Reconstitution of Squalus acanthias Chloride Transport into Liposomes

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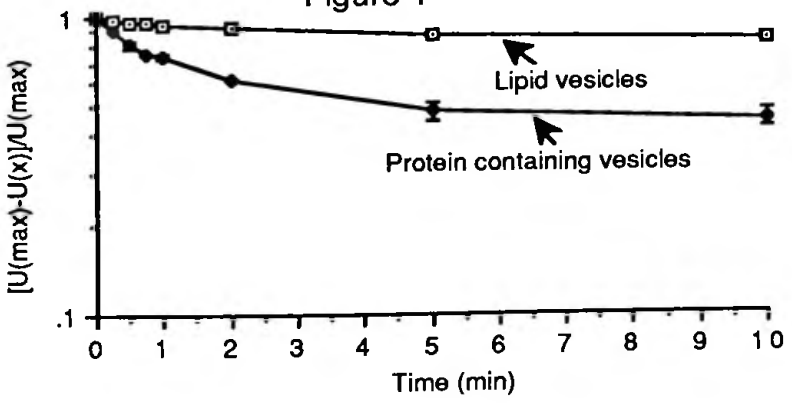
Squalus acanthias lives in an external environment with a markedly higher salt concentration than that of its own body fluids. To maintain normal internal salinity, the shark excretes excess NaCl via the rectal gland. This requires the movement of large amonts of NaCl across the rectal gland epithelial cells against a concentration gradient, with Na transport via the Na,K-ATPase as the active step and passive chloride movement with Na to maintain electrical neutrality. This chloride flux requires both basolateral and apical chloride transport mechanisms, making the squalus acanthias rectal gland a potentially rich source of chloride transport proteins for physiologic study and possibly for isolation. We chose to incorporate membrane proteins into liposomes in an attempt to study chloride transport without the internal milieu of the cell. This report describes the preliminary results of these experiments.

Membranes from Squalus acanthias rectal gland were prepared by differential centrifugation and stored at -70°C until usage. Liposomes were prepared by the detergent/dialysis technique of Racker (Racker, E. Methods in Enzymology, Vol LV. pp 699-711) using a mixture of phosphatidyl choline: cholesterol: dicetyl phosphate (5:2:0.2) previously shown by us to be relatively impermeant to anions (unpublished data). The lipids were sonicated into solution in the presence of the non-ionic detergent octyl glucoside in a buffer containing 280 mM NaCl, 5 mM KCl and 10 mM MES at pH 6.0. They were then dialyzed in a 10,000 molecular weight cut off dialysis bag for 48 hours against 280 mM NaCl, 5 mM KCl, 10 mM MES, pH 5.0 with two 1 liter bath changes at 4°C. If proteins were to be incorporated into the vesicles, tehn they were added to the detergent/lipid mixture after sonication at a ratio fo 1:0.35:0.0025 (detergent:lipid:protein). The vesicles were harvested and stored on ice until usage. Chloride flux was measured by adding ^{36}Cl at time zero and measuring uptake of chloride into the vesicles at time x by rapidly passing the vesicles over an ice cold anion exchange column (AG1 x 8 - BioRad) to remove any extravesicular chloride. The column effluent was then counted on a scintillation counter. Any inhibitor to be tested was added 10 minutes prior to the beginning of the experiment. All uptakes were done at room temperature with 280 mM NaCl and 5 mM KCL on both sides of the vesicle. Maximum uptake of ^{36}Cl by the vesicles was determined by adding the chloride ionophore, chlorotriphenyl tin, and incubating for 30 minutes.

Uptake of ^{36}Cl into the liposomes was linear with a $t_{1/2}$ of approximately 120 minutes. However, when rectal gland membranes were solubilized and reconstituted into the liposomes, there was an initial rapid uptake of chloride with a slower second component comparable to the rate of uptake in

Figure 1



lipid vesicles (figure 1). The $t_{\frac{1}{2}}$ of the rapid component is approximately 45 seconds. Several inhibitors of the chloride channel which have different mechanisms of action were tested on the lipid and protein containing vesicles. At 0.1 mM; furosemide, DIDS, and 9-anthracene carboxylic acid (which are known to inhibit the K:Na:2Cl cotransporter, the red cell Cl/HCO_3 exchanger, and the tracheal epithelial Cl channel, respectively) were not effective in inhibiting the rapid rate of chloride flux into the protein containing vesicles.

The incorporation of squalus acanthias rectal gland proteins into liposomes produced a rapid component of chloride uptake which was not inhibited by any of the chloride channel inhibitors tested. Previous experiments incorporating red cell membrane proteins into liposomes suggest that incorporation of non-chloride transporting proteins into liposomes does not result in an increased rate of chloride uptake. Combined, these data suggest that while a chloride transporting protein was successfully incorporated into these liposomes, inhibitor binding was prevented (either by inside-out incorporation or a detergent effect on the channel) or the appropriate inhibitor was not tested. Further studies with modification of the reconstitution protocol and testing of higher affinity inhibitors are warranted.

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