Table 1.--Myoglobin and Glycogen Content and Maximal Activity Levels of Enzymes of Energy Metabolism

	Ocean Pout		Sea Raven
	Heart	Skeletal muscle	Heart
Myoglobin (nmoles/g wet weight)	6.34 ± 2.89	--	64.1 ± 4.42
Glycogen (μmoles glucose/g wet weight)	86.11 ± 14.11	18.96 ± 2.69	33.99 ± 5.83
Hexokinase	2.45 ± 0.35	N.D.	2.52 ± 0.70
Phosphofructokinase	1.17 ± 0.15	2.51 ± 0.23	1.31 ± 0.11
Pyruvate kinase	36.34 ± 0.42	67.55 ± 5.9	--
Lactate dehydrogenase (.33 mM pyr)	41.34 ± 3.69	68.94 ± 2.99	83.68 ± 25.50
(10 mM pyr)	127.79 ± 4.16	177.4 ± 55	189.59 ± 66.91
Citrate synthase	12.78 ± 1.17	0.23 ± 0.01	--
α Ketoglutarate dehydrogenase	1.80 ± 0.13	N.D.	--
Cytochrome oxidase	34.64 ± 12.32	--	39.88 ± 8.97

Enzyme data are expressed as μmoles/gm wet weight·min at 10 C.

N.D. indicates not detected. The symbol - indicates that the assay has not been attempted. All values are expressed as mean ± S.E. on the basis of 3 - 8 individuals.

In conclusion, it is clear that the organization of energy metabolism in the ocean pout white heart is very different than that which occurs in white skeletal muscle. In keeping with the general findings from other systems the ocean pout heart appears to have a greater aerobic capability than the skeletal muscle; however, maximum rates of anaerobic glycolysis are higher in the skeletal muscle. The question still remains as to whether any quantitative differences exist between the red and white hearts. This problem is currently under investigation.

This work was supported in part by operating grants from the New Brunswick and Main Heart Foundations.

EFFECTS OF ETHACRYNIC ACID AND RELATED COMPOUNDS ON ACTIVE Cl SECRETION BY THE DOGFISH RECTAL GLAND

H. Clive Palfrey, Patricio Silva and Franklin H. Epstein, Department of Pharmacology, Yale University School of Medicine, New Haven, Ct., and Department of Medicine, Beth Israel Hospital, and Harvard Medical School, Boston, Ma.

"Loop" diuretics of the 5-sulfamoylbenzoic acid class, such as furosemide and bumetanide, inhibit cAMP-stimulated rectal gland secretion possibly by interacting with the putative NaCl cotransport system in the baso-lateral membrane (Silva et al., Amer. J. Physiol., 233: F298, 1977; Palfrey et al., Bull. MDIBL., 19: 58, 1979). The specificity of a series of related drugs of this type appears to be similar to that found for inhibition of Na/K/Cl cotransport in avian erythrocytes, and for diuretic activity in vivo in dogs (Palfrey et al., op cit.; Palfrey et al.,

Amer. J. Physiol., 238:C139, 1979). Recently, a chemically-distinct group of compounds, comprising ethacrynic acid (EA) and its -SH adducts, were tested on the avian erythrocyte system (Palfrey and Greengard, J. Gen. Physiol., 76:25a, 1980). The EA-(L)cysteine adduct was found to be about 50 times as effective as EA itself, in agreement with results obtained on perfusion of these two substances through isolated rabbit kidney tubules and observation of the corresponding reduction in transepithelial P.D., a reflection of active Cl transport in this preparation (Burg and Green, Kidney Int., 4: 301, 1973). Furthermore, it was found that the EA-(D)cysteine adduct was relatively inactive in the avian erythrocyte system, suggesting a stereospecific interaction of these agents with a putative "receptor," possibly the transport protein itself. In order to pursue the thesis that the rectal gland, kidney tubule and avian erythrocyte salt transport systems are similar, the effects of these compounds on active Cl secretion in the isolated perfused rectal gland were studied.

Rectal glands were dissected from adult dogfish, cannulated and perfused in the usual manner with dogfish Ringer's containing 0.05 mM dbcAMP and 0.25 mM theophylline (Silva et al., op cit.) to obtain a maximal secretion rate. Compounds to be tested were dissolved as 100 X stock solutions and diluted into the perfusate immediately prior to use. EA (Merck) stock solutions (50 mM) were made up in 100 mM $NaHCO_3$. To synthesize the (L)cysteine and (D)cysteine adducts, 50 mM solutions of these two amino acids were made up in 100 mM $NaHCO_3$ which had been flushed with N_2. These solutions were then mixed, in an N_2 atmosphere, with 50 mM EA solutions, in a ratio of 1.1:1 amino-acid:EA. The mixture was allowed to stand at room temperature for 1 hr then used immediately for an experiment. These conditions have been shown to lead to quantitative conversion of EA to the adducted forms, as judged by TLC (Palfrey, unpublished). Other compounds, MK-196 and dihydroethacrynic acid (DHEA) (Merck), were dissolved as described above.

EA-(L)cysteine proved to be a much more potent inhibitor of rectal gland secretion than EA itself or EA-(D)-cysteine. A typical experiment is shown in Fig. 1. Here, 10^{-4} M solutions of each of the three compounds were

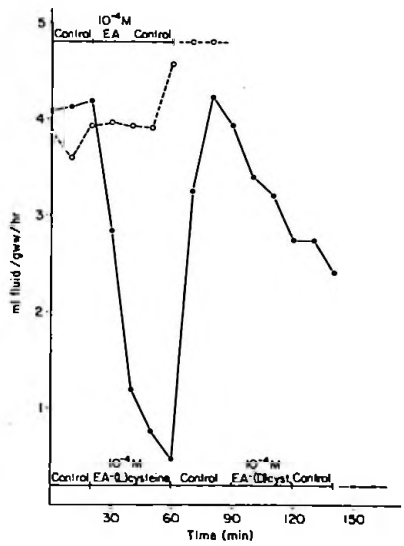

Figure 1

perfused for a 30-40 min period, collections of secreted fluid being made very 10 min. EA-(L)cysteine caused a profound reduction in both fluid and Cl secretion to about 12% of the control values; on replacement with drug-free Ringer's the control rate returned almost immediately. Subsequent perfusion with 10^{-4} M EA-(D)cysteine caused a slight decline in secretion rate, however, the control rate following drug removal was lower than the previous control, possibly indicating some deterioration in the gland. A separate experiment demonstrating the absence of an inhibitory effect of 10^{-4} M EA is also shown. It was found that EA only became effective in reducing fluid secretion at 10^{-3} M, and then only to about 50% of control values, confirming the results of Solomon et al (Bull. MDIBL, 18:13, 1978). At this concentration the saturated derivative of EA, DHEA, which has no -SH binding capacity, was about as effective. This observation, coupled with the ready reversibility of EA (Solomon et al., op cit.), suggests that EA is not binding to tissue -SH groups in inhibiting fluid secretion. A slightly different situation obtains in the avian erythrocyte, where EA is more effective than DHEA, but is "irreversible" (i.e., cannot be simply washed out, as can DHEA) implying an interaction with cellular -SH groups related to Na/K/Cl cotransport (Palfrey and Greengard, op cit.).

A preliminary attempt to define the dose-response relationship for inhibition of fluid and Cl secretion by EA-(L)cysteine suggested an IC_{50} value of $\sim 3 \times 10^{-5}$ M for this compound. This would make it about 30-40-fold more potent than the parent drug EA, in agreement with the differentials mentioned above for avian erythrocytes and perfused rabbit tubules. This data supports the hypothesis that the transport systems affected by these drugs in the three different tissues have similar properties. In this regard, it is of interest to note that we recently were able to demonstrate stoichiometric diuretic-sensitive movements of ^{36}Cl with cations following cAMP stimulation of avian erythrocytes (Palfrey and Greengard, unpublished results), directly analogous to results obtained with NaCl transporting epithelia.

CALCIUM AND RECTAL GLAND SECRETION

F.H. Epstein, J. Epstein, A. Stevens, M. Myers, R. Rosa, H.C. Palfrey and P. Silva, Charles A. Dana Research Institute, Harvard-Thorndike Laboratory of Beth Israel Hospital, Department of Medicine, Beth Israel Hospital and Harvard Medical School

Because calcium has beenshown to play a key role in stimulus-secretion coupling in a variety of tissues, we attempted to investigate its importance in stimulated chloride secretion of the isolated perfused rectal gland of Squalus acanthias, using pharmacological agents known to influence the cellular actions of calcium in other cells.

Rectal glands were perfused by the technique described by Solomon et al., (Bull, MDIBL 18:13-16, 1978). Duct fluid and venous effluent were collected every 10 minutes for a total of 60 - 90 min. Glands were usually stimulatedby the addition of 0.05 mM dibutyryl - cyclic-AMP and 0.25 mM theophylline to the perfusate. When vasoactive intestinal peptide was used to stimulate the gland, 10 μg were dissolved in 1-2 ml of shark Ringer's and injected directly into the artery of the gland at the previous rate of perfusion.

CALCIUM IONOPHORES

The divalent cation-selective ionophore A23187 was added to rectal gland perfusions at a concentration of 10^{-4}M (n = 3) or 10^{-5}M (n = 2), after 30 minutes of perfusion in the basal state, without producing a significant increase in chloride secretion. In two additional experiments, the concentration of calcium in the perfusate was increased from 2.5 mM to 10 mM during preliminary basal perfusion, and A23187 in a concentration of 10^{-6}M or 5×10^{-6}M was superimposed after 30 minutes. No change in rectal gland secretion was seen with the lower dose