OXYGEN CONSUMPTION OF THE DOGFISH RECTAL GLAND

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The past studies by Rosa et al. (Bulletin, MDIBL, 16:1976) have shown that there is a direct correlation between epithelial chloride transport and total oxygen consumption (QO_2) in the dogfish rectal gland. Following stimulation of the rectal gland with theophylline, dibutyryl cyclic AMP a marked increa in chloride secretion occurs with an increase in the QO_2 of this isolated tissue.

The purpose of this study was to measure the QO_2 of isolated tissue slices of the dogfish rectal gla under basal and theophylline, dibutyryl C-AMP stimulation and following ouabain inhibition of the Na/K ATPase.

Rectal glands were taken from spiny dogfish weighing 2 to 6 kg. Stadie-Riggs slices (200-500 µm thick) of the rectal gland were made and single slices were placed in 3 ml of Elasmobranch Ringers containing in mM:Na 290; k 5; Cl 270, HCO_3 8; urea 350; PO_4 1, Ca 2.5; mg 3; SO_4 0.5 and glucose 5 for basal QO_2 measurements. For QO_2 measurement following stimulation of chloride secretion 0.25 mM theophylline and 0.05 mM dibutyryl cyclic AMP were added to the Ringers solution. Ouabain was also added to the abo media to make a final concentration of 10^{-4} M.

The QO_2 was measured on adjacent slices using a chamber designed for corneas (Edelhauser, Exp. Eye Res. 19:317, 1974). Each chamber with 3 ml of Elasmobranch Ringer's contained 21.42 µl O_2 at 15°C and S conditions. This value was computed on the basis of an oxygen solubility at 15°C of 0.0340. Prior to Q determination, the incubation medium was equilibrated to an atmospheric PO_2 of 142 mm Hg resulting in a pH of 7.9. Three 20-minute QO_2 determinations were performed on each tissue slice, fresh Ringer's bein used for each measurement. Following QO_2 measurement, the rectal gland slices were removed from the chamber, blotted, weighed, and dried at 105°C for 24 hours to achieve a constant dry weight. The QO_2 wa then calculated on a dry weight as well as a wet weight basis.

Table 1 lists the measured QO_2 under basal and stimulated conditions. During the first 20-minute m urement, the QO_2 of the tissue slices in either the basal or stimulated media is similar; however, durin

Table 1

Oxygen Consumption (mean ± SE) of Shark Rectal Gland Tissue Slices+; Under Basal and Theophylline, Dibutyryl Cyclic AMP Stimulated States

	QO_2			
	µl O_2 mg^{-1} dry wt hr^{-1}		µMO_2 g^{-1} wet wt min^{-1}	
	Basal	Stimulated	Basal	Stimulated
0-20 minutes incubation	2.89 ± 0.31 (6)	3.60 ± 0.39 (7)	0.507	0.632
20-40 incubation	1.87 ± 0.19* (7)	2.81 ± 0.30 (8)	0.329	0.494
ouabain 10^{-4}M 40-60 minutes incubation	1.95 ± 0.26 (5)	2.18 ± 0.29 (7)	0.344	0.383

+wet wt = 18.06 mg; dry wt = 4.5 mg; % H_2O = 74.52 (N = 20)
( ) number of tissue slices
*Significantly lower than the 0- 20 min incubation period and the 20-40 min. TDb C-AMP stimulated period at $P<0.02$.

the second media change there is a significant decrease in the basal QO_2 but not in the Db C-AMP stimulated tissue. Following the addition of ouabain during the third incubation time period (40-60 min), there was no change in the basal QO_2; however, in the stimulated slice there is significant decrease from the 20-40 min time period.

The data from these QO_2 measurements illustrate that there is a basal non-transporting QO_2 in isolated dogfish rectal gland which can be increased following stimulation. These data also compare favorably with the studies of Rosa et al. (Bulletin, MDIBL 16:1976) who measured QO_2 by AV difference in isolated perfused rectal gland. The basal QO_2 from tissue slices of 0.3 to 0.5 μM O_2/min/gWW is similar to their data on whole glands. Following TDb C-AMP stimulation, the tissue slice values of 0.5 to 0.65 μM O_2/min/gWW are at the low end of the whole gland QP_2's but comparable. Ouabain inhibition lowered the tissue slice value to 0.38 μM O_2/min/gWW, a value similar to whole gland inhibition with ouabain.

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CELL VOLUME REGULATION BY SKATE HEART

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The little or hedgehog skate *Raja erinacea* is an imcomplete osmoconformer and, as such, is a useful model for studying mechanisms that regulate cell volume when interstitial fluids are osmotically perturned. This is a problem of interest both from an environmental and a clinical point of view. This elasmobranch is common in the outer estuaries of the American Atlantic coast from South Caroline to Nova Scotia and it has been collected at survey stations where salinities were as low as $20°/_{oo}$ (Fitz, E. S. Jr. and F. C. Daiber. Bull. Bing. Oceanogr. Coll. 18(3):69-97, 1963). In the laboratory they thrive well in sea water gradually diluted to half strength or lower causing plasma osmolality to drop 26-35%. In the current study on cardiac muscle we sought to differentiate between the osmoregulatory activities of atrial and ventricular fibers and to develop an in vitro method that would perhaps be useful later to examine transport kinetics, metabolic events and electrophysiology of the myocardium.

Methods. In vivo environmental dilution experiments were generally similar to those used in our earlier studies on the intracellular osmoregulatory role of amino acids and urea in skates and stingrays (Forster, R. P. and L. Goldstein. Am. J. Physiol. 230:925-931, 1976; Boyd, T. A., Chung-Ja Cha, R. P. Forster and L. Goldstein. J. Exp. Zool. 199:435-442, 1977). *Raja erinacea* of mixed sex with weights ranging from 1-2 Kg were collected by drag net from waters off Mount Desert Island. Gradual dilution of circulating sea water in the pool was accomplished by reducing the salinity by 10% per day down to 50% sea water, and the skates were then allowed to equilibrate for two days before samples were collected. Blood was taken from the caudal vessels and the skates then killed by swift transection of the spinal cord. The beating excised heart was left in a beaker of balanced isotonic elasmobranch medium gassed with 99% O_2 - 1% CO_2 (Forster et al. Comp. Biochem. Physiol. 42A:3-12, 1972) for 1-2 min until cleared of blood. The atrium and ventricle were surgically separated, the conus arteriosus removed, and the pericardium and adjoining supporting structures trimmed off. Samples of atrium and ventricle were quickly blotted lightly, weighed, placed in aluminum foil and frozen between blocks of dry ice. Tissue was ground to a powder by cold mortar and pestle on dry ice, and extracted. Extracts of tissue for analysis of ninhydrin-positive substances (NPS) were prepared by adding one volume of cold 50% trichloracetic acid (TCA) and 8 volumes of cold distilled water; then mixed, allowed to stand on ice for 30 min, and centrifuged. TCA was extracted 3 times with 3 volumes of hydrated ether. Plasma extracts were similarly prepared using equal volumes of plasma and 10% TCA. NPS determinations were made by the photometric method of Moore & Stein (J. Biol. Chem.