We conclude that in the isolated head preparation the gill effectively reduces free estrogen in the perfusate and prevents its accumulation in brain. Whether or not the gill also excretes conjugates is not known, although a substantial portion of the added substrate was lost somewhere in the perfusion system. Conjugated steroids have recently been identified as teleost pheromones and may enter the environment via this route. In contrast to estrogen, androgen apparently reaches the brain in amounts sufficient for aromatization. One explanation is that androgens are not as readily conjugated as estrogens. From studies in other vertebrates, it is known that conjugated estrogens are not acceptable ligands for receptor binding. It is likely, therefore, that central estrogen targets in the sculpin depend entirely on active hormone synthesized in close proximity to receptors. This hypothesis is depicted in Figure 1. An intriguing question is whether high brain aromatase activity in this species is a cause or a consequence of biological inactivation of estrogen in peripheral tissues (Supported by NSF PCM 78-23214/82-01975).

IDENTIFICATION OF AN ESTROGEN RECEPTOR IN THE TESTIS OF THE SHARK SQUALUS ACANTHIAS

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The shark Squalus acanthias is one of several non-mammalian vertebrates in which germ cells are topographically segregated within the testis according to their stage of development. This anatomical arrangement offers a unique opportunity to characterize biochemical changes occurring in conjunction with the cycle of the seminiferous epithelium. We previously reported distinct regional variations in the activities of several key steroidogenic enzymes leading to the formation of androgen and estrogen (Callard et al., The Bulletin 21:37, 1981). Since sex steroids have intratesticular sites of action in addition to their better known peripheral effects, we attempted to identify, characterize, and map the distribution pattern of an estrogen receptor in shark testis.

Using a dissecting microscope, testes were sectioned transversely and divided into four regions. Based on light microscopy, these were comprised of the following germ cell stages: I, spermatogonia; IIA, spermatocytes; IIB, round spermatids; III, mature spermatids. Epigonal tissues and mature spermatozoa collected from the vas deferens were used as control tissues. Cytosolic and nuclear extracts were prepared by differential centrifugation. All methods of receptor analysis are routinely used in this laboratory and have been described in detail (Mak et al., Biol. Reprod., in press, 1983). Unless otherwise stated, Zones I and IIA were used for characterizing the receptor. Nuclear extracts were labeled with ^{3}H-estradiol (5 nM) under exchange conditions (22° x 60 min, 4° x 15 min) to displace endogenous bound estrogen. Following separation of free and bound steroid on LH-20 columns, an estrogen binding component which could be displaced by 200-fold excess radioinert estradiol was detected. Comparison of displacement with estradiol and other steroids showed the following: estradiol (80%); diethylstilbestrol (57%); estrone (76%); estriol (61%); testosterone (38%); 5α-dihydrotestosterone (33%); progesterone (15%). When aliquots of nuclear extract were incubated with increasing concentrations of ^{3}H-estradiol (0.5 - 5.0 nM) $\pm$ 200-fold excess radioinert estradiol, specific binding approached saturation between 4 and 5 nM Figure 1). Scatchard analysis revealed a K_d of 1.53×10^{-9}M with a maximum binding capacity of 13.1 fmol per μg DNA (Figure 1). When nuclear extract labeled with ^{3}H-estradiol was analyzed by centrifugation on a high salt linear sucrose gradient (5-20%), a specific estrogen binding macromolecule having a sedimentation coefficient of 4.7 - 5S was detected. Measurement of binding capacity in different testicular regions revealed the following distribution (in fmol/ug DNA): Zone I (6.0); Zone IIA (3.5); Zone IIB (1.27); Zone III (0.69). No binding was found in epigonal tissue or in free spermatozoa.

Crude cytosol labeled by incubation with ^{3}H-estradiol $\pm$ 200-fold excess inert estradiol for 2 h at 4° was chromatographed on LH-20 and also on DNA-cellulose columns. DNA-cellulose affinity chromatography is useful for separating receptors (DNA-adhering) from low affinity, non-adhering estrogen binding molecules, usually serum contaminants. Crude cytosol was also subjected to a post-labeling method whereby samples chromatographed onto

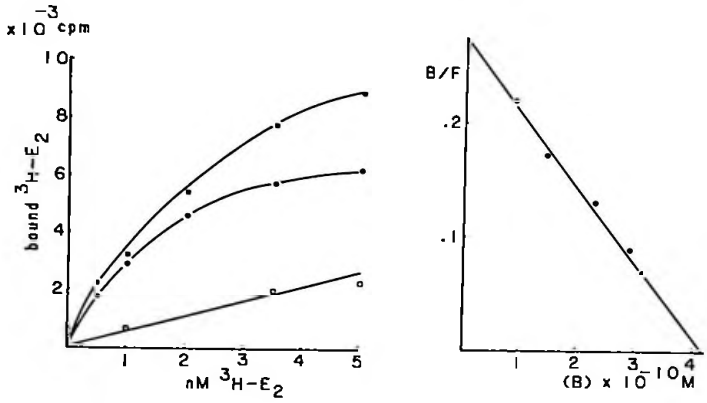

Figure 1.--On left, saturation analysis of estrogen binding activity in nuclear extracts of Squalus testis (Zone I): total binding (■——■); non-specific binding (□——□); specific binding (●——●). On right, Scatchard plot of the data presented on left: K_d = 1.53 x 10^{-9}M; maximum binding capacity = 13.1 fmol/μg DNA. DNA-cellulose columns were first washed extensively to remove non-adhering contaminants and then labeled in situ with ^{3}H-estradiol. This technique avoids possible metabolism of tracer by enzymes in crude cytosol. None of the methods used were successful in detecting specific estrogen binding in cytosol.

These results demonstrate the existence in Squalus testis of an estrogen binding macromolecule having the physicochemical characteristics of a classical estrogen receptor: high affinity, limited capacity, tissue and estrogen specificity. Its properties resemble those in mammals and other vertebrates, with the possible exception of its relatively low affinity for the non-steroidal estrogen, diethylstilbestrol. Although we failed to detect an estrogen binding component in crude cytosol, this was judged to be a technical problem since actual results were suggestive of contaminating proteases active even at the low temperatures used for incubation and chromatography (4°). Receptors in nuclear extracts, on the other hand, are relatively free of the many interfering components found in cytosol. Indeed, the presence of receptors in nuclear extracts obtained from untreated sharks is evidence that receptor occupancy by endogenous estrogen and trans-location of the complex to the nucleus are normal in vivo events. Although aromatase activity, and presumably local estrogen concentrations, are highest in Zone II, nuclear receptor is concentrated in Zone I. Whether this is a function of actual receptor numbers or an index of the rate at which endogenous estrogen is translocated cannot be determined until values for cytosolic receptors are obtained. Another consideration, however, is that the concentration of nuclear receptor may actually be a reflection of the proportion of somatic cell DNA to germ cell DNA in each region (I > II > III), prima facie evidence for localization of estrogen receptor in Sertoli cells. This would be unusual since Leydig cells are the cell type containing both aromatase and estrogen receptors in mammals and amphibians; however, typical vertebrate Leydig cells are not present in Squalus (Pudney et al., this issue). Possibly, their steroidogenic and other functions are assumed by the highly-developed Sertoli cells. Supported by NICHD 16715.

OPENING OF THE BLOOD-BRAIN BARRIER WITH OSMOTIC STRESS IN THE LITTLE SKATE, RAJA erinacea

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The permeability of the blood-brain barrier to polar solutes can be increased by exposure to hyperosmolar solutes, presumably by opening the junctional complexes between adjacent endothelial cells (Rapoport et al., Am. J. Physiol. 238:R421-R431, 1980). The osmotic stress is applied selectively to the central nervous system either by infusion into the carotid artery or by direct application to the surface of the brain of a highly hypertonic solution