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# Receptors for Androgen-binding Proteins: Internalization and Intracellular Signalling

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**In plasma, most steroid hormones are bound and transported by the specific binding protein, testosterone–estradiol-binding globulin (TeBG). For years, it was believed that the only function of this protein was to regulate the concentration of free steroids in plasma. However, a number of reports have provided evidence for the presence of specific TeBG receptors on plasma membranes. Furthermore, the interaction of TeBG with its receptor was shown to be inhibited when steroids are bound to TeBG, suggesting that TeBG is an allosteric protein. The purpose of this manuscript is to review the evidence that androgen-binding proteins bind to membrane receptors, and, in some cells, this binding stimulates cAMP accumulation, and transfer TeBG/ABP into tissue as a consequence of receptor mediated endocytosis. Recent studies from our laboratories have demonstrated binding and uptake of TeBG by MCF-7 breast cancer cells. The interaction of unligated rabbit TeBG with membranes from MCF-7 cells resulted in a time and concentration-dependent increase in adenylate cyclase activity. The TeBG alone also had a reproducible effect on intact cells by increasing cAMP accumulation by 30–35%. The addition of DHT to cells, after TeBG has been allowed to bind, resulted in increases in cAMP of greater than 4-fold. This effect was not blocked by antiandrogens. These data support the hypothesis that extracellular SHBG is a regulator of cellular function through a membrane receptor that is coupled to adenylate cyclase.**

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## INTRODUCTION

Androgen-binding proteins (ABPs) are present in the body fluids of most species. The dimeric structure of these proteins, their preferred ligands, and their proposed functions distinguish them from androgen receptors. The first androgen binding proteins to be discovered were those that are produced in liver and secreted into blood. Sex hormone binding globulin (SHBG) and testosterone–estradiol-binding globulin (TeBG) are among the many names given these serum binding proteins. ABP is the name given to a Sertoli cell product, 20% of which is secreted into the blood and the remaining 80% is secreted into the seminiferous tubular lumen. From there it is transported to the epididymis where it is taken up by the principal cells. Due to their different origins and minor structural differences, the hepatic and testicular ABPs were originally thought to be distinct. However, several studies showed that the hepatic and testicular proteins are derived from the same gene and that the heterogeneity within a single species relates to alternatively processed primary transcripts of this gene [1–3] and variations in carbohydrate composition [4–6]. In addition, the predicted amino acid sequences of the binding proteins from four species show extensive identity over long portions of their sequences. Since most studies have referred to the serum protein as TeBG (SHBG) and the testicular protein as ABP, respectively, this designation is maintained in this review. New evidence indicates that these proteins may have functions other than being regulators of free steroid concentration in blood. The purpose of this manuscript is to review the evidence that ABP and TeBG bind to membrane receptors, and, in some cells, this binding stimulates cAMP accumulation, and transfer of TeBG/ABP into tissue as a consequence of receptor mediated endocytosis.

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## RECEPTORS FOR ABPs

The first line of evidence suggesting ABP and TeBG may offer an advantage to species possessing them other than as serum transport proteins was provided by studies showing that TeBG added to prostate slices changed the pattern of testosterone metabolism from that observed with albumin in the incubation medium [7]. These latter findings suggested that TeBG has the ability to activate cellular metabolism by acting on the surface of the cell or after being internalized. Whether or not TeBG did act on the surface of prostate cells was not possible to determine at the time of these early studies due to indirect evidence suggesting that TeBG was in prostate cells [8].

The first high affinity binding sites for TeBG were reported on membranes from decidual endometrium [9]. Subsequently, studies of prostate cells indicated that TeBG bound to receptors that could be studied in intact cells, on cell membranes, or after solubilization from membranes. Importantly, it was shown that the binding of TeBG to this receptor occurred in the absence of steroid [10]. Steroid binding to TeBG inhibited its interaction with prostate membrane receptor with a relative inhibitory activity directly related to the binding affinity of individual steroids for TeBG [11]. There appear to be differences between the uterine and prostate receptors for TeBG that relate to binding affinity and the importance of the steroid for binding of TeBG to the membrane receptor [10, 12]. At present, it is not possible to determine whether these differences in TeBG receptor interactions in prostate and uterus are dependent upon the tissues or the techniques used. Recent studies from our laboratories on the binding of rabbit TeBG to MCF-7 breast cancer cells and membranes, as well as testicular membranes, demonstrated TeBG receptors [13–15]. These receptors were solubilized from both breast and testicular membranes. As outlined in this review, their physical and biological characteristics bear closer resemblance to receptors in prostate than in uterus. For example, saturating concentrations of androgen block the binding of rabbit TeBG to MCF-7 cells if the androgen and TeBG are added to the receptor preparation at the same time.

A sequence in TeBG that interacts with the receptor was identified following trypsinization of TeBG and isolation of the resulting peptides for competition assay and sequence determination [16]. A synthetic peptide corresponding to residues TeBG 48–57 inhibited TeBG binding to receptor. That this sequence is identical, except for one conservative amino acid substitution in mouse, in all species studied suggests that the binding site of the receptor may be highly conserved. Since the apparent affinity of TeBG 48–57 for the receptor is low, other regions of the protein must also contribute to receptor binding.

One other possible determinant of steroid binding protein–receptor interaction is the glycosylation of TeBG and ABP [6, 17–19]. There are two consensus sequences for N-linked oligosaccharides on TeBG and ABP, only one of which is conserved. Studies of TeBG show two N-linked and one O-linked carbohydrate chain [6, 17]. In ABP, there were no detectable O-linked sugars although their presence could not be excluded [19]. The fact that TeBG and ABP sequences have identical numbers of amino acid residues, but that ABP subunits have electrophoretic mobility with a smaller apparent molecular size than TeBG, is consistent with lack of an O-linked oligosaccharide chain in ABP. Chemical removal of carbohydrate from TeBG does not affect its immunochemical properties or steroid binding but does inhibit the binding of the steroid–protein complex to uterine receptor [9]. Although enzymatic removal of N-linked carbohydrate does not appreciably alter the immunochemical properties of ABP [20], there are no reports on the effect of carbohydrate on ABP interaction with its receptor.

## STIMULATION OF CELLS BY ABPs

Unliganded TeBG elicits a small increase in cAMP concentration when it binds to LNCaP prostate tumor cells [10]. The addition of DHT, T, or $E_2$ to the cells after human TeBG has been allowed to bind produces a further increase in cAMP concentration. Interestingly, 2-methoxy-$E_2$, which binds to TeBG, does not stimulate cAMP production by prostate membranes via the TeBG–receptor complex. The effects of sequential addition of TeBG followed by steroid is in marked contrast to TeBG that is saturated with DHT when added to cells; such a preparation does not bind or stimulate cAMP [11]. In LNCaP cells, both DHT and $E_2$ activated the TeBG–receptor–adenylate cyclase system, but $E_2$ was about 20-fold less potent [10], whereas only $E_2$ was active in cells derived from BPH tissue [21].

Experiments on the interaction of unliganded rabbit TeBG with membranes from MCF-7 breast cancer cells showed this protein produced a 160–400% increase in adenylate cyclase activity (Fig. 1). TeBG alone had a smaller but reproducible effect on intact cells by increasing cAMP accumulation by 30–35%. The addition of DHT to cells after TeBG was allowed to bind to its receptor resulted in increases in cAMP of greater than 4-fold. When TeBG and steroid were added sequentially to cells the response to DHT was dose-dependent; a detectable effect was observed with $10^{-8}$ M and a maximal effect was observed at $10^{-6}$ M (Fig. 2). This effect was not blocked by antiandrogens (Fig. 3). Estrogens, which do not bind well to rabbit TeBG, did not elicit a cAMP response. The results produced by rabbit TeBG on breast cells are in general agreement with those produced by human TeBG on prostate cells [10].

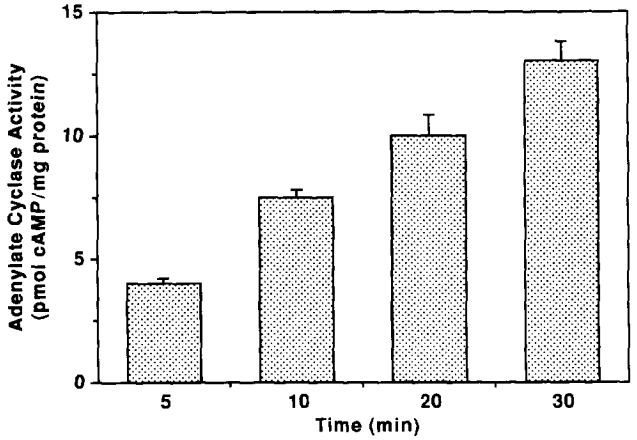


Fig. 1. Time-dependent increase in adenylate cyclase activity produced by rabbit TeBG (50 nM) on MCF-7 breast cancer cell membranes. Values expressed as mean ± SEM ($n = 4$ experiments).

Rosner and associates [10] have proposed the following mechanism to account for the action of the TeBG on cells. Since some TeBG is always unliganded at physiological testosterone concentrations, it is free to associate with its receptor. Free steroid from plasma, seminiferous tubular fluid, or epididymal fluids, can then bind to the TeBG/ABP receptor complex and stimulate cAMP accumulation. The fact that extracellular testosterone can increase cAMP accumulation in a cell type, which could also respond to intracellular action of testosterone via the androgen receptor, questions the purpose or the need for the TeBG-induced increases in cAMP. Studies on another steroid receptor suggest a mechanism by which the action of male sex hormone can be co-ordinated through free TeBG and androgen receptors. Recent studies have shown that actions of cAMP as well as progesterone can be mediated via the progesterone

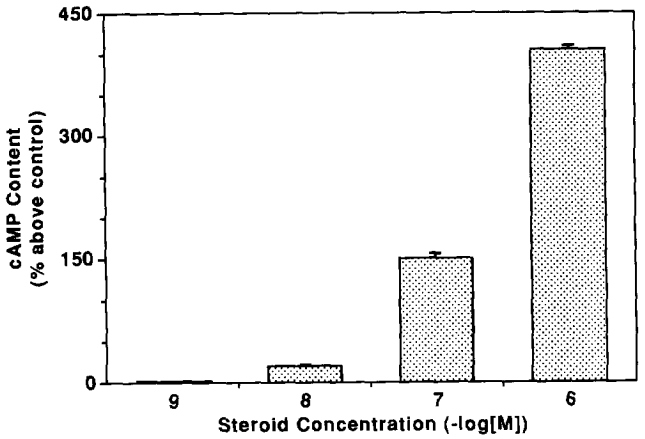


Fig. 2. Effect of increasing concentration of dihydrotestosterone (DHT) on intracellular cAMP content in MCF-7 cells that had been pre-incubated with TeBG. Cells were incubated with TeBG in the absence of steroid and then unbound TeBG was removed; the cells were then incubated with various steroid concentrations. In the presence of TeBG without steroid, the cAMP level was 16.2 ± 0.5 pmol/mg protein (control). Values expressed as mean ± SEM ($n = 5$ experiments).

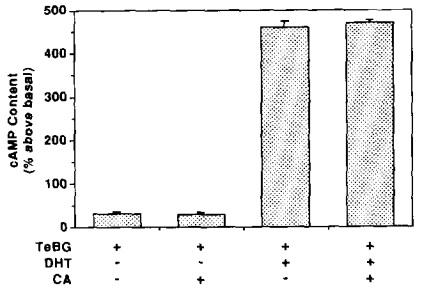


Fig. 3. Effect of the antiandrogen, cyproterone acetate (CA), on TeBG-induced increase in cAMP content in MCF-7 cells in the absence and presence of dihydrotestosterone (DHT). In this experiment TeBG and steroids were added sequentially as in Fig. 2. Values expressed as mean ± SEM ($n = 4$ experiments).

receptor [22]. Additional studies suggest that cAMP-mediated phosphorylation of the progesterone receptor, or other proteins in the transcription complex, can mimic and/or modulate progesterone receptor-mediated transcription. This finding suggests that TeBG and ABP-mediated stimulation of cells via membrane receptors could synergize with the steroid receptor-mediated hormone action [12].

## THE ENTRY OF BINDING PROTEINS INTO CELLS

One of the first studies showing that TeBG was in intact cells was the isolation of this protein from prostatic tissue in sufficiently large amounts so that its presence could not be accounted for by the blood and plasma in the extracellular space [8]. Immunostaining showed that TeBG was in primate prostate, epididymides, and other cells [23–25]. The entry of TeBG into cells was also demonstrated by studies showing that rabbit [$^3$H]TeBG infused into rats was rapidly cleared by testis and prostate [26]. These studies also suggested that almost all TeBG-bound testosterone was available to the testis and prostate by virtue of TeBG uptake into these organs. These observations explain why the action of testosterone in the rat is not influenced by infused TeBG in spite of the fact that the free testosterone level is markedly decreased in TeBG-treated animals [27].

In parallel studies, it was shown that ABP produced in the testis is transported to the epididymis where it is taken up by epithelial cells [28]. Although all the principal cells of the epididymis have the ability to take up ABP, this occurs most rapidly in the proximal segment of the caput epididymis [29]. Several morphological studies suggested that ABP is taken up in epididymal principal cells by receptor-mediated endocytosis, both *in vivo* and *in vitro* [29, 30]. Immunostainable ABP was present in the principal epididymal cells as granules in the supranuclear cytoplasm. Ultrastructural immunocytochemistry analysis revealed a reaction product in small coated vesicles. The vesicles were clustered in multiple vesicular bodies above the Golgi

region. These results are consistent with the uptake of ABP by epididymal epithelia by receptor-mediated endocytosis in coated vesicles and the subsequent transfer of this protein into the lysosomal system of these cells [30]. This view was confirmed by studies using gold-labeled ABP and rabbit TeBG in cultured epididymal cells (i.e. membrane, coated vesicles, and lysosome localization) [31]. The uptake and transport of ABP was the same for that of rabbit TeBG, consistent with the hypothesis that the portion of these proteins that bind to receptors is conserved among species. Studies of [$^{125}$I]TeBG confirmed that there was specific binding to epididymal cells (but not to kidney cells) and temperature-dependent internalization [31]. Perfusion studies showed that caput epididymal tubules accumulated [$^{3}$H]testosterone more efficiently from the luminal surface in the presence of ABP [32]. A number of investigators have proposed that ABP is responsible for delivering testosterone to the luminal side of epididymal cells, thus providing an explanation for why the caput epididymis cannot be maintained by large doses of androgens following efferent duct ligation [33]. The studies reviewed above suggest that ABP–membrane interactions could have an effect on epididymal cells other than via passive delivery of testosterone.

Our laboratory has also demonstrated binding and uptake of TeBG by MCF-7 breast cancer cells. Specific, temperature-dependent cellular binding and uptake of [$^{125}$I]TeBG reached a plateau in 6 h [13]. The mechanism by which gold-labeled TeBG was internalized was studied by electron microscopy. TeBG first bound to the plasmalemma and then appeared respectively in receptosomes, multivesicular endosomes, and lysosomes. It was not possible, using either cell release experiments or electron microscopy, to determine whether a portion of the internalized TeBG was released back into media. These observations are consistent with a TeBG binding site on MCF-7 cells and with TeBG uptake by receptor-mediated endocytosis, similar to that of the ABP uptake by the epididymis.

Although TeBG/ABP is taken up by testis and epididymis, it is not known whether or how the binding proteins stimulate these cells. Human prostate tissue and MCF-7 cells both show evidence for binding, stimulation, and internalization. Studies using human prostate cancer cells that bind but do not internalize TeBG show the latter process is not essential for TeBG-mediated stimulation of cAMP [10]. Thus, there is a need to understand whether all cells that bind TeBG or ABP can be stimulated by these proteins, and whether internalization is for TeBG degradation and steroid delivery, or is an essential step in cell activation.

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