Characterization and developmental expression patterns of testicular androgen-binding protein in the Djungarian hamster (*Phodopus sungorus*)

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Studies were conducted to characterize Djungarian hamster androgen-binding protein and examine its expression during development. The cDNA encoding the full length testicular androgen-binding protein was cloned and, except for two suspected polymorphisms, shared a common primary sequence with hepatic sex hormone-binding globulin. A single androgen-binding protein/sex hormone-binding globulin gene was identified and a 1.7 kb mRNA encoding androgen-binding protein/sex hormone-binding globulin was present in both the testis and liver. Testicular homogenates contained specific 5 α -dihydrotestosterone-binding activity that was identified as androgen-binding protein. In the prepubertal testis, immunoreactive androgen-binding protein/sex hormone-binding globulin subunits ranged from 46 kDa to 60 kDa, with the majority of isoforms > 50 kDa. These subunits were distinct from the major 55 kDa and 51 kDa isoforms of sex hormone-binding globulin present in the serum of prepubertal hamsters. Deglycosylation studies demonstrated that size heterogeneities were the result of developmentally specific glycosylation patterns. Expression of androgen-binding protein by the testis was upregulated during puberty and coincided with a decline in serum sex hormone-binding globulin activity. The tissue- and age-dependent expression of specific androgen-binding protein and sex hormone-binding globulin variants suggests that these proteins play different roles in steroid-mediated sexual development.

Introduction

Androgen-binding protein (ABP) is a glycoprotein produced by the Sertoli cells of the testis and secreted primarily into the lumen of the seminiferous tubule (Joseph, 1994). ABP binds certain androgens and oestrogens with high affinity and is internalized by spermatogenic and epididymal cells via specific receptors (Joseph, 1994; Gérard, 1995; Porto *et al.*, 1995). A plasma protein with similar steroid-binding properties is produced by the liver and is commonly designated as sex hormone-binding globulin (SHBG) or sex steroid-binding protein. Both ABP and SHBG are considered to be products of the same gene, but are glycosylated differently (Joseph, 1994). These proteins can influence sex steroid action by altering the uptake and metabolism of their ligands as well as by interacting directly with specific receptors on target cells (Rosner *et al.*, 1992; Joseph, 1994; Gérard, 1995).

Significant changes in the expression of ABP and SHBG have been observed during important stages of reproductive development, such as the perinatal and pubertal periods (Damassa and Cates, 1995). In primates, serum SHBG activity increases shortly after birth in both males and females, and remains high until just before puberty (Chaussain *et al.*, 1978;

Forest *et al.*, 1986; Westphal, 1986a). In contrast, testicular ABP concentrations are low during the perinatal period and increase during puberty (Westphal, 1986b; Keeping *et al.*, 1990; Rey *et al.*, 1993). Similar developmental patterns of ABP and SHBG expression have been observed in rabbits (Weddington *et al.*, 1974; Berger *et al.*, 1980; Westphal, 1986a, b). Unlike primates and rabbits, most rodent species (including rats, mice and golden hamsters) do not express SHBG after birth (Westphal, 1986a; Gustafson *et al.*, 1989; Sullivan *et al.*, 1991; Joseph, 1994). As in other species examined, ABP expression is upregulated during puberty in rats; however, the testicular concentration of ABP varies greatly from species to species (Carreau, 1986; Forest *et al.*, 1986; Westphal, 1986b; Joseph, 1994). In rats, large amounts of ABP activity are measured during early puberty (Danzo and Eller, 1985; Westphal, 1986b; Joseph, 1994) whereas, in the mouse testis, ABP concentrations are much lower and detected only by sensitive immunological methods (Westphal, 1986b; Wang *et al.*, 1989; Joseph, 1994).

The Djungarian hamster has become an important rodent model for the study of testicular development. Unlike other rodents, puberty and testicular activation can be delayed in hamsters exposed to short photoperiod (Hoffmann, 1978; Yellon and Goldman, 1984; Van Haaster *et al.*, 1993). This species is also unique among rodents in expressing SHBG. As in primates, serum SHBG concentrations in both male and

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female hamsters increase markedly after birth and decrease at puberty (Gustafson *et al.*, 1989; Cates *et al.*, 1995). We have previously characterized SHBG in this species and observed age-related changes in SHBG glycosylation patterns (Cates *et al.*, 1995). Such changes in glycosylation have been implicated in regulating the interactions of SHBG with the cellular SHBG-receptor (Avvakumov *et al.*, 1988; Joseph, 1994). Despite the importance of the Djungarian hamster in the study of testicular development, little is known about the molecular structure and expression patterns of testicular ABP in this species. Therefore, Djungarian hamster ABP was cloned, characterized, and its molecular structure compared with those of other species. In addition, the developmental patterns of ABP expression and glycosylation were examined and compared with those of SHBG.

Materials and Methods

Tissue and serum samples

Djungarian hamsters (*Phodopus sungorus*) obtained from our breeding colony were housed as mating pairs and maintained on a long photoperiod (16 h light:8 h dark); lights on at 06:00 h. Tissues and serum samples were collected from individual animals as described by Cates *et al.* (1995).

Cloning of Djungarian hamster androgen-binding protein

A pubertal testis (3 weeks of age) cDNA library was constructed in gt22A with the SuperScript Lambda System (GIBCO-BRL, Gaithersburg, MD) using RNA pooled from two hamsters. The library was screened using a 51-mer degenerate SHBG oligonucleotide based on the N-terminal amino acid sequence of Djungarian hamster SHBG (Cates *et al.*, 1995; Damassa *et al.*, 1996). Ten positive plaques were isolated and rescreened; all isolates were true positives.

Individual resuspended plaques were used as templates for PCR amplification of cDNA inserts using gt11 primers. All ten amplified cDNAs encoding ABP were approximately 1.4 kb in size. The products were then subcloned into pSV-Sport1 (GIBCO-BRL, Gaithersburg, MD), and both strands were sequenced as described by Cates *et al.* (1995). A consensus sequence of cDNA encoding ABP was constructed from the compilation of at least three independent sequencing reactions of six clones of cDNA encoding ABP.

The longest cDNA encoding ABP was nearly full length, extending into the signal peptide coding region, and was designated pSV-ABP-5. The remaining coding sequence and the 5' untranslated region (UTR) were cloned via 5' rapid amplification of cDNA ends (5'RACE Kit, GIBCO-BRL, Gaithersburg, MD) of pubertal hamster testis polyadenylated RNA. Synthesis of testis cDNA was primed with a gene-specific antisense oligonucleotide primer located 314–293 base pairs (bp) downstream from the 5' end of pSV-ABP-5. After poly-dC tailing, the cDNA was amplified using a nested antisense gene-specific primer located 292–269 bp from the above reference point. The resulting 5'RACE product was then cloned into pNoTA/T7 (5Prime–3Prime Inc., Boulder, CO). Six positive clones were isolated and sequenced as above.

Computer-based sequence assembly, as well as sequence similarity determinations and structural analyses, were made using the GCG software package (Genetics Computer Group, Inc., Madison, WI) and Entrez (NCBI, Bethesda, MD).

Southern analysis

Genomic DNA was isolated from Djungarian hamster liver (GNome DNA Isolation Kit, Ambion Inc., Austin, TX) and was subjected to Southern analysis (Ausubel *et al.*, 1989). Restricted DNA (approximately 20 µg) was hybridized with a 1.2 kb partial Djungarian hamster cDNA encoding SHBG (Cates *et al.*, 1995) labelled by random priming (DECAprime Random Priming DNA Labeling Kit, Ambion Inc., Austin, TX).

Northern blotting

RNA was isolated by guanidine thiocyanate–phenol–chloroform extraction and subjected to northern analysis (30 µg total RNA) (Ausubel *et al.*, 1989) using the 1.2 kb partial Djungarian hamster cDNA encoding SHBG as described by Cates *et al.* (1995). A complete developmental series was included on each blot. Densitometric analysis of ethidium bromide-stained 18S rRNA was used to normalize samples for differences in sample loading and transfer (see below).

Steady-state native PAGE

Samples were preincubated with 2.0 nmol [³H]5α-dihydrotestosterone (DHT) l⁻¹ and fractionated by steady-state native PAGE as described by Gustafson *et al.* (1989). Nonspecific binding was assessed on duplicate gels containing 100-fold excess of cold DHT. To identify ABP, protein was eluted from gel slices containing DHT-binding activity, precipitated with 80% acetone and subjected to western analysis using an anti-SHBG/ABP peptide antiserum.

Deglycosylation analysis

Proteins in serum or testis homogenates (50 µg) were precipitated with 80% acetone and resuspended in 10 µl of digestion buffer (50 mmol sodium phosphate l⁻¹ (pH 7.5), 50 mmol β-mercaptoethanol l⁻¹, 0.1% (w/v) SDS) and denatured at 100°C for 15 min. After brief centrifugation at 12 000 g for 10 s and cooling, 5 µl of 7.5% Nonidet P-40 was added to the samples (to give a final concentration of 0.7% w/v). N-glycanase (6 U) (Genzyme Corp., Cambridge, MA) was then added to the samples and sterile water was used to bring the final volume to 25 µl. Reactions were carried out for 16 h at 37°C. Duplicate samples were incubated without added N-glycanase. The reactions were terminated by acetone precipitation and samples were resuspended in 10 µl of SDS buffer (62 mmol Tris-HCl l⁻¹ (pH 6.8), 0.1% SDS, 1.0% β-mercaptoethanol, 10% glycerol) in preparation for western blot analysis.

Western blotting

Western blots were performed as described by Cates *et al.* (1995) and Damassa *et al.* (1996). Samples of serum or testis

homogenates containing 50 µg protein were precipitated with 80% acetone and resuspended in SDS buffer for reducing SDS-PAGE. A previously characterized rabbit anti-SHBG peptide antisera (R2383-5) was used in immunoblot analyses (Cates *et al.*, 1995; Damassa *et al.*, 1996).

Image analysis

Measurements of the integrated optical density (IOD) of protein and RNA bands, as well as the determination of the relative molecular sizes of these bands, were made using computer-based image analysis (BioImage Model 110S-2D system with Whole-Band Analysis software, Millipore, Bedford, MA).

Statistical analysis

Measurements of RNA encoding SHBG and SHBG immunoactivity are reported as means $\pm$ SEM. Comparisons among means of the various age groups were made using Fisher's test and one-way analysis of variance (ANOVA). For statistical purposes, samples with undetectable activity were assigned values equal to the sensitivity limit of the appropriate assay. Differences were regarded as significant when $P < 0.05$. All other studies were repeated at least twice.

Results

Sequence analysis of Djungarian hamster androgen-binding protein

A comparison of the deduced amino acid sequence of Djungarian hamster ABP with those obtained for other species is shown (Fig. 1). There is a high degree of sequence identity among the few species that have been studied (66–78%). The Djungarian hamster cDNA encoding ABP contains an open reading frame of 1197 bases, which encodes a presumed leader peptide of 29 residues and the 370 amino acids of mature ABP (Fig. 1). The full length Djungarian hamster testicular clones of cDNA encoding ABP differed at only two positions from a cDNA encoding SHBG previously isolated from Djungarian hamster liver by PCR cloning. Both nucleotide substitutions encode conservative amino acid changes (I-260 and V-295 in Djungarian hamster ABP are V-260 and G-295 in Djungarian hamster SHBG).

All four cysteine residues shown to participate in disulfide bonds in human SHBG are conserved in the hamster cDNA (Fig. 1). There are five potential sites of *N*-glycosylation in hamster ABP. Two sites (N-241 and N-364) are conserved across all species examined. The N-351 site in human SHBG/ABP is found in Djungarian hamster ABP at N-348, but not in other species. The remaining two potential *N*-glycosylation sites (N-131 and N-324) appear to be unique to Djungarian hamster ABP. A potential *O*-glycosylation site is present in Djungarian hamster ABP (T-5) and human ABP (T-7), but not in rat or mouse ABP (Fig. 1).

A single 1.7 kb mRNA encoding ABP, identical in size to the liver SHBG transcript, was identified in the pubertal testis by northern analysis (Fig. 2). Southern analysis of Djungarian hamster genomic DNA indicated that a single copy gene

encodes both SHBG and ABP (Fig. 3). The entire SHBG/ABP gene is encoded within a 5.85 kb BamHI fragment and a 6.0 kb HindIII fragment. Digestion of Djungarian hamster genomic DNA with SstI results in two bands of 13.5 and 16.0 kb, consistent with the one predicted SstI site in the hamster cDNA encoding ABP. Although there are two EcoRI digestion sites in the hamster cDNA encoding ABP, EcoRI digestion gives only two bands (9.0 and 1.4 kb) owing to the proximity of the upstream site to the 5' terminus of the cDNA probe.

Identification of testicular androgen-binding protein

Analysis of [³H]DHT-binding in adult testicular homogenates by 'steady-state' native PAGE revealed a single broad peak of specific activity with a slightly lower mobility than that of Djungarian hamster SHBG (Fig. 4). Addition of 100-fold molar excess of cold DHT eliminated the binding of [³H]DHT to proteins within the low mobility peaks, but not to serum albumin (data not shown). The absence of [³H]DHT-binding by albumin in testicular homogenates suggests minimal plasma protein contamination.

When eluted from the native gel and separated by SDS-PAGE, the peak of specific DHT-binding activity in adult testicular homogenates migrated as a single 48 kDa band that was recognized by a peptide antiserum generated against the common N-terminal 17 amino acids of Djungarian hamster SHBG/ABP. A similar predominant 48 kDa band was also identified in immunoblots of adult testis homogenates (Fig. 5).

Testicular androgen-binding protein isoforms

Western blot analysis of testis protein from 1 week old hamsters revealed 55 kDa and 51 kDa isoforms, as well as minor 60 kDa and 48 kDa bands of immunoactivity (Fig. 5). The 55 kDa and 51 kDa isoforms are the same sizes as Djungarian hamster SHBG isoforms, but the relative abundances of these two isoforms were opposite to those found in hamster serum. After a transitional isoform pattern observed at 2 weeks of age, testicular ABP immunoactivity migrated as a major 48 kDa band and minor 44 and 41 kDa bands. As the hamsters age and enter adulthood (6 weeks), the intensity of immunostaining of all ABP isoforms decreased (Fig. 5). Control blots using preimmune serum instead of the anti-SHBG serum were consistently negative (data not shown).

Deglycosylation of androgen-binding protein

The contribution of differential glycosylation to the electrophoretic microheterogeneity observed among Djungarian hamster SHBG/ABP isoforms was examined by digestion of the proteins with *N*-glycanase. The 55 and 51 kDa isoforms of serum SHBG, the 48 kDa ABP isoform in pubertal testes, and the heterogeneous isoforms of ABP observed in prepubertal hamster testes were all converted into a major immunoreactive band of 44 kDa (Fig. 5). A minor band of 41 kDa was also observed in *N*-glycanase-digested testicular homogenates, but not in serum that had been similarly treated.

Hamster	-29	MENRDSVASLLLLLLPPPHTHQGVLRHVPTQNSQDSPARYLSN	18
Human	-29	MESRGPLATSR-----LLR--R--WA--P-L--SAH-P--VH----	20
Rat	-30	MEKG-VASLRRCR-----TL-P---RT---ID-I-SA--K-----	20
Mouse	-30	MEKRDSVALHWR-----M--P---RA---ID-I-SA--P--K-----	20
Hamster	19	GPGQEPVAVMTIDLTQMSKPYSSFEFRTL DPEGVIFYG DTNTKDDWFMLG	68
Human	21	-----I---F---KIT-TS---V--W-----P-----	70
Rat	21	-----T-L---KI---S-----W-----E-----	70
Mouse	21	-----M-----KI---H-----W-----E---L--	70
Hamster	69	LRDQGLEIQMHNPWAQLTVGFGRPLNDGRWHQVELKMSGDSLQLWVDGKE	118
Human	71	---RP---L--H-----A---D-----V--E---VL-E---E-	120
Rat	71	-----L--L--R-----P-----N-----L-----	120
Mouse	71	--A-----L--A--R-----D---P---N---L-----	120
Hamster	119	LLCLRQISGTLANNSWPSMRIALGGLLLPTSSLRFPPLVPALDGLRRDTW	168
Human	121	V-R---V--P-TSKRH-I-----F-A-N--L-----S-	170
Rat	121	M-----V-AS--DHPQL-----K-----I--I-	170
Mouse	121	M-----AS--DH-QR-----K-----I--I-	170
Hamster	169	LGHQVHLSPSP#NLGNCVDVLDQPGLFPPQGTAEFSLQDIPQPRTPDW	217
Human	171	-DK-AEI-A---TS-RS---ESN--I-L-P--Q---N-R---HAE--A	220
Rat	171	---AQ--T--RTS-----P-----H---T	220
Mouse	171	---AQ--A-PTS-----P-----HA---T	220
Hamster	218	FSLELGLKLVDGSGCLLALGTRTNSSWLSLHLQDQKVVLSSGIEPKLVLA	267
Human	221	---D---QAA--H---PE-P-----SG-G-D-P	270
Rat	221	-----F---A-R--T---G-----T-----T---EA---A-P	270
Mouse	221	-----F-----Q-----G-----NI---N-S---EA---V--P	270
Hamster	268	LDMGLPLQLKLDILKVVLSSQGPKEVLVASASRLAALRTLWSHPQGLLSL	317
Human	271	-VL-----SMR-----S-MKA-ALPPLG--P-LN--AK---R-F-	320
Rat	271	-AV-----VF--A-----M---ST-LL--S-WR-----H---	320
Mouse	271	--V-----T--RV-----M---SM-LL-P-S-WR-----H---	320
Hamster	318	GALAGDNSSASFCLSDLVVQGRQLDIDQALNRSQNIWTHSCPHSPNNVSH	367
Human	321	---P-ED--T---NG--A-----V-----HE-----Q--G-GTD	370
Rat	321	---P-ED-----K--S---D-----Q--S-DT-	370
Mouse	321	---P-ES-----F-----S---D-----QR-S-DTR	370
Hamster	368	ISH	370
Human	371	A--	373
Rat	371	T--	373
Mouse	371	T--	373

Fig. 1. Sequence comparison of Djungarian hamster androgen-binding protein (ABP) with those of other species. The deduced amino acid sequence of Djungarian hamster ABP is compared with human sex hormone-binding globulin (SHBG)/ABP (Walsh *et al.*, 1986; Gershagen *et al.*, 1987a) and the predicted sequences derived from the rat (Joseph, 1994) and mouse (Wang *et al.*, 1989) cDNAs encoding ABP. The rabbit SHBG sequence is not included since it is only partial length (Griffin *et al.*, 1989) and there are some sequence discrepancies (Wang *et al.*, 1989). Numbers refer to the position in relation to the first amino acid of mature SHBG (□) determined by direct amino acid sequencing (Damassa *et al.*, 1996). The putative SHBG-receptor binding site (Khan *et al.*, 1990) is marked in bold type and the steroid-binding region (Joseph, 1994) is double-underlined. S, conserved cysteine residues participating in disulfide bonds in human and rat SHBG; ●, potential N-linked glycosylation sites; *, potential O-linked glycosylation site (Joseph, 1994; Walsh *et al.*, 1986); #, deleted residue in hamster ABP.

Developmental patterns of testicular expression of androgen-binding protein and serum sex hormone-binding protein

Neonatal hamster testes were too small to analyse individually; however, mRNA encoding ABP was detected at all other ages examined. Transcript concentrations were low (40–60% of maximum concentrations) in 1 and 2 week old hamsters, but peaked at 3 weeks of age (Fig. 6). The amount of message decreased to 40% and 20% of peak concentrations by 4 and 6 weeks of age, respectively.

The predominant 48 kDa immunoactive isoform of ABP was expressed at very low concentrations in the testis until 3 weeks

of age, when concentrations increased significantly (Fig. 6). Testicular concentrations of the 48 kDa isoform in late pubertal–early adult hamsters (6 weeks of age) were significantly lower than the peak values observed in mid-pubertal (4–5 weeks of age) males (Fig. 6). Owing to the greater contribution of heavier SHBG-like isoforms in the prepubertal testis, the amount of total ABP immunoactivity (the sum of the IODs of all detected isoforms) remained relatively constant from 1 week until 4 weeks of age (data not shown).

In serum, immunoactive SHBG/ABP isoforms were detected in males and females throughout prepubertal development (Fig. 7). The previously identified 55 and 51 kDa SHBG isoforms

Fig. 2. Northern blot analysis of Djungarian hamster liver and testis sex hormone-binding globulin (SHBG)/androgen-binding protein (ABP). Polyadenylated RNA (10 µg) isolated from testes of peripubertal (3 week) hamsters and the liver of postnatal (1 week) hamsters was probed with a partial Djungarian hamster cDNA encoding SHBG/ABP. A single specific 1.7 kb mRNA encoding SHBG/ABP was detected in both tissues. The same blot was stripped and re-probed with a partial human cDNA encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to assess loading and transfer efficiencies.

were present at high concentrations from birth until 2 weeks of age in all animals. During this postnatal period, trace amounts of 48 and 44 kDa isoforms were also found in serum of both males and females, indicating that the testes were not the source of the immunoactivity. After 2 weeks of age, there was an overall decrease in all SHBG/ABP isoforms in serum from both sexes (Fig. 7).

Discussion

Djungarian hamster ABP and SHBG were found to be products of a single gene, as has been shown for human and rat ABP/SHBG (Gershagen *et al.*, 1989; Sullivan *et al.*, 1991; Joseph, 1994). The two nucleotide differences in the testis clones of cDNA encoding ABP relative to that reported for a Djungarian hamster liver clone of cDNA encoding SHBG could reflect polymorphism in the hamster gene pool or PCR-cloning artifacts (Cates *et al.*, 1995). The conservative amino acid differences predicted by the two discrepancies between the ABP and SHBG cDNAs suggest that these

Fig. 3. Southern blot analysis of Djungarian hamster genomic DNA. Djungarian hamster genomic DNA (15 µg) was restricted with the indicated enzymes and probed with a partial Djungarian hamster cDNA encoding sex hormone-binding globulin (SHBG)/androgen-binding protein (ABP). The positions of λ -Hind III digested DNA markers are shown on the left.

are polymorphisms, with at least two alleles encoding Djungarian hamster SHBG/ABP. Genetic variants of SHBG have also been observed in humans (Gershagen *et al.*, 1987b; Power *et al.*, 1992; Joseph, 1994; Larrea *et al.*, 1995; Hardy *et al.*, 1995).

The overall homology of Djungarian hamster SHBG/ABP with that of other mammals is high (66–78%). Long stretches of extensive identity interspersed with short regions of divergent sequence are particularly notable upon comparison of the aligned sequences of several species. This high degree of conservation may indicate regions with strict secondary structure that are critical for SHBG/ABP function (such as dimerization, steroid binding, and recognition of cell surface receptors) flanked by less conserved, possibly species-specific, structural regions (Khan *et al.*, 1990; Joseph, 1994; Hammond and Bocchinfuso, 1995).

The major isoform of ABP in the mature hamster testis is 48 kDa, in contrast to the principal 55 and 51 kDa variants of serum SHBG. Differences in the molecular weights of ABP and

Fig. 4. 'Steady-state' PAGE of testicular homogenates and serum from Djungarian hamsters. Serum of 2 week-old female hamsters (○) and homogenates of adult hamster testes (●) were preincubated with 2.0 nmol [^3H]5 α -dihydrotestosterone (DHT) l^{-1} and then separated on native acrylamide gels containing 1.0 nmol [^3H]DHT l^{-1} . The d.p.m. of [^3H]DHT in each gel slice is plotted against gel slice number (beginning at the origin). ■, Position of serum albumin, determined by staining gel slices with bromocresyl green.

Fig. 5. Western blot analysis of Djungarian hamster sex hormone-binding globulin (SHBG)/androgen-binding protein (ABP) and effects of deglycosylation. Testis homogenates from hamsters aged 1, 2, 4, and 6 weeks of age and serum from 2 week old female hamsters (S) were separated by SDS-PAGE. Selected samples were treated with *N*-glycanase (+) or with the reaction buffer only (−). The anti-SHBG/ABP peptide antisera recognized multiple molecular weight isoforms of ABP in prepubertal testis, including the serum SHBG-like 55 and 51 kDa isoforms. A 48 kDa immunoreactive protein (large arrow) became the predominant ABP isoform as males reached puberty (4 weeks) and older (6 weeks). Removal of *N*-linked carbohydrates from ABP of prepubertal and pubertal testis and from SHBG of postnatal female serum resulted in a major 44 kDa immunoactive species. The mobilities of the molecular mass standards (kDa) are shown on the left and computer-determined molecular weight estimates for the predominant isoforms are shown on the right.

SHBG isoforms have also been reported in other species. In adult rabbits, as in Djungarian hamsters, the predominant serum SHBG isoform is larger than testicular ABP (Westphal, 1986a, b; Joseph, 1994); in contrast, the human ABP variants (54 kDa and 50 kDa) are larger than those of human SHBG (52 kDa and 48 kDa) (Westphal, 1986b; Joseph, 1994).

Variable degrees of glycosylation appear to account for the differences observed in the relative molecular weights of the

Fig. 6. Developmental patterns of testicular androgen-binding protein (ABP) expression in Djungarian hamsters. Data are presented as the percentage of the density (IOD) of the hybridization signal relative to that measured at 3 weeks of age (100%) of mRNA encoding ABP, after normalization to the amount of 18S rRNA transferred to the filter (●). Measurements of the IOD of immunoblots of the major ABP isoform (48 kDa) were made using computerized image analysis (□). Data are expressed as means $\pm$ SEM; $n = 3$ –6 animals per age group. B, birth.

SHBG/ABP subunits in Djungarian hamsters, as has been shown for other species (Danzo *et al.*, 1991; Petra *et al.*, 1992; Joseph, 1994). The predicted core molecular weight of Djungarian hamster SHBG/ABP based on the deduced amino acid sequence is 40.6 kDa. Therefore, the minor 41 kDa isoform produced by *N*-glycanase treatment of testicular extracts probably represents completely deglycosylated ABP, whereas the major 44 kDa isoform indicates the presence of an *O*-linked glycan on ABP (presumably at T-5, analogous to T-7 in human SHBG) (Walsh *et al.*, 1986; Joseph, 1994). In general, ABP isoforms in the immature testis are larger than those found in the adult testis. In particular, ABP isoforms in early postnatal testes appear to resemble those of serum SHBG. The SHBG-like immunoactivity in the neonatal testis is not due to gross plasma contamination since [^3H]DHT-binding activity attributable to serum albumin was not detectable in testicular extracts. In addition, the ratio of the 55 and 51 kDa molecular weight isoforms was different in testicular extracts compared with serum. Since testicular ABP transcripts are detected at these ages, the immature Sertoli cells may be glycosylating ABP in 'SHBG-like' patterns (Cheng *et al.*, 1986; Danzo *et al.*, 1991; Joseph, 1994). Alternatively, the testis may selectively sequester certain SHBG isoforms during the early postnatal period (Larrea *et al.*, 1981; Sakiyama *et al.*, 1988; Gérard *et al.*, 1994). Thus, an exogenous source of the testicular SHBG-like isoforms cannot be ruled out, and further investigation is necessary to clarify the origin of ABP immunoactivity in the neonatal testis.

Whereas the extent of glycosylation of SHBG in liver and serum increases during early postnatal development, the

Fig. 7. Western blot analysis of Djungarian hamster serum sex hormone-binding globulin (SHBG) in males and females during development. Serum (1.0 μ l) from male or female hamsters aged 0 (1 day), 2, 4, and 6 weeks of age were separated by SDS-PAGE. Anti-SHBG/androgen-binding protein (ABP) antisera recognized the major 55 kDa and 51 kDa isoforms in animals up to 4 weeks of age. Minor 48 kDa and 41 kDa bands were seen in sera from both males and females at 2 weeks of age or younger. The mobilities of the molecular weight standards (kDa) are shown on the left.

number and size of oligosaccharides on ABP decreases during the same period. Thus, it appears that both the testis and liver of Djungarian hamsters are capable of generating similar molecular weight isoforms of ABP and SHBG, but that these processes are independently regulated and follow age-dependent patterns in each tissue. The significance of shifting isoform ratios is unclear. Differential glycosylation does not appear to influence the steroid-binding properties of SHBG (Bocchinfuso *et al.*, 1991; Petra *et al.*, 1992; Joseph, 1994), but may affect the interaction of SHBG with steroid-responsive cells (Avvakumov *et al.*, 1988; Rosner *et al.*, 1992; Joseph, 1994; Damassa and Cates, 1995; Porto *et al.*, 1995; Gérard, 1995) or target specific isoforms to different secretory pathways (Cheng *et al.*, 1986; Danzo *et al.*, 1991; Joseph, 1994). Analysis of glycosylation variants by differences of molecular weight measured by PAGE is rather crude, and the nature and number of glycans added to Djungarian hamster SHBG and ABP are unknown. Determining what differences in glycosylation are responsible for the generation of different molecular weight variants of ABP and SHBG would require a more detailed analysis of specific isoforms. However, on the basis of ratios of the relative abundance of the major 44 kDa isoform to the minor 41 kDa isoform of ABP and SHBG subjected to N-deglycosylation, it may be surmised that the vast majority of both these proteins are glycosylated via an O-linkage, similar to human SHBG (Walsh *et al.*, 1986; Joseph, 1994).

In Djungarian hamsters, ABP expression is minimal during early postnatal development. Conversely, hepatic expression of serum SHBG peaks at this time. ABP message concentrations peak early in puberty (at 3 weeks of age); the reduction in concentrations of ABP and mRNA encoding ABP that occur thereafter may be due either to hormonal insensitivity of

mature Sertoli cells or to dilution of Sertoli cell products by an increasing germ cell mass during puberty (Hall *et al.*, 1990). Testicular ABP concentrations increase at puberty but there is a coincident decrease in hepatic SHBG expression and circulating androgen-binding activity. Similar patterns have been observed in pubertal monkeys and rabbits (Weddington *et al.*, 1974; Berger *et al.*, 1980; Forest *et al.*, 1986; Westphal, 1986a, b; Keeping *et al.*, 1990). Although other rodents also upregulate ABP at puberty, they do not exhibit the postnatal surge in hepatic SHBG expression observed in Djungarian hamsters (Weddington *et al.*, 1974; Berger *et al.*, 1980; Westphal, 1986b; Keeping *et al.*, 1987; Gustafson *et al.*, 1989; Cates *et al.*, 1995).

The reasons for species differences in ABP and SHBG expression and their implications regarding the actions of these proteins are unknown, but may be related to the development and maturation of spermatzoa and other steroid target tissues. Djungarian hamsters raised under a long photoperiod (18 h light:6 h dark) normally enter puberty shortly after birth, at around 3 weeks of age (Van Haaster *et al.*, 1993). However, when they are raised under a short photoperiod (6 h light:18 h dark), puberty is delayed for up to a year (Hoffmann, 1978; Yellon and Goldman, 1984; Van Haaster *et al.*, 1993). In contrast, rodent species that lack postnatal increases in SHBG, such as mice, rats and golden hamsters, show a rapid onset of puberty even when raised under a short photoperiod. Whether SHBG, acting through its receptor, influences the timing of the maturational increases in FSH or the activation of steroid sensitive tissues in Djungarian hamsters remains to be determined.

Rao *et al.* (1995) have reported that male Djungarian hamsters exposed to a short photoperiod from birth upregulated testicular ABP expression at the appropriate postnatal age and continued to express high concentrations of mRNA encoding ABP beyond 3 weeks of age. In contrast, concentrations of mRNA encoding ABP in hamsters reared under a long photoperiod decreased at puberty. How these differences in concentrations of mRNA encoding ABP are related to changes in the cellular composition of the testis or to ABP protein production is not known. Therefore, it is difficult to postulate relationships between ABP and short photoperiod-induced delay of testicular development. Further study of the physiological consequences of species-specific differences in the expression and glycosylation of testicular ABP and hepatic SHBG throughout development are needed to characterize the role of these proteins in modulating steroid action at target tissues.

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