LITERATURE CITED

1. V. A. Belitser and T. V. Varetskaya, Thrombos Res., 3, 265 (1973).
2. V. S. Davydov, F. B. Subkhankulova, R. I. Litvinov, and D. M. Zubairov, Vopr. Med. Khimii, No. 4, 83 (1982).
3. D. M. Zubairov, Kazan' Med. Zh., No. 1, 62 (1976).
4. I. K. Esipova (ed.), The Normal Lung [in Russian], Novosibirsk (1975).
5. K. L. Shakhanina, Immunologic Analysis [in Russian], ed. by L. A. Zil'ber, Moscow (1968), pp. 202-223.
6. C. L. Geczy, Haemostasis, 14, No. 5, 400 (1984).
7. R. A. Kekwik, M. M. Mackay, M. H. Nance, et al., Biochem. J., 60, 671 (1955).
8. T. Lyberg, I. Ivhed, H. Prydz, and K. Nilsson, Br. J. Haematol., 53, No. 2, 327 (1983).
9. Q. Myrvik, E. S. Leake, and B. Fariss, J. Immunol., 86, No. 2, 128 (1961).
10. H. Rothberger and M. R. McGee, J. Exp. Med., 60, No. 6, 1880 (1984).

OXIDOREDUCTASE ACTIVITY OF AN UNUSUAL ESTROGEN-BINDING PROTEIN OF RAT LIVER

A. N. Smirnov and E. G. Kazanova

UDC 616.36-008.936.6:577.175.64]-02:577.152.53]-092.9

KEY WORDS: unusual estrogen-binding protein; oxidoreductase activity; steroid hormones; liver.

The writers previously found a sex-dependent protein in the liver cytosol of male rats which, unlike sex hormone receptors, interacts specifically and to a comparative degree with two groups of steroids at once: estrogens and androgens [3]. Later this unusual estrogen-binding protein (UEBP) was fully purified by affinity chromatography on estradiol-sepharose and described [4, 8]. The results of experiments in vitro and in vivo point to the functioning of this protein as a modulator of reception and metabolism of its ligands by a process of labile deposition [1, 5, 7, 9, 12]. With regard to those ^{3}H-steroids that have been investigated (estradiol, estrone, estriol, testosterone, and androstenedione) UEBP did not exhibit hydroxysteroid-dehydrogenase and reductase activity under the conditions used [7]. By the use of chromatographic procedures to purify UEBP in order to isolate an estrophilic nonreceptor protein from the soluble fraction of rabbit liver, we obtained a highly purified preparation of steroidophilic hydroxysteroid dehydrogenase (NADP-dependent), active on androgens and gestagens as substrate.

Accordingly, in the present investigation the presence of oxidoreductase activity was again tested in UEBP, using a broad spectrum of steroid compounds as potential substrates.

EXPERIMENTAL METHOD

A highly purified preparation of UEBP was isolated from the liver cytosol of mature male rats by fractionation with ammonium sulfate, gel-filtration, and ion-exchange, affinity chromatography on estradiol-sepharose, as described previously [4, 8]. The resulting protein preparation preserved its hormone-binding properties characteristic of UEBP. Purity of the preparation was verified by polyacrylamide gel electrophoresis under denaturing conditions [10] and revealed the presence of only one polypeptide band, staining with Coomassie, with mol. wt. of about 31,000. The enzyme activity of the preparation was determined by measuring fluorescence of NADPH formed during oxidation, in the presence of NAD^+, $NADP^+$, NADH, or NADPH ("Reanal," Hungary), utilized for reduction of steroid substrates. Depending on the structure of the steroid substrates (on the presence of hydroxy and keto groups or of a double

Laboratory of Endocrinology, Faculty of Biology, M. V. Lomonosov Moscow University.
(Presented by Academician of the Academy of Medical Sciences of the USSR I. I. Ashmarin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 3, pp. 337-339, March, 1989. Original article submitted January 4, 1988.

TABLE 1. Determination of Oxidoreductase Activity of UEBP with respect to Formation or Utilization of NADPH

Steroid	Reaction velocity, nmoles/min				RCA, %*
	NAD	NADP	NADH	NADPH	
Estrane series:					
1,3,5(10)estratriene-3,17 β-diol (estradiol 17β)	0**	0**	–	–	100
1,3,5(10)estratriene-3-16 α,17 β-triol (estriol)	0	0	–	–	170
3-hydroxy-1,3,5(10)estratrien-17-one (estrone)	–	–	0***	0***	12.6
1,3,5(10)estratriene-3,17 α-diol (epiestradiol)	0	0	–	–	0.8
Androstane series:					
5α-androstane-3 α,17 β-diol	0	0	–	–	13.8
17β -hydroxy-4-androsten-3-one (testosterone)	0	0	0	0	5.9
17β -hydroxy-5 β-androsten-3-one (5 β-dihydrotestosterone)	0	0	0	0	2.2
17β -hydroxy-5 α-androstan-3-one (5 α-dihydrotestosterone)	0	0	0	0	2.2
17α -hydroxy-4-androsten-3-one (epitestosterone)	0	0	0	0	1.0
5 β -androstane-3α ,17β -diol	0	0	–	–	~1.0
5β-androstane-3 β,17 β-diol	0	0	–	–	~0.5
3β-hydroxy-5-androsten-17-one (dihydroepiandrosterone)	0	0	0	0	~0.5
5 α -androstane-3β ,17β -diol	0	0	–	–	<0.5
3β-hydroxy-5α -androstan-17-one (androsterone)	0	0	0	0	<0.5
3 α -hydroxy-5 α-androstan-17-one (epiandrosterone)	0	–	0	0	–
3 β -hydroxy-5 β-androstan-17-one (etiocholanolone)	0	0	0	0	<0.5
3 β -hydroxy-5 β-androstan-17-one	0	0	0	0	<0.5
4-androstene-3,17-dione	–	–	0	0	<0.5
5α-androstane-3,17-dione	–	–	0	0	–
5 β -androstane-3,17-dione	–	–	0	0	<0.5
4-androstene-3,11,17-trione	–	–	0	0	<0.5
5-androstene-3 β,17 β-diol	0	0	0	0	–
Pregnane series:					
4-pregnene-3,20-dione (progesterone)	–	–	0	0	<0.5
5α-pregnane-3,20-dione	–	–	0	0	–
5 β -pregnane-3,20-dione	–	–	0	0	–
5α-pregnane-3 β,20 β-diol	0	0	–	–	–
21-hydroxy-4-pregnene-3,20-dione (deoxycorticosterone)	–	–	0	0	–
11β ,21-dihydroxy-4-pregnene-3,20-dione (corticosterone)	0	0	0	0	<0.5
11β ,17α ,21-trihydroxy-4-pregnene-3,20-dione (cortisol)	0	0	0	0	–
3αβ ,17α ,21-trihydroxy-5 β-pregnane-11,20-dione (tetrahydrocortisone)	0	0	0	0	–
11β ,21-dihydroxy-4-pregnen-18-also-3,20-dione (aldosterone)	0	0	0	0	–

Legend. *Relative competitive activity (RCA) of compound taken from [6, 11]; **sensitivity of method 5 pmoles/min; ***) sensitivity of method 25 pmoles/min.

bond) the oxidized or reduced forms of the coenzyme were used. For this purpose 5 μg of steroids in 2 ml of 10 mM Tris-HCl buffer, pH 7.5, containing 10 mM KCl and 1 mM EDTA, was mixed with 10 μl of a 5 mM solution of the coenzyme. The reaction was initiated by addition of 2 μg of the UEBP preparation in 10 μl of buffer. The level of fluorescence of the sample was recorded continuously for 8-10 min at 25°C on an MPF-4 spectrofluorometer ("Hitachi," Japan). The temperature conditions used favor preservation of the hormone-binding activity of UEBP. The remaining experimental conditions were such that the hydroxysteroid-dehydrogenase activity of the estrophilic enzyme from rabbit liver could be recorded. The wavelength of excitation was 300 nm and of emission 460 nm, corresponding to maxima of absorption and emission of NADPH, with a transmission bandwidth of 10 nm. Solutions of NADPH of known concentration were used for calibration.

EXPERIMENTAL RESULTS

Table 1 gives the results of determination of the oxidoreductase activity of UEBP relative to the steroid belonging to the estrane, androstane, and pregnane series. For comparison, data are given on the relative competitive activity of the same compounds based on displacement of ^{3}H-estradiol from complexes with UEBP. None of the steroids tested, irrespective

of the degree of its affinity for protein, can act as substrate, within the limits of sensitivity of the method and under the experimental conditions used, for the presumptive oxidoreductase activity of UEBP.

The results are further confirmation of the regulatory function of UEBP, realized through temporary exclusion of protein ligands from the general hormonal biodynamics. The fact that UEBP is found in the soluble fraction of cells rules out the likelihood of mono-oxygenase activity of this protein [2]. Meanwhile there remains the possibility that UEBP possesses a different type of enzyme activity (transferase, for example), relative to its ligands.

LITERATURE CITED

1. M. L. Miroshnichenko, A. N. Smirnov, T. A. Shchelkunova, and V. B. Rozen, Probl. Éndokrinol., 31, No. 3, 61 (1985).
2. B. V. Pokrovskii, Hormone Biochemistry and Hormonal Regulation [in Russian], ed. by N. A. Yudaev, Moscow (1976), pp. 246-299.
3. A. N. Smirnov, O. V. Smirnova, and V. B. Rozen, Biokhimiya, 42, No. 3, 560 (1977).
4. A. N. Smirnov, T. A. Shchelkunova, V. V. Egorova, et al., Byull. Éksp. Biol. Med., 98, No. 9, 377 (1984).
5. A. N. Smirnov, T. A. Shchelkunova, and V. B. Rozen, Biokhimiya, 50, No. 9, 1395 (1985).
6. A. N. Smirnov, T. A. Shchelkunova, and V. B. Rozen, Biokhimiya, 51, No. 5, 751 (1986).
7. T. A. Shchelkunova, A. N. Smirnov, and V. B. Rozen, Byull. Éksp. Biol. Med., 100, No. 8, 186 (1985).
8. T. A. Shchelkunova, A. N. Smirnov, and V. B. Rozen, Biokhimiya, 51, No. 6, 937 (1986).
9. R. F. Aten, M. J. Weinberger, and A. J. Eisenfeld, Endocrinology, 106, No. 4, 1127 (1980).
10. U. K. Laemmli and M. Favre, J. Mol. Biol., 80, No. 2, 575 (1973).
11. M. L. Miroshnichenko, O. V. Smirnova, A. N. Smirnov, and V. B. Rozen, J. Steroid Biochem., 18, No. 4, 403 (1983).
12. W. Powell-Jones, C. Tompson, S. N. Nayfeh, and G. W. Lucier, J. Steroid Biochem., 13, No. 2, 219 (1980).

EFFECT OF INHIBITORS OF POLYAMINE BIOSYNTHESIS AND OF EXOGENOUS POLYAMINES ON MITOGENIC EFFECT OF EPIDERMAL GROWTH FACTOR IN PRIMARY HEPATOCYTE CULTURE

L. A. Osipova, N. I. Nemlii, N. Ya. Gridina, and V. A. Shlyakhovenko

UDC 616.36-018.1-02:547.425.5]-092.4-07

KEY WORDS: epidermal growth factor; polyamines; hepatocyte culture.

Activity of the enzymes of polyamine biosynthesis correlates positively with the proliferation rate of cells. It has been shown in the case of the regenerating liver that stimulation of proliferative processes is accompanied by increased activity of the key enzyme of polyamine synthesis, namely ornithine decarboxylase (ODC), during the hours immediately after the operation [4]. Temporary inhibition of ODC prevents the cells of the regenerating liver from entering the S phase of the mitotic cycle [9]. However, contradictory results have been obtained on primary cultures of hepatocytes [7, 8]. It has been shown, in particular, that several hormones possess a stimulating action on ODC, including some which are not inducers of cell proliferation [6]. There are likewise conflicting data on the effect of amines on proliferative processes, although it is known that an increase in the intensity of polyamine

R. E. Kavetskii Institute for Problmes in Oncology, Academy of Sciences of the Ukrainian SSR, Kiev.

(Presented by Academician of the Academy of Medical Sciences of the USSR T. T. Berezov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 107, No. 3, pp. 339-342, March, 1989. Original article submitted January 14, 1988.

0007-4888/89/0003-0375$12.50