DETERMINATION OF THE OXYGEN COST OF CHLORIDE TRANSPORT IN THE RECTAL GLAND OF THE DOGFISH *Squalus acanthias*

Robert M. Rosa, Patricio Silva, Katherine Spokes, Barbara Kent and Franklin H. Epstein, Department of Medicine and Thorndike Laboratory of Harvard Medical School at Beth Israel Hospital, Boston, Massachusetts, Department of Surgery, Mount Sinai School of Medicine, New York.

Determination of the oxygen cost of electrolyte transport across epithelia has preoccupied physiologists for many years, but is beset by many problems. Although transporting epithelia expend energy to move electrolytes and water across the cells, they also require energy to maintain cellular structure. Total oxygen consumption of transport cells is thus the sum of "basal consumption" and that additional oxygen required for transport. Further problems arise from the fact that some epithelia actively transport more than one electrolyte. The determination of oxygen tension is not easy to perform in live animals where the measurements have to be performed by catheterizing both artery and vein of the organ in question, a task compounded by the presence of red cells which narrows the arteriovenous oxygen difference, thereby increasing the probability of error. Finally, the rate of electrolyte transport in most organs can be varied only over a narrow range, thus increasing the difficulty of obtaining meaningful correlations between oxygen utilization and electrolyte transport.

The isolated perfused rectal gland model can be used advantageously to study the relation between oxyten consumption and electrolyte transport. The rectal gland can be perfused in vitro and remains viable for long periods of time. It actively transports chloride into the glandular lumen accompanied by sodium and very small quantities of other electrolytes. The gland is perfused with an artificial medium lacking red cells, through a single artery, and mixed effluent can be sampled from a single vein. Furthermore, the rate of electrolyte transport can be varied at will by stimulating the gland with theophylline, dibutyryl cyclic AMP, or both.

Rectal glands were taken from spiny dogfish weighing 2 to 6 kg. The rectal gland artery, vein and duct were cannulated using PE90 tubing. The glands were then placed in a plexiglass and aluminum chamber kept at 15° to 17°C by running seawater. Perfusion was maintained by gravity at a pressure of 4 mm Hg. The perfusion medium contained in mM: Na 290; K 5; Cl 270; HCO_3 8; urea 350; phosphate 1; Ca 2.5; Mg 3; sulfate 0.5; pH 7.6, when gassed with 99% O_2 1% CO_2. Glucose 5 mM was used as the sole exogenous substrate. Collections of arterial perfusate were obtained through a self-sealing rubber connector close to the arterial cannula. Rectal gland-vein collections were taken anaerobically from the venous catheter. Ductal fluid was collected in 1.5 ml conical centrifuge tubes. Oxygen determinations were made using a polarographic oxygen electrode with a water jacket kept at 15°C. Chloride determinations were done in a Buchler Cotlove Chloridometer. Sodium and potassium measurements were made in an IL343 flame photometer.

The oxygen tension of the arterial perfusate ranged from 300 to 500 mm Hg. Arteriovenous oxygen difference varied from 30 to 150 mm Hg. The rectal gland secretes an average of 6.38 ± 3.57 μEq of mm Hg. The rectal gland secretes an average of 6.38 ± 3.57 μEq. of Cl-/min/g wet weight in the resting state. At this rate of secretion the oxygen consumption averages 0.41 ± 0.19 μM of O /min/g wet weight ranging from 0.10 to 0.62. When the chloride secretion of the gland is stimulated by the addition to the perfusion medium of 0.25 mM theophylline and 0.05 mM dibutyryl cyclic AMP the chloride secretion rate increases to 21.56 ± 5.77 and concomitantly the oxygen consumption rises to 1.12 + 0.42 ($p < 0.005$). In three experiments oxygen consumption was measured during a control resting period and again after stimulation with 0.25 mM theophylline

and 0.05 mM dibutyryl cyclic AMP. Chloride secretion rose from 5.87 ± 1.86 to 20.74 ± 4.42 μEq/min/g wet weight. Oxygen uptake increased 0.54 ± 0.33 μM O_2/min/g wet weight rising from 0.47 ± 0.11 during the control resting period to 1.01 ± 0.37 during stimulation.

Figure 1 shows the relation between oxyten consumption and chloride secretion in 12 rectal glands during resting periods and also after stimulation with theophylline and cyclic AMP. The graph shows a clear linear relation between both variables. The slope of the curve indicates that for each μM of O_2 consumed 20.0 μEq of Cl^- are secreted. This figure is remarkably similar to that found in other transporting epithelia like the kidney. The intercept of the line is not different from the origin, suggesting that the basal oxygen consumption in the nontransporting state is negligible.

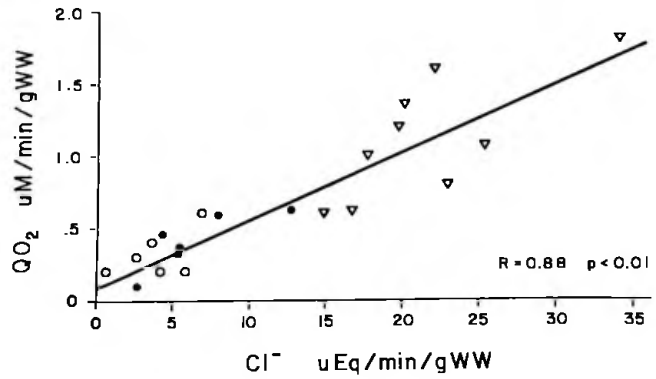

Figure 1. There is a clear linear relation between oxygen consumption and chloride secretion in the isolated perfused rectal gland. Both chloride secretion and oxygen consumption increase when the gland is stimulated with 0.25 mM theophylline and 0.05 mM dibutyryl cyclic AMP, and decrease when secretion is inhibited with ouabain 0.1 mM. ● resting state, ▽ stimulation with theophylline and dibutyryl cyclic AMP, O ouabain 0.1 mM after stimulation.

The addition of ouabain 10^{-4} M to the perfusate reduced both chloride secretion and oxygen consumption to the same extent. Chloride secretion fell from 21.97 ± 6.69 to 3.93 ± 2.05 μEq/min/g wet weight and QO_2 from 1.17 ± .46 to 0.55 ± 0.46 μM O_2/min/g wet weight ($p < 0.005$). After inhibition with ouabain, the points relating chloride secretion with oxygen utilization fell to the same range as those seen during resting conditions (Figure 1), illustrating that ouabain does not inhibit resting secretion in the rectal gland.

These experiments demonstrate that oxygen utilization is linearly related to chloride secretion in the rectal gland. The cost of chloride secretion calculated from the slope of the line in Figure 1 is 20.0 μEq/μM of O_2, a figure remarkably close to that found for sodium transport in the kidney. It is a very interesting fact that in the isolated perfused rectal gland, no basal or nontransporting oxygen consumption could be demonstrated.