken in low, i.e. 0.4 $\mu$g, doses, $E_2$ decreased the UEBP content only after multiple injections (Fig. 2A, B). Higher doses of $E_2$, e.g., 10 $\mu$g, were effective also on a single administration. In the latter case, however, the effect was observed after a long lag period, i.e. 3 days (Fig. 2B).

The return of the UEBP content to the initial level both after a single and multiple injections of $E_2$ to pubertal males was observed only 10–12 days after the development of the maximal effect (Fig. 2).

The inhibitory effect of $E_2$ on the UEBP content

Table 3. Effects of removal of some endocrine glands on the effectiveness of androgen induction of UEBP level in the liver of pubertal female rats

| Experimental group | $N_{UEBP}$, pmol/mg protein (M ± m) | | |
|---|---|---|---|
| | Without hormone | After TP injections | $P$* |
| Intact | <0.05 (7) | 0.39 ± 0.10 (10) | |
| adr/ect | <0.05 (3) | 0.36 ± 0.13 (18) | >0.1 |
| thr/ect | <0.05 (4) | 0.44 ± 0.06 (7) | >0.1 |
| sham hp/ect | <0.05 (3) | 0.31 ± 0.06 (7) | >0.1 |
| hp/ect | <0.05 (6) | <0.05 (11) | — |
| ov/ect | 0.39 ± 0.29 (23) | 1.92 ± 0.49 (24) | <0.001 |

*Significance of differences is given in relation to the TP-induced level of UEBP in the liver of pubertal intact female rats.
†TP was injected as indicated in footnote ‡ to Table 1.

Table 4. Effects of estrogens on androgen induction of UEBP level in female rate liver

| Experimental group | $N_{UEBP}$, pmol/mg protein (M ± m) |
|---|---|
| Pubertal | <0.05 (7) |
| Pubertal ov/ect | 0.39 ± 0.29 (23) |
| Pubertal ov/ect after TP injection* | 1.88 ± 0.39 (11) |
| Pubertal ov/ect after $E_2$ injection prior to TP injection† | 1.71 ± 0.68 (6) |
| Pubertal ov/ect after simultaneous injection of $E_2$‡ and TP* | 0.52 ± 0.24 (10) |
| Pubertal ov/ect after $E_2$ injection‡ | 0.22 ± 0.08 (6) |

*TP (3.0 mg) was injected in a single dose; the UEBP content was determined after 7 days.
†$E_2$ (10 μg) was injected for 6 days followed by TP (3.0 mg) injections for 3 days; the UEBP content was determined 1 day after cessation of TP injections (control injections of TP alone—see Table 1).
‡$E_2$ (10 μg) was injected in a single dose; the UEBP content was determined after 7 days.

Table 5. Effects of estrogens on the UEBP content in the liver of rats with different endocrine status

| Experimental group | $N_{UEBP}$, pmol/mg protein (M ± m) | | |
|---|---|---|---|
| | Initial level | After $E_2$ injections | $P$* |
| Pubertal males | 6.83 ± 0.49 (48) | 1.37 ± 0.65 (10)† | <0.002 |
| | | 0.90 ± 0.12 (5)‡ | <0.001 |
| Castrated males | 3.74 ± 0.69 (30) | 0.36 ± 0.09 (14)† | <0.001 |
| Ov/ect females with TP-induced UEBP | 1.43 ± 0.31 (7) | 0.50 ± 0.35 (8)† | <0.05 |
| Hp/ect males | 3.64 ± 1.09 (22) | 3.44 ± 0.77 (8)§ | >0.1 |

The UEBP content was determined 1 day after cessation of $E_2$ injections. All hormones were injected for 6 days.
*Significance of differences is given in relation to initial UEBP content.
†$E_2$ (0.4 μg).
‡Hex (10.0 μg).
§$E_2$ (10.0 μg).

was observed also in animals with a strong deficiency in endogenous androgens (Table 5). Presumably, the $E_2$-induced decrease of the UEBP content is not the result of depletion of the androgen level in the blood by $E_2$.

*Regulatory effects of androgens.* The UEBP concentration rose with maturation of the animals (Fig. 3, curve 1). During this period TP exerted a stimulating effect on the UEBP level only in prepubertal males (Fig. 3B). The lack of sensitivity to the regulatory influence of TP in the liver of immature males (Fig. 3A) may be accounted for the underdevelopment of the hepatic androgen–receptor system in the animals of this age group. In pubertal males the administration of TP did not further increase the UEBP level (Fig. 3C), apparently due to the fact that under effects of endogenous androgens the UEBP level in pubertal males reaches its maximum.

The decrease of the concentration of endogenous androgens in castrated or hp/ect males was accompanied by a reduction of the UEBP content ($P < 0.01$). Under these conditions, TP injections to pubertal males were effective and fully compensated for the decrease in the UEBP level caused by changes of the activity of the pituitary–gonadal system (Fig. 3D, E, F).

Besides, androgens can modulate in pubertal animals the effectiveness of the inhibitory effect of $E_2$. TP prevented the appearance of the inhibitory effect of $E_2$ on the UEBP content when injected simultaneously with it (Fig. 4A) and the development of this effect when injected after $E_2$ (Fig. 4B).

Hence, androgens regulate the UEBP level in two ways. On the one hand, these hormones stimulate the synthesis of this protein, but only at low initial concentration of UEBP. The reduction of the UEBP

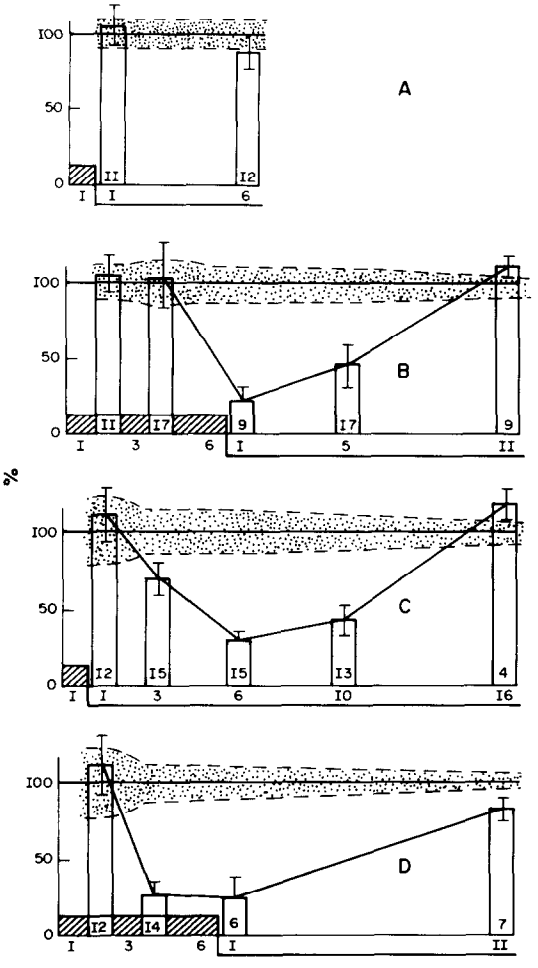


Fig. 2. Dynamics of changes in the UEBP content (M ± m) in male rat liver cytosol depending on the dose and duration of $E_2$ injections and the time elapsed after cessation of hormone administration. A—single injection; B—multiple injections of $E_2$ (0.4 μg); C—single injection; D—multiple injections of $E_2$ (10.0 μg) to pubertal male rats. The interrupted line delineates the UEBP level (M ± m) in control animals. Ordinate: $N_{UEBP}$ after $E_2$ injections (% of control). Abscissa: hatched area—duration of $E_2$ injections (days); unhatched area—time elapsed after cessation of $E_2$ injections (days). Here and further on the figures inside the columns designate the number of experiments.

content may be caused by several reasons, e.g. deficiency of endogenous androgens or inhibition by $E_2$. In all these cases, the regulatory effects of androgens are direct to the restoration and maintenance of the level of UEBP similar to that observed in pubertal males. On the other hand, androgenization, which itself does not affect the UEBP content in pubertal males, can provide for the stabilization of the normal UEBP level in these animals by diminishing the inhibitory effect of $E_2$.

*The role of hypophysis in regulation of UEBP level and realization of sex steroid effects*

Hypophysectomy of pubertal males resulted in the reduction of the UEBP content down to the level

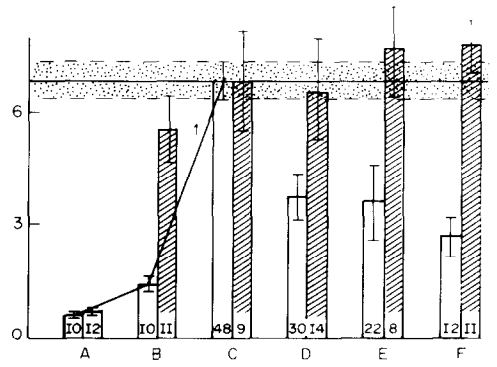


Fig. 3. Effects of androgens on the UEBP content in the liver of male rats with different endocrine status. Ordinate—$N_{UEBP}$ (M ± m) in pmol/mg of protein in male rat liver cytosol 24 h after 3-day injections of TP, 3.0 mg (harched columns) and in the absence of the hormone (unhatched columns). A—nonpubertal; B—prepubertal; C—pubertal; D—castrated; E—hp/ect; F—castrated hp/ect males. Here and further the interrupted lines delineate the UEBP level (M ± m) in pubertal males.

similar to that in castrated animals ($P > 0.1$, Table 6). At the same time, hypophysectomy of castrated males resulted only in a slight decrease of the UEBP content in comparison with $N_{UEBP}$ in castrated animals. It seems likely, therefore, that the inhibitory effect of hypophysectomy on the UEBP level in pubertal animals is mainly due to the absence of stimulatory influence of gonadotropins on androgen secretion by the testes. Evidence for this assumption can be obtained from the ability of TP to restore the initial level of UEBP both after castration and hypophysectomy. One should also not overlook the possibility of direct influence on the UEBP level of some hypophyseal factors, whose action can be simulated by androgens.

It seems quite probable that the hypophysis does not play any essential role in the realization of the stimulating regulatory effect of androgens on the UEBP level in male rat liver. On the other hand, it has been mentioned already that hypophysis is necessary for the realization of androgen induction of the UEBP level in female rats (Tables 3, 6). Evidently, the mechanisms of TP-dependent induction of the high UEBP level in females and of the stimulating regulatory effect of TP on the UEBP content in males are different.

The data presented in Table 6 suggest that the inhibition by $E_2$ of the UEBP level can take place only in animals with intact hypophysis. The lack of the inhibitory effect of $E_2$ on the UEBP content in hp/ect males may be due to the considerable decrease of the $E_2$ receptor content in the liver of hp/ect animals [20, 21].

## DISCUSSION

The present study demonstrates that the mechanisms of hormonal regulation of sex-related

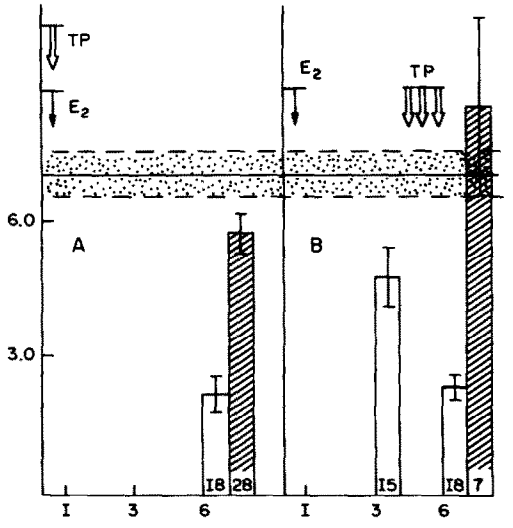


Fig. 4. Effects of androgens on the inhibition by $E_2$ of the UEBP content in pubertal male rat liver cytosol. Ordinate—$N_{UEBP}$ ($M \pm m$), pmol/mg of protein, in the liver of pubertal male rats after injections of $E_2$ (unhatched columns) and $E_2$ + TP (hatched columns). Abscissa—time elapsed after injections of $E_2$ (days). A—simultaneous single injections of $E_2$ (10.0 $\mu$g) and TP (3.0 mg); UEBP content was determined after 6 days; B—consecutive injection of $E_2$ (10.0 $\mu$g, single dose) and TP (3.0 mg, 3 days daily beginning with the 4th day after $E_2$ injection); UEBP content was determined prior to TP injections and 1 day after cessation of TP injections. Other designations as in legends to Figs 2 and 3.

differences in certain hepatic metabolic systems both have some common features and possess a number of important individual peculiarities. The general principles of regulation of the content of known sex-dependent liver proteins, including UEBP, appear to be as follows: (1) necessity of primed androgen determination; (2) subsequent reciprocal regulatory influence of estrogens and androgens; (3) participation of hypophysis in these processes [5, 7–9, 11–14, 22]. The results presented above provide evidence for some peculiarities and additional characteristic features of UEBP regulation by sex steroids. They are: (1) possibility of primed androgen induction of high UEBP level in females during long time intervals (from neonatal stage up to the pubertation period); (2) necessity of hypophysis only for realization of primed androgen determination and estrogen regulation of the UEBP content but not for the regulatory action of androgens; (3) realization of the stimulating regulatory influence of androgens only in the case of depletion of the UEBP content from normal level in contrast with estrogens acted at any initial level of UEBP.

The data on the presence of comparable amounts of sex steroids in the blood of male and female rats [11] may serve as evidence for the existence of estrogen–androgen control of the UEBP content under natural conditions.

And, finally, the observed peculiarities in sex steroid regulation of the UEBP content are directed at the maintenance of sex differences in the UEBP level in rat liver. The necessity of existence and maintenance of the elevated UEBP content only in males appear to be connected with its own special functions. According to our findings (in press), the presence of UEBP exerts an inhibitory action both on the hepatic estrogen–receptor interaction and on the metabolism of estrogens and androgens in the liver. Taking into account the ability of UEBP to interact with steroids of both sexes and their metabolites [4], it seems very probable that this protein is capable of mediating in male hepatocytes the influence of one of these steroids on the dynamics of reception and metabolism of others. It may be proposed that the main function of UEBP in males is to serve as an additional factor providing the dynamic maintenance of sex differences in the metabolism and reception of androgens and estrogens in the liver.

It may be concluded that at the level of the whole organism sex steroids act as factors, controlling the UEBP content and thus regulating sex-related differences in liver cell functions mentioned above.

Table 6. The role of hypophysis in the regulation of UEBP content and in the realization of sex steroid effects on UEBP level in the liver of male and female rats

| Sex | Experimental group | $N_{UEBP}$, pmol/mg protein ($M \pm m$) | | |
|---|---|---|---|---|
| | | Initial level | After TP injections* | After $E_2$ injections† |
| Males | Pubertal | $6.83 \pm 0.49$ (48) | $6.81 \pm 1.54$ (9) | $2.26 \pm 0.47$ (18) |
| | | | $P_1 > 0.1$‡ | $P_1 < 0.01$ |
| | hp/ect | $3.64 \pm 1.09$ (22) | $7.67 \pm 1.30$ (8) | $3.44 \pm 0.77$ (8) |
| | | $P_1 < 0.01$ | $P_2 < 0.01$ | $P_2 > 0.1$ |
| | Castrated | $3.74 \pm 0.69$ (30) | $6.60 \pm 1.39$ (14) | $0.66 \pm 0.09$ (14) |
| | | $P_2 > 0.1$ | $P_2 < 0.01$ | |
| | Castrated hp/ect | $2.75 \pm 0.62$ (12) | $7.77 \pm 0.68$ (11) | — |
| | | $P_2 > 0.1$ | $P_2 < 0.01$ | |
| Females | Pubertal | <0.05 (7) | $0.39 \pm 0.10$ (10) | — |
| | hp/ect | <0.05 (6) | <0.05 (11) | — |

*TP was injected as indicated in footnote ‡ to Table 1.
†$E_2$ (10 $\mu$g) was injected for 6 days; the UEBP content was determined 1 day after cessation of $E_2$ injections.
‡$P_1$: significance of differences in relation to $N_{UEBP}$ in the liver of pubertal male rats; $P_2$: the same in relation to $N_{UEBP}$ in the liver of HP/ect male rats.

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