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# Sex hormone-binding globulin mediates steroid hormone signal transduction at the plasma membrane ☆

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**Abstract**

Sex hormone-binding globulin is a plasma glycoprotein that binds certain estrogens and androgens with high affinity. Over the past several years it has been shown that, in addition to functioning as a regulator of the free concentration of a number of steroid hormones, SHBG plays a central role in permitting certain steroid hormones to act without entering the cell. The system is complex. SHBG interacts with a specific, high affinity receptor ($R_{SHBG}$) on cell membranes that appears to transduce its signal via a G protein. The SHBG–$R_{SHBG}$ complex causes the activation of adenylyl cyclase and the generation of cAMP within a matter of minutes after exposure to an appropriate steroid. Only steroids that bind to SHBG can activate SHBG–$R_{SHBG}$, but not all steroids that bind have this function, e.g. are agonists. All steroids that bind to SHBG but do not activate adenylyl cyclase are antagonists. The signals generated by the steroid-SHBG–$R_{SHBG}$ complex generate messages that have effects on the transcriptional activity of classic, intracellular receptors for steroid hormones. These and other downstream effects of this system are reviewed. 

## 1. Introduction

Sex hormone-binding globulin owed its initial description to its ability to bind certain androgens and estrogens with high affinity [1–3]. Subsequent studies of its function concentrated on the physiologic consequences of the binding of the involved steroids. The driving hypothesis in most of these studies was that SHBG hindered the access to cells of the steroids that it bound. This model of the function of SHBG visualized it as a relatively passive participant in the overall scheme of steroid hormone signaling. This model had to be amended when it was discovered that the plasma membranes of a number of different cells were able to bind SHBG specifically and with high affinity. In this communication we will review the model that has emerged of the place of SHBG in furnishing an infrastructure for selected steroids to generate second messengers by interacting with SHBG at the plasma membrane.

## 2. A receptor for SHBG ($R_{SHBG}$) on cell membranes

Fig. 1 is a cartoon that shows how SHBG interacts with its membrane receptor ($R_{SHBG}$). SHBG has two binding sites, one site binds steroids and the other binds to $R_{SHBG}$. Binding of a steroid induces a change in the SHBG membrane-binding domain that inhibits SHBG from binding to cell membranes. The inhibition is non-competitive, and the potency of a steroid in preventing receptor binding correlates with the strength of its interaction with SHBG. Further, the affinity of a steroid for SHBG is not necessarily an indication of its

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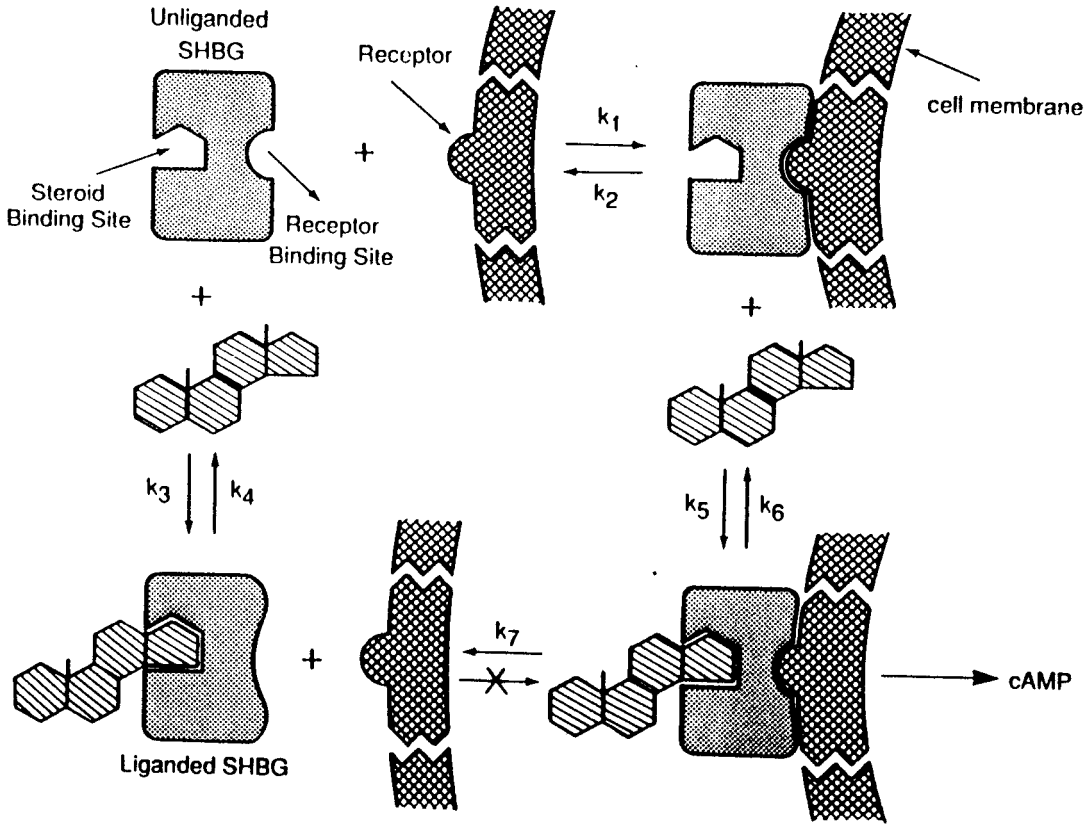


Fig. 1. Cartoon of the steroid-SHBG-$R_{SHBG}$ signaling system (adapted from Hryb et al. [5]). See text for discussion.

biologic potency. For instance, 2-methoxyestradiol is a biologically inert metabolite of estradiol but it binds more tightly to SHBG than does either testosterone or estradiol [4,5]. It also inhibits SHBG binding to $R_{SHBG}$ more strongly than either of these two steroids [5]. Although occupancy of its steroid-binding site prevents the interaction of SHBG with $R_{SHBG}$, unliganded, membrane-bound SHBG can bind steroids. Although binding of a steroid to the SHBG–$R_{SHBG}$ complex initiates dissociation of the complex, the rate of dissociation is extraordinarily slow, with a half-life $>30$ h. Thus, there is more than ample time for a steroid to initiate events leading to the generation of a second messenger. We will not review it here, but the biochemical/biophysical nature of the SHBG–$R_{SHBG}$ interaction has been thoroughly examined; the kinetic and equilibrium constants at physiological temperatures are known [5,6]. The model described above was developed in human prostatic tissue, but it appears to be equally applicable to the breast cancer cell line, MCF-7 [7]. Additionally, SHBG has shown to bind to tissue derived from normal endometrium, endometrial carcinoma, liver and epididymis, but neither striated muscle nor colonic mucosa [8].

## 3. $R_{SHBG}$ and second messengers

$R_{SHBG}$ is an integral part of a signal transduction system that allows certain steroids to affect cell function without entering the cell. It participates in the initiation of a cascade that signals through adenylyl cyclase and cAMP. Unlike most plasma membrane receptors for peptides, signaling is not initiated when the ligand, SHBG, is bound. $R_{SHBG}$ requires not only the initial binding of its ligand, SHBG, but the subsequent binding of a steroid to the SHBG–$R_{SHBG}$ complex for a downstream signal to be initiated. The binding of an appropriate steroid is necessary to activate adenylyl cyclase and cause an increase in intracellular cAMP. This tripartite system (steroid–SHBG–$R_{SHBG}$) is reminiscent of the way that steroids initiate signaling within the cell. Steroids bind to their cognate receptors (functionally homologous to SHBG in this analogy) activating them (functionally homologous to activating SHBG–$R_{SHBG}$), and permitting them to bind to steroid response elements and activate transcription (functionally homologous to activating adenylyl cyclase).

We first observed activation of adenylyl cyclase by

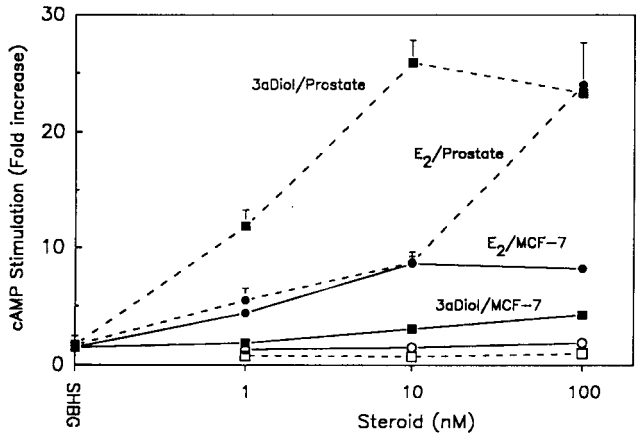


Fig. 2. Generation of cAMP in prostate explants and MCF-7 cells. To saturate SHBG receptors, 50 nM, highly purified SHBG was added to tissues/cells, in serum free medium, for 3 h at 37°C. SHBG in the medium was then removed by a single wash, after which there was a 15 min incubation with vehicle or the indicated concentration of steroids and isobutyl-methylxanthine (0.1 mM). At the end of this second incubation, cAMP was extracted and determined by a commercial ELISA. The data on prostate were recalculated from Nakhla et al. [11]. The data on MCF-7 cells are from unpublished studies from this laboratory (Nakhla, A. M., Leonard, J. and Rosner, W.). Open symbols—no preincubation with SHBG; Closed symbols—preincubation with SHBG; Circles—5α-androstan, 3α, 17β-diol (3αDiol); Squares—estradiol ($E_2$). Data are ±SEM. Absence of an error bar indicates it was smaller than the symbol.

the $R_{SHBG}$ system in the human prostate carcinoma cell line, LNCaP [9]. In those cells, both dihydrotestosterone (DHT) and estradiol caused an increase in intracellular cAMP, in the presence, but not the absence, of SHBG–$R_{SHBG}$. Subsequent experiments in human [10] and canine [11] prostate explants revealed a number of interesting differences from the LNCaP cell line. First, the most robust increases in cAMP were observed in stromal, not epithelial cells. Second, although estradiol-SHBG activated adenylyl cyclase, as it did in LNCaP cells, the quintessential prostatic androgen, DHT, did not activate the SHBG–$R_{SHBG}$ complex to initiate increases in cAMP. On the contrary, it antagonized the activation of SHBG–$R_{SHBG}$ by estradiol. We screened other steroids known to bind to SHBG, and found the DHT 'metabolite' 5α-androstan, 3α, 17β-diol to be an extremely potent agonist in the stimulation of cAMP accumulation in prostate explants [11]. It is more potent than estradiol and its concentration at half maximal potency (1 nM) is equal to its concentration in male plasma [12,13]. Before this observation, this steroid was thought to be an inactive metabolite of DHT. These observations made it clear that it was a hormone in its own right, and it subsequently has been shown to be a physiologic hormone in the guinea pig [14]. Further evidence of the tissue specificity of this system is demonstrated by the agonist effect of estradiol in human breast cancer cells (MCF-7), and the lack of agonist activity of testosterone and DHT in this same tissue [7]. We have confirmed the agonistic activity of estradiol and also shown that 5α-androstan, 3α, 17β-diol is a weak agonist in MCF-7 cells (Nakhla, A. M., Leonard, J., Rosner, W., unpublished), Fig. 2. As shown in Fig. 2, although estradiol is an agonist in both the prostate and MCF-7 cells, one-half maximal stimulation of cAMP occurs at about 1 nM estradiol in the breast cancer cells, but at about 20 nM estradiol in the prostate. Conversely, for 5α-androstan, 3α, 17β-diol, one-half maximal stimulation occurs at about 1 nM in the prostate and 10 nM in the breast. Thus, as has been described for classic pathways of steroid hormone action [15], there are cell specific outcomes of membrane-mediated effects of steroids.

Table 1
Steroid activation of SHBG-$R_{SHBG}$ prostate explants[a]

| Compound | Binds | Agonist | Antagonist |
|---|---|---|---|
| Dihydrotestosterone | +++ | ++ | +++ |
| 5α Androstan, 3α, 17β-diol | ++ | +++ | 0 |
| Estradiol | + | ++ | 0 |
| Testosterone | ++ | 0 | ++ |
| 5α Androstan 3β, 17β-diol | ++ | 0 | ++ |
| 5β Androstan 3α, 17β-diol | 0 | 0 | 0 |
| 5β Androstan 3β, 17β-diol | 0 | 0 | 0 |
| 2 Methoxyestradiol | ++ | 0 | ++ |

[a] Each compound has been examined for its ability to stimulate SHBG–$R_{SHBG}$ to increase the generation of cAMP in prostate explants. Steroids that do not bind to SHBG, i.e. the two 5β-androstanes are neither agonists nor antagonists. DHT may either be an agonist or antagonist, depending upon the tissue in which effects are sought. From Rosner et al. [26].

In human prostate tissue, screening of the steroids that bind to SHBG, as well as structurally related compounds that do not bind, led to the generalization that if a steroid binds to SHBG it is either an agonist or antagonist in this system (Table 1).

## 4. Biology of the SHBG–$R_{SHBG}$ system

cAMP is widely distributed and functions as a second messenger for a variety of hormones. Its effects are partitioned so that its accumulation does not lead to promiscuous activation of numerous pathways, but rather bears a specific relationship to the stimulus which caused its appearance. To date, three studies have addressed the biology of the SHBG–$R_{SHBG}$ system. Two of these are concerned with growth, and one with interactions with the classic intracellular androgen receptor (AR). We will comment briefly on the former and more extensively on the latter, as this mechanism might prove to be more general.

### 4.1. Effects on growth

In the prostate cancer cell line, ALVA-41, we have shown that, in *serum-free* medium, both DHT and estradiol increase growth in the presence, but not the absence, of SHBG. This increase in growth also follows the addition of cAMP to the cells and is equivalent to that seen with DHT in serum-containing medium (DHT alone does not increase growth in serum-free medium). The DHT–SHBG mediated growth was enhanced by inhibiting protein dephosphorylation with the protein phosphatase inhibitor, okadaic acid. We concluded that cAMP causes increased growth in this prostate cancer cell line, and that both DHT–SHBG and estradiol–SHBG can regulate intracellular cAMP and hence growth in these cells [16].

Unlike the increase in growth seen in prostate cells, estradiol-induced cell proliferation was inhibited by both cAMP and SHBG–estradiol in the MCF-7 breast cancer cell line [17]. The published data support the authors' conclusion that the inhibition of growth by SHBG–estradiol was not caused by the sequestration of estradiol by SHBG, but rather by activation of $R_{SHBG}$ [17].

The discrepancy in the foregoing studies is more apparent than real. The apparent inconsistency between the observations that cAMP retards growth in MCF-cells, as well as in an androgen-independent prostate carcinoma cell line (PC-3) [18], whilst enhancing it in ALVA-41 cells is consistent with the known pleiotropic effects of cAMP on growth [19]. More generally, it is becoming increasingly clear that there are cell- and promoter-specific actions of steroid hormones that allow the same ligand and the same receptor to give rise to different, and sometimes opposite, effects in a variety of tissues [15]. The $R_{SHBG}$ system appears also to participate in this same kind of behavior. For instance, in the ALVA-41 and LNCaP cell lines, both DHT and estradiol are agonists for the generation of cAMP via $R_{SHBG}$. However, in non-malignant prostatic tissue, from both humans and dogs, not only is DHT–SHBG inactive in causing increases in cAMP, but it inhibits the activation of SHBG–$R_{SHBG}$ by estradiol [10,11].

### 4.2. Interaction with the androgen receptor

We postulated that the effects initiated by SHBG–$R_{SHBG}$ might be mediated, at least in part, by an interaction with a classic steroid hormone receptor. To test this hypothesis, we chose an androgen-responsive model, stimulation of prostate specific antigen (PSA) secretion in human prostate explants [20]. Because the PSA gene contains an androgen response element and is activated by DHT [21], it represents a likely target for cross-talk with membrane-initiated steroid effects.

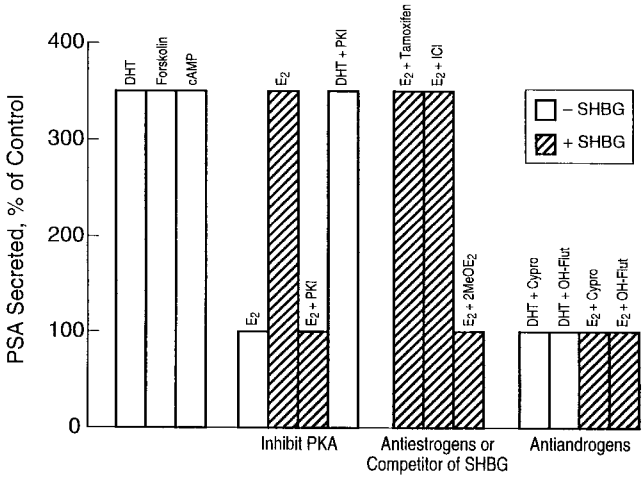


Fig. 3. Estradiol–SHBG–$R_{SHBG}$ activation of PSA secretion. This is a composite Figure based on data from Nakhla et al. [20]. The Figure is idealized. The actual differences in bar height may have been greater or smaller than those represented. PSA secretion was measured after the indicated treatment of primary cultures of human prostate tissue in serum free medium. PSA, prostate specific antigen; DHT, dihydrotestosterone; $E_2$, estradiol; PKI, inhibitor of protein kinase A; ICI 164,384 (a pure antiestrogen); $2MeOE_2$, 2 methoxyestradiol; Cypro, cyproterone acetate, OH-Flut, hydroxyflutamide. See text for details.

The experiments we conducted are summarized in a single Figure (Fig. 3). In brief, PSA secretion, as expected, was stimulated by DHT. As has been pointed out [21], the increase from baseline is modest compared to that seen in cultured LNCaP cells. Estradiol alone had no effect on PSA secretion, but it caused an increase equal to DHT if the prostate tissue was first loaded with SHBG, e.g. if $R_{SHBG}$ was occupied by SHBG. Because estradiol–SHBG increases intracellular cAMP, we ascertained whether other compounds that raise cAMP (forskolin), or cAMP itself, could increase PSA secretion. Such was the case. Downstream effects of cAMP require that it activate protein kinase A (PKA). Hence, if estradiol–SHBG increases PSA secretion by a mechanism involving cAMP, inhibition of PKA should block estradiol–SHBG-initiated PSA secretion. That also was the case. Inhibition of PKA by PKI caused estradiol–SHBG to fail to stimulate PSA. On the other hand, DHT-stimulated PSA secretion, which does not involve activation of PKA, was not inhibited by PKI. The lack of inhibition of estrogen-stimulated PSA secretion by two anti-estrogens (tamoxifen and ICI 164,384) demonstrates that the effect of estradiol–SHBG was independent of the estrogen receptor.

As already stated the promoter of the PSA gene has an androgen response element [22,23], and both PSA secretion and the expression of PSA mRNA are andro-

gen-regulated [21,24]. Thus we examined the effect of anti-androgens, hydroxyflutamide and cyproterone acetate, on the $E_2$–SHBG-mediated increase in PSA secretion. As expected, both antiandrogens blocked the effect of DHT on PSA secretion. Surprisingly, they also blocked the effect of $E_2$–SHBG on PSA secretion. $E_2$ does not bind to the AR. Therefore, the $E_2$-induced secretion of PSA observed in this study has to be considered a ligand-independent activation of the AR [25]. Thus, estradiol activates a typical AR-mediated event, PSA synthesis and secretion, by activating SHBG–$R_{SHBG}$ and demonstrates cross-talk between a steroid hormone-engendered event at the cell membrane and a classic intracellular steroid hormone receptor.

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