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EFFECTS OF HORMONES ON SBP mRNA LEVELS IN HUMAN CANCER CELLS

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Summary—The human plasma sex steroid binding protein (SBP) has been previously shown to be synthesized in liver cells. The hormonal regulation studies of hepatic SBP mRNA demonstrate that it is controlled by estradiol, antiestrogen tamoxifen, dihydrotestosterone, triiodothyronine and insulin in a similar way as secreted SBP. The metabolic inhibitor cycloheximide was unable to prevent the estrogen or thyroid hormone induced increase in SBP mRNA. The slight stimulation of SBP synthesis by estradiol suggests that non-steroidal factors may be involved in its regulation and that the estrogen regulatory mechanism could also be partly post-transcriptional. In endometrial (Ishikawa cells) and prostatic (LNCaP cells) carcinoma cells, SBP mRNA has been detected suggesting that SBP may play a role in the uptake and intracellular mechanism of action of sex steroid in target cells.

INTRODUCTION

Sex steroid binding protein (SBP) or sex hormone binding globulin (SHBG) is a glycoprotein which binds in the human plasma, the most biologically active steroids, testosterone (T), dihydrotestosterone (DHT) and estradiol (E_2) [1, 2].

SBP has been shown to be synthesized in the monkey [3] and human [4] hepatocytes by indirect immunofluorescence studies as well as by the detection of a mRNA coding for SBP [4] in the liver. SBP was also shown to be secreted by the human hepatocarcinoma cell line, Hep G2 [5] and by a clone H5A derived from these cells [4].

In vivo evidence suggests that SBP synthesis by the liver is stimulated by estrogens and thyroid hormones and inhibited by androgens [6]. In some physiological events, the impact of the net androgen/estrogen/thyroid hormone balance on plasma SBP levels is apparent but it is not so obvious in others. Thus it is not known if these hormones exert their effects directly or indirectly on the hepatic SBP synthesis. *In vitro*, SBP secretion by Hep G2/H5A cells is clearly increased by thyroid hormones, thyroxine (T_4) or triiodothyronine (T_3) [7, 4], while only slightly induced by E_2 [4] but also by high concentrations of DHT [8]. In this cell-line, SBP mRNA accumulation is regulated by E_2 and T_3 in a similar way as secreted SBP [9].

The immunocytochemical localization of SBP in the human prostate, endometrium [4] and breast [10], which are target organs for steroid hormone action, asks the question of SBP origin. SBP may be available for transfer across cell membranes, possibly by a receptor mediated process; for instance, human decidual endometrial [11] and prostatic membranes [12] would interact specifically with SBP. Alternatively, SBP could be synthesized in the target cells. The presence of SBP in the target organs either by endocytosis or by synthesis *in situ*, leads us to propose that in addition to the classical role of reservoir regulating the biologically active free fraction of hormones in plasma. SBP may participate in the uptake and the intracellular mechanism of action of sex steroids.

In the present work, the regulation of SBP mRNA accumulation in H5A cells by steroids and their antagonists, as well as by thyroid hormone and insulin has been investigated, as has the presence of SBP mRNA in human endometrium, prostate and breast cancer cell lines.

EXPERIMENTAL

Cell lines

The different human cell lines under study have been originated from:

—hepatocarcinoma: H5A, a clone isolated from the Hep G2 cell line [13]

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—breast cancer: MCF-7 cells [14]
—endometrium adenocarcinoma: RL95-2 [15] and Ishikawa cells [16]
—prostate carcinoma: LNCaP [17]

Cell cultures

Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat inactivated fetal calf serum (Gibco, Grand Island, NY, U.S.A.), insulin (3 μg/ml; Organon), penicillin (100 μ/ml) and streptomycin (100 μg/ml; Eurobio, France). One week before the onset of each experiment, cells were transferred to the same medium but supplemented with 10% charcoal-dextran treated fetal calf serum (DCC-FCS), as described previously [4]. When a subconfluent state was reached, cells were switched for 24 h to phenol red-free DMEM without any additive. Treatment time with hormones was 60 h in phenol red-free DMEM supplemented with 10% DCC-FCS. Steroids (Roussel-Uclaf, France) in 0.1% ethanol, T_3 (Sigma, St Louis, MO, U.S.A.) in 0.1% NaOH M or insulin were added every 12 h in order to maintain hormone levels. Solvent alone was added to the control cells.

Poly (A)$^+$ RNA preparation

Total RNA was selectively precipitated in high yield from cell pellets homogenized with an ultra-Turrax (IKA Labor Technik) in LiCl 3 M/urea 6 M, as described previously [9]. After precipitation overnight at 4°C, RNA was separated from DNA and proteins by centrifugation 90 min at 12,000 *g* (4°C). The RNA pellets were carefully dissolved in 10 mM Tris–HCl, 1 mM EDTA, sodium dodecyl sulfate (SDS) 0.5%, pH 7.5 and freed from residual proteins by successive extractions with saturated phenol (v/v), phenol/chloroform–isoamyl alcohol (25/24:1, v/v), chloroform–isoamyl alcohol (24:1, v/v). After addition of 1/10 vol 3 M sodium acetate, pH 5.2, RNA was precipitated at −20°C with 2.5 vol absolute ethanol.

Poly (A)$^+$ RNAs were selected by one cycle chromatography of total RNA on oligo (dT)-cellulose using a mRNA purification kit (Pharmacia, Uppsala, Sweden), quantified by u.v. adsorption at 260 and 280 nm and precipitated as described previously.

Electrophoresis and Northern blot analysis

Partially purified poly (A)$^+$ RNAs (15 μg) were denatured with formaldehyde at 65°C for 5 min and separated by electrophoresis on 1% agarose gels containing 6% formaldehyde. To ensure that relatively equal amounts of RNAs were loaded, ethidium bromide (5 μg/ml) was added to the samples before heat denaturation. RNA molecular weight standards ranging from 9.49 to 0.24 kb (BRL) were run in parallel.

Poly (A)$^+$ RNAs were transferred to Hybond-N (Amersham) membrane after partial alkaline hydrolysis with Vacugene XL (Pharmacia LKB Biotechnology) in 20 × SSC (SSC: 0.15 M NaCl, 0.015 M sodium citrate).

The SBP cDNA probe was obtained by G. Hammond *et al.* [18]. This clone of 0.55 kb long represents the 3′ most part of the SBP cDNA up to the central Eco RI site. A β-actin cDNA was used as an ubiquitous probe since it is not regulated by steroid hormones. Both SBP and β-actin cDNAs were ^{32}P-labeled by a multiprime DNA labeling kit (Amersham). Radioactive probes were washed from free ^{32}P by chromatography on NACS-Prepac column (BRL) according to the recommendations of the manufacturer. Membranes were prehybridized for 4 h at 42°C and then hybridized overnight in 50% formamide at 42°C with 2×10^5 cpm/cm^2 of radioactive probe. After hybridization, membranes were rinsed at room temperature for 30 min in 5 × SCC, 0.1% SDS and twice for 30 min in 2 × SSC, 0.1% SDS, then 3 times for 30 min in 0.2 × SCC, 0.1% SDS at 68°C. Filters were air dried and exposed at −70°C to Kodak X-Omat AR-5 films using intensifying screens for 7 days. Northern blots were later stripped of probe by washing 1 h at 62°C in prehybridization buffer and reprobed with β-actin cDNA to normalize all message levels to those of β-actin. Autoradiograms were analyzed densitometrically using a Delsi controlled Vernon densitometer and mRNA levels were estimated by calculating the area under the curve with an integrator program.

RESULTS

Regulation of SBP mRNA concentration by E_2 and T_3 in H5A cells

SBP cDNA clone [18] was used to study the regulation of SBP mRNA in the human hepatoma cell line HepG2/H5A. A Northern transfer hybridization analysis of mRNAs from H5A cells grown in the absence of hormones (control cells, C) (Fig. 1) demonstrates that SBP cDNA hybridizes, under high stringency washing conditions, to a low abundance mRNA of

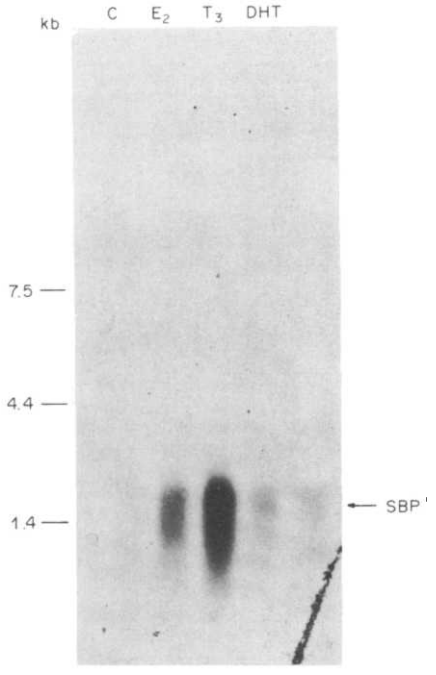

Fig. 1. E_2 and T_3 effect on SBP mRNA in H5A cells. After 1 week growth in 10% DCC-FCS-DMEM, H5A cells were treated for 60 h with red phenol-free DCC-FCS-DMEM only (C) or in the presence of 100 nM E_2 or T_3. Hormones were added every 12 h; mRNAs (15 μg) were separated by electrophoresis on 1% agarose-6% formaldehyde gels, transferred on Hybond N and hybridized with a random primed ^{32}P-SBP cDNA probe. The RNA ladder used for size standards was indicated at the left.

approx. 1.6 kb which agrees with the length of SBP mRNA in human liver [4]. The relative intensity of the signal varies from one experiment to another but does not change during the process of increasing the washing stringency; such a variation could reflect different degrees of confluence of the cells.

The steady state level of SBP mRNA was then measured in H5A cells which were previously treated for 2 days 1/2 with E_2 or T_3 at a concentration of 100 nM. The rapid metabolism of these hormones in the liver has prompted us to refeed the cell cultures every 12 h with E_2 or T_3. To carefully examine SBP mRNA regulation by hormone, it is important to maintain cells in an environment in which estrogenic activity is minimized. Since the estrogenic effects of phenol red, contained in the culture medium, have been documented [19], cells were maintained in DMEM lacking this pH indicator. When compared to the cells grown in the absence of hormone, SBP mRNA accumulation is increased after stimulation by E_2 but the amplitude of the response only remains 1.3–1.5 times the control level. The most potent induction is observed after T_3 treatment (Fig. 1). Therefore E_2 and T_3 increase the steady state level of SBP mRNA at concentrations previously shown to stimulate the secretion of SBP from H5A cells [4].

Inhibition of protein synthesis does not prevent mRNA induction in H5A cells

The close correlations of E_2 and T_3 regulation on both SBP secretion and SBP mRNA levels in H5A cells suggest that the effect of hormones is related to an increase of SBP synthesis.

The effect of cycloheximide (CHX), a protein synthesis inhibitor, on SBP mRNA accumulation in H5A cells was studied in order to determine whether E_2 or T_3 induction is a primary response or requires the synthesis of additional regulatory factors. Half of the cells were pretreated for 1 h with 50 μM CHX; all the cells were then stimulated for 7 h with or without 100 nM E_2 or T_3, in the presence or absence of the protein synthesis inhibitor. mRNAs were analyzed by Northern blot hybridization, normalized for equal loading by reprobing with β-actin cDNA probe and quantitated by densitometry scanning.

As shown in Fig. 2, CHX does not affect the induction of SBP mRNA by E_2 or T_3 suggesting

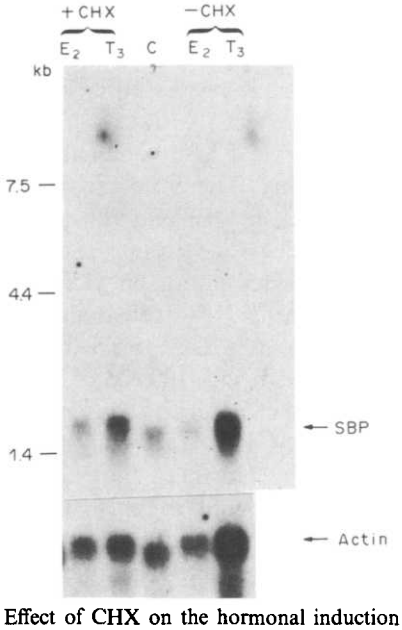

Fig. 2. Effect of CHX on the hormonal induction of SBP mRNA in H5A cells. H5A cells grown in phenol red-free and steroid-stripped medium were incubated for 1 h with (+CHX) or without (−CHX) 50 μM CHX. E_2, T_3 (100 nM) or solvent alone (C) were then added for 7 h. mRNAs (15 μg) were analyzed by Northern blot hybridization as described in Fig. 1. A control β-actin RNA which was not regulated by estrogens was detected by a β-actin cDNA probe. The amount of mRNA loaded on each line was evaluated relative to β-actin mRNA content by densitometric analysis.

that hormonal effect is independent of new protein synthesis.

Inhibition of SBP mRNA accumulation in H5A cells by insulin

Insulin has been found to decrease SBP secretion from HepG2 cells at a concentration of 10 nM [20] or 10 μM [21]. The effect of insulin on SBP synthesis has been investigated in H5A cells.

H5A cells were maintained for 60 h on phenol red-free 10% DCC-FCS medium in the presence or absence of 0.5 μM insulin, with or without 100 nM E_2 and the steady state levels of SBP mRNA were examined by Northern blot hybridization. Changes in SBP mRNA were quantified by scanning densitometry and the data presented as percent of E_2 induction in insulin deprived cells, after normalization for equal RNA loadings. In control cells grown without E_2 insulin decreases SBP mRNA level (Fig. 3). However the 1.5 times induction of SBP mRNA accumulation by E_2 is not affected by addition of 0.5 μM insulin.

Regulation of SBP mRNA level by steroids and antagonists

Plasma SBP concentrations are markedly increased by natural (E_2) or synthetic (ethinylestradiol) estrogens while androgens (T, DHT) and some progestational agents have a depressive effect. However the precise hormonal control of SBP is actually equivocal and unconvincing. Therefore the effects of E_2, DHT, progesterone (P) and their antagonists tamoxifen, cyproterone acetate and RU486 on SBP mRNA accumulation in H5A cells have been investigated. H5A cells maintained on phenol red-free 10% DCC-FCS medium were stimulated for 60 h with 100 nM of hormones or antagonists. The natural steroids were supplemented every 12 h. mRNAs were isolated and analyzed by Northern blot hybridization.

The relative amounts of SBP mRNA, after scanning densitometry and normalization for equal RNA loadings with β-actin cDNA probe, have been expressed as percent of E_2 induction (Fig. 4). P and its antagonist RU486, along with the antiandrogen cyproterone acetate have no effect and SBP mRNA remains at the control level. DHT seems to lower SBP mRNA accumulation but the significance of this has to be precised. The antioestrogen tamoxifen clearly induces SBP mRNA at a higher extent than E_2 itself.

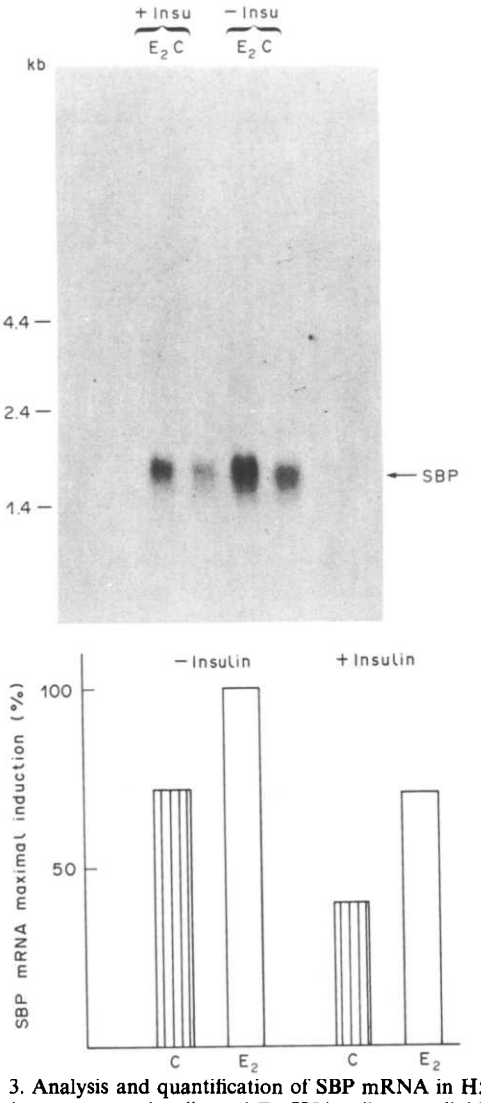

Fig. 3. Analysis and quantification of SBP mRNA in H5A cells in response to insulin and E_2. H5A cells were divided into 4 groups and treated in phenol red-free DCC-FCS-DMEM for 60 h as follows: E_2 100 nM + insulin 0.5 μM, insulin 0.5 μM, E_2 100 nM and no hormones (C). Hormones were renewed every 12 h; mRNAs were isolated and quantified as described in Figs 1 and 2. Upper panel: Northern blot hybridization; lower panel: the amount of mRNAs was evaluated by densitometry scanning and, after normalization for equal loadings, expressed as percent of maximal induction. 100%: E_2 induction in the absence of insulin.

SBP mRNA in breast cancer cells MCF-7

SBP-like antigen has been observed, by indirect immunofluorescence in some human mammary carcinoma [22] as well as in normal breast tissues [10]. The presence of SBP mRNA in the human breast cancer cell line MCF-7 has been investigated.

MCF-7 cells maintained on phenol red-free DMEM supplemented with 10% DCC-FCS were grown for 60 h in the presence or absence of 100 nM E_2 or T_3. H5A cells were studied in

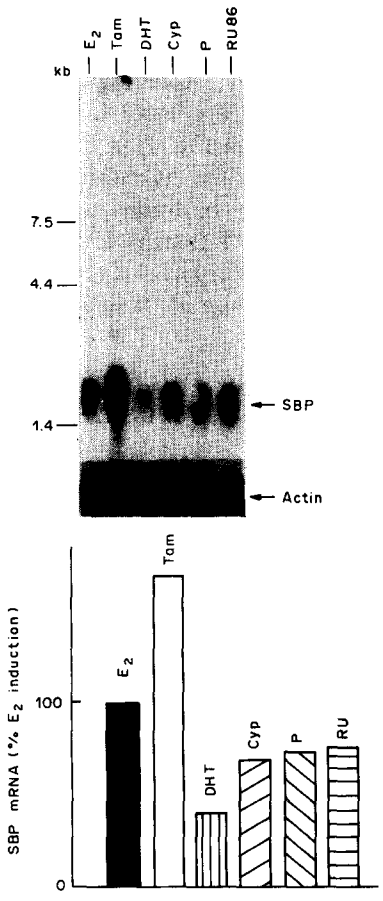

Fig. 4. Regulation of SBP mRNA in H5A cells by steroids and antagonists. H5A cells were grown in phenol red-free DCC-FCS-DMEM, divided in 6 groups and treated for 60 h as follows: E_2 100 nM, Tamoxifen (Tam) 100 nM, DHT 100 nM, cyproterone acetate (Cyp) 100 nM, P 100 nM and RU486 100 nM. Natural hormones were renewed every 12 h. mRNAs were isolated and analyzed as described in Figs 1–3. Upper panel: Northern blot hybridization; lower panel: densitometry quantification.

parallel as a control. Cells were then harvested; mRNA were isolated and analyzed by Northern blot hybridization.

As seen in Fig. 5, in control as well as in hormone treated MCF-7 cells, SBP mRNA could not be detected while it is present in H5A cells.

SBP mRNA in endometrial carcinoma cells RL95-2 and Ishikawa

In the human uterus, immunofluorescence studies have revealed SBP-like antigen only localized in endometrium [4]. The presence of SBP mRNA was initially investigated in a human endometrial cancer cell line RL95-2 [9]. Whatever the hormonal stimulation, it was not possible to detect SBP mRNA in these cells maintained in serum-free conditions.

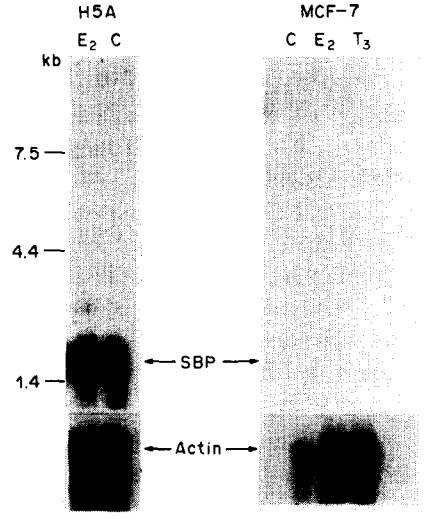

Fig. 5. SBP mRNA investigation in the breast cancer cell line MCF-7. MCF-7 cells and H5A cells (studied as a positive control) were grown for 60 h in phenol red-free DCC-FCS-DMEM in the absence (C) or presence of E_2 or T_3 (100 nM). Hormones were renewed every 12 h. mRNAs (20 μg) were isolated and analyzed as described in Figs 1 and 2.

RL95-2 were therefore grown for 60 h in phenol red-free DMEM supplemented with 10% DCC-FCS in the absence or presence of 100 nM E_2 or T_3. H5A cells were studied in parallel as a control. Cells were harvested; mRNAs were isolated and analyzed by Northern blot hybridization. As seen in Fig. 6, SBP mRNA is not observed, in control or in hormone-treated RL95-2 cells, even if large

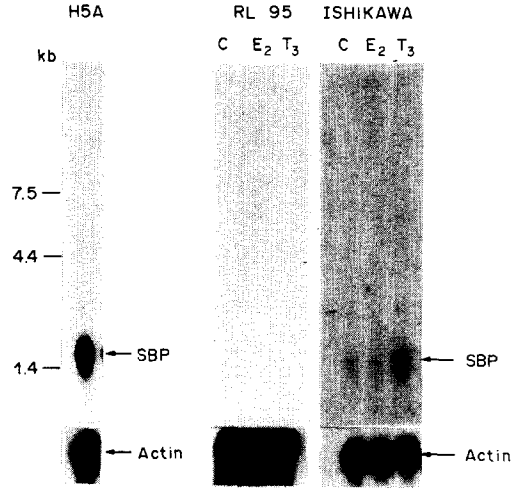

Fig. 6. Analysis of SBP mRNA in two endometrial carcinoma cell lines, RL95-2 and Ishikawa. RL95-2 and Ishikawa were grown in phenol red-free DCC-FCS-DMEM and exposed for 60 h to hormones (E_2, T_3, 100 nM) or to a vehicle solvent (C). H5A cells were studied as a positive control. RL95-2 mRNAs (40 μg) and Ishikawa mRNAs (15 μg) were analyzed as described in Figs 1 and 2.

amounts, up to 25 μg, of mRNA are studied, as shown by β-actin cDNA signals.

Since SBP mRNA could be lost during the transformation of RL95-2 cells, a second cell line, Ishikawa cells, isolated from a human endometrium adenocarcinoma, was investigated. Ishikawa cells were maintained in the same culture conditions, RL95-2 cells and mRNAs were analyzed as mentioned before. Under high stringency washing conditions, in the control cells as in the E_2- or T_3-treated Ishikawa cells, a SBP mRNA which is essentially the same size as in H5A cells is present (Fig. 6). T_3 seems to better induce SBP mRNA accumulation but the significance of this increase has to be confirmed.

SBP mRNA in prostate adenocarcinoma cells LNCaP

Immunocytochemical studies have shown [4] that a SBP-like antigen was only present in the glandular epithelium of the human prostate while absent in the prostatic stroma. In order to answer the question of prostate SBP origin which could be synthesized *in situ* or may enter the cells by endocytosis, SBP mRNA has been investigated in the human prostatic carcinoma cell line LNCaP.

LnCaP cells were maintained for 60 h in phenol red-free DMEM supplemented with 10% DCC-FCS in the absence or presence of E_2 or T (100 nM). Cells were then harvested; mRNAs were purified and analyzed by Northern blot hybridization. H5A were grown under the same conditions as a control for SBP mRNA detection. As seen in Fig. 7, in E_2-treated cells, the SBP cDNA probe hybridizes under high stringency washing conditions to a mRNA of 2–2.2 kb while, in T-treated LNCaP cells, to 3 mRNAs species of approx. 2–2.2, 1.4–1.6 and 1 kb. However the size of these mRNAs has to be precise.

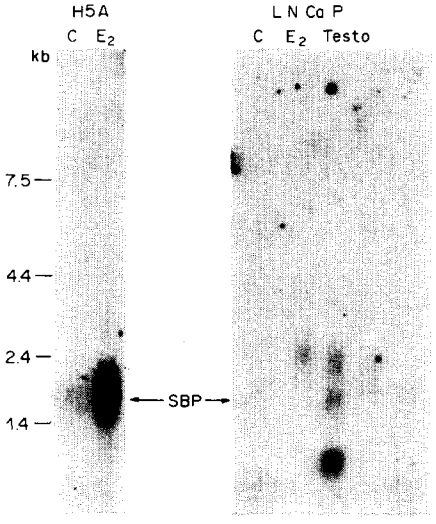

Fig. 7. Northern analysis of SBP mRNA in the prostate carcinoma cell line, LNCaP. LNCaP cells were grown in phenol red-free DCC-FCS-DMEM and exposed for 60 h to E_2 or T 100 nM or to a vehicle solvent (C). Hormones were supplemented every 12 h; H5A cells were stimulated in parallel as a positive control. mRNAs were purified and analyzed as described in Figs 1 and 2.

DISCUSSION

In vivo the hormonal regulation of human plasma SBP levels under physiological and pathological conditions has been largely reviewed [1, 6, 23] and an attempt has been made to relate plasma SBP variations to stimulation or inhibition of the SBP synthesis in the liver. However this assumption precludes any further comment on the hepatic SBP synthesis control by steroids, thyroid hormones and insulin which has not previously been investigated. Therefore, the present study deals with the regulation of the steady state level of SBP mRNA in hepatoma cells, H5A, by hormones which are known to promote changes in plasma SBP concentrations.

The presence of SBP mRNA in hepatoma cells grown in medium supplemented by DCC-FCS is a direct evidence that the liver is the site of SBP synthesis [4]. The small concentration of SBP message in the control cells reflects the poor representation of 0.0013% recently reported for SBP mRNA in the human liver [24]. One mRNA of 1.6 kb is present in H5A cells; the human liver also contains in addition an approx. 2.5 kb mRNA, the identity of which is unknown but it might represent incompletely processed SBP mRNA [25]. Plasma SBP concentration is known to rise markedly during the first half of pregnancy under the influence of rapidly increasing estrogen concentration; however in the second half of pregnancy SBP increase is not so clearly correlated with estrogen levels. In H5A cells, E_2 stimulates to a lesser extent both SBP mRNA and SBP secretion. Hep G2/H5A cells have been shown to possess nuclear estrogen receptor but the correlations between E_2 and inducible proteins seem relatively weak. So that other non-steroidal factors may be involved in the regulation of SBP synthesis. Finally, as reported for the plasma thyroxine binding globulin [26], estrogen could also modify the sialylation of

SBP which would be cleared from the plasma more slowly.

The induction of SBP mRNA accumulation by T_3 in H5A cells reflects the effect of both T_4 [7] or T_3 [4] on SBP secretion and the elevated level of plasma SBP found in thyreotoxicosis [6]. The pretreatment of the cells by CHX does not affect E_2 and T_3 stimulation of SBP mRNA accumulation suggesting that no protein synthesis is required for hormonal induction. However the effect of E_2 and T_3 on SBP mRNA transcription and stability has to be investigated.

Insulin has been reported to decrease SBP secretion from Hep G2 cells [20, 21] but there is some controversy in the literature concerning the efficient concentrations and the mitogenic effect. Insulin (0.5 μM) decreases the basal level of SBP mRNA in control H5A cells without any change in the percent of E_2 induction. This effect of insulin on SBP synthesis could partly explain the small level of plasma SBP seen in massive obesity. However work is actually in progress to elucidate the mechanism of action of insulin which could be mediated either by its own receptor or by the IGFI receptor.

The study of the regulation of SBP mRNA accumulation by steroids and their antagonist has shown that progesterone and the antagonist RU486 along with the antiandrogen cyproterone acetate have no effect. Exogenous progesterone does not change either plasma SBP concentration [27] or SBP secretion from H5A cells [4]. In contrast, cyproterone acetate which has been reported to break down plasma SBP levels [28] in hirsute women treated with androcur, would not act on SBP synthesis. DHT seems to decrease SBP mRNA accumulation; this result is in agreement with the antagonistic effect of the administration of androgen on plasma SBP concentration [6]. However SBP secretion from Hep G2 cells was elevated after DHT stimulation [8] but, at micromolar concentrations, DHT induces estrogen-sensitive proteins via the estrogen receptor [29]. Finally the induction of SBP mRNA by tamoxifen reflects the partial agonist activity of this antiestrogen which has already been reported on plasma SBP [23] and secreted SBP [4] from H5A cells.

The presence of SBP-like antigen in human prostate epithelium and endometrium [4] as well as in mammary glands [10] raises the question of SBP origin. SBP may enter the cells by endocytosis or be synthesized *in situ*. Thus SBP mRNA has been investigated in some human cancer cell lines isolated from breast (MCF-7), endometrium (RL95-2 and Ishikawa cells) and prostate (LNCaP).

In MCF-7 cells, SBP mRNA could not be detected. This result might have been expected since SBP-like antigen has been localized in MCF-7 cells cytoplasm [3] only after addition of pure SBP in the culture medium. However, SBP mRNA could be absent in transformed cells and the search of the message should be undertaken in normal mammary glands and in other breast cancer cell lines.

The human endometrial carcinoma cell line RL95-2 was derived from a moderately differentiated adenosquamous carcinoma of endometrium and possessed E_2 receptor [15]. In serum-free conditions [9] or in medium supplemented with 10% DCC-FCS, irrespective of the hormonal addition and of the amount of loaded poly $(A)^+$ RNAs, no SBP mRNA was observed. Estrogen responsiveness of these cells has not been clearly established; therefore the Ishikawa cell line [16], also isolated from a human endometrium adenocarcinoma, was investigated. These cells do contain estrogen and P receptors, are partly responsive to E_2 in terms of proliferation rate and P receptor induction while unresponsive on cathepsin D secretion [30]. In contrast to RL95-2, a mRNA of 1.6 kb coding for SBP, as suggested by the hybridization under stringent conditions, is present in Ishikawa cells grown with or without E_2 or T_3 (100 nM) and the hormonal regulation study of which is actually in progress. This result is the first to demonstrate that SBP is synthesized within some target organs for steroid hormones and tends to suggest that SBP may have an additional role, that of a reservoir function.

The human prostate carcinoma cell line LNCaP has been reported to be androgen responsive in terms of increased growth rate and secretion of androgen-sensitive protein [31]. However the androgen receptor has a point mutation which changes the hormonal specificity. The SBP cDNA probe hybridizes with one mRNA in control and E_2-treated LNCaP cells while in T-stimulated cells, three messages are present, the exact size of which has to be confirmed. That all of the mRNA species code for SBP or closely related proteins is suggested by their hybridization under high stringency conditions. In the human liver, SBP mRNAs were resolved into two bands of

hybridization [25]. Androgen-binding protein (ABP) was closely related to SBP and the analysis of ABP mRNA isolated from rat Sertoli cells showed two species which appeared to be differentially regulated by follicle-stimulating hormone (FSH) [32]. Differential regulation could occur by the use of alternate promoters of RNA transcription or of alternative splicing. Since one SBP mRNA is present in liver, endometrium and E_2-treated prostate cancer cells, prostatic SBP mRNAs species may be tissue specific and differentially regulated by T. However clearly additional studies are needed, especially on normal human endometrium and prostate, to understand the hormonal regulation of SBP mRNAs.

In conclusion, factors controlling SBP synthesis in the human liver are, until now, poorly understood. E_2 which slightly increases hepatic SBP mRNA accumulation may also act at a post-transcriptional level on the sialylation of the protein. The interesting observation of SBP mRNA within target organs for steroid action provides evidence that in addition to the classical role of a buffer system regulating the free fraction of hormones in the plasma and along with the characterization of SBP membrane receptors [11, 12], SBP may be involved in their uptake by the target organs and in their intracellular mechanism action.

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