plasma and rectal gland secretion and that intracellular sodium and chloride are both low, though chloride is higher than sodium. The active step in the transport of sodium chloride from plasma to rectal gland duct is likely to be at the luminal border of the cell rather than at the basal border since diffusion of both sodium and chloride from intracellular fluid into duct fluid against a large chemical gradient seems improbable.

1974 #34

QUANTITATIVE ASPECTS OF THE INHIBITION OF GILL Na-K-ATPase BY INTERNAL AND EXTERNAL OUABAIN IN SEAWATER EELS, *Anguilla rostrata*

PATRICIO SILVA, FRANKLIN H. EPSTEIN, KATHERINE SPOKES, SARA EPSTEIN AND RICHARD SOLOMON. DEPARTMENT OF MEDICINE AND THORNDIKE LABORATORY, HARVARD MEDICAL SCHOOL, BETH ISRAEL HOSPITAL, BOSTON, MASSACHUSETTS

A critical question in the physiology of ion transport by teleost gills is the anatomical position of the sodium pump. Much evidence favors an important role for Na-K-ATPase located in chloride cells in the active extrusion of sodium across the gill in fish adapted to seawater. It is not clear however whether the Na-K-ATPase of gill chloride cells "faces" outward so as to transport Na^+ from the cell to the sea or inward so as to transfer Na^+ from chloride cell cytoplasm to the intracytoplasmic tubules which are believed to be continuous with extracellular fluid. Since ouabain binds to Na-K-ATPase only on that side of the membrane toward which Na^+ is transported, one way to approach the problem is to see whether ouabain inhibits gill Na-K-ATPase when applied from the blood side or the sea side of the gill.

Eels (*Anguilla rostrata*) adapted to seawater for at least three weeks were used. Varying amounts of ouabain were placed in the external aerated

Fig 1

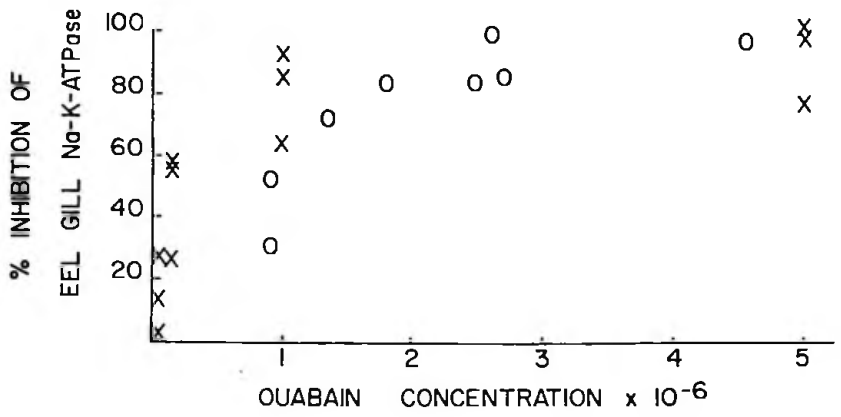

Figure 1: Effect of ouabain on eel gill Na-K-ATPase. This figure shows the effect of increasing concentrations of ouabain on eel gill Na-K-ATPase when injected intraperitoneally to live eels (o) or added to the incubation media in vitro (x). Each point represents one experiment, and corresponds to the percent inhibition found in enzyme activity as compared to samples obtained prior to injection in the in vivo experiment or controls without ouabain in the in vitro group.

seawater bath or injected intraperitoneally together with tracer quantities of ^{3}H ouabain. After one hour the eels were sacrificed and in some experiments, the concentration of ouabain in plasma was estimated from the radioactivity of plasma, making use of the known specific activity of the injected ouabain. Gill filaments and kidneys removed from the eels were homogenized and assayed for Na-K-ATPase.

5×10^{-6} M ouabain was found to inhibit Na-K-ATPase completely in vitro in gill homogenates of salt water adapted eels (Figure 1). However when this concentration of ouabain was added to potassium-free aerated seawater (15°C)

in which eels were freely swimming no change was found in the enzyme activity of the gill. Na-K-ATPase of gill biopsies sampled before and one hour after the fish was placed in a bath containing 10^{-5} M ouabain was likewise unchanged. When 10^{-4} M ouabain was placed on the outside gill Na-K-ATPase was found to be 45 percent inhibited (11.8±5.51 before immersing in ouabain vs 6.48±5.87 after). However, kidney Na-K-ATPase sampled at the end of the experiment was also found to be inhibited as compared with normal controls sacrificed at the same time indicating that ouabain had found its way into the circulatory system of the fish. Increasing the outside concentration of ouabain to 10^{-3} M resulted in further Na-K-ATPase inhibition of approximately the same magnitude in both gill and kidney (8.2±5.1 experimental vs 18.01±4.5 control in gills; 9.2±3.8 experimental vs 17.2±7.8 control in kidneys).

Ouabain was then administered intraperitoneally in amounts producing plasma concentrations of 10^{-6} M to 5×10^{-6} after one hour. Gill filaments removed surgically prior to the ouabain injection, and one hour after the administration of the drug were assayed for Na-K-ATPase activity. A close correlation between the plasma concentration of ouabain and the percent inhibition of gill Na-K-ATPase was observed closely paralleling the *in vitro* ouabain inhibition curve of eel gill Na-K-ATPase (Figure 1). An intrapertoneal dose of 0.25 mg ouabain per 100g body weight was found to produce a plasma concentration of ouabain of about 2.5×10^{-6} M and to result in 90 to 100 percent inhibition of gill Na-K-ATPase. When gill Na-K-ATPase was inhibited plasma sodium was found to be elevated: 186.5±9.3 mEq/l and plasma potassium was also high: 6.0±3.0 mEq/l (normal values found in separate controls Na: 167.9±5, K: 2.2±0.4). These experiments indicate that ouabain circulating in blood inhibits Na-K-ATPase in the eel gill at a concentration in the range of that necessary for inhibition *in vitro*. A much higher concentration is

required when ouabain is applied to the exterior of the gill and inhibition by external ouabain occurs only when the drug gains access to the interior of the fish.

The functional effect of complete inhibition of gill Na-K-ATPase was tested by measuring the efflux of Na^{22}, Cl^{36} and tritiated water in seawater eels after intraperitoneal injection of 0.25 mg of ouabain per 100g body weight. After ouabain the efflux of Na^{22} was reduced to nine percent. The efflux of Cl^{36} was similarly inhibited to 11 percent of normal. These changes could not be attributed to the general circulatory effects of ouabain since the efflux of THO was much less reduced, to 60 percent of normal.

Inhibition of gill Na-K-ATPase by ouabain in intact salt water eels thus results in almost complete inhibition of Na efflux. Surprisingly Cl efflux is markedly inhibited as well though in other systems Na-K-ATPase is not thought to participate in the active transport of Cl or its carrier-mediated diffusion. The results are consistent with the theory that the Na-K-ATPase of chloride cells faces inward, lining intracytoplasmic tubular channels, and contributing to a high concentration of NaCl within these channels that in turn enhances the outward transport of NaCl across the gill.

1974 #35

STUDIES OF RECTAL GLAND FUNCTION IN *Squalus acanthias* IN VIVO AND IN VITRO

RICHARD SOLOMON, LAURIE HAYS, SARA EPSTEIN, JOHN FORREST, JR., PATRICIO SILVA AND FRANKLIN H. EPSTEIN. HARVARD MEDICAL SCHOOL, BOSTON, MASSACHUSETTS; AND YALE UNIVERSITY SCHOOL OF MEDICINE, NEW HAVEN, CONNECTICUT

The role of the rectal gland in maintaining the chemical composition of the plasma of elasmobranchs is unclear. Following extirpation of the rectal