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SHORT COMMUNICATION

ANDROGEN RECEPTORS IN THE RAT EPIDIDYMIS DO NOT DISAPPEAR AFTER CASTRATION

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SUMMARY

Binding of [^{3}H]-dihydrotestosterone to cytoplasmic androgen receptors in the rat epididymis was studied at varying times after castration (1, 5, 9, 33, 50 and 90 days), using two different methods: sucrose gradient centrifugation and polyacrylamide gel electrophoresis. Cytoplasmic androgen receptors were shown to be present in the epididymis at all times after castration. Earlier reports which describe the disappearance of cytoplasmic androgen receptors in the epididymis after castration are probably the result of methodological artifacts. Polyacrylamide gel electrophoresis was a more reliable technique than sucrose gradient centrifugation for demonstrating the presence of epididymal androgen receptors.

The importance of androgens for the maintenance of the morphological and functional integrity of the epididymis has been well documented [1]. It is a general belief, that steroid actions on target tissues are mediated through the formation of a cytoplasmic hormone–receptor complex followed by translocation of this complex into the nuclear compartment.

The presence of such an androgen receptor system in the epididymis is well documented [2–5]. Calandra *et al.* [6], showed a disappearance of the 8*S* epididymal cytosol receptor, by sucrose gradient centrifugation technique (SGC), after long-term (30 days) castration. Furthermore, androgen substitution to such long-term castrated rats, prevented the disappearance of the 8*S* androgen–receptor complex. The conclusion drawn from this study, was that binding of androgens to the epididymal cytoplasmic receptor is under androgenic control. However, since long-term castrated animals are still responsive to androgens this would imply that the androgen receptors are not essential for the stimulatory effects of testosterone on epididymal growth and development. Another possibility was that the epididymal androgen receptors were simply not demonstrable with the technique which was used. We therefore re-investigated the possible presence of cytoplasmic androgen receptors in the epididymis by two different methods: SGC and agarose-acrylamide gel electrophoresis (PAGE). The latter technique has been used to demonstrate androgen receptors in a number of tissues [7, 9].

Male Sprague–Dawley rats, weighing 260–320 g, were anesthetized with ether and castrated via a scrotal incision at different times before the experiments. Groups of 6–11 animals, were used at 1, 3, 5, 9, 33, 50 and 90 days, following castration. The rats were killed and the epididymides were dissected out and cleaned of surrounding fat. The organs were then weighed and homogenized in 4 vol. (w/v) of 50 mM Tris–HCl Buffer, pH 7.4 at 4°C, containing 1.0 mM EDTA, 0.5 mM β-mercaptoethanol and 10% glycerol. The homogenates were centrifuged at 105,000 *g* for 1 h at 0–2°C. An aliquot of the cytosol fraction was taken for protein determination [8]. Aliquots of cytosol, adjusted to a protein concentration between 4–5 mg/ml, were incubated with [1,2,4,5,6,7-^{3}H]-5α-dihydrotestosterone ([^{3}H]-DHT; 100 Ci/mmol) at a concentration of 2.5 nM, for 16 h at 0°C. Following the incubation period, aliquots of the labelled cytosols were layered over 5–20% (w/v) linear sucrose gradients containing 10% (v/v) glycerol and centrifuged at 246,000 *g* (av), for 20 h, at 0°C, in a SW 56 rotor (Spinco L2-65B). Gradients were punctured at the bottom and fractionated into 30 fractions. Bovine serum albumin (BSA) and human immunoglobulin G (IgG) were used as reference standards. Parallel aliquots of the [^{3}H]-labelled cytosol (100, 200 and 300 μl) were run on gels containing 3.5% acrylamide and 0.5% agarose, prepared as described earlier by Dingman and Peacock [9], with the modifications of Naess *et al.* [9]. Gels were run in glass tubes (80 × 7 mm) at 2.5 mA/tube and 0–2°C. Bromophenol Blue (BBP) was added as a marker. Following electrophoresis, gels were sliced into 2.3 mm thick slices and radioactivity extracted with 10 ml of scintillation solution (0.5%, 2-5-diphenyloxazole and 0.005% 1,4-*bis*-2-(4-methyl-5-phenyloazolyl)-benzene in toluene), overnight at room temperature. Radioactivity was counted in a Nuclear Chicago Mark II liquid scintillation spectrometer.

Cytoplasmic androgen–receptor complexes, demonstrated as 7–8 *S* peaks by SGC and as a peak of bound dihydrotestosterone with a mobility relative to BPB (*Rx* 0.53) by PAGE, were demonstrated in male Sprague–Dawley rats castrated 1, 5 and 33 days (Fig. 1). One day after castration (Fig. 1A), considerable quantities of testicular androgen binding protein (ABP) are present in the epididymal cytosol as shown by the big 4.6*S* complex by SGC and the rapid moving complex (*Rx* 0.66) by PAGE. After longer periods of castration ABP disappears from the epididymis (Fig. 1B and C), as shown previously [11]. At days 3, 9 and 50 after castration, the results were almost identical to those shown in Fig. 1B and C. At 90 days of castration, the 7–8 *S* complex was greatly reduced, probably due to a polydispersed aggregation of the androgen–receptor complex. Sharp peaks with the same electrophoretic mobility could still be demonstrated by PAGE (not shown).

The androgen–receptor complex (*Rx* 0.53) demonstrated by PAGE in the 33 days old rats was further characterized and shown to possess physico-chemical properties and steroid binding properties identical to those of the 7–8*S* androgen–receptor complexes shown by SGC. The heat lability, sulphydryl-dependence, high affinity for testosterone and dihydrotestosterone ($2–4 \times 10^{-10}$ M) and very slow dissociation of the bound ligand ($t_{\frac{1}{2}}0°C > 48$ h) are similar to that previously published for androgen receptors in a variety of androgen responsive tissues [11].

It should be noted that the binding observed by SGC an atrophy and metaplasia of the epithelial cells (contain-

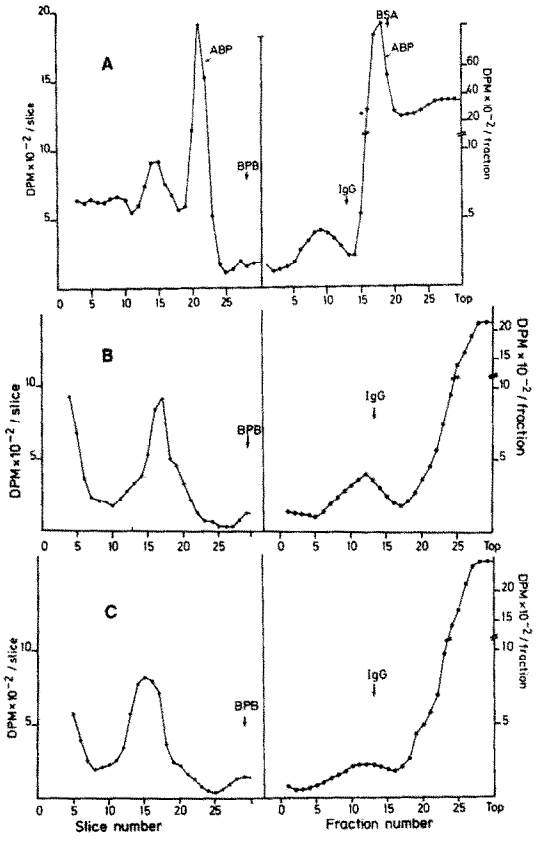

Fig. 1. Epididymal cytoplasmic androgen receptors in 1(A), 5(B) and 33(C) days castrated rats. *Left*: Binding activity analysed by electrophoresis in 3.5% acrylamide gels containing 0.5% agarose. BPB; bromophenol blue. *Right*: Sedimentation profile in 5–20% sucrose gradients. BSA; bovine serum albumin. IgG; human immunoglobulin G. ABP; androgen binding protein.

was consistently lower than that found by PAGE. Although neither SGC nor PAGE are well suited for quantitative measurements of epididymal androgen receptors, we have a general impression that receptor binding expressed per mg of cytosol protein, show a small decrease after prolonged time of castration. However, the decrease is not greater than what should be expected from the change in epithelial/fibrous tissue ratio, following prolonged periods of orchiectomy. After castration, there is ing androgen receptors) and a relative increase in fibromuscular stroma (without receptor) [11].

Several reports have appeared in the literature claiming that androgen receptors disappear after short-term [12–15] or long-term [6] castration. The high proteolytic activity in the tissue extract present a short-time after castration and the instability of the SGC technique are probably the explanation for these findings.

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REFERENCES

1. Hamilton D. W.: The Mammalian Epididymis. *In Reproductive Biology*. (Edited by H. Balin, and S. Glasser). Excerpta Medica, Amsterdam (1972) pp. 237–268.
2. Blaquier J. A.: *Biochem. biophys. Res. Commun.* **45** (1971) 1076–1082.
3. Hansson V. and Tveter K.: *Acta endocr., Copenh.* **66** (1971) 745–755.
4. Tindall D. J., French F. S. and Nayfeh S. N.: *Biochem. biophys. Res. Commun.* **49** (1972) 1391–1397.
5. Blaquier J. A. and Calandra R. S.: *Endocrinology* **93** (1973) 51–60.
6. Calandra R. S., Blaquier J. A., Del Castillo E. J. and Rivarola M. A.: *Biochem. biophys. Res. Commun.* **67** (1975) 97–102.
7. Tindall D. J., Hansson V., McLean W. S., Ritzén E. M., Nayfeh S. N. and French F. S.: *Molec. cell. Endocr.* **3** (1975) 83–101.
8. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: *J. biol. Chem.* **193** (1951) 265–275.
9. Naess O., Hansson V., Djoeseland O. and Attramadal A.: *Endocrinology* **97** (1975) 1355–1363.
10. Hansson V., Djoeseland O., Reusch E., Attramadal A. and Torgersen O.: *Steroids* **22** (1973) 19–33.
11. Hansson V., Ritzén E. M., French F. S. and Nayfeh S. N., Androgen Transport and receptor mechanisms in testis and epididymis. In *Handbook of Physiology*, Section 7, Vol. 5, (Edited by D. W. Hamilton and R. O. Greep). Am. Physiol. Soc. (1975) pp. 173–200.
12. Jung I. and Baulieu E. E.: *Biochimie* **53** (1971) 807–817.
13. Bruchosvky N. and Craven S.: *Biochem. biophys. Res. Commun.* **62** (1975) 837–843.
14. Mainwaring W. I. P. and Mangan F. R.: *J. Endocr.* **59** (1973) 121–139.
15. Sullivan J. N. and Strott C. A.: *J. biol. Chem.* **248** (1973) 3202–3208.