A Human Sex Hormone-binding Globulin Isoform Accumulates in the Acrosome during Spermatogenesis*

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Human sex hormone-binding globulin (SHBG) binds estradiol and testosterone with high affinity. Plasma SHBG is produced by hepatocytes, but the human SHBG gene is also expressed in the testis. Little is known about SHBG gene expression in the human testis, but human SHBG transcripts accumulate in a spermatogenic stage-dependent manner in the testes of mice containing an 11-kb human SHBG transgene. We have now found that human SHBG transcripts containing an alternative exon 1 sequence are located specifically in the testicular germ cells of these transgenic mice, whereas murine SHBG transcripts are confined to Sertoli cells. In addition, we have detected immunoreactive human SHBG in the acrosome during all stages of spermiogenesis in mice containing an 11-kb human SHBG transgene. Western blots of germ cell extracts from these transgenic mice and from human sperm indicate that the immunoreactive human SHBG in the acrosome composes electrophoretic variants, which are 3–5 kDa smaller than the major electrophoretic isoforms of human SHBG in the blood. This apparent size difference is due in part to differences in glycosylation of plasma and acrosomal SHBG isoforms. The function of the human SHBG isoform in the acrosome is unknown, but it binds steroid ligands with high affinity. This is the first demonstration that human SHBG transcripts encode an SHBG isoform that remains within a cellular compartment.

Mammalian genes encoding sex hormone-binding globulin (SHBG)3 contain at least two transcription units (1, 2). In humans, the transcription unit responsible for the production of plasma SHBG by hepatocytes consists of eight exons that span ~3.2 kb on chromosome 17 (1, 3) and is under the control of a promoter sequence that contains several well defined binding sites for liver-enriched transcription factors (4, 5). The human SHBG gene contains a second transcription unit that consists of an alternative exon 1 sequence that replaces the exon 1 sequence present in the SHBG mRNA found in the liver. As a consequence, these differentially spliced SHBG transcripts lack the secretion signal sequence associated with the plasma SHBG precursor polypeptide.

The expression of the SHBG gene in the testis has been studied extensively in the rat (2). In this species, the SHBG gene is expressed in Sertoli cells (6, 7) and encodes the SHBG homologue that is generally known as the testicular androgen-binding protein (ABP). The ABP produced by rat Sertoli cells is secreted into the lumen of seminiferous tubules where it is thought to serve primarily as a carrier of testosterone throughout the male reproductive tract (2). Although SHBG transcripts are present in the human testis (1), virtually nothing is known about their function or how they are regulated, and evidence that they encode a precursor polypeptide containing a leader sequence for secretion is lacking. In fact, all the available evidence suggests that the human testis contains several alternative SHBG transcripts comprising a non-coding alternative exon 1 sequence and some of them also lack exon 7 sequences (1, 8). Differentially spliced human SHBG transcripts lacking exon 7 sequences have also been identified in several other tissues (9, 10), but their 5′-sequences have not been characterized. Like the human SHBG gene, the rat SHBG gene produces transcripts that consist of alternative exon 1 sequences, and these have been identified in the rat brain (11) and the fetal rat liver (12). There is no obvious sequence similarity between the alternative exon 1 sequences associated with various SHBG transcripts in different species, but there is evidence they encode SHBG isoforms comprising subcellular localization signals within a unique amino-terminal sequence (13).

To study the tissue-specific expression of various human SHBG transcripts, we have produced several lines of transgenic mice (14) containing either a 4-or an 11-kb human SHBG transgene. The 4-kb transgene consists of the eight exons encoding plasma SHBG (1) and 0.9 kb of 5′-flanking DNA that includes the promoter utilized in the liver (4), whereas the 11-kb human SHBG transgene contains an additional 5′-flanking DNA sequence that includes an alternative exon 1 sequence associated with the SHBG transcripts present in the human testis (1, 8). We have shown previously that only the 11-kb human SHBG transgene is expressed in the mouse testis, as evidenced by the presence of human SHBG transcripts in the seminiferous epithelium (14). These studies also indicated that the human SHBG transcripts accumulate in a spermatogenic cycle stage-dependent manner in this location, but the cell type in which they were located could not be clearly identified, and their protein products eluded detection (14). We have therefore re-examined this issue, and we have found that the majority of human SHBG transcripts in the testis of these mice consist of...
the alternative exon 1 sequence associated with SHBG cDNAs from a human testis library (1). Furthermore, these alternatively spliced human SHBG transcripts are confined to testicular germ cells and an immunoreactive human SHBG isoform accumulates in the acrosome of developing spermatids and immature sperm in the transgenic mice. We have also obtained direct evidence that this acrosomal SHBG isoform binds steroids and is also present in human sperm.

EXPERIMENTAL PROCEDURES

Animals—Transgenic mice containing 11-kb (lines shbg 11-a and shbg 11-b) or 4-kb (lines shbg 4-a and shbg 4-b) regions of human SHBG gene have been characterized previously (14, 15). Animals were housed under standard conditions and provided with food and water ad libitum. At ~10 weeks of age, mice were sacrificed for the isolation of Sertoli cells and/or germ cells for protein and RNA analysis (see below).

For immunohistochemistry, mice were perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (14). All procedures were approved by the Animal Use Subcommittee of the University Council on Animal Care (University of Western Ontario, Canada).

Immunohistochemistry—The anti-human SHBG antibodies for immunohistochemistry were purified from a rabbit antiserum by immunoaffinity chromatography using an N-hydroxysuccinimide-activated HiTrap column coupled to purified human SHBG (16), according to instructions provided by Amersham Biosciences. Testes from perfused mice (see above) were further fixed in 4% paraformaldehyde at 4 °C for 24 h, subsequently dehydrated with a series of ethanol solutions, and embedded in paraffin. The paraffin sections were de-waxed and incubated at high power in a microwave oven for 10 min in citrate buffer, pH 9.9. The sections were then cooled at room temperature for 20 min and treated with a 0.03% hydrogen peroxide solution for 7 min, prior to incubation (overnight at 4 °C) with affinity-purified rabbit antibodies against human SHBG. The immunoreactive human SHBG was detected using the EnVision™ + System, HRP (DAB) from DAKO ( Carpinteria, CA).

Sperm from transgenic mice (14) were spread on slides, fixed for 5 min in 4% paraformaldehyde, and washed with PBS. After incubation in citrate buffer, pH 9.9, for 30 min at room temperature, the sections were treated with 0.03% hydrogen peroxide solution for 7 min at room temperature. The sections were incubated with the affinity-purified antibodies against human SHBG overnight at 4 °C, and immunoreactivity was detected using the EnVision™ + System ( DAKO).

Sertoli Cell and Germ Cell Isolation—A mixed population of Sertoli cells and germ cells was isolated from the testes of wild-type and transgenic mice (1). Briefly, the testes were excised and washed in Dulbecco’s modified Eagle’s medium/NUT mix F-12 culture medium (Invitrogen Canada, Burlington, Canada) supplemented with penicillin, streptomycin, and amphotericin (12). The testes were minced in the PBS solution. The medium was removed, and the remaining testicular fragments were digested in PBS containing trypsin (80 mg/ml) at 33 °C for 10 min. The reaction was stopped by adding 25 mg/ml trypsin inhibitor, and the resulting solution was treated with deoxyribonuclease I (0.4 mg/ml) at room temperature for 5 min. The isolated tubules were subjected to several rounds of mincing and filtration, as described previously (17, 18). After centrifugation, the pellet was resuspended in 15 ml of Dulbecco’s modified Eagle’s medium/NUT mix F-12 culture medium (Invitrogen) supplemented with 10% fetal bovine serum and incubated in a tissue culture flask for 5 h. The supernatant containing germ cells without Sertoli cells was recovered and centrifuged. After two washes with PBS, the pellet was frozen in aliquots for protein or RNA extraction.

Western Blot Analysis—Soluble protein was extracted from mixed populations of Sertoli cells and germ cells and from isolated germ cells (see above) with 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS at 4 °C for 12 h. Human donor sperm samples were centrifuged (350 × g for 10 min) to fractionate seminal plasma and sperm, and sperm samples were either washed in HTF culture medium (Irvine Scientific, Santa Ana, CA) or purified by Percoll density gradient centrifugation prior to extraction with 0.25 M Tris-HCl, pH 8.0, by sonication in a water bath and three freeze-thaw cycles. Samples were heat-denatured in loading buffer and subjected to discontinuous SDS-PAGE with 4 and 10% polyacrylamide in the stacking and resolving gels, respectively. Proteins in the gel were transferred (19) to Hybond ECL nitrocellulose membranes (Amersham Biosciences). The membranes were first blocked for 1 h in PBS containing 0.01% Tween 20 and 5% skim milk and were then incubated overnight at 4 °C with primary antibodies against human SHBG (DAKO; kindly provided by Dr. Francina Munell) in the same buffer. The blots were washed (3 ×) containing 0.01% Tween 20, and excess antibody, and specific antibody-antigen complexes were identified using a horseradish peroxidase-labeled donkey anti-rabbit IgG and chemiluminescent substrates (Fierce) by exposure to x-ray film.

To assess the influence of glycosylation on the electrophoretic mobility of immunoreactive human SHBG in different samples (i.e. diluted serum and protein extracts from isolated testicular cells), we performed a similar Western blotting experiment. For this purpose, protein was extracted from isolated testicular cells with 0.25 M Tris-HCl, pH 8.0, by sonication in a water bath and three freeze-thaw cycles, and cell debris was removed by centrifugation. Samples were then treated with N-glycosidase F (Roche Diagnostics) at 37 °C overnight, as recommended by the enzyme supplier, prior to analysis by SDS-PAGE and Western blotting, as described above.

RNA Analysis—Total RNA was extracted from mouse testicular cells and liver using TRIzol reagent (Invitrogen), separated by electrophoresis on a 1% agarose gel in the presence of formaldehyde, and transferred to a Zeta- Probe nylon membrane (Bio-Rad). The hybrid was hybridized with various 32P-labeled human SHBG cDNAs, i.e. the 3′ EcoRI fragment spanning exons 6–8 (20), the SHBG exon 1 sequence encoding the leader sequence for secretion of SHBG, and the SHBG alternative exon 1 sequence (1). In addition, cDNAs for mouse vimentin and transfection protein 1 were used as markers for somatic cells and germ cells, respectively. In some experiments, a mouse SHBG cosmid probe was used to examine exon 6–8 sequences was used as a probe, and a cDNA for 18 S ribosomal RNA was also used as an additional control for RNA loading and transfer (15).

We also used the total RNA from isolated germ cells to further analyze the human SHBG transcripts. To accomplish this, reverse transcription (RT) was performed at 42 °C for 50 min using 3 μg of total RNA and 200 units of Superscript II together with an oligo(dT) primer and reagents provided by Invitrogen. An aliquot (1 μl) of the RT product was amplified in a 20-μl reaction in the presence of 1 unit of Taq polymerase, 0.05 mM dGTP, 1.25 μM of each dNTP, and 0.2 μM of each oligonucleotide primer. For this purpose, we used the following primer (A sequence, 5′-GGGCTTCTGGAGTCCTCC (corresponding to a 5′-sequence 5′-TGGCCCTTGTGGAGGCCC) in the SHBG alternative exon 1 and a reverse primer complementary to a sequence (5′-TGGCCCTTGTGGAGGCCC) within exon 8 of the human SHBG gene (1). The PCR was performed for 40 cycles at 94 °C for 30 min, 65 °C for 30 s, and 72 °C for 1 min. A mouse transition protein 1 cDNA was amplified by RT-PCR under the same conditions using two specific primers (5′-CCACCGCCAAAGCTGAGCTCATGC and 5′-AGCTATTGGCCGACATCAAGTGGG) to control for the integrity and relative amounts of germ cell mRNA in the samples. The PCR products were resolved by electrophoresis in a 1% agarose gel and purified using the GenElute Gel Extraction kit (Sigma). They were then cloned using the Zero Blunt TOPO PCR Cloning kit (Invitrogen), and plasmids containing PCR products were sequenced.

SHBG Steroid Binding Assays—To determine whether the immunoreactive SHBG extracted from testicular cells binds steroids, we used a saturation ligand binding assay (23). In this assay, the endogenous steroids were first removed from protein extracts (25 μg/ml) by dilution (1:3) in a dextran-coated charcoal (DCC) suspension and incubation for 30 min at room temperature. After centrifugation, the supernatant was further diluted (1:3) and incubated at room temperature for 1 h with 10 nM [3H]dihydrotestosterone (PerkinElmer Life Sciences) followed by an additional incubation (30 min) at 0 °C. Nonspecific binding was estimated in the presence of excess unlabeled 5α-dihydrotestosterone (DHT). Free ligand was removed (10 min) with an ice-cold charcoal and following separation of the DCC by centrifugation, the supernatant containing SHBG-bound ligand was taken for radioactivity measurements (21). A similar protocol was used to determine the steroid-binding properties of the immunoreactive SHBG extracted from the
Human SHBG in the Acrosome

### Results

#### The 11-kb Human SHBG Transgene Is Expressed in Germ Cells of the Mouse Testis—A single Northern blot was used to measure the relative abundance of human SHBG transcripts in mixed populations of Sertoli cells and germ cells, as well as a pure population of germ cells isolated from the testes of wild-type mice and mice containing 4- or 11-kb human SHBG transgenes (Fig. 1). When a human SHBG cDNA that recognizes exon 6–8 sequences was used as probe, human SHBG transcripts were only detected in RNA extracts of testicular cells from mice containing the 11-kb human SHBG transgene. The use of mouse vimentin (a marker of somatic cells) and transition protein 1 (a germ cell specific marker) cDNAs allowed us to demonstrate the purity of the germ cells isolated from the testes of transgenic mice (Fig. 1, lanes 7 and 8). These data indicate that similar numbers of germ cells were present in all samples, as evidenced by the presence of similar amounts of transition protein 1 mRNA. Therefore, because the relative abundance of human SHBG transcripts in the germ cell-only preparation was similar to that in the mixed population of Sertoli cells and germ cells, this suggested that the SHBG transcripts in the testis of 11-kb human SHBG transgenic mice cannot be derived from Sertoli cells.

To characterize further the human SHBG transcripts that accumulate in testicular germ cells, a Northern blot of total RNA extracts of germ cells, isolated from transgenic mice containing either the 4- or 11-kb human SHBG transgenes, was performed using exon 1- and alternative exon 1-specific cDNA probes. As a control, we also included a similar amount of total RNA from an 11-kb human SHBG transgenic mouse liver (Fig. 2). When comparing the ratios of signals obtained using these exon 1-specific cDNAs with those obtained using a cDNA corresponding to human SHBG exon 6–8 sequences (Fig. 2), it is again apparent that SHBG transcripts are only present in germ cells from the 11-kb human SHBG transgenic mice. As expected, the SHBG transcript in the liver RNA sample comprises predominantly the exon 1 sequence containing the translation initiation codon for the SHBG precursor polypeptide and the leader sequence for secretion. By contrast, the SHBG transcript in the germ cells from mice containing the 11-kb human SHBG transgene can only be detected using the cDNAs that recognize the alternative exon 1 sequence and sequences corresponding to exons 6–8.

The human alternative SHBG transcripts were also analyzed in germ cells from transgenic mice by an RT-PCR with specific primers for human SHBG alternative exon 1 and human SHBG exon 8, and mouse transition protein 1-specific primers were again used in an RT-PCR as a positive control for the presence of intact mRNA species. Human SHBG transcripts were detected only in the germ cells of the 11-kb human SHBG transgenic mice, whereas mouse transition protein 1 was amplified in all samples including those from 4-kb human SHBG transgenic mice and wild-type mice (Fig. 3). When the two differently sized RT-PCR products amplified using human SHBG-specific primers were cloned and sequenced, the ~1.1-kb RT-PCR product (Fig. 3) was found to contain the alternative exon 1 sequence followed by the sequences of exons 2–8, whereas the smaller and less abundant RT-PCR product (~0.9 kb) contained the alternative exon 1 sequence followed by the sequences of exons 2–6 and 8.

Expression of the endogenous mouse SHBG gene was examined in the testicular cell preparations from wild-type mice by Northern blotting, and mouse vimentin and transition protein 1 cDNAs were again used to monitor for the presence of somatic cells and germ cells, respectively (Fig. 4). When we used a cDNA corresponding to mouse SHBG exon 6–8 sequences, murine SHBG transcripts were only detected in the mixed population of Sertoli cells and germ cells. Although similar levels of transition protein 1 mRNA levels were detected in the two different isolated testicular cell preparations, murine SHBG transcripts could not be detected in the purified germ cells (Fig. 4). These data therefore indicate that the mouse SHBG gene is expressed in Sertoli cells rather than in germ cells.

#### Immunoreactive Human SHBG Accumulates in the Acrosome during Spermiogenesis in Mice Expressing the 11-kb Human SHBG Transgene—The immunofinity-purified rabbit antibodies against human SHBG do not detect antigens in the testes of wild-type mice, and this illustrates the specificity of the immunoreactivity observed at low power magnification (10×) in the sections of testes from transgenic mice containing the 4- or 11-kb human SHBG transgenes (Fig. 5A). In these sections, similar amounts of immunoreactive human SHBG are present in the interstitial compartment of both transgenic mouse lines, irrespective of the size of the human SHBG transgene. Because we have been unable to detect any human SHBG transcripts in the testes of mice containing the 4-kb human SHBG transgene, and because the plasma levels of human SHBG are similar in these two lines of transgenic mice (14), the immunoreactivity in the interstitial compartment reflects the sequestration of SHBG from the plasma. In contrast to previous studies (14), in which we were not able to detect any immunoreactive human SHBG within the seminiferous tubules of mice containing human SHBG transgenes, microwave pretreatment of histology sections in a high pH buffer most likely exposed human SHBG epitopes within the semi-
niferous tubules of the 11-kb human SHBG transgenic mouse testis (Fig. 5A). At higher power magnification, this immunoreactive human SHBG could be detected within the acrosome of spermatids during spermiogenesis only in the seminiferous tubules of transgenic mice containing the 11-kb human SHBG transgene (Fig. 5B). Moreover, this immunoreactivity could be detected in the acrosome as soon as it begins to form on spermatids (stage VII of spermiogenesis) and persists in the acrosome as it develops throughout the elongation stages (stages IX–XII) of spermiogenesis (Fig. 5B).

To investigate whether the immunoreactive human SHBG remains within the acrosome after sperm are released into the male reproductive tract, we performed immunohistochemistry on sperm isolated from the epididymis of transgenic mice containing the 11-kb human SHBG transgene (Fig. 5C), and this clearly shows that immunoreactive human SHBG is only present in the acrosome of sperm from the 11-kb human SHBG transgenic mice (Fig. 5C).

Biochemical Characteristics of the Immunoreactive Human SHBG Extracted from Testicular Cells of Transgenic Mice and Human Sperm—To determine the molecular size of the immunoreactive SHBG within the acrosome of 11-kb human SHBG transgenic mouse germ cells, we used Western blotting to examine protein extracts isolated from testicular cells, i.e. a mixed population of Sertoli cells and germ cells or isolated germ cells. The lack of any immunoreactive molecules in the protein extract of testicular cells from wild-type mice confirms the specificity of the anti-human SHBG antibodies used for this purpose (Fig. 6). In addition, there was no immunoreactivity in the protein extract of isolated testicular cells from mice containing a 4-kb human SHBG transgene (Fig. 6), despite the fact
FIG. 5. Immunoreactive human SHBG is present in the acrosome of germ cells within the 11-kb human SHBG transgenic mouse testis. A, immunoreactive human SHBG (brown stain) is present in the interstitial compartment of the testis from 4- and 11-kb human SHBG transgenic mice (×10 magnification), whereas the testis of wild-type (WT) mice was completely devoid of any immunoreactivity. B, at higher magnification (×60), immunoreactive human SHBG (brown stain) can be detected only within the seminiferous tubules of 11-kb human SHBG transgenic mice, and it can be seen to accumulate in the acrosome (arrowheads) of the germ cells during stages VII–XII of spermatogenesis. C, immunoreactive human SHBG (brown stain) is specifically located in the acrosome (arrowhead) of epididymal spermatozoa taken from 11-kb human SHBG transgenic mice (×100).
the positions of protein size markers are shown on the left.

The steroid-binding affinities of plasma SHBG (closed circles) and acrosomal SHBG (open circles) from 11-kb human SHBG transgenic mice are very similar. The steroid-binding affinities of plasma SHBG \( (K_d = 1.74 \text{ nm}) \) and acrosomal SHBG \( (K_d = 2.25 \text{ nm}) \) were determined using \([^{3}H]DHT\) as labeled ligand. The bound over free \([^{3}H]DHT\) ratio \((B/F)\) was plotted against the amount of \([^{3}H]DHT\) bound specifically to SHBG.

**TABLE I**

Comparison of the relative binding affinity of steroid ligands for serum and acrosomal SHBG isoforms

| SHBG isoform | DHT | Testosterone | Estradiol |
|--------------|-----|--------------|-----------|
| Serum        | 100 | 30.4         | 2.1       |
| Acrosomal    | 100 | 33.5         | 2.9       |

* Determined as a ratio of the concentration of steroid competitor resulting in 50% reduction in specific binding of \([^{3}H]DHT\) to the concentration of DHT required to produce the same effect multiplied by 100.

that the testes of these animals contain appreciable amounts of immunoreactivity in the interstitial cell compartment (Fig. 5A). Thus, the 45-kDa immunoreactive protein observed in the isolated testicular cell extracts of mice containing the 11-kb transgene cannot be accounted for by contamination of SHBG from blood or the interstitial cell compartment.

In preliminary Western blotting experiments of the testicular protein extracts, we noticed that the apparent molecular size of the immunoreactive human SHBG in these samples was slightly smaller than that in a serum sample from the same animals and that its electrophoretic heterogeneity was different from that associated with human SHBG purified from serum. To explore this further, we compared the electrophoretic behavior of immunoreactive SHBG in serum and testicular cell extracts from the 11-kb human SHBG transgenic mice. For this experiment, aliquots of the samples were also treated with N-glycosidase F to remove N-linked oligosaccharides prior to Western blot analysis (Fig. 7). This confirmed that the apparent molecular size of immunoreactive human SHBG in the testicular protein extract (44–46 kDa) is smaller and electrophoretically more heterogeneous than the major electrophoretic isoform of SHBG in serum (50–51 kDa). However, treatment with N-glycosidase F clearly reduces the electrophoretic heterogeneity of immunoreactive SHBG in both samples and results in similarly sized immunoreactive products of about 43 kDa for deglycosylated serum SHBG and 42 kDa for deglycosylated acrosomal SHBG (Fig. 7).

The steroid-binding properties of the immunoreactive human SHBG in the protein extracts from isolated testicular cells were also examined using a steroid-binding capacity assay. Our experiments indicated that specific binding could only be detected in the extracts from mice containing the 11-kb human SHBG transgene and that the highest levels were present in one particular line (shbg 11-b) of these mice (14). We therefore prepared protein extracts of mixed populations of Sertoli cells and germ cells from these mice to study the steroid binding characteristics of the SHBG extracted from the acrosome. A Scatchard analysis using \([^{3}H]DHT\) as labeled ligand indicates that the affinity constant of the acrosomal SHBG is essentially the same as SHBG in serum (Fig. 8). Furthermore, a competition analysis with other SHBG ligands indicates that testosterone and estradiol compete equally as effectively for the binding of \([^{3}H]DHT\) to the acrosomal SHBG, as compared with serum SHBG (Table I).

Our finding that expression of alternative human SHBG transcripts in the testis of transgenic mice results in accumulation of an SHBG isoform in the acrosome of epididymal sperm led us to determine whether SHBG is also present in human sperm. To accomplish this, we compared the electrophoretic mobility of human SHBG in a serum sample with human SHBG in seminal plasma, unwashed sperm in seminal plasma, washed sperm, and sperm that had been purified by Percoll® density gradient centrifugation (Fig. 9). This Western blot demonstrates that the apparent molecular size...
(Mₐ) of the immunoreactive SHBG in human sperm is about 5 kDa smaller than SHBG in either blood or seminal plasma. Furthermore, the Mₐ of immunoreactive SHBG in human sperm is similar to that of the immunoreactive SHBG extracted from the testicular cells of 11-kb human SHBG transgenic mice but is less heterogeneous with respect to its electrophoretic mobility (Fig. 9).

**DISCUSSION**

Based on extensive studies of ABP production by the rat testis (2), it is generally assumed that the presence of an SHBG-like protein in the epididymis of other mammalian species is the result of SHBG gene expression in Sertoli cells (23). In rats, the transcription unit responsible for plasma SHBG production by the fetal liver is also expressed in the testis of sexually mature animals (2), with the highest levels of expression occurring in Sertoli cells during sexual development (6, 24). In contrast, the 4-kb human SHBG transcription unit that is expressed in the liver of transgenic mice is not expressed in the testis (14), despite the fact that the corresponding rat SHBG transcription unit with a similar 5′-regulatory region is expressed strongly as a transgene in the mouse testis (25). These observations provided an indication that there are species-specific differences in the way the SHBG gene is expressed in the testis.

Our results confirm this because they clearly show that the human and rodent SHBG genes are expressed in different cell types within the testis. Like the rat, expression of the mouse SHBG gene appears to be confined to Sertoli cells. However, expression of human SHBG transgenes in the mouse testis occurs in germ cells, and the resulting transcripts consist of an alternative exon 1 sequence identical to that present in several human tissues, and in many cases these transcripts lack exon 7 sequences (1, 9, 10). Analysis of human SHBG transcripts in the germ cells of our transgenic mice by RT-PCR also indicate that some alternative exon 1 containing human SHBG transcripts lack exon 7 sequences. This type of alternatively spliced transcript would result in a premature termination of the open reading frame encoding an SHBG isoform, and this type of product would most likely fold abnormally and undergo rapid degradation, as shown recently (27) for a human SHBG variant encoded by an abnormal allele with a premature stop codon in exon 8. In addition, if human SHBG transcripts lacking exon 7 sequences produce a carboxyl-terminally truncated form of SHBG, this protein would lack consensus sites for N-glycosylation, and our Western blot analysis indicates that the immunoreactive human SHBG in the mouse germ cells is N-glycosylated. We therefore conclude that the human SHBG isoform we have identified in the acrosome cannot be the product of a transcript lacking exon 7.

Our data also indicate that the most abundant human SHBG transcript in the mouse germ cells consists of the alternative exon 1 sequence followed by a sequence corresponding to human SHBG exons 2–8 (1). The 5′-end of this alternative exon 1 sequence has not yet been identified, but preliminary primer extension analysis and comparisons with the published human SHBG sequence (1) indicate that the complete alternative exon 1 sequence lacks an in-frame AUG codon. This might imply that the first conventional translation initiation codon is the AUG codon for Met-30 in the mature SHBG protein sequence, which is located in exon 2 (1). However, because the size of the acrosomal SHBG isoform after de-glycosylation is within 1–2 kDa of the size of serum SHBG treated in the same way, it is unlikely that its amino terminus corresponds to Met-30 in SHBG because the de-glycosylated acrosomal SHBG isoform would then be at least 3 kDa smaller than the deglycosylated serum SHBG. Furthermore, the acrosomal SHBG isoform accumulates in the proacrosomal vesicle, which forms from granules that originate from the Golgi apparatus, and it must therefore consist of a leader sequence that is removed as
the nascent protein undergoes translocation through the rough endoplasmic reticulum. Thus, we speculate that translation of the major alternative human SHBG transcript in testicular germ cells might start from a non-conventional translation initiation codon as part of an internal ribosome entry site (28) and that the precursor polypeptide it encodes comprises a novel amino-terminal leader sequence.

The electrophoretic mobility of acrosomal SHBG is more heterogeneous than SHBG in serum when analyzed by SDS-PAGE, and our data indicate that this is due to a difference in the extent of N-glycosylation. This might also be attributed to the fact that spermatids at all stages of development were used for the extraction of acrosomal SHBG, and the fact that the carbohydrate composition of glycoproteins in the acrosome changes throughout spermiogenesis in a species-specific manner (29). Although the functional significance of N-linked carbohydrates within the carboxyl-terminal domain of SHBG remains obscure, it has been shown previously (30) that the glycosylation has no influence on its steroid binding activity. However, one particular N-glycosylation site is invariably conserved across a wide variety of mammalian species and is likely to be functionally important (31). It could for instance influence the ability of SHBG to interact with other proteins on the surface of specific cell types (32), and this might be relevant to its function in the acrosome.

Given the morphological differences in the testes of humans and rodents, and the fact that rodents lack SHBG in the blood, it is not surprising that there are differences in the way the human and rodent SHBG genes are expressed in the testis. Previous studies have shown that the overexpression of a rat SHBG transgene in the Sertoli cells of the mouse testis results in an increase in germ cell apoptosis (18), but this does not occur in 11-kb human SHBG transgenic mice in which the transgene is expressed within the germ cells. Furthermore, there are marked differences in the levels of SHBG gene expression in the testis of mice and rats (33), and attempts to demonstrate that human Sertoli cells secrete a protein with steroid-binding properties similar to SHBG have not been successful (34). In this context, there is also no reason to assume that SHBG gene products function similarly in the human and rodent testis. Based on the observation that human SHBG accumulates in the acrosome of sperm in our transgenic mice, we examined human sperm samples and confirmed that an SHBG isoform, which can be distinguished from plasma SHBG by gel electrophoresis, is present in ejaculated human sperm when compared with the SHBG extracted from transgenic mouse testicular cells, and this suggests that SHBG gene products function similarly in the human and rodent testis.

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