IDENTIFICATION AND STAGE-RELATED SYNTHESIS OF 11-DEOXY-CORTICOSTERONE (DOC) BY THE DOGFISH (SQUALUS ACANTHIAS) TESTIS

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In the testes of the dogfish shark, Squalus acanthias, germ cells and associated Sertoli cells at different developmental stages are topographically segregated. Previous studies using this animal model have shown stage-related variations in key steroidogenic enzymes (Callard et al., 1985, Endocrinol. 117:1328-1335) and the steroid secretory appearance of Sertoli cells (Pudney and Callard, 1984, Anat. Rec. 209:311). Moreover, patterns of blood flow within the testis suggest that steroids produced by more mature stages may have a parahormonal role in regulating the developmental progression of earlier stages. During these studies, two major polar products of [3H]progesterone (-P) metabolism were detected in increased amounts in areas with mature germ cells. In this study, we positively identified and quantified these unknowns.

Testes were sectioned transversely and cross-sections subdivided into premeiotic (zone I), meiotic (zone II) and post-meiotic (zone III) stages. Microsomes were prepared (11,000-100,000 x g pellet) and incubated with [3H]substrate (final conc.=10 μM, SA=0.1 Ci/mmole) plus 2 mM NADPH as previously described (Callard et al., op. cit.). In the first experiment, the products of a large-scale incubation (25 ml, 1 mg/ml protein) with [3H]P or [3H]17α-hydroxyP (-17αP) were separated by reverse-phase high performance liquid chromatography (HPLC, mobile phase=60% MeOH) after ether extraction. The retention times of the major products corresponded exactly to the 21-hydroxylated forms of P and 17αP: respectively, 11-deoxycorticosterone (DOC) and 11-deoxycortisol (Compound S) (data not shown). The 20β-hydroxylated metabolites of [3H]P and [3H]17αP were only minor products in these incubations. Similar results were obtained when [3H]products were separated by thin layer chromatography (TLC) using the solvent systems benzene:acetone (4:1) and chloroform:ethyl acetate (2:1). Radioactivity coeluting with DOC after HPLC and TLC was diluted with authentic DOC and recrystallized to constant specific activity. This verified its identity.

In subsequent experiments, 50 to 500 μg of microsomal protein was incubated with [3H]P in a final volume of 0.5 ml. The products of 21-hydroxylase (P450-21) action (DOC) and 17α-hydroxylase (P450-17α) action (17αP + androstenedione + testosterone) were separated by TLC (benzene:acetone, 4:1) and quantified by liquid scintillation spectroscopy. When compared to earlier stages, significantly higher yields of DOC were produced by microsomes from the post-meiotic stage of spermatogenesis comprising round spermatids and spermatozoa (Fig. 1). Androgen production also was highest in the most mature stage as previously reported, but did not reach values obtained for DOC. In three separate testicular preparations stage-related patterns were similar to the experiment shown in Fig. 1, but absolute product yields and the ratio of androgen to DOC varied.

Kinetic studies indicated that P450-21 had a higher affinity for P than P450-17α (apparent Km = 0.5 μM vs. 1-4 μM, respectively in three separate experiments). Linear Michaelis-Menten kinetics for P450-17α were obtained only with short incubations (2-6 min). Apparent Vmax values for P450-21 and P450-17α confirmed that DOC is produced at greater rates than androgens in most preparations (eg. 231 vs. 71 pmole/mg/min in a representative experiment).

DOC has been identified previously in high concentrations in Squalus semen (Simpson et al., 1963, J. Endocrinol. 26:489) and in [14C]P incubates of Squalus testicular homogenates (Simpson et al., 1963, J. Endocrinol. 31:29) and semen (Simpson et al., 1964, J. Endocrinol., 31:11). The present report confirms and extends these earlier studies. It is uncertain what functional role DOC plays in Squalus testis. One possibility is that the 21-hydroxylating pathway limits androgen production by competing for available P substrate. There is also the possibility that the products of P450-21 action competitively inhibit P450-17α, which could

explain why we were unable to get linear reaction kinetics at longer incubation times. As an alternative to these direct enzymatic effects, DOC may regulate spermatogenesis via a classical nuclear receptor mechanism. It is noteworthy here that glucocorticoid receptors have recently been identified in rat Sertoli cells (Levy et al., 1989, Endocrinol. 124:430-437.). Studies in teleost fish suggest that C20-hydroxylated progestin metabolites, which are produced in increased amounts at spermiation, may be involved in controlling sperm maturation, spermiation, and spawning behavior. Finally, DOC derived from the testis may regulate peripheral metabolism and/or salt and water balance during the shark's reproductive migration, a role reminiscent of that in migratory birds and salmonids. These possibilities are currently under investigation.

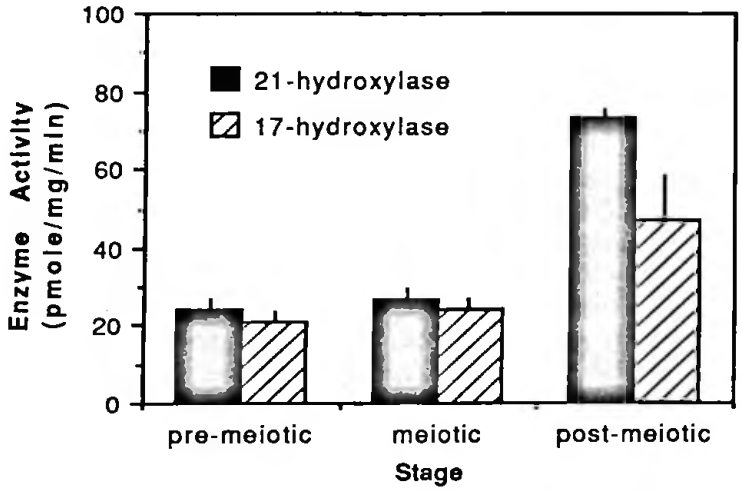

Fig. 1. Zonal distribution of microsomal 21-hydroxylase (P450-21) and 17α-hydroxylase (P450-17α) activities in the testis of the dogfish shark, Squalus acanthias. Enzyme activities were measured by conversion of [3H]P to DOC (21-hydroxylase) or to 17αP + androstenedione + testosterone (17α-hydroxylase). Bars show mean values and range of quadruplicate incubations and are representative of three separate experiments.

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