INHIBITION OF SHARK RECTAL GLAND BY AN ENDOGENOUS DIGITALIS-LIKE COMPOUND

F.H. Epstein, A. Stevens, K. Spokes, P. Silva and D. Lichtstein, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts

The ubiquitous presence on transporting epithelia of receptors for ouabain-like steroids that inhibit Na-K-ATPase binding has generated speculation that endogenous compounds might act as inhibitors and modulators of Na-K-ATPase activity. A compound with ouabain-like properties has been extracted from the abdominal and back skin of the toad Bufo veridis. Partial purification was achieved by methanol and trichloracetic acid extraction followed by separation using high performance liquid chromatography. The compound (termed endigin in this communication) is neither a steroid nor a peptide, has a molecular weight less than 400 and is polar. It resembles ouabain in several bioassays including inhibition of ^{3}H-ouabain binding to rat synaptosomes, and inhibition of Na-K-ATPase activity and of monensin-induced hyperpolarization of chick embryo fibroblasts. The material used in our experiments had been bioassayed by measuring its inhibition of ^{3}H-ouabain binding to rat brain synaptosomes and its potency is expressed as ouabain-equivalents in that assay in which 6×10^{-5}M ouabain inhibits 50% of binding. The present experiments were undertaken to examine the action of this material on the rectal gland of Squalus acanthias, since activation of Na-K-ATPase plays such a key role in the maintenance of active ion secretion by the gland.

Isolated cells were prepared from fresh minced rectal glands by the procedure of Silva et al., suspended in shark Ringers solution with 10 mM glucose, 10 mM pyruvate and 10 mM acetate buffered at pH 7.6 with 40 mM Hepes, and equilibrated with room air (Silva et al., this issue). Respiration was determined using a Yellow Springs oxygen monitor; the chamber volume was 2 ml and cells were maintained at 25°C. Oxygen tension in the chamber was recorded continuously and respiration rates calculated from the slopes of the traces obtained. After a steady state was reached, the basal Q_{O_2} was determined over a period of at least 15 min, following which dibutyryl cAMP, 0.5 mM and theophylline 2.5 mM were added in a volume of 40 µl Ringers to stimulate respiration maximally. Successive increments of ouabain or endigin were then added to determine the inhibitory effect of each on respiration.

In 3 separate experiments endigin was found to inhibit the respiration of rectal gland cells in a dose-dependent manner. A representative experiment is shown in Figure 1, which also depicts the dose-related inhibition produced by

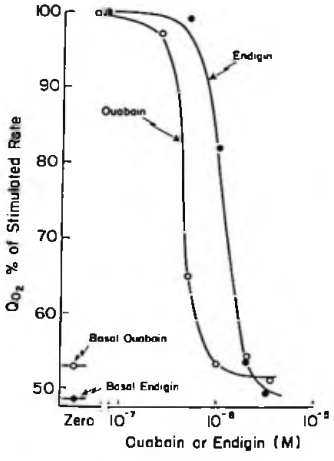

Figure 1.--Dose-dependent inhibition of respiration of isolated rectal gland cells by endigin and ouabain. In this experiment the peak (100%) Q_{O_2} of cells in the stimulated state was 45 $\mu MO_2/hr/g$ for cells subsequently exposed to endigin, and 51 $\mu MO_2/hr/g$ for cells subsequently exposed to ouabain.

ouabain. The Ki for endigin in this experiment was 1.1×10^{-6}M, whereas that for ouabain was 4×10^{-7}M. The effects of ouabain and endigin were overlapping, rather than additive. Full inhibitory doses of ouabain 1.2×10^{-5}M did not depress respiration further when added to a full inhibitory concentration of endigin (1.2×10^{-5}M), and vice versa.

The effect of endigin on binding of ^{3}H-ouabain to slices of rectal gland in the course of a 300 min incubation in Ringers solution was also tested (Bull. MDIBL, 21:103-108, 1981). Endigin (5×10^{-5}M) almost completely inhibited the binding of 10^{-9}M ^{3}H-ouabain, reducing it by 95%.

These experiments supply additional evidence that the endogenous non-steroidal digitalis-like compound found in anuran tissues may bind to ouabain receptors in the cell membranes of animal species widely separate in evolutionary development, and might therefore represent an example of an endogenous regulator of Na-K-ATPase.

MORPHOLOGICAL IDENTIFICATION OF STEROID PRODUCING CELLS IN THE TESTIS OF SQUALUS ACANTHIAS

Jeffrey Pudney, Gloria V. Callard, and Jacob Canick, Department of Biology, Boston, Ma., Department of Pathology, Warren and Children's Hospital, Brown University, Providence, RI.

In previous studies we have demonstrated that the presence and activities of certain enzymes involved in the synthesis of both androgens and estrogens varied topographically in the testis of Squalus acanthias, according to the stage of spermatogenesis examined (Callard, G.V. and Petro, Z., The Bulletin, MDIBL 19:38, 1979; Callard, G.V. et al., The Bulletin, MDIBL 21:37, 1981). This implies that the steroidal microenvironment associated with these different phases of germ cell maturation may be quite specific. Light microscopic observations of the testis, at this time, indicated that the interstitial tissue, which in all vertebrate species so far studied contains the steroid-producing Leydig cells, appeared undifferentiated. The Sertoli cells, however, were well developed, which was taken as good evidence that these cells were responsible for the steroidogenic activity displayed by Squalus testis. To identify more precisely the cellular site of steroid production and to determine whether Leydig cells were present or absent in the testis of Squalus, tissue was also examined by means of electron microscopy.

The testes of pithed male sharks were perfused by cannulation of either the conus arteriosus or celiac artery. Perfusion was initiated by physiological saline containing 1% heparin which was allowed to flow for 10 mins. This was immediately followed by 500 ml of half-strength Karnovsky's fluid (Karnovsky, M.J., J. Cell Biol. 27:137A, 1965). Egress of the perfusate was accomplished by section of the atrium. The testes were then removed, sliced into small pieces and further fixed in Karnovsky's fluid for 24 hrs. Post-fixation was carried out by immersion in an aqueous 1:1 mixture of 2% osmium tetroxide and 3% potassium ferrocyanide for 1 hr. (Karnovsky, M.J., J. Cell Biol. 284A, 1971). The tissue was then processed for electron microscopy by conventional procedures.

Initial observations demonstrated that cells containing organelles known to be involved in the synthesis of steroids, e.g., smooth endoplasmic reticulum, lipid droplets, and mitochondria with tubulo-vesicular cristae, were present in the interstitial tissue of Squalus testis (Figure 1). These cells, however, were undifferentiated and possessed

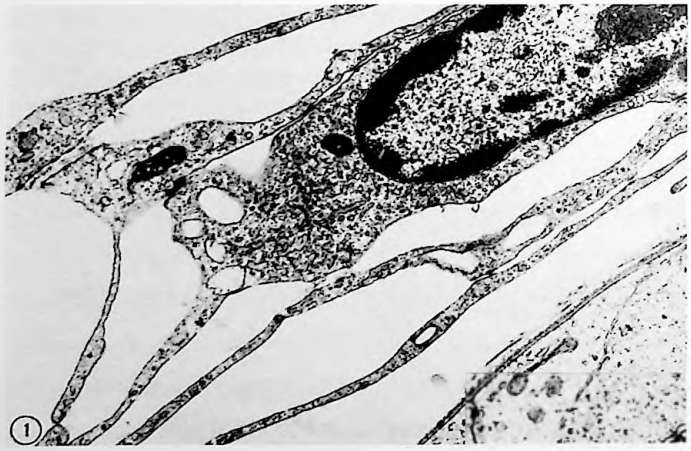

Figure 1.--Electron micrograph of "Leydig-like" cell present in the interstitial tissue of Squalus testis. These cells are fusiform in shape and produce long attenuated cytoplasmic processes. They are relatively undifferentiated and contain a few lipid droplets, mitochondria, and a small amount of smooth endoplasmic reticulum.