Distribution of 5 α -reductase in the epididymis of the tammar wallaby (*Macropus eugenii*) and dependence of the epididymis on systemic testosterone and luminal fluids from the testis

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Summary. The activity of 5 α -reductase was much higher in the caput and corpus epididymidis than in the cauda epididymidis. Orchidectomy caused a reduction in 5 α -reductase activity in the caput and corpus epididymidis, and regression of the epithelium and reduction in mass of all regions of the epididymis. Subsequent testosterone therapy caused a substantial increase in amount of epithelium and overall mass of the cauda epididymidis but showed little or no increase in any of the responses measured in the caput and corpus epididymidis.

We concluded that the caput and corpus epididymidis of the tammar respond to factors other than testosterone, probably some constituent in the luminal fluid, and therefore are homologous with the initial segments of the epididymis in eutherians.

Keywords: epididymis; initial segment; tammar; 5 α -reductase

Introduction

Jones *et al.* (1984) described the structural anatomy of the epididymal epithelium of the tammar, and concluded that the organ is differentiated into 3 main segments which correspond with its anatomical division into the caput, corpus and cauda epididymidis. It was also concluded that the caput epididymidis has some features which characterize the initial segment described for the epididymis of eutherians and the echidna, a monotreme. It is generally recognized that these features are a tall epithelium with long stereocilia and a low concentration of spermatozoa in the lumen (Benoit, 1926; Nicander & Glover, 1973). However, there is some doubt about the homology of the initial segment in the tammar and other mammals because, for example, the epithelium lining the caput epididymidis in the tammar is lower and has shorter stereocilia than does that of other species which have been studied. Also, the epithelium lining the caput epididymidis of the tammar is not much taller and the stereocilia are no longer than in the corpus epididymidis. Consequently, criteria other than structure are required to determine the extent of the initial segment in the tammar and its homology with the initial segment in eutherians. It is considered that two appropriate criteria, which have been shown to be characteristic of the initial segment in eutherians, would be the dependence of the duct mucosa on luminal fluids originating from the testis (Gustaffson, 1966; Brooks, 1977; Moniem *et al.*, 1978; Fawcett & Hoffer, 1979; Jones *et al.*, 1981), and the occurrence in the duct mucosa of high levels of 5 α -reductase which are dependent on luminal fluids originating from the testis (Aafjes & Vreeburg, 1972; Robaire *et al.*, 1977, 1981). This report examines these criteria in a study on the effect of bilateral orchidectomy and subsequent

replacement therapy with testosterone on the caput, corpus and cauda epididymidis of the tammar.

Materials and Methods

Adult tammar were obtained from 'Gungahlin', Canberra, ACT, in a breeding colony which originated from stock captured on Kangaroo Island, South Australia. The experiment was a randomized block design testing the three treatments shown in Tables 1 and 2. Bilateral orchidectomy was carried out under general anaesthesia induced by injection into the lateral tail vein of 4% (w/v) sodium thiamylal (Surital, Parke, Davis & Co., Ann Arbor, MI, U.S.A.) at a rate of about 25 mg/kg. At 4 weeks after orchidectomy Silastic implants (length 35 mm, i.d. 3.3 mm, o.d. 4.6 mm; Dow Corning Corp., Midland, MI, U.S.A.) containing 0.14 g testosterone propionate (Sigma Chemical Co., St Louis, MO, U.S.A.) were implanted under the skin of the belly during anaesthesia (as above). The animals were killed by an overdose of anaesthetic 2 weeks later.

Blood samples were collected before surgery and at weekly intervals until slaughter. Plasma from the samples was frozen at -15°C until assayed for testosterone using the radioimmunoassay as described by Catling & Sutherland (1980) and testosterone antiserum 6050 (a gift from Dr R. I. Cox, CSIRO, Sydney). The antiserum, raised in sheep, cross-reacted 31% with 5 α -dihydrotestosterone, 30% with 4-androsten-3 β ,17 β -diol, 3.5% with 4-androsten-17 β ,19-diol-3-one, 1.3% with androstenedione and <0.1% with other steroids. Samples (200 μl) were extracted twice with freshly redistilled diethyl ether. The efficiency of extraction of [^3H]testosterone from plasma was 97–102%. The standard curve represented by the equation $y = 1.682 - 0.013x$ ($r = 0.942$; logit transformation) ranged from 5 to 500 pg/tube and the least detectable concentration was 10 pg/tube (120 pg/ml plasma). Displacement of 50% of [^3H]testosterone occurred at a dose of 80 pg/tube. The slope of the curve ($y = 1.754 - 0.012x$; $r = 0.96$) obtained by diluting a plasma high in testosterone was not different from that of the standard curve ($P > 0.3$). Recovery of testosterone from plasma containing endogenous concentrations of 0.38 ng/ml was highly correlated with the amount added ($y = 1.01 + 0.4x$, $r = 0.991$; $P < 0.001$; $n = 21$) and was always within 12% of that expected. Intra-assay coefficients of variation for 2 pools of plasma containing 4.05 ng/ml and 0.38 ng/ml were 7.9% and 10.5% respectively. Inter-assay coefficients of variation for the same pools of plasma were 14% and 12.4% respectively.

At slaughter, the epididymis, prostate and Cowper's glands were excised, dissected free of adventitia and weighed. One epididymis was fixed in Bouin's fluid and used to prepare paraffin-wax sections (5–7 μm) which were stained with haematoxylin and eosin. An ocular micrometer was used to measure epithelial height and diameter of the ductus epididymidis (3 cross-sections per animal) and these parameters were used to calculate the area of epithelium in a cross-section of the ductus.

Samples from the unfixed epididymis were taken to determine the activity of 5 α -reductase. About 200 mg from the caput, corpus and cauda epididymidis were minced into cubes about 1 mm³ and incubated in a shaking water bath (60 oscillations/min) at 32°C for 1 h in 2 ml Tris buffer (10 mM-Tris, 10 mM-KCl, 1 mM-EDTA, pH 7.4) containing 10 nM-[^3H]testosterone or [^3H]dihydrotestosterone. After incubation the samples were frozen in a solid CO₂-ethanol bath and stored at -39°C . As incubation of whole tissue could result in further metabolism of dihydrotestosterone produced during the incubation, the residual level of the substrate, testosterone, only was measured.

After the addition of 100 μg testosterone and 5 α -dihydrotestosterone as carriers the incubates were extracted with 30 ml chloroform:methanol (2:1, v/v) and the extract was subjected to thin-layer chromatography on silica gel using benzene:ethyl acetate (2:1, v/v) in a saturated system as the developing solvent. The peak of radioactivity that corresponded to testosterone and the androstane diols was acetylated overnight at room temperature in pyridine:acetic anhydride (1:1, v/v), rechromatographed and the resulting testosterone acetate was saponified in 1 mol KOH/l methanol. Samples to estimate recovery of radioactivity were taken at all stages. Authentic testosterone (40 mg) was added to the saponified testosterone fraction and the whole was recrystallized three times, by dissolving in a minimal volume of methanol and adding water dropwise until crystallization was initiated, to constant specific activity for positive identification.

Analyses of variance were used to test the statistical significance of the effects of treatment. As the within-treatment variance was heterogeneous for measures of testosterone concentration and mass of prostate and Cowper's gland, these values were transformed before analyses using the following transformation: transformed response = $(x_i - \bar{x})/s$ where x_i was the response for the i th animal, $\bar{x}$ was the overall mean response and s was the standard deviation from the pooled, within-treatment variance. Results are presented as means $\pm$ s.e. calculated from untransformed data.

Results

Mean testosterone concentrations in plasma samples collected before surgery were similar for all treatment groups. Samples from the untreated group contained 1.4 ± 0.59 ng testosterone/ml (4 animals) and the concentrations remained unchanged for the subsequent 6 weeks. Table 1 shows the mean testosterone concentrations and masses of accessory organs at slaughter. Orchidectomy

Table 1. The effects of orchidectomy and orchidectomy followed by replacement therapy with a Silastic implant of testosterone propionate on blood plasma testosterone levels on the day of autopsy and mass of the prostate and Cowper's gland

Treatment	Testosterone (ng/ml) (N = 4)	Prostate (g) (N = 5)	Cowper's gland (g) (N = 5)	Epididymis (g) (N = 5)
(a) Untreated	1.03 ± 0.52	41.4 ± 19.8	18.4 ± 2.7	3.37 ± 0.25
(b) Orchidectomized	0.17 ± 0.02	16.7 ± 1.0	14.1 ± 0.7	1.33 ± 0.23
(c) Orchidectomized + testosterone propionate	6.02 ± 0.88	54.5 ± 7.1	25.6 ± 1.3	1.88 ± 0.12
a vs b	*	***	ns	***
b vs c	***	***	***	ns

Values are means ± s.e.m. for N tammaris.
ns, not significant; * $P < 0.05$; *** $P < 0.001$.

caused a large reduction in mean testosterone concentration and mass of the prostate and epididymis, but only a small reduction in the mean mass of the Cowper's glands. The copious, viscous secretion which was present in the prostates of the untreated animals was absent in the prostates of the orchidectomized animals. Testosterone therapy substantially increased mean plasma testosterone concentration, and the mass of the Cowper's glands and the prostate gland. The latter also contained the copious, viscous secretion like those in untreated animals. Testosterone treatment (Table 2) produced a small increase in mass of the caput and corpus (21% and 12% respectively of the post-castration fall) but there was a substantial increase in the mass of the cauda epididymidis (42% of the post-castration fall).

The histological studies (Table 2) showed that the whole of the ductus epididymidis regressed after orchidectomy, and that testosterone therapy induced a slight recovery in the caput and corpus epididymidis and substantial recovery in the cauda epididymidis. Part of the histological response to orchidectomy and subsequent testosterone therapy involved collapse of the wall of the ductus into the lumen, and then recovery. The changes were similar to those shown for the rat in Fig. 19 by Fawcett & Hoffer (1979). The regression of the duct epithelium could be demonstrated as a reduction in cross-sectional area of epithelium in all regions and a reduction in epithelial height in the caput and corpus epididymidis. However, there was no reduction in the height of the epithelium in the cauda epididymidis as the epithelial cells became narrower and longer when the duct collapsed. The histological response to testosterone therapy was measured as a relatively small increase in cross-sectional area of epithelium in the caput (25% of post-castration fall) and corpus (21% of post-castration fall) and a substantial increase in the cauda epididymidis (77% of post-castration fall). This was due to an increase in epithelial height in all regions and an increase in duct diameter in the caput and cauda epididymidis.

Measurement of 5 α -reductase activity showed (Table 2) that it was much higher in the caput and corpus epididymidis than the cauda epididymidis. Orchidectomy caused a reduction of its activity in the caput and corpus epididymidis to the levels in the cauda epididymidis, and testosterone therapy had no effect on the enzyme activity in any region of the epididymis.

Discussion

All of the characteristics measured in this study indicated that the function of the epididymal epithelium in the caput and corpus epididymidis of the tammar is dependent on luminal fluids originating from the testis. Consequently, if luminal dependence is accepted as a distinguishing feature of the initial segments (see 'Introduction') it is concluded that, in this marsupial, these regions are homologous with the initial segments of the epididymis of eutherian mammals (Benoit,

Table 2. The effects on the epididymis of the tammar of orchidectomy and orchidectomy followed 4 weeks later by a Silastic implant of testosterone propionate (T) for 2 weeks before slaughter

Region*/treatment	Duct dimensions				5 α -reductase activity (pmol T metabolized g ⁻¹ .h ⁻¹) (N = 3)
	Mass (mg) (N = 5)	Epithelial height (μ m) (N = 5)	Duct diam. (μ m) (N = 5)	Cross-sectional area of epithelium (μ m ²) (N = 5)	
<i>Caput (1-8)</i>					
(a) Untreated	787	40.7	257	27 668	143
(b) Orchidectomized	265	18.1	75	3237	18
(c) Orchidectomized + T	385	28.8	131	9251	51
a vs b	***	***	***	***	***
b vs c	ns	***	**	***	ns
<i>Corpus (9-18)</i>					
(a) Untreated	1079	35.5	245	23 374	112
(b) Orchidectomized	368	14.0	110	4224	22
(c) Orchidectomized + T	454	30.6	116	8213	18
a vs b	***	***	***	***	***
b vs c	ns	***	ns	**	ns
<i>Cauda (19-22)</i>					
(a) Untreated	1506	29.5	419	36 112	17
(b) Orchidectomized	695	26.9	218	16 156	26
(c) Orchidectomized + T	1038	42.7	277	31 443	23
a vs b	***	ns	***	***	ns
b vs c	***	***	**	***	ns
<i>Pooled se</i>	<i>60</i>	<i>1.81</i>	<i>1.2</i>	<i>68</i>	<i>11.8</i>

Values are means for N tammar.

*Numbers in parentheses refer to the regions described in Fig. 1 of Jones *et al.* (1984).

ns, not significant; ** $P < 0.01$; *** $P < 0.001$.

1926; Glover & Nicander, 1971; Nicander & Glover, 1973; Fawcett & Hoffer, 1979; Robaire *et al.*, 1977, 1981). According to the classification proposed by Fawcett & Hoffer (1979) the caput epididymidis is the initial segment proper and the corpus epididymidis, which consists of several structurally distinct regions (Jones *et al.*, 1984), constitutes the distal initial segments. This classification means that sperm maturation in the epididymis of the tammar occurs in the initial segments (Jones *et al.*, 1984) and that these segments are responsible for secreting most of the protein (Jones, 1987) and most of the modification of the luminal milieu which occurs in the epididymis (Jones & Clulow, 1987). Furthermore, in terms of the classification of the epididymis which was proposed by Glover & Nicander (1971), it means that there is no middle segment in the epididymis of the tammar or that Glover & Nicander's (1971) classification should be revised to account for new developments in our understanding of the regulation of the epididymis.

Whilst the mass and 5 α -reductase activity of the caput and corpus epididymidis showed little response to testosterone therapy, the histological studies showed a significant response. The histological response involving a collapse of the duct after orchidectomy and then recovery with testosterone therapy was probably due to an effect of testosterone resulting in a net transport of fluid and solute into the lumen of the duct (Wong & Yeung, 1977) and it occurred in the cauda epididymidis as well as the proximal regions. It is not possible to decide whether it is an action of testosterone or dihydrotestosterone, as some 5 α -reductase activity remained in the duct after castration.

The magnitude of the responses to testosterone therapy differed considerably between the cauda epididymidis, the prostate and Cowper's gland. Part of this difference is probably due to differences in sensitivity of the organs to testosterone (Prasad *et al.*, 1973). Part of the difference must also be due to different proportions of epithelium in the organs. In this respect the response was greatest in the prostate which contains a greater proportion of glandular tissue than does Cowper's gland (Rodger & Hughes, 1973) and the epididymis (Djakiew & Jones, 1982; Jones *et al.*, 1984). It is also suggested that part of the difference in response between the prostate and the cauda epididymidis may be because the testis regulates the cauda epididymidis by a factor other than testosterone.

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