Effects of photoperiod on androgen-binding protein and sperm fertilizing ability in the hamster

M. K. Holland, B. J. Rogers, M.-C. Orgebin-Crist and B. J. Danzo

Departments of Obstetrics & Gynecology, Biochemistry, and Cell Biology and the Center for Reproductive Biology Research, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, U.S.A.

Summary. Androgen binding protein (ABP) was detected in both the testis and epididymis of golden hamsters exposed to a long photoperiod (16L:8D). The concentration of ABP in the testis rose from 0-1 pmol/g testis in 2-week-old animals to attain maximum values (3-9 pmol/g testis) at 6–7 weeks, then declined to adult values (1.8 ± 0.4 pmol/g testis) after 10–11 weeks of age. In contrast, the ABP concentration of the caput epididymidis reached maximum values at 4–7 weeks of age (14 pmol/g tissue) and declined to adult values (4.8 ± 1.5 pmol/g tissue) by 10–11 weeks of age. ABP content of the corpus epididymidis was maximal (1.0 pmol/g tissue) at 2 weeks of age and thereafter declined to below detectable levels by 10–11 weeks. No ABP could be detected in the cauda epididymidis from animals of any age examined.

Hamster ABP analysed by steady-state polyacrylamide gel electrophoresis had a relative mobility ($R_s$) of 0.33 compared to 0.41 for rabbit ABP. Sucrose gradient analysis of hamster ABP indicated a sedimentation coefficient of about 4 S. The binding of $[^3H]5\alpha$-dihydrotestosterone ($[^3H]5\alpha$-DHT) to hamster ABP was very rapid with equilibrium occurring within 10 min. The dissociation of $[^3H]5\alpha$-DHT from hamster ABP was also rapid ($t_1 = 2.77$ min). Saturation analysis of ABP from mature animals yielded an apparent dissociation constant of 6.4 nm and an ABP concentration of 1.2 ± 0.2 pmol/mg protein. The binding of $[^3H]5\alpha$-DHT to hamster ABP was inhibited by $5\alpha$-DHT > testosterone >> cyproterone acetate.

Exposure of mature hamsters to a short photoperiod (8L:16D) for 3 weeks resulted in a 42% drop in epididymal ABP levels (10:3 to 4.3 pmol/g tissue). Epididymal ABP further declined so that after 15 weeks in a short photoperiod it was 4% (0.4 pmol/g) of initial values. Accompanying this decrease in epididymal ABP concentrations was a decline in the fertilizing ability of spermatozoa from the distal cauda. When hamsters were transferred from a short to a long photoperiod (16L:8D), epididymal ABP content returned to about 50% of control values within 3 weeks. However, the fertilizing ability of spermatozoa from the cauda epididymidis of these animals did not return to control values after a 9-week exposure to a stimulatory photoperiod.

Introduction

The reproductive organs of the male hamster undergo an annual cycle of regression and reactivation which affects sperm production (Hoffman & Reiter, 1965; Gaston & Menaker, 1967). The endocrine mechanisms which underly and control these events have been well studied (Berndtson & Desjardins, 1974; Turek et al., 1975), but there are relatively few data on the changes in the accessory sex glands (Payne & Bennett, 1976) and on the correlation between these changes and fertility. This study was undertaken to determine whether (1) androgen-binding protein (ABP), an index of Sertoli cell function in other species, was present in the hamster testis and epididymis, (2) it
was photoperiodically regulated, and (3) there was any correlation between photoperiod-induced fluctuations in fertility and ABP levels.

Materials and Methods

Animals. Golden hamsters (Mesocricetus auratus) and Sprague-Dawley rats were obtained from Harlan, Inc. (Indianapolis, IN, U.S.A.). Frozen hamster testes and epididymides were obtained through Bio-Trol (Indianapolis, IN, U.S.A.). New Zealand White rabbits, at least 6 months of age, were purchased from Myrtle’s Rabbitry (Franklin, TN, U.S.A.). Hamsters were maintained in a long (16 h light:8 h dark; 16L:8D) or short (8L:16D) photoperiod. All animals had free access to food and water.

Chemicals. All chemicals utilized were of reagent or analytical grade and purchased from Fisher (Fairlawn, NJ, U.S.A.) or Sigma (St. Louis, MO, U.S.A.).

Electrophoresis. Steady-state polyacrylamide gel electrophoresis (SS-PAGE) was conducted by the method of Ritzen et al. (1974) with modifications previously described (Taylor et al., 1980). Cytosol (50 µl) was incubated with 4 nM-[3H]5α-dihydrotestosterone (DHT) alone or with a 100-fold excess of unlabelled 5α-DHT for 1 h on ice. Bromophenol blue and sucrose were added to the samples and were applied to 7% (w/v) polyacrylamide gels containing 4 nM-[3H]5α-DHT alone or with a 100-fold excess of unlabelled 5α-DHT. After electrophoresis, the gels were sliced into 2 mm segments and counted in a scintillation mixture of Spectrafluor (Amersham, Arlington Heights, IL, U.S.A.) and toluene (100:2365, v/v).

Sucrose gradient analysis

A full description of this technique has previously been published (Danzo et al., 1973). Briefly, cytosol samples were incubated with 4 nM-[3H]5α-DHT alone or with 400 nM-unlabelled 5α-DHT for 1 h on ice in a final volume of 0.5 ml. The samples were then extracted for 6 sec with the pellet from 0.5 ml of a charcoal–dextran suspension (0.5% charcoal, Norit I, 0.05% dextran 60 C). The charcoal was pelleted (5 min at 1500 g) and 300 µl of the supernatant were applied to 5–20% linear sucrose gradients. Gradients were centrifuged for 16–17 h at 189,000 g, the base of each tube was pierced, and samples (250 µl) were collected and counted.

Charcoal assay. The 6-sec charcoal assay (Danzo & Eller, 1975a) was used routinely to estimate the quantity of ABP present in various samples. In brief, triplicate samples containing ABP were incubated with a saturating concentration of [3H]5α-DHT (4 nM) alone or with a 100-fold excess of unlabelled 5α-DHT (to estimate non-specific binding) or, with various potential inhibitors, in a final volume of 0.5 ml. After incubation, 0.5 ml of the charcoal–dextran suspension was added to the tubes which were vortexed for 6 sec, centrifuged (1500 g for 5 min) and the supernatant counted in a scintillation fluid containing Spectrafluor, Triton X-100, and toluene (100:1230:2460, by vol.). Specific binding was determined by subtracting the counts present in the sample with the 100-fold excess of unlabelled 5α-DHT from those present in the other samples. When a molar concentration of ABP is given, it was calculated by converting specifically bound counts to moles of steroid bound, assuming one binding site for [3H]5α-DHT per ABP molecule. This was taken to be a measure of ABP.

All studies with ABP were conducted at 0–4°C.

Testosterone assay. Testosterone concentrations in unextracted serum samples were measured by using a commercial solid-phase radioimmunoassay and [125I]-labelled testosterone tracer (Diagnostic Products Corporation, Los Angeles, CA, U.S.A.). Testosterone standards were prepared from stock solutions dissolved in ethanol and calibrated by measuring absorption at 241 nm (Engel, 1963). The assay procedure followed the manufacturer’s protocol. The samples were counted in a Micromedics Systems Apex gamma counter (Micromedics Systems, Horsham, PA, U.S.A.).

The antibody used shows 8% cross-reactivity with dihydrotestosterone calculated as % cross-reaction = (100 × A)/B, where A is the mass of non-radioactive testosterone needed to effect a 50% displacement of tracer and B is the mass of tested steroid required to effect the same displacement. Cross-reactions with other naturally occurring steroids are negligible.

The limit of detection of the assay, estimated from Bl/Bo = 0.8 on the standard curve, is 13 pg/tube or, using our standard 50-µl sample, 0.2 ng/ml. Interassay co-efficients of variation for a commercially prepared low (1.3 ng/ml) and high (5.4 ng/ml) standard were 6% and 2.6%, respectively. Intra-assay co-efficients of variation were 8.5% at the low (0.66 ng/ml) and 4.7% in the middle (3.1 ng/ml) range of the assay. The results using this assay have been corrected with the procedure of Abraham et al. (1977), which relies on ether extraction and celite column chromatography. Over a range (0.2 to 4.0 ng/ml) of serum testosterone concentrations, the correlation co-efficient was r = 0.92.

Photoperiod experiments. Hamsters were housed in a long photoperiod (16L:8D) unless otherwise specifically indicated. Testicular regression was initiated by transferring animals to a short photoperiod (8L:16D). ABP and fertilizing ability of spermatozoa from animals undergoing photoperiod-induced testicular regression were measured at the intervals indicated in Tables 2 and 3. After 15 weeks in the short photoperiod, animals were transferred back to
the long photoperiod (16L:8D) and again ABP and sperm fertilizing ability were measured at the intervals indicated (Tables 2 and 4).

In-vitro fertilization. The method used was that of B. D. Bavister (personal communication). Spermatozoa were obtained from a nick in the cauda epididymidis and diluted into a modified Tyrode's solution designated TALP (Bavister & Yanagimachi, 1977). Only samples with good motility were used. The spermatozoa were incubated in TALP containing penicillamine (20 µM), hypotaurine (100 µM), and adrenaline (1 µM) at 37°C under 5% CO₂ in air for 1 h. During this time the cumulus mass was collected from female hamsters which had been stimulated to ovulate 15½ h earlier by the administration of 25 i.u. hCG in TALP buffered with 5 mM-Hepes. The cumulus was divided approximately equally and dispersed into 90 µl droplets of TALP under mineral oil. Spermatozoa (1–2 × 10⁶) were added to each drop as was penicillamine (20 µM), hypotaurine (100 µM), and adrenaline (1 µM) and the dishes were incubated for 3 h at 37°C under 5% CO₂ in air. At this time eggs were removed, washed 3 times in 2 ml TALP-Hepes to remove all loosely attached spermatozoa. The zona pellucida was then removed with trypsin (13 000 units/ml). The eggs were deposited on a slide, compressed with a coverslip, and the numbers of decondensed sperm heads in the vitellus were counted. To ensure that any decline in the number of fertilized ova was a consequence of poor fertility related to the photoperiodic exposure, each experiment always included a control in which spermatozoa were obtained from an animal maintained in a photoperiod appropriate for breeding.

Results

Demonstration of ABP in the hamster testis and epididymis

Cytosol prepared from homogenates of whole epididymis from adult animals kept in 16L:8D showed very low specific binding of androgener using the charcoal assay (data not shown). When cytosol was prepared from the major anatomical subdivisions of the epididymis (caput, corpus and cauda) specific androgen binding could be detected only in caput cytosol (Table 1). Since testosterone binding globulin (TeBG) is not demonstrable in hamster blood (Corvol & Bardin, 1973), the binding that we detect is attributed to ABP. Dilution of ABP-containing tissue by that not containing ABP obscured the presence of ABP in whole organ homogenates. The concentration of ABP in the caput epididymidis was maximal in 4–7-week-old animals and declined as the animals matured (Table 1). A small quantity of ABP was present in the corpus of young animals, but by the time animals had reached 10–11 weeks of age, no ABP could be detected in that segment (Table 1). No ABP could be detected in the cauda of any age group. The concentration of ABP in the testis rose from low values in young (2–3 week) animals to reach a maximum in 6–7-week-old hamsters before declining to adult values in 10–11-week-old animals (Table 1).

<table>
<thead>
<tr>
<th>Table 1. The effect of age on ABP levels in the hamster epididymis and testis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (weeks)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>6–7</td>
</tr>
<tr>
<td>10–11</td>
</tr>
<tr>
<td>Adult</td>
</tr>
</tbody>
</table>

N.D. = not detectable.

*The values represent the means of triplicate determinations on cytosol prepared from pooled tissues in each age group except the adult. The value shown for the adult is the mean ± s.e.m. of triplicate determinations from 5 separate pools of animals. The charcoal assay (using 4 nm-[³H]5α-DHT) was used to estimate ABP levels; for the quantitation of ABP, it was assumed that each mole of ABP binds one mole of [³H]5α-DHT.
Characteristics of hamster ABP

Polyacrylamide gel electrophoresis. Samples of adult epididymal cytosol analysed for androgen binding activity of SS-PAGE yielded a peak of $[^{3}H]5\alpha$-DHT binding with a relative mobility ($R_f$) of 0.33 (Fig. 1). Samples incubated with $[^{3}H]5\alpha$-DHT and a 100-fold excess of unlabelled $5\alpha$-DHT subjected to electrophoresis on gels containing the same ratio of labelled to unlabelled $5\alpha$-DHT failed to show a peak with an $R_f$ 0.33, indicating that binding of $[^{3}H]5\alpha$-DHT was occurring to a limited number of sites (Fig. 1). Albumin, indicated by its ability to bind the tracking dye bromophenol blue, was frequently detected in hamster epididymal cytosols (Fig. 1). It is assumed that albumin arises from serum contamination. Binding of label to albumin was unaffected by excess unlabelled $5\alpha$-DHT. When an equivalent quantity of rabbit epididymal cytosol was analysed under identical conditions, about 5 times more ABP than in the hamster was detected (2.02 pmol in the rabbit; 0.47 pmol in the hamster). In addition, rabbit ABP ran further into the gel ($R_f = 0.41$) than did hamster ABP (Fig. 1).

Sucrose gradient analysis. The analysis of adult hamster caput cytosol on low ionic strength (0.01 M-KCl) sucrose gradients indicated a peak of macromolecular bound $[^{3}H]5\alpha$-DHT having a sedimentation coefficient of about 4 S relative to bovine serum albumin (Fig. 2). As the sample was stripped with charcoal before sedimentation analysis, the presence of free hormone in the gradient indicated that dissociation of $[^{3}H]5\alpha$-DHT from ABP occurred during centrifugation. Incubation with excess unlabelled $5\alpha$-DHT eliminated the peak, indicating that binding of $[^{3}H]5\alpha$-DHT was occurring to a limited number of sites (Fig. 2).

Association and dissociation of $[^{3}H]5\alpha$-DHT with hamster ABP. A study of the time course of $[^{3}H]5\alpha$-DHT binding to, hamster caput cytosol showed that binding equilibrium was attained before the first time examined, 5 min (Fig. 3). Although there was no statistically significant difference in the amount of label bound during the first 2 h of incubation, the amount bound at the 4-h point was lower than at the 10-min point ($P < 0.01$) (Fig. 3). This implies a lack of stability of hamster ABP at 0–4°C. Whether the instability of hamster ABP is an inherent property of the molecule or is the result of the presence of proteolytic enzymes in the extract is under investigation. However, we have established that the presence of 10 mM-p-methylsulphonylfluoride, an inhibitor of serine proteases, does not affect the stability (data not shown). Dissociation of $[^{3}H]5\alpha$-DHT
**Fig. 2.** Sucrose gradient analysis of $[^3H]5\alpha$-DHT binding to adult hamster caput cytosol. Cytosol prepared from a pool of caputs from adult hamster epididymides was incubated with $4 \text{nM-}[^3H]5\alpha$-DHT alone (●) or with $400 \text{nM}$ unlabelled $5\alpha$-DHT (○) extracted with charcoal to remove unbound label, and analysed on low ionic strength (0.01 M-KCl) 5–20% sucrose gradients. $[^{14}C]$Bovine serum albumin sediments at fraction 7.

**Fig. 3.** Time-course of $[^3H]5\alpha$-DHT binding to hamster ABP. Cytosol was prepared from a pool of caputs from adult hamster epididymides. Two sets of tubes were set up containing cytosol and buffer. $[^3H]5\alpha$-DHT alone was added to one set and $4 \text{nM-}[^3H]5\alpha$-DHT and $400 \text{nM}$ unlabelled $5\alpha$-DHT (to determine non-specific binding) were added to the other set. At the indicated times after the addition of steroid, charcoal was added to the samples; they were mixed on a vortex for 6 sec and centrifuged. The supernatants were counted. The values plotted are the mean specific binding ± s.e.m. of triplicate determinations.
from hamster ABP also occurs rapidly. Adult hamster caput cytosol was incubated with $[^3]H$5α-DHT for 30 min to achieve binding equilibrium. A 1000-fold excess of unlabelled 5α-DHT was added and binding was measured by the charcoal assay (Fig. 4). The decline in the amount of $[^3]H$5α-DHT bound to ABP was exponential, apparently of first order kinetics, and occurred with a dissociation half-time ($t_{1/2}$) of 2:80 min. When the study was repeated, but the samples were incubated for the indicated lengths of time with charcoal (Fig. 4) in the absence of unlabelled 5α-DHT, the dissociation of $[^3]H$5α-DHT from ABP slowed to 5:12 min. Therefore, despite the rapid rate of dissociation of steroid from hamster ABP, the 6-sec charcoal assay should yield a reasonably quantitative measurement of the amount of ABP present in the preparations.

**Fig. 4.** Dissociation of $[^3]H$5α-DHT from hamster ABP. Adult hamster caput cytosol was incubated with 4 nm-$[^3]H$5α-DHT alone or with 400 nm unlabelled 5α-DHT for 30 min to allow binding equilibrium to occur. Then 4000 nm unlabelled 5α-DHT or charcoal was added to the sets of tubes. At the indicated times after the addition of unlabelled 5α-DHT, charcoal was added to the tubes, they were vortexed for 6 sec and centrifuged. The supernatants were counted. The sets of tubes to which charcoal alone had been added were vortexed for 6 sec at the indicated times after the addition of charcoal, centrifuged, and the supernatants were counted. The specific binding of assay triplicates is plotted.

**Saturation analysis of $[^3]H$5α-DHT binding to hamster ABP.** Incubation of a fixed quantity (0:45 mg protein/tube) of adult hamster caput cytosol with various concentrations of $[^3]H$5α-DHT in the range of 0:5–8:6 nM resulted in saturation of binding sites (Fig. 5, inset). Scatchard (1949) analysis of the data (Fig. 5) yielded a straight line, indicating a single class of high-affinity binding sites for $[^3]H$5α-DHT on ABP. Linear regression analysis gave a correlation coefficient of 0:995 and a dissociation constant ($K_d$) of 6:4 nM). The concentration of binding sites was 1:2 ± 0:2 (mean ± s.e.m. for 3 separate experiments) pmol/mg caput protein.

**Steroid specificity of hamster ABP.** The specificity of binding of steroids to hamster ABP was assessed by incubating caput cytosol with a fixed (4 nm) concentration of $[^3]H$5α-DHT alone (control) or with a 100-fold excess of unlabelled potential competitor. Binding was assessed with the charcoal assay. Both 5α-DHT and testosterone completely inhibited $[^3]H$5α-DHT binding to hamster ABP. Oestradiol and cyproterone acetate caused some inhibition of binding, but were much less effective than the androgens (Fig. 6). A generally similar pattern was obtained with both
Fig. 5. Saturation analysis of $[^3]H$5α-DHT binding to ABP. Caput cytosol from hamster epididymides was incubated with 0.5-8.6 nM $[^3]H$5α-DHT alone or with a 100-fold excess of unlabelled 5α-DHT for 1 h. Specific binding was determined using the 6-sec charcoal assay. The values plotted are the means of assay triplicates.

$r = 0.995$
$y = -1.563 \times 10^8 + 0.137$
$K_d = 6.4 \times 10^{-9}$

Fig. 6. Specificity of steroid binding to ABPs. Cytosol was prepared from the caput of hamster or rabbit epididymides, or from the entire rat epididymis. Cytosols were then incubated with 4 nM $[^3]H$5α-DHT alone (control) or with 400 nM unlabelled 5α-DHT (DHT), testosterone (T), oestradiol (E2) or cyproterone acetate (CA). Specific binding was determined by the 6-sec charcoal assay. The values plotted for the hamster are the means ± s.e.m. of 3 separate experiments. The values plotted for the rabbit and rat are the means of assay triplicates.
rat and rabbit ABP, although oestradiol was a more effective competitor for androgen binding sites on rat than on rabbit ABP. Cyproterone acetate was the least effective competitor in all three species.

**General characteristics.** The concentration of ABP in frozen hamster epididymal tissue did not differ from that from fresh tissue (data not shown). Indeed, tissue could be frozen, thawed on ice, fat and connective tissue removed, and the epididymis refrozen for later thawing and homogenizing without influencing the concentration of ABP. However, once cytosol was prepared, it could not be frozen and stored even for 1 day without losing activity (data not shown). These data further substantiate the relative instability of the hamster ABP molecule. Charcoal extraction of testicular or epididymal cytosols to remove possible endogenous steroids failed to lead to any measurable change in ABP levels (data not shown).

**Effect of short photoperiod on the ABP concentration of the hamster epididymis and on the fertilizing ability of cauda spermatozoa**

Data showing that less ABP was measured in caput cytosol (Fig. 7) from 20–22-week-old hamsters exposed to a short photoperiod (8L:16D) than in caput cytosol from animals maintained on a long photoperiod (16L:8D) prompted us to examine the time course of ABP decline. Within 3 weeks of being transferred from a long to a short photoperiod, ABP concentration in the caput epididymidis (Table 2) had decreased. Subsequently, the decline in caput epididymal ABP values was linear, by 15 weeks after transfer to the short photoperiod only 0.4 pmol ABP/g caput tissue was detected. The decline in testicular ABP was similar to that observed in the epididymis until Week 12. At Week 15, testicular ABP was increasing (Table 2). When the decline in epididymal ABP concentration with time between Weeks 0 and 15 was evaluated by linear regression analysis,
Table 2. The effect of photoperiod on ABP levels in the hamster caput epididymidis and testis and on circulating testosterone concentrations

<table>
<thead>
<tr>
<th>Duration of study (weeks)</th>
<th>pmol ABP/g tissue</th>
<th>Testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caput</td>
<td>Testis</td>
</tr>
<tr>
<td>Regression phase (8L:16D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10.3</td>
<td>3.0</td>
</tr>
<tr>
<td>3</td>
<td>4.3</td>
<td>1.3</td>
</tr>
<tr>
<td>6</td>
<td>3.8</td>
<td>1.1</td>
</tr>
<tr>
<td>9</td>
<td>2.3</td>
<td>–†</td>
</tr>
<tr>
<td>12</td>
<td>1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>15</td>
<td>0.4</td>
<td>1.6</td>
</tr>
<tr>
<td>Recovery phase (16L:8D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2.5 (5.1)*</td>
<td>–†</td>
</tr>
<tr>
<td>5</td>
<td>1.6 (3.0)</td>
<td>–†</td>
</tr>
<tr>
<td>7</td>
<td>2.9 (4.4)</td>
<td>–†</td>
</tr>
<tr>
<td>8</td>
<td>2.0 (3.6)</td>
<td>–†</td>
</tr>
</tbody>
</table>

*The values in parentheses indicate age matched controls kept throughout the experiment in 16L:8D. ABP was measured as indicated in Table 1.
†Not measured.

Table 3. The fertilizing ability of spermatozoa from hamsters exposed to a short photoperiod

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>No. of eggs fertilized/total no. of eggs recovered</th>
<th>16L:8D</th>
<th>8L:16D</th>
</tr>
</thead>
<tbody>
<tr>
<td>0*</td>
<td>27/32 (84%)</td>
<td>46/56 (82%)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>21/26 (81%)</td>
<td>27/48 (56%)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>25/28 (89%)</td>
<td>2/44 (4%)</td>
<td></td>
</tr>
</tbody>
</table>

*Two males were used for each time point.

ABP concentration in the caput epididymidis and fertilizing ability of cauda spermatozoa after transfer of hamsters from short to long days.

Animals which had been in a 8L:16D for 15 weeks were transferred to a stimulatory photoperiod (16L:8D). After 3 weeks of reversal, the ABP concentration of the caput epididymidis was significantly greater than after 15 weeks in 8L:16D. However, ABP values did not return to normal during the recovery period studied (Table 2). Circulating testosterone returned to normal values by a t₁ of 5.3 weeks (r = 0.949) for the disappearance of ABP was calculated. Testicular ABP declined with a t₁ of 7.7 weeks (r = 0.954) for the 0–12-week interval. Circulating testosterone concentrations declined over the 15-week period and the decrease paralleled the ABP decline (Table 2).

The sperm fertilizing capacity of hamsters transferred to a short photoperiod also declined with time (Table 3). The percentage of eggs penetrated in vitro declined from 56% after 3 weeks to 4% after 6 weeks exposure to the short photoperiod. From 9 to 15 weeks, insufficient motile spermatozoa could be recovered from the cauda epididymidis to allow fertility to be evaluated.

ABP concentration in the caput epididymidis and fertilizing ability of cauda spermatozoa after transfer of hamsters from short to long days.
5 weeks (Table 2). By 3 weeks, the caudal sperm reserves had not been replenished and sperm fertilizing ability could not be tested. Although spermatozoa could be recovered from the epididymis at 5 and 6 weeks, they were not able to fertilize. Spermatozoa capable of penetrating eggs (at a reduced rate) were recovered from the epididymis at 7 and 9 weeks after return to a stimulatory photoperiod (Table 4).

Table 4. Recovery of sperm fertilizing ability of hamsters transferred from a short to a long photoperiod

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>No. of eggs fertilized/total no. of eggs recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16L:8D</td>
</tr>
<tr>
<td>3</td>
<td>27/30 (90%)</td>
</tr>
<tr>
<td>5</td>
<td>48/50 (96%)</td>
</tr>
<tr>
<td>6</td>
<td>21/25 (84%)</td>
</tr>
<tr>
<td>7</td>
<td>13/15 (87%)</td>
</tr>
<tr>
<td>9</td>
<td>23/25 (92%)</td>
</tr>
<tr>
<td></td>
<td>8L:16D</td>
</tr>
<tr>
<td></td>
<td>0/38 (0%)</td>
</tr>
<tr>
<td></td>
<td>0/25 (0%)</td>
</tr>
<tr>
<td></td>
<td>6/21 (29%)</td>
</tr>
<tr>
<td></td>
<td>19/33 (58%)</td>
</tr>
</tbody>
</table>

Control animals were maintained in a long (16L:8D) photoperiod while treated animals were kept for 15 weeks in a short photoperiod (8L:16D) to ensure testicular regression before being transferred to the long photoperiod for the length of time indicated. Spermatozoa from 2 animals were used for each time point. Insufficient spermatozoa were obtained from treated animals at 3 weeks to permit fertility to be assessed.

Discussion

Androgen-binding protein (ABP) is a Sertoli cell product that has been identified in several species (Steinberger et al., 1975; Schmidt et al., 1981; Danzo et al., 1973, 1982; Hansson et al., 1975; Jegou et al., 1978). Although its physiological role has yet to be discovered, various investigators have proposed that ABP, and/or its bound androgens, may be involved in the regulation of spermatogenesis, of sperm maturation and/or of epididymal function. Since ABP is readily measured by various techniques (Danzo et al., 1973; Ritzén et al., 1974; Gunsalus et al., 1978), it has been used to monitor Sertoli cell function.

This report is the first demonstration of the presence of ABP in the hamster testis and epididymis. In the testis, ABP concentrations increase 39-fold between 2 and 6 weeks of age at the time when the testis becomes responsive to the inhibitory effects of short days (Ellis & Turek, 1979). There is a regional distribution of ABP along the epididymal duct similar to that reported for the hamster (Danzo et al., 1973), rat (Hansson et al., 1975), or guinea-pig (Danzo et al., 1982). The change in regional distribution of ABP with age observed in the hamster epididymis is reminiscent of previous observations (Danzo et al., 1975) for immature rabbits in which ABP was detected along the length of the duct (Danzo et al., 1975) at a time when the epithelium was in an undifferentiated low columnar state (Danzo et al., 1975). When the epithelium had assumed its fully differentiated adult appearance, ABP was detectable only in the caput. A small quantity of ABP could, however, be measured in fluids collected from the cauda (Danzo et al., 1977). The reason for this
age-dependent difference in the regional distribution of ABP in the epididymis is not clear; however, we postulate that ABP and/or the androgens which it transports may be important for the differentiation of the epithelium of the entire epididymis in young animals. The presence of ABP primarily in the more proximal regions of the epididymal duct in adult animals may be due to a restriction of its utilization to that region as the animals mature. This would be consistent with the fact that immunochemical localization of ABP was confined to the caput epithelium in the rat (Feldman et al., 1981; Pelliniemi et al., 1981). However, the possibility that ABP is destroyed in more distal regions of the duct cannot be ruled out.

Hamster ABP is similar in many of its characteristics to ABP from other species. The sedimentation coefficient (4 S) and the apparent equilibrium dissociation constant (6-4 nm) of hamster ABP are similar to the values reported for other species (Danzo et al., 1973, 1982; Hansson et al., 1975; Schmidt et al., 1981).

The androgen specificity of hamster ABP was similar to that of other species, but its affinity for oestradiol was intermediate between the low affinity exhibited by the rabbit and the high affinity shown by the rat and guinea-pig (Danzo et al., 1973, 1982). These differences in steroid affinity of the various ABPs may indicate differences in their binding sites. Cyproterone acetate is a poor competitor for binding sites on hamster ABP as it is for binding sites on other ABPs and TeBG (Danzo & Eller, 1975a; b; Danzo et al., 1975, 1982), but it is an excellent competitor for the androgen receptor (Stern & Eisenfeld, 1969; Danzo & Eller, 1975b). These data emphasize that the steroid binding site on the transport proteins ABP and TeBG are similar to each other, but distinct from the binding site on the androgen receptor.

The association of [3H]5a-DHT with hamster ABP is very rapid in contrast to guinea-pig ABP (Danzo et al., 1982). Dissociation of [3H]5a-DHT from hamster ABP (t1/2 = 2-77 min), as measured after addition of unlabelled 5a-DHT to samples at binding equilibrium, is more rapid than dissociation from rabbit or rat ABP, 4-6 min (Danzo et al., 1975; Schmidt et al., 1981). The t1/2 measured in the presence of charcoal alone was slower than when both unlabelled hormone and charcoal were present (t1/2 = 5-2 min), a phenomenon similar to that observed with TeBG (Danzo & Eller, 1975a). The physiological significance of these similarities and differences in association and dissociation of [3H]5a-DHT from ABP among the various species is unclear.

In the hamster, testicular function is controlled by the relative day-length with regression occurring as the days shorten and reactivation during increasing daylength (Hoffman & Reiter, 1965; Gaston & Menaker, 1967). The endocrine mechanisms which underly and control these changes in testicular function have been well studied (Berndtson & Desjardins, 1974), but few data exist on the changes in the accessory sex glands. Short photoperiods in male Syrian hamsters result in low circulating androgen concentrations which cause regression in the weight of accessory sex glands, similar to that observed after castration (Reiter & Hester, 1966), and lower testosterone metabolism by the prostate (Lucini et al., 1983). However, the pattern of prostatic regression in the hamster exposed to short photoperiod is quite different from that reported after castration, suggesting that the regression observed is not entirely due to the lower concentrations of circulating androgens (Buzzell et al., 1984, 1985). We are not aware of studies on the effect of the photoperiod on the hamster epididymis or ABP levels. In the ram, 5α-DHT and ABP concentrations in rete testis fluid were significantly lower in the non-breeding season, although there was no significant decrease in circulating and rete testis testosterone concentrations (Jegou et al., 1978).

In the present study, exposure of hamsters to a short photoperiod resulted in a linear decline in the ABP concentration of the caput epididymidis. The half time of disappearance was about 5 weeks. This disappearance probably reflects decreased Sertoli cell production of ABP rather than an alteration in its degradation rate since ABP is essentially cleared from the epididymis by 3 days after ligation of the ductuli efferentes (Danzo et al., 1974), and its clearance from the blood is biphasic with t1/2 values of 4 and 14 h (Danzo & Eller, 1984).

Our observation that the concentration of ABP in the testis was returning to normal after 15 weeks in a short photoperiod is consistent with previous observations that spontaneous testicular
reactivation in the hamster begins at 14–16 weeks, even when animals are exposed to a constant
short photoperiod (Reiter, 1975). The fact that the concentration of ABP in the epididymis was
lower at 15 weeks in the face of rising testicular ABP may be due to decreased fluid flow from the
testis to the epididymis. Jegou et al. (1978) have reported a decrease in the flow rate of rete testis
fluid during the breeding and non-breeding season in the ram.

We have previously used two animal models to assess the influence of Sertoli cell function,
determined by measuring ABP levels, on sperm fertilizing ability (Anthony et al., 1984a, b). One
model, the restricted (H+) rat, displays a declining ABP secretion and fertility after puberty. In the
other model, the hypophysectomized rat treated with pregnenolone, circulating testosterone con-
centrations are low, but rete testis androgen and epididymal ABP values increase in response to
pregnenolone in a dose-dependent manner up to normal values. With both models, we dem-
strated a correlation between sperm fertilizing ability and ABP levels, suggesting that the Sertoli
cells may influence sperm fertilizing ability.

In adult male hamsters exposed to short days, changes in hypothalamic–pituitary sensitivity
occur within 10–20 days (Ellis & Turek, 1979) and testicular regression occurs within 5–7 weeks
(Turek et al., 1975). In this study, we found that epididymal ABP levels decreased by half during
the first 3–5 weeks and epididymal sperm fertilizing ability decreased by 90%. Taking into account
that the cycle of the seminiferous epithelium in the hamster is 8–7 days (Clermont & Trott, 1969)
and that epididymal transit time is 15–6 days (Amann et al., 1976), by 3 weeks after exposure to
a short photoperiod the spermatozoa that fertilized 56% of the eggs had arisen from elongated spermatids at a time when ABP concentrations in the testis and epididymis varied from 100% to
40% of normal values. At 6 weeks after exposure to the short photoperiod, the spermatozoa that
fertilized only 4% of the eggs had arisen from spermatocytes which developed from the prelepto-
tene stage to testicular spermatozoa at a time when ABP values were 100 to 40% of normal and
from testicular to cauda spermatozoa at a time when ABP concentrations were only 40% of
normal. The main difference between the two sperm populations appears to be their exposure to a
lower concentration of ABP and other intraluminal factors during epididymal transit. However, we
cannot rule out the possibility that the declining ABP levels during early spermatogenesis may have
had an effect.

After return to long days, recovery of epididymal ABP levels was rapid. However, ABP concen-
trations were only 50% of normal throughout the recovery period. Therefore, the difference in
sperm fertilizing ability 5 and 7–8 weeks after exposure to long days does not appear to be related
to differences in epididymal ABP or other intraluminal factors, but may reflect photoperiod-related
changes during early spermatogenesis. Our data also indicate that the re-establishment of normal
circulating concentrations of testosterone is not a sufficient cause for the re-establishment of
normal fertilizing ability. Furthermore, the data indicate that a protracted period of time is
required after a photoperiod-induced regression for the normalization of Sertoli cell function.

We thank John Strode and Tina Krivaka for excellent technical assistance. These studies were
supported by NIH grants HD13813 (BJD) and HD03820 (MCOC) and a grant from the Andrew
W. Mellon Foundation. The Center for Reproductive Biology Research is supported by NIH grant
HD05797.

References

dymal spermatozoal reserves and transit time of spermatozoa through the epididymis of the rhesus
sperm fertilizing ability and androgen-binding protein in the restricted rat. Endocrinology 114,
1413–1418.


Danzo, B.J., Cooper, T.G. & Orgebin-Crist, M.-C. (1977) Androgen binding protein (ABP) in fluids collected from the rete testis and cauda epididymidis of sexually mature and immature rabbits and observations on morphological changes in the epididymis following ligation of the ductuli efferentes. Biol. Reprod. 17, 64–77.


Received 5 January 1987