PREPARATION OF LENS FIBERS MEMBRANE VESICLES OF DOGFISH *SQUALUS ACANTHIAS* EYES

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Information currently available concerning the synthesis, functional capacity and turnover of transport proteins in the lens is limited as very few membrane carriers have been identified and none have been characterized and studied in isolation in artificial membrane systems (Alcala, J. and H. Maisel: *In* "The Ocular Lens, Structure, Function and Pathology", H. Maisel, editor, New York, Marcel Dekker, 1985, pp 169-222). Majority of the interest has been focused on the lens epithelium; this is possibly due to the sparse distribution of other proteins in the fibers with the exception of the main intrinsic polypeptide (MIP26) (Beneditti, E.L., Dunia, I., Bentzel, C-J., Vermoken, A.K.M., Kibbelaar, M. and H. Bloemendahl: Biochim. Biophys. Acta 457:353, 1976).

The lens of the eye is comprised of the epithelium, cortex and nucleus. The nucleus contains fetal lens fibers overlaid by adult lens fibers, the youngest ones being in the area of the cortex and lying adjacent to the epithelium. The lens fibers are very closely packed to limit light scattering, the distance separating adjacent cells is 100-200 A° so the extracellular spaces are small and composed of long clefts that are open to the aqueous and vitreous. These restricted clefts create a high-resistance pathway for diffusion and current flow. The cells of the lens exhibit extensive cell-to-cell coupling by gap junctions (A. I. Cohen: Invest. Opthalmol. (Copen) 37: 36, 1959b).

Abnormal electrophysiologic factors in the formation of cataracts disrupts the ability of the lens to regulate cell fiber permeability as well as causes the loss of integrity of cell-to-cell communication through gap junctions. It is of evolutionary interest to understand the lens of the shark in comparison to the lenses of vertebrates. It has been shown that despite vast differences in habitat, the shark lenses are remarkably similar to the mammalian species; the structural lens proteins found in the shark are conserved in higher species (Bloemendahl, H. and W.W. deJong: TIBS, 4: 137, 1979). In addition, aging causes the formation of the senile cortical cataract (Bellows et al.: Cataract and abnormalities of the Lens, pg. 303. Bellows, J.G., Ed., Grune and Stratton, NY, 1975). The opacification of lens tissue, an inevitable consequence of aging, has also been known to be precipitated by radiation, excessive amounts of glucocorticoids, and high blood glucose levels as seen in individuals with diabetes mellitus. It is for these medically relevant ends that the function of the fibers with respect to glucose and ion transport must be investigated.

The primary aim of our experiments was to determine whether viable vesicles could be produced from lens fibers of *Squalus Acanthias* and whether these vesicles would transport glucose. The lens fibres from the cortex were used for our procedures.

Spiny Dogfish *Squalus acanthias* of either sex were caught by trawling off the coast of Maine during July and August. After capture they were held for no more than four days until use. Only eyes that

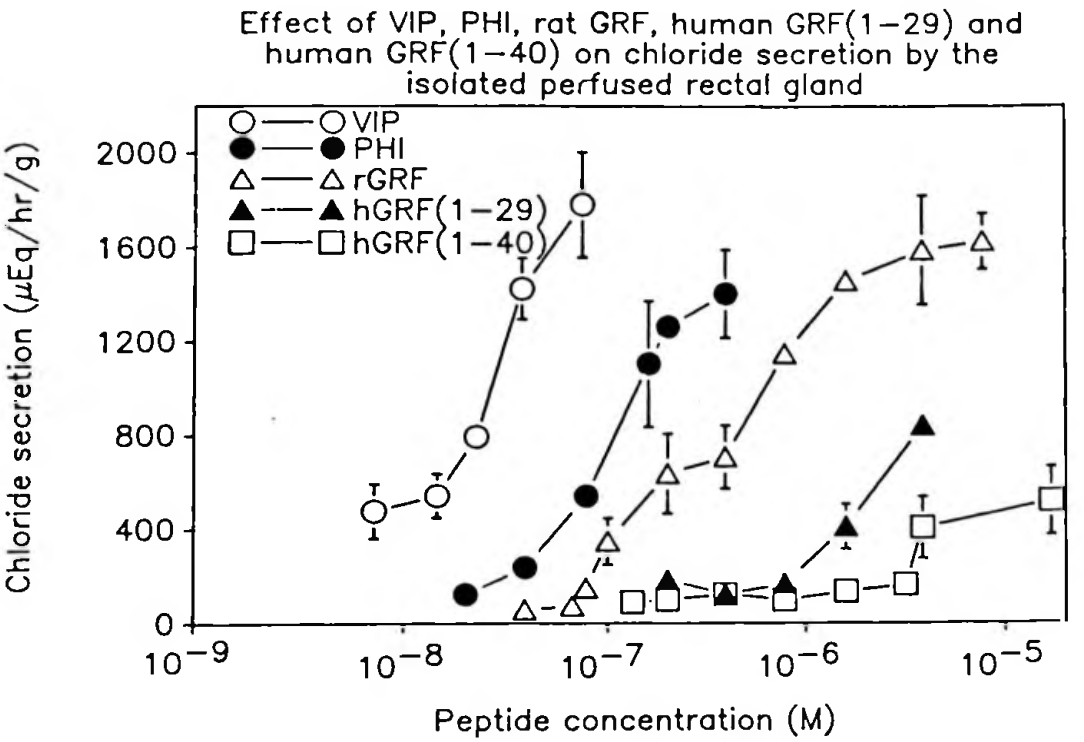
Effect of VIP, PHI, rat GRF, human GRF(1–29) and human GRF(1–40) on chloride secretion by the isolated perfused rectal gland
VIP
PHI
rGRF
hGRF(1–29)
hGRF(1–40)
Chloride secretion (μEq/hr/g)
2000
1600
1200
800
400
0
10⁻⁹
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
Peptide concentration (M)

Figure 1

appeared healthy on visual inspection were used.

Enucleated eyes were dissected and the lenses were carefully separated from the ciliary body and iris. The epithelium of the lens was discarded and the cortex was used for vesicle preparation. Approximately 20 lenses were homogenized (cold) at full speed in a Waring blender in 120 ml of ice cold 500 mM Tris pH 8.0 for 30 sec. The homogenate was poured into centrifuge tubes and the hard, unblended cores were discarded. The cell contents were separated from the plasma membranes by centrifugation in a refrigerated Sorval at 1000 x g for 15 mins. The supernatant was saved. The pellet was washed once with buffer and then discarded. The two supernatants were combined. The plasma membranes were obtained by centrifugation in a refrigerated Sorval at 17,5000 x g for 20 mins. The pellet, washed twice with Tris buffer to decrease the crystallines, finally was suspended in vesicle buffer (0.2M mannitol, 0.001M $Mg(NO_3)_2$, 0.02M HEPES).

The uptake of [^{14}C] glucose in isolated vesicles was measured by the rapid filtration method (first described by Hopfer at al. 1975). After isolation the vesicles were suspended in a small volume of vesicle buffer (0.2M Mannitol, 0.001M $Mg(NO_3)_2$ and 0.02M HEPES adjusted to pH 6.5 with Tris) and used immediately for transport studies. Glucose uptake was measured by rapid filtration technique in a medium containing 0.2M Mannitol, 0.001M $Mg(NO_3)_2$, 0.02M HEPES pH 6.5 and 170 mM NaCl (170 mM KCl for sodium free) and 10 µCi D glucose (or L-glucose) with a final concentration of 0.1 mM D-glucose (or L-glucose). Uptake was measured at 15°C where 50 µl of vesicles in vesicle buffer were added to 100 µl of transport medium. The uptake was terminated after periods of 15", 1', 1'45" and 2'30" by diluting 20 µl of the reaction mixture into 1 ml of ice cold stop solution (0.24M Mannitol, 0.2M KNO_3, 0.0012M $Mg(NO_3)_2$ and 0.02M HEPES pH 6.5). After dilution the membrane suspension was rapidly filtered through a filter which was kept under vaccum (Millipore HAWP, pore size 0.45 µ); the filter was washed once with 3.5 ml stop solution. For the controls vesicle buffer was substitued for membranes. The readings were obtained by liquid scintillation counting of the filters dissolved in Optifluor (R) (Packard Instruments). The controls were subtracted from the time points to correct for the background.

The final pellet obtained from the lens was white and slightly sticky. Electron micrographs of the vesicles showed circular sealed vesicles approximately 2000 A° in size. The uptake of [^{14}C] D-glucose increased proportionally at every time point from 15" to 2'30" to 90' (equilibrium). The uptake of [^{14}C] glucose when measured as a function of pH, was found to double from pH 8.1 to 6.5 throughout all the time points and at equilibrium. To verify whether the vesicles were sealed and to distinguish between uptake and passive diffusion, the uptake of both [^{14}C] D and L-glucose was measured. The uptake of L-glucose was less than 20% of the value obtained for D-glucose.

These studies indicated that (a) membrane vesicles could be prepared from shark lens fibers, (b) they were viable, (c) glucose uptake was measured, it was not passive diffusion and uptake increased from 15" to equilibrium, (d) glucose uptake was favored by acid pH and (e) the uptake was specific for D-glucose. These experiments show that the fibers do transport sugar and that there is a carrier for glucose in the fibers.

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