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Identification of Androgen Binding-Protein (ABP) From Testis and Epididymis of the Seasonal Rodent, *Octodon degus* (Molina, 1872)

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ABP, a Sertoli cell secretory product, was identified in the seasonal rodent *Octodon degus* (Molina, 1872). It was shown to be present in cytosols from the testis and epididymis. It migrated with an Rf of 0.37 on nondenaturing polyacrylamide gels. Ligation of the vas efferens caused the disappearance of ABP from the epididymis and its accumulation in the testis, indicating its testicular origin. Binding to [^{3}H]5α-DHT was specific and completely reversible, with an apparent Kd of 3.5 $\pm$ 0.4 $\times 10^{-9}$ M. Half-times of association and dissociation were at 15 and 120 minutes, respectively. Binding equilibrium was achieved at 120 minutes. Steroid affinity relative to the best competitor, 5α-DHT, was 0.27 for testosterone, 0.06 for 17β-estradiol, and 0.01 for cyproterone acetate. The presence and similar characteristics of ABP in a wide variety of mammals, including those with special reproductive strategies such as seasonal breeding, suggests that this protein may play a general role in the mechanisms regulating spermatogenesis, probably affecting the transport and concentration of androgens in the testis and epididymis.

Key words: Androgen binding protein, testis, epididymis, seasonal breeder.

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Spermatogenesis is a complex process in which anatomic and physiologic relationships between somatic and germ cells occur, making possible the production of differentiated sperm cells with the potential for fertilization (Russell, 1980; Tindall et al, 1985).

The study of somatic-germ cell interactions have focused chiefly on the characterization of secretory products originating in the Sertoli cell that are believed to play a role in spermatogenesis. Among these products are a number of proteins secreted exclusively by the Sertoli cell, of which androgen binding protein (ABP) is the best characterized. It is present in a number of species including the rat (Ritzen et al, 1971; French and Ritzen, 1973a, 1973b), rabbit (Danzo et al, 1973), human (Hsu and Troen, 1978), sheep (Carreau et al, 1979), guinea pig (Danzo et al, 1982), Cynomolgus monkey (Keeping et al, 1985), and hamster (Holland et al, 1987).

In all these species, ABP shares common characteristics. This protein is produced by Sertoli cells

and is transported to the epididymis where it is degraded (French and Ritzen, 1973a, 1973b). ABP has a higher affinity for androgens than estrogens, its dissociation constant for 5α-dihydrotestosterone (5α-DHT) is in the range of 1.2 to 7.8 × 10^{-9} M among the different species (Ritzen et al, 1971; French and Ritzen, 1973a; Danzo et al, 1973; 1982; Hsu and Troen, 1978; Carreau et al, 1979; Keeping et al, 1985; Holland et al, 1987). At 0°C, the equilibrium time for binding of 5α-DHT has been measured to be between 10 minutes in the golden hamster (Holland et al, 1987) and 160 minutes in the guinea pig (Danzo et al, 1982), while the half-time of dissociation has been determined to be from 2.8 minutes in the golden hamster (Holland et al, 1987) to approximately 300 minutes in the guinea pig (Danzo et al, 1982).

These data, particularly those on the kinetic properties of ABP and its presence in a wide variety of species, support the concept that this protein plays a role in spermatogenesis, probably as a carrier for androgens, acting at the level of the testis and/or the epididymis (French and Ritzen, 1973b; Hansson et al, 1974). Its absence in some species such as the boar, in which unusually high levels of testosterone (T) are found in serum, supports an indirect more than a direct involvement of ABP in spermatogenesis, for example, through its action as a carrier enhancing the supply of androgens to the target tissues (Hansson et al, 1975).

More definitive evidence on the role of ABP requires adequate *in vivo* models to study its properties during physiologically significant changes in spermatogenesis. In this regard it is important to extend the study of ABP to other species where particular hormonal conditions occur, such as seasonal mammals. In these species, cyclic variations in gonadotropin and sex steroid hormones take place associated with changes in gonadal activity in response to environmental conditions such as photoperiod (Lincoln, 1981). Studies on ABP in seasonal mammals are scarce and detailed characterization has been made only for sheep and golden hamster ABP (Carreau et al, 1979; Holland et al, 1987).

O. degus is a wild short-day breeder inhabiting the central valley of Chile (28 to 35° S). The mating period under natural conditions occurs in winter and mid-spring, pups being born in the spring and early summer after a 3-month gestation (Weir, 1970; 1974). In this communication, the presence and binding properties of ABP in *O. degus* are reported.

Materials and Methods

Animals

The animals were sexually active males from our own stock that has been maintained for at least 30 years under an outbred reproduction schedule. They were about 12 to 18 months old and weighed 200 to 300 g. They were given food and water *ad libitum* under a stimulatory light schedule (10 hr light: 14 hr darkness).

Testicular and Epididymal Cytosols

Testicular and epididymal tissues were homogenized in 2 and 1 ml of Tris, EDTA, Mercaptoetuanol, Glicerol (TEMG) buffer per organ, respectively (10 mM Tris, 1.5 mM Ethylenediamine tetraacetic acid (EDTA), 2 mM 2-mercaptoethanol, 10% glycerol, pH 7.4), at 0°C and centrifuged at 4°C at 2,500 × g (10 min), 10,000 × g (10 min), and finally at 105,000 × g (90 min). Supernatants were obtained (cytosol preparation) and stored at −20°C until assay. Testicular cytosols for studying the binding properties of ABP were obtained 3 days after ligation of the vas efferens, which facilitated the procurement of increased amounts of ABP from the testis.

Charcoal-Dextran Assay

A dextran-coated charcoal assay was used for determining the binding properties of ABP (Danzo et al, 1982). Fixed volumes of testicular cytosol were incubated with variable concentrations or 3 nM of [^{3}H]5α-DHT for 3 hr at 0°C in a final volume of 0.5 ml. The free and bound steroid were separated by adding to each tube 0.5 ml of chilled 0.5% charcoal, 0.05% dextran in TEMG buffer, vortexing for 6 seconds and centrifugation at 1500 × g at 4°C (10 min). The radioactivity remaining in the supernatant (bound [^{3}H]5α-DHT) was determined in a Beckman L-50 liquid scintillation counter using a mixture of 67% toluol, 33% Triton X-100, 0.4% PPO, and 0.005% POPOP.

SDS-Page Assay

Steady-state polyacrylamide gel electrophoresis (SS-PAGE) was used for detection of ABP (Ritzen, 1974). Cylindrical 6% polyacrylamide gels were made in 14 × 0.5-cm glass tubes with 2 cm of a 3% stacking gel in TEMG buffer. Before polymerization, [^{3}H]5α-DHT was added at a concentration of 2 or 3 nM. Variable amounts of cytosol were electrophoresed in 0.025 M Tris-0.192 M glycine buffer, pH 8.6, for 3 hours at 5 mA per tube (4°C). After electrophoresis, the gels were frozen and cut into 2.4-mm slices. Each slice was added to 3 ml of scintillation liquid and the radioactivity was counted.

Reagents

All reagents for charcoal-dextran and SS-PAGE assays were purchased from Sigma (St. Louis, MO). (1,2[^{3}H])DHT (5αandrostan-17β-ol-3-one) with a specific activity of 51.6 Ci/mmol was from New England Nuclear (Boston, MA), radioinert 5α-DHT, testosterone and 17β-

estradiol (E_2) were from Sigma and cyproterone acetate (Androcur®) was obtained from Schering Laboratories (Berlin).

Results

Identification of ABP in Epididymis and Testis of O. degus

Two androgen binding activities (peak I and peak II) with different electrophoretic mobilities were demonstrated by SS-PAGE in epididymal and testicular cytosols. The Rfs of the peaks were 0.37 and 0.74, respectively (Figs. 1A, 1B). The electrophoretic pattern in serum revealed high levels of only peak II, suggesting that this activity may correspond to albumin (data not shown). The specificity of the binding of [^{3}H]5α-DHT to *O. degus* ABP was evaluated by SS-PAGE of testicular cytosol in the presence of a 100-fold excess of radioinert 5α-DHT (Fig. 1C). Such treatment abolished completely the binding to ABP but not to albumin. This is in agreement with the specific nature of the binding of 5α-DHT to ABP seen in other species, and the low specificity and high capacity that characterize steroid binding to albumin.

Effects of Ligation of the Vas Efferens on Epididymal and Testicular ABP Levels

With the objective of determining the organ of origin of the component that specifically binds [^{3}H]5α-DHT, the vas efferens was ligated in three groups of animals and the levels of ABP in epididymal and testicular cytosols were studied 1, 2, and 4 days after surgery.

Figure 2 shows ABP levels in cytosols from ligated and contralateral, nonligated organs 1 day post-surgery. A high peak of ABP was seen in the nonligated epididymis (Fig. 2B), while in the ligated organ this activity had virtually disappeared (Fig. 2A). On the other hand, ABP levels in the testis from the ligated side were very increased (Fig. 2C), while the nonligated side had low ABP levels corresponding to the normal testis (Fig. 2D). Similar results were obtained at 2 and 4 days after surgery (data not shown), indicating that the increased activities resulted from the ligation procedure. Peak II corresponding to albumin was used as an internal control in this experiment and was not altered by ligation, suggesting a specific effect of this procedure on ABP levels and the absence of major changes in the blood supply of the testis (Fig. 2).

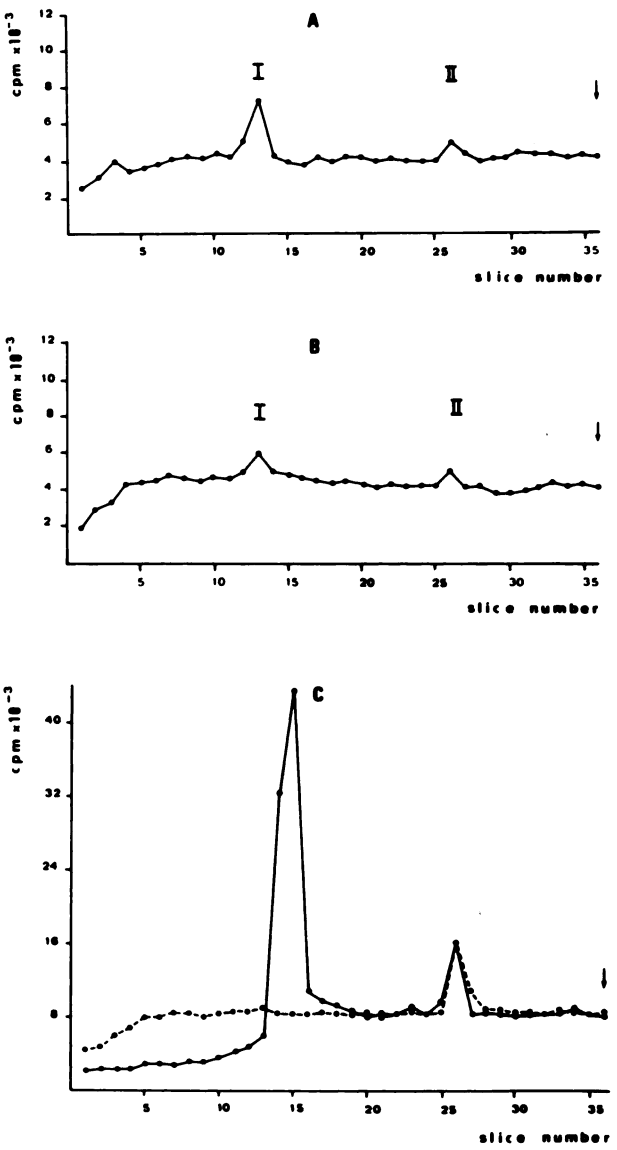

Fig. 1. Identification of ABP in epididymal and testicular cytosols. (A) Epididymal cytosol. (B) Testicular cytosol. 0.05 ml of epididymal or testicular cytosol was brought to 0.2 ml with TEMG buffer. SS-PAGE was performed in gels containing 2 nM [^{3}H]5α-DHT. Peaks I and II correspond to ABP and albumin, respectively. (C) Binding specificity. 0.2 ml of testicular cytosol obtained 3 days after efferent duct ligation was brought to 0.4 ml with TEMG buffer. SS-PAGE was performed as described above. The slower and faster migrating binding activities correspond to ABP and albumin, respectively. ■—■ 2 nM [^{3}H]5α-DHT; ■---■ 2 nM [^{3}H]5α-DHT plus a 100-fold excess of radioinert 5α-DHT. Arrows show the position of the tracking dye (bromophenol blue).

Analysis of the Binding of [^{3}H]5α-DHT to O. degus *ABP*

Equilibrium Binding Analysis. A typical saturation

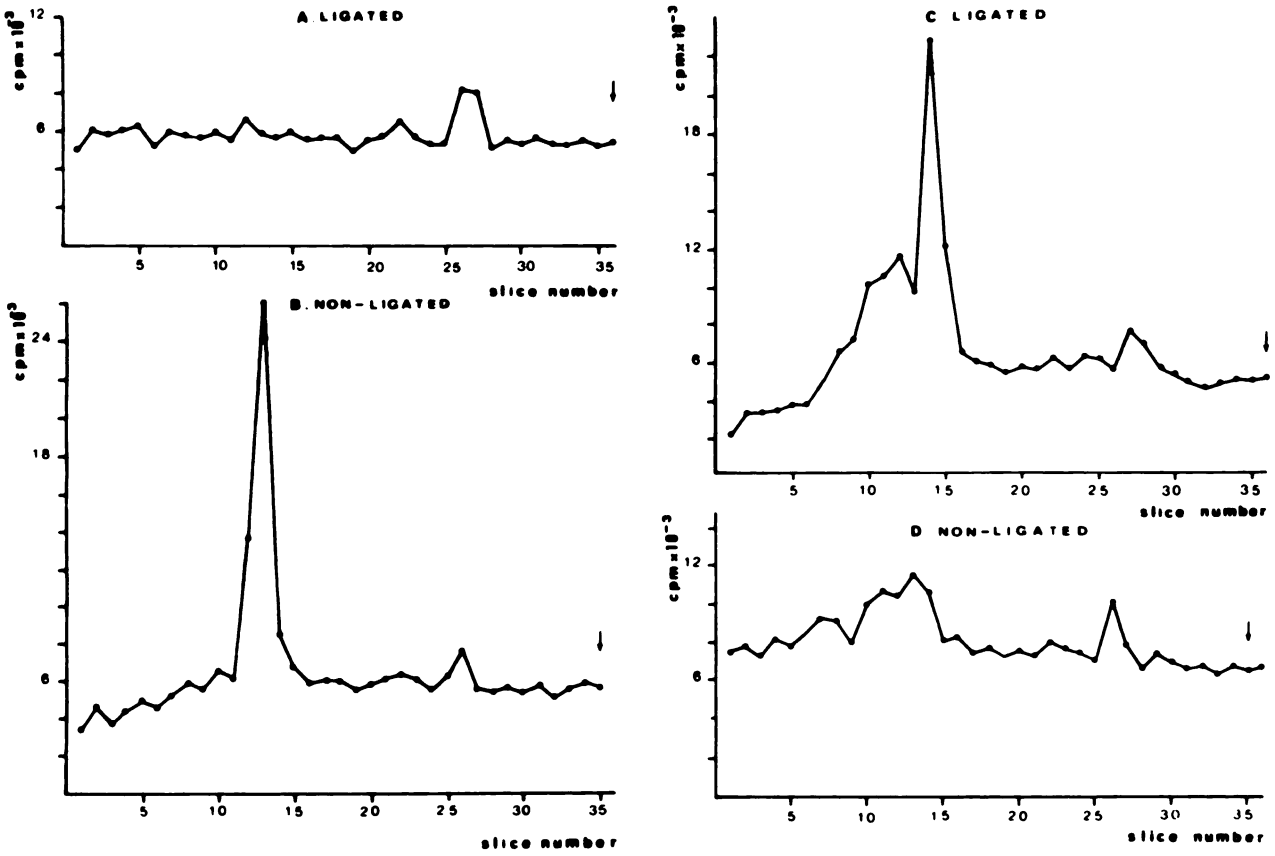

Fig. 2. ABP levels in the epididymis and testis after vas efferens ligation. (A, B) Epididymal cytosol. (C, D) Testicular cytosol. 0.2 ml of epididymal or testicular cytosol from both the ligated and nonligated side of each animal was brought to 0.4 ml with TEMG buffer and subjected to SS-PAGE in gels containing 2 nM [^{3}H]5α-DHT. The figure shows the results obtained 1 day after surgery. Arrows show the position of the tracking dye (bromophenol blue).

curve was obtained when a fixed volume of testicular cytosol was incubated with increasing concentrations of [^{3}H]5α-DHT (2.4 to 48.0 × 10^{-9} M), indicating the specific nature and limited capacity of this binding activity (Fig. 3). A linear plot was obtained by Scatchard analysis (Scatchard, 1949) that suggested the presence of a single class of sites with an apparent Kd of 3.5 ± 0.4 × 10^{-9} M (Fig. 3 inset).

Association Rate. To define the time required for achieving equilibrium, fixed quantities of testicular cytosol and [^{3}H]5α-DHT were incubated at different time intervals and the specific binding was calculated. Figure 4A shows the time-course of the binding, the equilibrium being reached at approximately 120 minutes; the half-time of association was 15 minutes.

Dissociation Rate. For determining the rate of dissociation of [^{3}H]5α-DHT from ABP, displacement of this steroid by an excess of radioinert 5α-DHT was measured after reaching equilibrium. The dissociation curve in Figure 4B indicated that this binding is completely reversible, exhibiting a half-time of dissociation of 120 minutes.

Steroid Affinity. The ability of different steroids to inhibit the binding of [^{3}H]5α-DHT to testicular cytosol was studied. Figure 5 shows that 5α-DHT was the most potent inhibitor followed by T and then E_2, while cyproterone acetate caused very little inhibition. The relative affinities calculated from the mass of cold steroid required for a 50% displacement were: 5α-DHT, 1.00; T, 0.27; E_2, 0.06; and cyproterone acetate, 0.01.

Discussion

In this report the presence and some characteristics of ABP in *O. degus*, an unusual seasonal rodent, were established. ABP in both testis and epididymis had similar electrophoretic mobilities. The relative mobility for *O. degus* ABP was lower than for rat ABP measured under similar conditions. Such

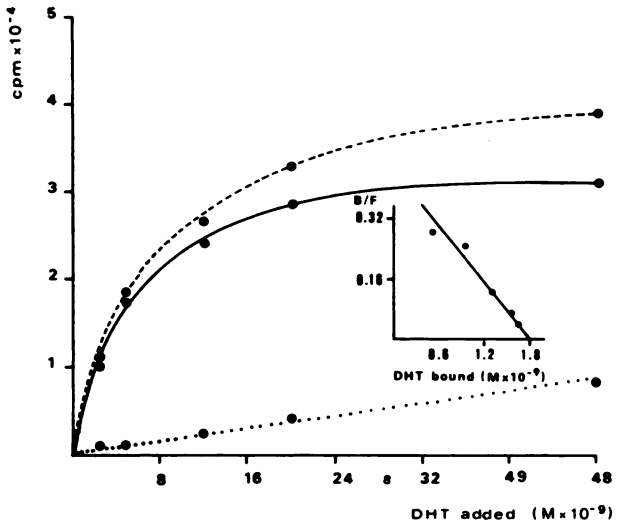

Fig. 3. Equilibrium binding analysis. 0.025 ml of cytosol was incubated in triplicate with 2.4 to 48.0 nM [³H]5α-DHT. The free and bound steroid were separated using charcoal-dextran. Specific binding was calculated by subtracting from the total binding the nonspecific binding determined in a parallel set of tubes containing a 100-fold excess of radioinert 5α-DHT. The Scatchard analysis (inset) was based on data obtained from three experiments. ■---■ total binding; ■—■ specific binding; ■···■ nonspecific binding.

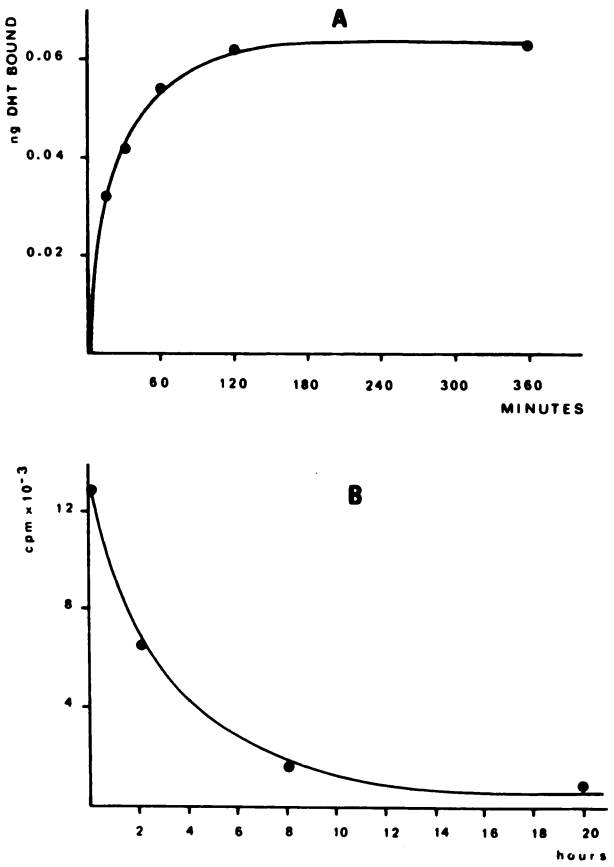

Fig. 4. Association and dissociation rates. (A) Association rate. 0.05 ml of testicular cytosol was incubated in triplicate with 3 nM [³H]5α-DHT. Specific binding at various time intervals was determined by the charcoal-dextran method. (B) Dissociation rate. 0.05 ml of testicular cytosol was incubated in triplicate under conditions similar to those described above. At 3 hours of incubation, a 100-fold excess of radioinert 5α-DHT was added to each tube and [³H]5α-DHT specifically bound at each time point was determined by the charcoal-dextran method.

differences may indicate physicochemical variations between the ABP from these two species.

The testicular origin of *O. degus* ABP was demonstrated by ligation of the efferent ducts. Under this condition, ABP accumulated in the testis and disappeared from the epididymis as rapidly as the 1st day after surgery. This result was more impressive in *O. degus* than that reported in the rat, where after 3 days of efferent duct ligation some ABP still remains, especially in the cauda epididymidis (French and Ritzen, 1973b). The more rapid disappearance of ABP in *O. degus* may indicate a higher degradative activity at the level of the epididymis and/or an enhanced transport to the distal segments of the seminal ducts. This subject is presently under investigation in our laboratory.

The binding properties of *O. degus* ABP to 5α-DHT indicated that this activity was specific and saturable, with an apparent Kd of $3.5 \pm 0.4 \times 10^{-9}$ M, which is in the range of the data available for other species (Ritzen et al, 1971; French and Ritzen, 1973a, 1973b; Danzo et al, 1973, 1982; Hsu and Troen, 1978; Carreau et al, 1979; Schmidt et al, 1981; Keeping et al, 1985; Holland et al, 1987).

The half-time of dissociation of [³H]5α-DHT from *O. degus* ABP was 120 minutes with equilibrium being achieved at 120 minutes at 0°C, indicating that this binding is a relatively fast process, compatible with the putative role of androgen carrier proposed for this protein. In this regard, there are some differences from the ABP of other species, such as the golden hamster, rat, and guinea pig. Golden hamster ABP has the most rapid dissociation half-time (2.8 minutes) and the most rapid equilibrium time (10 minutes at 0°C; Holland et al, 1987). Rat ABP has a dissociation half-time of 6 minutes at

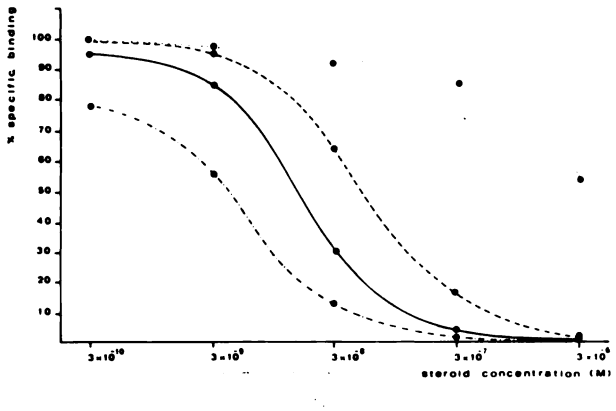

Fig. 5. Steroid affinity. 0.025 ml of testicular cytosol was incubated in triplicate with 3 nM of [^{3}H]5α-DHT in tubes containing variable concentrations of different radioinert steroids. After 3 hours of incubation, the specific binding of [^{3}H]5α-DHT was determined by the charcoal-dextran method. ■-·-·-■ 5α-DHT; ■—■ testosterone; ■---■ 17β-estradiol; ■····■ cyproterone acetate.

0°C and an equilibrium time of less than 30 minutes at 4°C (Sanborn, et al, 1975; Hansson et al, 1976; Schmidt et al, 1981). Guinea pig ABP, on the other hand, has a dissociation half-time of 300 minutes, equilibrium being reached after 160 minutes of incubation at 0°C (Danzo et al, 1982). These variations may be based on a different accessibility of androgens to the binding sites on ABP, which would suggest differences in the conformation of these proteins.

The relative affinity of *O. degus* ABP was higher for 5α-DHT than for T and E_2 and negligible for cyproterone acetate. These characteristics, together with the rapid association and dissociation rates for 5α-DHT, establish a clear difference from the cytosolic receptor for androgens present principally in prostate, testis, and epididymis, which has a high affinity for cyproterone acetate and an estimated dissociation half-time of 35 hours (Hansson et al, 1976).

O. degus is a short-day seasonal breeder. Seasonality in mammals has been viewed as an advantageous experimental model for the study of spermatogenesis. Animals presenting gonadal activity adjusted to cyclic environmental signals necessarily have had to develop mechanisms directed to turn spermatogenesis on and off under defined environmental conditions. The elucidation of these control mechanisms may contribute greatly to the general understanding of the regulation of spermatogenesis in mammals.

It is a general concern that the biologic responses of individuals from naturally occurring populations are far from uniform. Recent evidence in deer mice shows that animals exposed to defined photoperiods are capable of multiple neuroendocrine adjustments with different consequences for testicular function (Blank and Desjardins, 1986). These findings differ from the more homogeneous responses obtained in seasonal species classically used in research such as the hamster and sheep, which are, to a large extent, adapted to laboratory conditions through artificial selection. We were therefore interested in studying the presence and characteristics of ABP, a protein widely distributed in mammals, in a wild species such as *O. degus*. Individual animals in captivity maintain many of the characteristics found in natural populations, including a heterogeneous response to photoperiod.

Our findings demonstrate clearly the presence of ABP in *O. degus*. Its ABP has some slight and not significant variations from ABP in other species as distant phylogenetically as man and sheep, indicating that ABP has some highly conserved characteristics, such as its high affinity for androgens and its steroid specificity. In addition, the physicochemical similarities found between the ABP from different species support the concept that ABP may be involved in some of the mechanisms regulating spermatogenesis. However, the definitive role of this protein remains to be established.

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