Journal of Steroid Biochemistry, 1974, Vol. 5, pp. 151–154. Pergamon Press. Printed in Great Britain

DEMONSTRATION OF AN ANDROGEN BINDING PROTEIN (ABP) IN RABBIT TESTIS: SECRETION IN EFFERENT DUCT FLUID AND PASSAGE INTO EPIDIDYMIS

E. M. RITZÉN and F. S. FRENCH*

Department of Pediatrics and Reproductive Endocrinology Unit, Karolinska sjukhuset, S-104 01 Stockholm 60, Sweden

(*Received* 15 *June* 1973)

SUMMARY

An androgen binding protein (called ABP) is present in 105,000 g_{av} supernatants of rabbit testis and caput epididymidis but not in cauda. Testicular efferent duct fluid contains ABP in a concentration of $1{\cdot}5 \times 10^{-7}$ M, assuming one binding site per molecule of protein. Ligation of the efferent ducts causes ABP to increase in testis supernatant and disappear from supernatant of caput epididymidis. An androgen binding protein similar to ABP is present in rabbit serum; however, the concentrations of ABP per mg protein are much higher in efferent duct fluid and caput supernatant than in serum. These findings indicate that ABP is secreted in the testicular efferent duct fluid and may serve as a carrier of androgen from testis to epididymis.

INTRODUCTION

It has been demonstrated recently that a high affinity androgen binding protein (ABP†) is secreted in the efferent duct fluid of rat testis and carried into the epididymis [1–5]. The concentration of ABP binding sites in rat efferent duct fluid approximates that of testosterone and it has been suggested that the protein may serve as a carrier of androgen from testis to epididymis. Rabbit testis has also been found to contain a similar androgen binding protein and the studies reported herein demonstrate its presence in rabbit efferent duct fluid and passage into caput epididymidis.

MATERIALS AND METHODS

Adult male rabbits weighing 3500–4500 g were anesthesized with phenobarbital (30–50 mg/kg), and the efferent ducts were ligated 2–3 mm from the testis. Eighteen to 24 h later, the rabbits were killed by cervical concussion, the efferent ducts were incised, and the fluid suctioned into Pasteur pipettes; 0·05 to 0·15 ml fluid could be collected from each testis by this technique. Fluid was collected in test tubes on ice, centrifuged to remove spermatozoa and frozen at −20°C. In some experiments, testis and epididymis on one side were perfused with 30–40 ml ice-cold saline prior to removal. The perfusion was performed via a catheter in the abdominal aorta, after clamping the renal, the mesenteric and the iliac arteries. One testis and epididymis was removed before the perfusion. Testis and epididymis were homogenized in 3 vol. 10 mM Tris–HCl buffer, pH 7·4, containing 1·5 mM EDTA and 1·0 mM 2-mercaptoethanol (TEM buffer). Homogenates were centrifuged at 105,000 g_{av} for 1 hr at 0°C. Glycerol was added to the supernatants to 10% v/v. Steroids contained in the supernatants were adsorbed on charcoal, 1 mg/mg protein for 10 h at 0°C and the charcoal was removed by centrifugation. Polyacrylamide gel electrophoresis (PAGE) was performed on nonlabelled [6] and on prelabelled gels [3]. By the latter method, labelled steroid was dissolved in the acrylamide solution prior to polymerization. Stacking gels were omitted and unlabelled samples were applied directly to the running gels. The labelled steroid was distributed uniformly in the gel and was bound by the protein as it moved through the gel. Sample size and steroid concentration within the gel were adjusted to

* Present address: Department of Pediatrics and Laboratories for Reproductive Biology, University of North Carolina, Chapel Hill, N.C. 27514, U.S.A.

† Abbreviations used: ABP = Testicular androgen binding protein; DHT = 17β-hydroxy-5α-androstan-3-one (dihydrotestosterone); PAGE = polyacrylamide gel electrophoresis; TEM = 10 mM Tris buffer containing 1·5 mM EDTA, 2 mM 2-mercaptoethanol, pH 7·4; TEMG = TEM buffer made 10% v/v in glycerol.

enable bound and free steroid to reach equilibrium. Equilibrium between bound and free steroid was obtained when the concentration of free radioactivity remaining behind the peak of bound radioactivity equalled the concentration of free steroid in front of the peak. (Fig. 2a). Under equilibrium conditions it was possible to compare quantitatively the amount of ABP in different samples [3]. A full description of this technique is being published elsewhere [13]. All other materials and methods were as previously described [3, 6]. [1,2-^{3}H]-Dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) and other steroids were obtained from New England Nuclear.

RESULTS

Binding of ^{3}H-DHT to ABP after equilibration *in vitro* with charcoal adsorbed supernatant fractions of rabbit testis, caput and cauda epididymis is shown in Fig. 1. Larger amounts of ABP were present in caput than in testis, and ABP could not be demonstrated in cauda.

Efferent duct fluid contained an abundance of spermatozoa and was completely free of blood contamination. After centrifugation, supernatants were clear and contained about 2·5 mg/ml total protein. Gel electrophoresis of efferent duct fluid revealed the presence of an androgen binding protein with a mobility identical to the ABPs in testis and epididymis (Fig. 2a). The concentration of ABP in efferent duct fluid measured by saturation analysis using prelabelled gels (Fig. 2b) was 60 pmol/mg protein or $1{\cdot}3 \times 10^{-7}$ M.

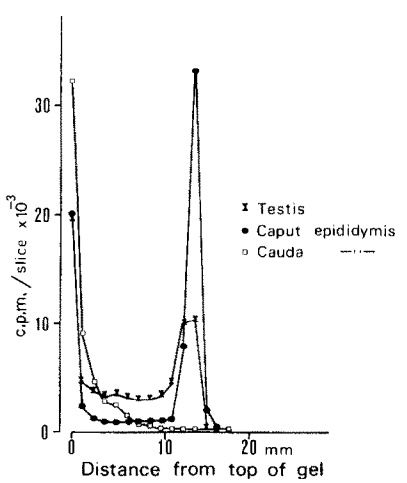

Fig. 1. Binding of ^{3}H-DHT in 105,000 g_{av} supernatants from rabbit testis and epididymis. Supernatants containing 7·0–7·5 mg/ml total protein were charcoal extracted and 0·6 ml aliquots were equilibrated with ^{3}H-DHT (0·15 μCi, 1·0 ng) for 2 h, and layered over 6·5% polyacrylamide gels. The electrophoresis was run at 5 mA/tube for 3 h, in Tris–glycine buffer, 0–2°C, pH 8·6. Gels were sliced and the radioactivity counted in a triton: toluene 1:2 scintillation mixture [6].

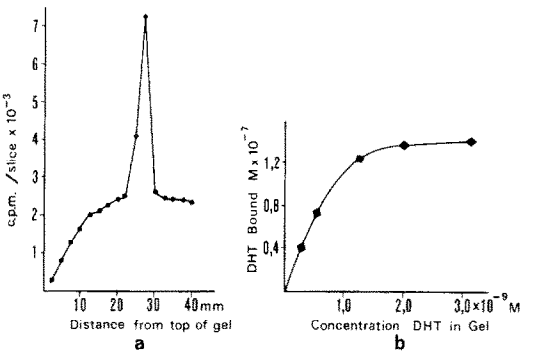

Fig. 2. Binding of ^{3}H-DHT in rabbit efferent duct fluid. The fluid was diluted 1:10 with TEMG buffer and shaken with 1 vol. TEMG buffer containing 0·5% charcoal and 0·5% gelatin (final dilution 1:20). Charcoal was removed by centrifugation and aliquots were applied to 6·5% gels (4 ml) and run at 4 mA/tube for 2·5 h. The gels were then transversely sliced into 2·4 mm slices, and the radioactivity in each slice measured. (a) Gel contains ^{3}H-DHT (0·1 μCi, 0·67 ng) and 100 μl diluted fluid was applied. A single binding component (ABP) is shown by the peak of radioactivity. (b) Saturation curves of binding to ABP. Gels contain ^{3}H-DHT (0·1 μCi, 0·67 ng) and 0, 0·5, 1·0, 2·0, 3·0, 6·0 ng amounts of unlabelled DHT. 100 μl diluted efferent duct fluid was applied to each gel. Total binding in each gel was calculated from radioactivity in the peak after subtraction of "background" radioactivity and divided by the specific radioactivity of DHT in each gel.

Thus, ABP was present in the fluid that normally enters the epididymis. To establish further that ABP is secreted in efferent duct fluid and passes through the epididymis, efferent ducts were ligated unilaterally and the amounts of ABP in supernatants of testis and caput epididymidis were compared on the ligated and nonligated sides using PAGE in prelabelled gels (Fig. 3). Within 16 h after efferent duct ligation, ABP was markedly increased in testis supernatant and almost disappeared from caput supernatant.

Rabbit serum when fractionated by PAGE on gels prelabelled with ^{3}H-DHT formed a peak of radioactivity with mobility identical to ABP (Fig. 4). However, there was a much larger amount of ABP in caput supernatant than in serum diluted to contain an equal concentration of total protein. Binding to albumin in epididymis was negligible in comparison with albumin binding in serum.

The amounts of ABP in testis and caput epididymidis supernatants were only slightly influenced by perfusion of tissues to remove intravascular serum proteins (Fig. 5). ABP was measured as the amount of bound steroid in prelabelled gels containing ^{3}H-DHT and additional unlabelled DHT in amounts sufficient to saturate ABP binding sites. ABP concentrations

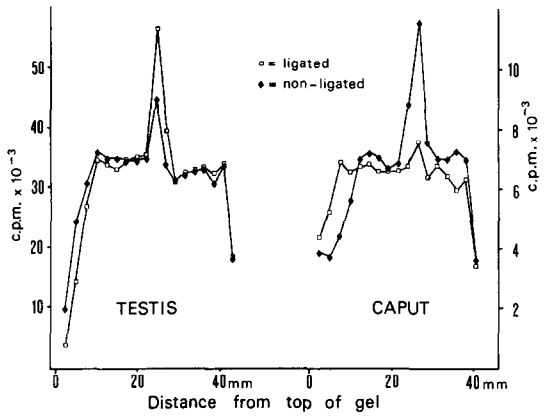

Fig. 3. Effect of unilateral efferent duct ligation on ABP in supernatants of testis and caput epididymidis. Testes and epididymides from ligated and nonligated sides were removed 16 h after ligation. Testes were homogenized in 3 vol. TEMG buffer and endogenous steroids in the supernatants were extracted with dry charcoal 1 mg/mg protein. Caputs were homogenized in 6 vol. buffer and the steroids in the supernatants were extracted with equal vol. TEMG buffer containing 0·5% charcoal and 0·5% gelatin. Testis supernatants (0·2 ml) were run in 4 ml gels containing $1{\cdot}1 \times 10^{-8}$ M ^{3}H-DHT, caput supernatants (0·05 ml) were run in gels containing 202×10^{-9} M ^{3}H-DHT.

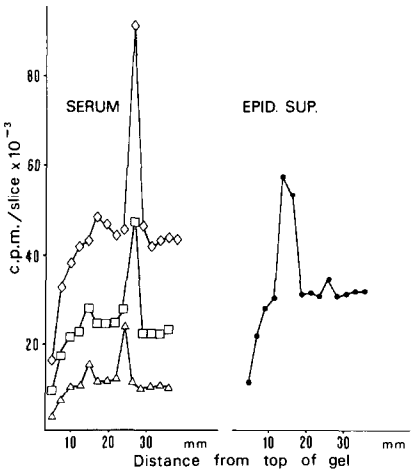

Fig. 4. Binding of ^{3}H-DHT to ABP in caput epididymis supernatant in comparison with the androgen binding proteins in rabbit serum. Samples were extracted overnight in 1·5 vol. 0·5% charcoal in TEMG buffer containing 0·5% gelatin. Charcoal was removed by centrifugation and 0·2 ml aliquots were fractionated by PAGE on 4 ml prelabelled gels containing ^{3}H-DHT and unlabelled DHT. Total steroid concentrations in the gels: Serum $0{\cdot}7 \times 10^{-8}$ M (△—△), $1{\cdot}4 \times 10^{-8}$ M (□—□) and $2{\cdot}8 \times 10^{-8}$ M (◇—◇), epididymis supernatant $2{\cdot}0 \times 10^{-8}$ M (●—●). The serum androgen binding protein corresponding to caput ABP was saturated at those steroid concentrations, while serum albumin showed no saturation. Protein concentration in caput supernatant was 7·37 mg/ml; and in serum diluted 1:10 with TEM buffer containing 10% glycerol (TEMG) it was 8 mg/ml.

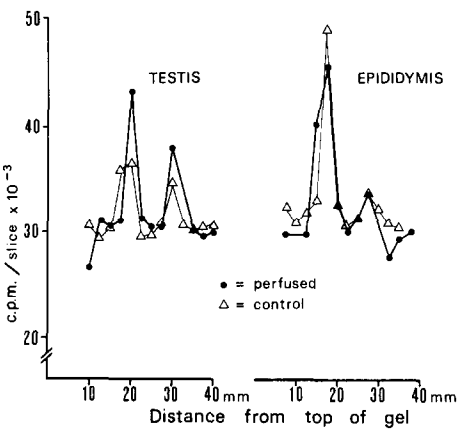

Fig. 5. Binding of ^{3}H-DHT in supernatants from perfused and nonperfused rabbit testis and epididymis. Supernatants were charcoal extracted and 0·2 ml aliquots were layered over 6·5% gels containing saturating amounts of ^{3}H-DHT and unlabelled DHT (total concentration $1{\cdot}8 \times 10^{-8}$ M for testis and $3{\cdot}0 \times 10^{-8}$ M for epididymis). The electrophoresis was run for 2 h as indicated under Fig. 1. ABP concentrations were calculated from the radioactivity in the ABP peak divided by the specific activity of ^{3}H-DHT, assuming one binding site per molecule. Total protein concentrations (mg/ml) in the supernatants were: testis perfused 6·67, nonperfused 7·15; caput perfused 6·18, nonperfused 7·37. (pmol/mg protein) in supernatants were: testis perfused 0·54, nonperfused 0·52, caput perfused 5·84, nonperfused 6·71.

DISCUSSION

These studies show that rabbit efferent duct fluid contains an androgen binding protein which passes through the efferent ducts and into the caput epididymidis. It has been shown previously that the production of testicular fluid is unimpaired by efferent duct ligation and that the ligation does not interfere with testicular blood flow or lymphatic drainage [7]. Neither is epididymal blood flow changed within the first several days after efferent duct ligation [8]. Since efferent duct fluid is produced largely by the seminiferous tubules, tubular epithelium would appear to be the most likely source of ABP. It seems unlikely that ABP in rabbit efferent duct fluid could be derived from serum because its concentration is so much higher than the androgen binding protein in serum, and large molecules have difficulty passing the blood testis barrier [9]. Furthermore, Hansson *et al.* [10] have been able to separate the rabbit serum androgen binding protein from ABP using either isoelectric focusing in acrylamide gels or chromatography on DEAE cellulose and have shown that the serum protein is not present in efferent duct fluid. The failure to remove ABP from epididymis by perfusion, the much higher

amount of ABP in caput epididymis supernatant than in serum and its absence in the cauda epididymis clearly establish that the ABP measured in caput supernatant does not result from serum contamination.

Studies on the characterization of rabbit ABP indicate that it is a specific androgen binding protein with a high affinity for dihydrotestosterone ($Kd = 1{\cdot}4 \times 10^{-9}$ M) and lesser affinities for testosterone and 5α-androstane-3α,17β-diol. It has a sedimentation coefficient of 4–5 S, Stokes radius of 43 Å, mol. wt. of 70,000–80,000, and isoelectric pH of 4·5–5·0 [10, 11].

Danzo *et al.*, have demonstrated a 4 S androgen binding protein in supernatant of caput epididymidis from noncastrated rabbits. Although it has been described as a cytoplasmic 5α-dihydrotestosterone receptor [12] of rabbit epididymis, this protein has a binding affinity for dihydrotestosterone and testosterone which is similar to the rabbit testis ABP, and, like ABP, it is also found in caput but not in cauda epididymidis.

It seems likely that rabbit testis ABP is a carrier of androgen from testis to epididymis. Studies in the rat have indicated that a major portion of ABP is either destroyed in the lumen as it passes through the caput or is taken up by the epithelium [2]. Failure to find ABP in rabbit cauda suggests that in the rabbit ABP may be degraded or taken up even more rapidly as it flows through the lumen of caput epididymidis.

Acknowledgements—Supported by Swedish Medical Research Council Grant 3168, USPHS Grant HDO4466, the Swedish Medical Research Council Visiting Scientist Program, and the Medical Research Council of the Swedish Life Insurance Offices. FSF was the recipient of a Kenan Research Leave from the University of North Carolina.

REFERENCES

1. French F. S. and Ritzén E. M.: *J. Reprod. Fert.* **32** (1973) 479.
2. French F. S. and Ritzén E. M.: *Endocrinology* **93** (1973) 88.
3. Ritzén E. M., Dobbins M. C., Tindall D. J., French F. S. and Nayfeh S. N.: *Steroids* **20** (1973) 593.
4. Hansson V.: *Steroids* **20** (1973) 575.
5. Hansson V., Djösland O., Reusch E., Attramadal A. and Torgersen O.: *Steroids* **21** (1973) 457.
6. Ritzén E. M., Nayfeh S. N., French F. S. and Dobbins M. C.: *Endocrinology* **89** (1971) 143.
7. Setchell B. P. and Waites G. M. H.: *J. Reprod. Fert.* **13** (1973) 15.
8. Brown P. D. C. and Waites G. M. H.: *J. Reprod. Fert.* **28** (1972) 221.
9. Setchell B. P.: In *The Testis* (Edited by G. Johnson and Van Demark), Academic Press, New York, Vol. 1 (1971) p. 101.
10. Hansson V., Ritzén E. M., French F. S., Attramadal A. and Torgersen O.: Program of the IX *Acta Endocrinologica Congress*, 1973, p. 7.
11. Ritzén E. M., Hansson V. and French F. S.: 55th Program of the Endocrine Society, 1973.
12. Danzo B. J., Orbegin-Crist M. C. and Toft D. O.: *Endocrinology* **92** (1973) 310.
13. Ritzén E. M., French F. S., Weddington S. C., Nayteh S. N. and Hansson V.: Submitted to *J. biol. Chem.*