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TESTOSTERONE-ESTRADIOL-BINDING GLOBULIN BINDS TO HUMAN PROSTATIC CELL MEMBRANES

Daniel J. HRYB, M. Saeed KHAN, and William ROSNER*

Department of Medicine, St. Luke's-Roosevelt Hospital Center, and Columbia University, College of Physicians and Surgeons, 428 West 59th street, New York, New York 10019

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Specific binding sites for human testosterone-estradiol-binding globulin have been found on human prostatic cell membranes. Scatchard analysis reveals both a high and a low affinity binding site for [^{125}I]testosterone-estradiol-binding globulin. The high affinity site is specific for testosterone-estradiol-binding globulin, whereas the low affinity site also binds human corticosteroid-binding globulin and human transferrin.

In human plasma, steroid hormones are largely bound to the specific steroid hormone-binding proteins testosterone-estradiol-binding globulin (TeBG) and corticosteroid-binding globulin (CBG) (1-5). According to the current model of steroid hormone action, the free fraction (non-protein bound) of the hormone diffuses from the capillary bed and then across cell membranes (6), where it binds to a high affinity, specific receptor in the cytoplasm of target cells (7,8). An alternative to the free-hormone model is suggested by data purporting to demonstrate TeBG and CBG in various steroid hormone target organs. There have been reports on the immunocytochemical demonstration of TeBG: in monkey prostate, epididymis, and testis (9); in human testis and epididymis (10); and in mammary carcinoma cells (11); and of CBG: in liver (12), uterus (3), kidney (3), and lymphocytes (13). A number of biochemically based studies support the presence of CBG or a CBG-like binder in various glucocorticoid and progesterone target

*To whom correspondence should be addressed.

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organs: pituitary (14,15); kidney (16); uterus (17-19); muscle (20); lung (21); breast cancer (22); and lymphocytes (13). A TeBG-like binder was also reported in human prostatic cytosol (23). Some of these reports have been flawed by the omission of critical controls and/or a lack of evidence that the observations were not due to contamination with plasma (24,25). More recently, however, in a very well controlled study, guinea pig CBG has been specifically localized in pituitary corticotrophs (26).

All these observations suggest that TeBG and CBG, like some other serum proteins, might bind to specific membrane receptors and be internalized via receptor-mediated endocytosis (27).

We report here experimental evidence demonstrating the specific binding of TeBG to human prostatic cell membranes.

MATERIALS AND METHODS

Prostatic tissue was obtained from men undergoing trans-urethral prostatectomy for benign prostatic hyperplasia. Plasma membranes were prepared using a modification of a previously described method (28-30). After rinsing the tissue with ice cold 0.3M sucrose, it was minced, diluted with 5 vol of ice cold 0.3M sucrose, and homogenized with a Brinkmann Polytron homogenizer (PT 10-35; 3 x 20 sec bursts at setting 7). The homogenate was centrifuged at 1000 x g for 10 min, and the resultant pellet was resuspended in 4 vol 0.3M sucrose, rehomogenized and centrifuged as before. The combined supernatants were centrifuged at 15,000 x g for 20 min, after which the 15,000 x g supernatant was centrifuged at 100,000 x g for 1 hr. The 100,000 x g pellet was resuspended in 2 vol 0.3M sucrose, with the aid of a glass/teflon homogenizer, and centrifuged again. The final 100,000 x g pellet (prostatic membranes) was resuspended in 1 vol of 25 mM Tris-HCl, pH 7.4, 10 mM $CaCl_2$, 0.2% bovine serum albumin (BSA) (Buffer A), and used for binding experiments immediately, or stored at -70 C. Results were the same when membranes were prepared from fresh tissue or frozen tissue (2-4 weeks).

TeBG was isolated from pregnancy plasma by a modification (31) of our original proceedure (32). Its purity was verified by polyacrylamide gel electrophoresis, sodium dodecyl sulfate gel electrophoresis, and immunoelectrophoresis against anti-whole human antiserum. It was iodinated as previously described (31) except that after passage through Bio-Gel P-60, it was adsorbed to Concanavilin A, washed with 50 mM Tris-HCl, pH 7.4, 50 mM $CaCl_2$, and then eluted with alpha methylglucoside. The specific activity of the [^{125}I]TeBG varied from 0.3-0.5 mol ^{125}I/mol TeBG. Human CBG and albumin were isolated as previously described (33,34). Human transferrin was purchased from Sigma Chemical Co.

Binding took place in 1.5 ml polypropylene, Eppendorf centrifuge tubes that were pretreated with 1.0 ml buffer A for 1 hr at 37C to decrease test tube blanks. [^{125}I]TeBG (8-10 ng, about 160,000 cpm/tube), plus or minus competitor, was incubated with 0.2 mg/ml of prostatic membranes, in 35 mM Tris-HCl, pH 7.4, 25 mM $CaCl_2$, and 0.12% BSA in a final volume of 0.25 ml. After incubation, the membranes were centrifuged in an Eppendorf centrifuge for 10 min at 4C. After aspirating the supernatants, the tip of each tube was cut 0.5 cm from the bottom and counted at an efficiency of 72%. Test tubes without membranes served as blanks.

RESULTS

Initially, we explored the binding of [^{125}I]TeBG to prostatic membranes as a function of time and temperature (Fig 1). Specific

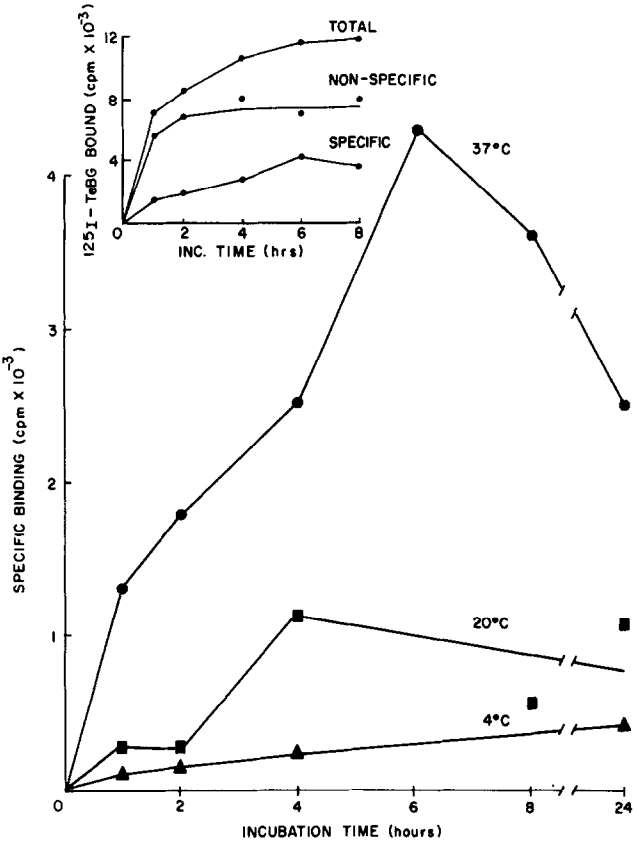

FIGURE 1. Time-course of binding of [^{125}I]TeBG to prostatic membranes. [^{125}I]TeBG (150,000 cpm, 10 ng) was added to prostatic membranes (0.2 mg/ml), with or without radioinert TeBG (12.5 μg), and incubated at three temperatures, 37C, 20C, and 4C. The main figure indicates only specific binding; the insert shows total and non-specific, as well as specific binding at a single temperature, 37C. Generally about 2-3% of the added [^{125}I]TeBG bound specifically to the prostatic membranes after 6 hours of incubation at 37C. Specific binding is the difference between total binding (6-8% of added [^{125}I] TeBG) and non-specific binding, binding in the presence of 1000 to 3000-fold excess of radioinert TeBG (usually about 65% of total binding).

binding was markedly higher at 37C than at 20C and 4C. Although it is possible that the increase in binding as a function of temperature represents activation of a receptor, it seems more reasonable to hypothesize that the data reflect an exchange phenomenon (35).

Scatchard analysis (36) of specific TeBG binding to prostatic membranes at 37C indicates binding to two sites (Fig 2). We used the curve-fitting program LIGAND (37,38) to analyze the nonlinear Scatchard plots. The simultaneous analysis of several experiments yields an association constant (Ka) of 6.53 ± 2.32 (SE) x 10^7 L/M and a binding capacity of 972 fmol/mg of membrane protein for the high-

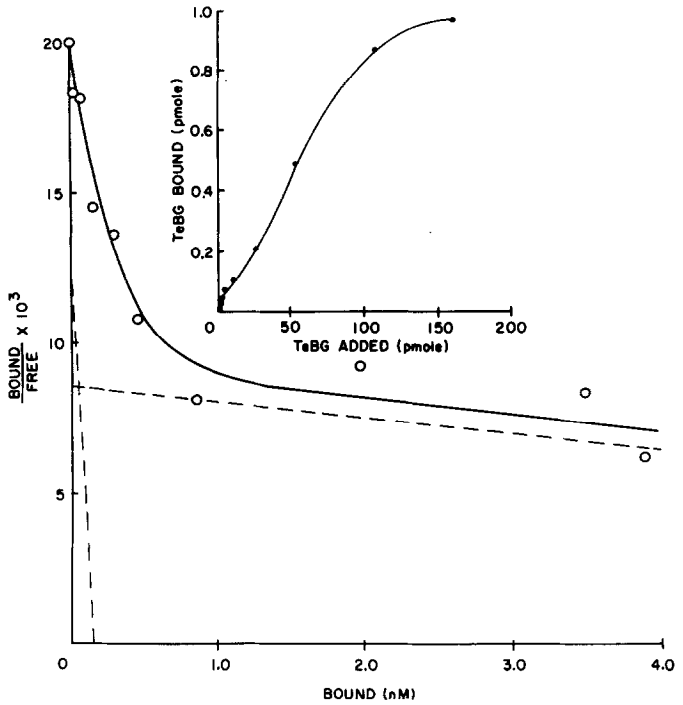

FIGURE 2. Scatchard analysis. [^{125}I]TeBG (160,000 cpm, 0.38 nM) was incubated with prostatic membranes (0.2 mg/ml) in the presence of increasing amounts of radioinert TeBG (0 to 850 nM) at 37C for 6 hours. The insert is a saturation plot of specific TeBG binding. Both plots are corrected for non-specific binding using a 3000-fold excess of radioinert TeBG (1300 nM). The above data was analyzed using the curve-fitting program LIGAND (37). The results of the analysis (dashed lines) indicate that the data is best fit by a two binding site model (see text).

affinity site, and a Ka of $1.23 \pm 0.43 \times 10^5$ L/M and a binding capacity of 1.78 nmol/mg of membrane protein for the low-affinity site.

To determine the ligand specificity of the receptor, the ability of radioinert TeBG to compete with the binding of [^{125}I]TeBG was compared with that of another human plasma steroid hormone binding protein, CBG, and two other human plasma proteins, transferrin and albumin, Fig 3. The effect of albumin in reducing the binding of [^{125}I]TeBG appears to be a non-specific one, while both CBG and transferrin compete for specific sites. The inhibition of [^{125}I]TeBG binding by CBG and transferrin was analyzed in detail with the LIGAND program. The analysis indicates that neither CBG nor transferrin binds to the high affinity site, but that they both bind to the low affinity site with association constants approximately equal that of TeBG to this site.

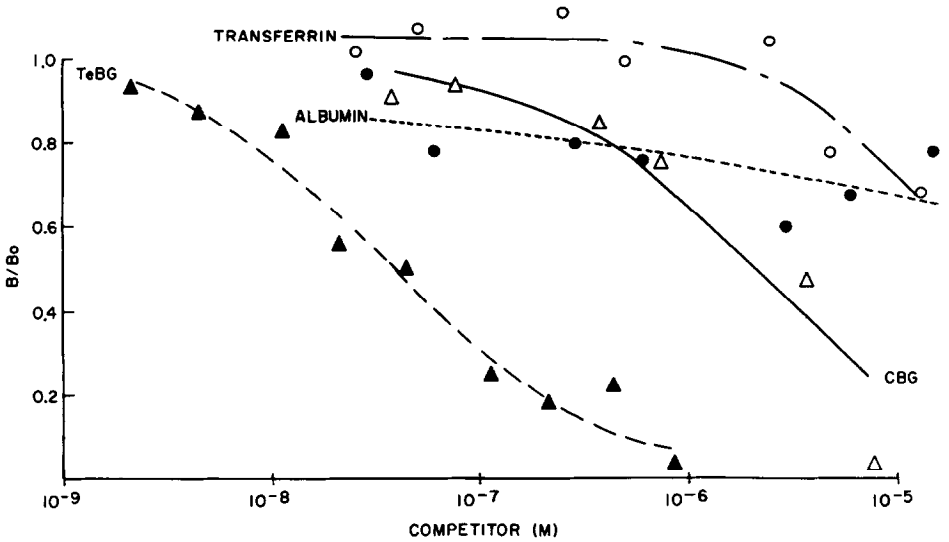

FIGURE 3. Competition by human TeBG, CBG, transferrin, and albumin for [^{125}I]TeBG binding to prostatic membranes. [^{125}I]TeBG was incubated with prostatic membranes in the presence of increasing amounts of: 1) Radioinert human TeBG (▲); 2) Human CBG (△); 3) Human transferrin (○); and 4) Human albumin (●). B_0, specific binding of [^{125}I]TeBG in the absence of competitor; B, specific binding of [^{125}I]TeBG in the presence of competitor.

DISCUSSION

The studies described in this report demonstrate the existence of specific binding sites for TeBG on prostatic membranes; the binding sites are typical of membrane receptors. They are saturable, specific, have high affinity for TeBG (Kd=15 nM), and have a concentration, 972 fmoles/mg of membrane protein, within the range reported for many membrane receptors (39). There are receptors for other plasma transport proteins, such as the transferrin receptor (40) and the low density lipoprotein (LDL) receptor (41). A major difference between these two protein-receptors systems and the one for TeBG, is that the plasma concentration of transferrin and LDL exceeds their receptor dissociation constants by several orders of magnitude, whereas the plasma concentration of TeBG (35 nM in men and 70 nM in women) is approximately equal to its receptor dissociation constant. An important consequence of these relationships is that the density of LDL and transferrin on the surface of cells, within a wide range of plasma concentrations, is determined by receptor concentration, whereas the density of TeBG could be altered by small changes either in its plasma concentration, or in receptor concentration. This kind of regulation is typical of the binding of plasma peptide hormones to cell membranes. Such a system is "hormone - receptor" sensitive, thus allowing for two levels of control at the binding stage of hormone action.

Plasma steroid hormone-binding protein receptors have been hypothesized (3,5). Indeed, there is a report of a putative CBG receptor on human liver membranes (42). However, although specific binding of [^{125}I]asialo-CBG to membranes was shown, the binding activity can be attributed to the presence of the well-known liver membrane asialoglycoprotein receptor (43). Although we have demonstrated the existence of specific binding of TeBG to prostatic

membranes, we have shown no function for it beyond that of binding. The literature on intracellular TeBG and CBG (9-23,26), taken together with our results, seriously raises the possibility that TeBG, and more speculatively CBG, may serve to transport steroid hormones into certain tissues via a specific receptor. This hypothesis is strengthened by previous observations which indicate that there are circumstances in which cortisol bound to CBG is biologically more active than unbound cortisol (44,45). Moreover, it has been reported that TeBG is able to enter the cytoplasm of cultured MCF-7 cells (9). Receptor-mediated endocytosis could furnish a mechanism for such entry.

Finally, there has been a recent important challenge to the classic model (two-step) of steroid hormone action . New evidence indicates that, at least for estrogens, the cytosolic receptor may be an artifact of cell disruption and that all of the receptor is nuclear (46-48). Hence, we would be left without a direct mechanism to create a positive steroid concentration gradient between the cytoplasm and the plasma free hormone. Steroid hormone action could proceed without such a mechanism, but a substitute one would lend an additional degree of specificity and control. For instance, one could envision a small, steady, diffusion of free steroid hormones into cells, augmented, as necessary, by the internalization of bound steroids into target cells via membrane receptors for TeBG or CBG. The transport of steroid hormones into cells by TeBG and CBG by way of specific membrane receptors, which in turn are signposts or markers for target cells, would furnish a fine-tuned control system, and fractionate the effects of the prevailing plasma hormone concentration on a tissue by tissue basis.

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