1971), however it has not been successfully tested in intact fishes to ascertain its functions on ion translocation in the gills because of technical difficulties. The findings reported here of a muscarinic receptor indicate that this transmitter has a definite function in cell and internal medium osmoregulation in the teleost fish. Support for this work by the following funds is gratefully acknowledged: Student fellowship for George M. Rowing of the Science Research Council of Great Britain and NIH Research grants GM 25002 and EY 07009.

SATURATION OF THE OCULAR TRANSPORT MECHANISM FOR D-GLUCOSE IN THE SPINY DOGFISH, *Squalus acanthias*

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Work on the transport properties of the ocular barriers of the dogfish was continued. As regards D-glucose transport from the blood to the ocular humor, if one postulates a "carrier-mediated" facilitated diffusion transport mechanism, certain classical criteria can be tested. A number of these criteria, namely that D-glucose transport is downhill and faster than it should be on the basis of molecular size and lipid solubility, and that glucose transport is stereospecific with D-glucose being greatly preferred to its L-isomer, have been previously reported by us in this bulletin (Bull. MDIBL 17:106-110, 1977). This summer we addressed ourselves to the remaining question of whether this transport system is saturable.

Essentially our method consisted of elevating the blood D-glucose level, via intravenous infusion of unlabeled D-glucose solutions, for a period of 30 minutes and subsequently determining the cold glucose level at 10-minute intervals from plasma samples obtained; with the circulating plasma at a relatively stable, elevated glucose level, we then performed our determination of ocular D-glucose transport rate utilizing ^{3}H D-glucose and methods previously described (Bull. MDIBL 17:106-110, 1977).

Dogfish weighing from 1.7 to 4.9 kg were restrained on a wooden rack, head submerged nearly flat in tank of freshly flowing native seawater. The dorsal aorta was cannulated and fitted with a 2-way stopcock allowing free access to the dogfish plasma (the fish was heparinized to avoid clotting). The cold D-glucose solutions were infused using a constant delivery volume peristaltic pump which was calibrated, and run at pumping rates of .005 to .020 ml/min. We took care to infuse no more than 3 ml of solution/kg of fish during the whole course of the experiment in an effort to not excessively raise the plasma volume. When the total volume of blood samples withdrawn are considered, the infusion elevated the plasma volume only approximately 2-3%. At 10-minute intervals, 100 µl samples of whole blood were withdrawn and the concentration of D-glucose determined by the enzymatic colorimetric procedure using glucose oxidase and peroxidase supplied in kit form by Sigma Chemical Co. When the plasma glucose was sufficiently elevated, the pumping rate was lowered to a maintenance level and a final blood sample taken from which we could estimate the cold glucose concentration before injection of the labeled D-glucose. We also determined the cold D-glucose concentration at the end of the ocular rate transport determination and thus were able to estimate a mean D-glucose concentration, $\bar{c}$, during a particular experimental period for the determination of the ocular rate transport constants, Ki and Ko.

After the D-glucose infusion period, a bolus injection of (^{3}H) D-glucose was introduced into the central plasma compartment of the test animal at time 0. At intervals during the experimental period beginning with time 0, small samples of blood were taken and the concentration of radioactively labeled test substance determined. The times used were roughly: 2, 4, 6, 9, 12, 15, 20, 25, 30 minutes for a 30-minute experiment. From these data a mathematical description of concentration as a function of time was constructed having the form:

$$C_p = A + Be^{-b_2 t} + Ce^{-b_1 t}$$

The constants A, B, C, b_1, b_2 were determined and served to define the function for the test period from 0 to t. At the end of the test period, the animal was sacrificed and the concentration of test substance in the ocular humors determined via liquid scintillation counting. In the dogfish, mean concentrations C_a and D_v were determined (the aqueous and vitreous humors); the vitreous was further divided into posterior, middle and anterior regions giving more specific information. With the determined plasma function and the particular ocular concentration at the end of the test period, use was made of a computer solution to the model equations to calculate K_i and K_o, the net entering and exiting rate constants for that particular experiment. The model considers three types of transport; passive, active and bulk absorption. The rate equation resulting from our model is:

$$\frac{dCa}{dt} = K_d C_p - K_d C_a + K_f n C_p - K_f C_a = K_i C_p - K_o C_a$$

where C_a and C_p are concentration variables for plasma (P) and ocular humor (A); K_d is the diffusion rate constant; K_f is the secretion constant which is assumed to be equal to the bulk absorption constant since the eye compartment volume remains relatively constant. The concentration of newly secreted fluid is taken to be nC_p, which is a simple linear function of plasma concentration.

Both L and D-glucose, are not actively secreted into the ocular compartments; therefore n=1 and $K_i=K_o$. Thus, knowing $C_p(t)$, one can derive an expression for $C_a(t)$ and calculate K_i and K_o from specific knowledge of C_a, C_p at a particular time. Details can be found in last year's bulletin as mentioned.

Thus, experiments were performed which give an estimate of the blood-ocular ^{3}H D-glucose transport rate at various plasma levels ($\bar{c}$) of unlabeled D-glucose. Figures 1 and 2 illustrate the results of these experiments. The mean plasma D-glucose concentration without infusion was found by us to be 8.6 ± .05 mM.

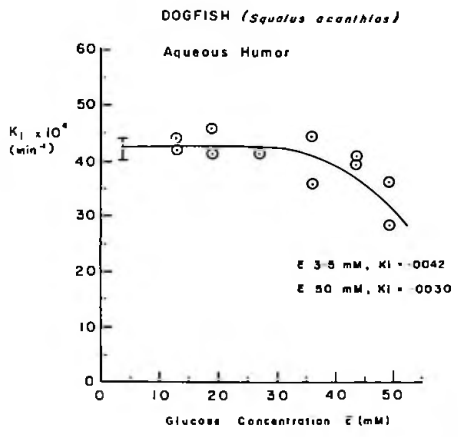

Figure 1. Aqueous humor D-glucose rate transport constant (K_i) vs. plasma glucose concentration ($\bar{c}$) in *Squalus acanthias*.

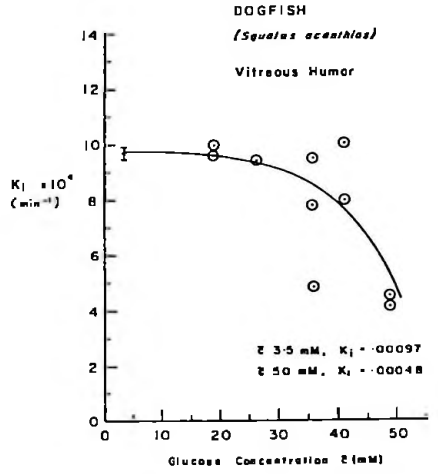

Figure 2. Vitreous humor D-glucose rate transport constant (K_i) vs. plasma glucose concentration ($\bar{c}$) in *Squalus acanthias*

Without infusion, the rate transport constants Ki, for D-glucose was .0042 ± .0002 min^{-1} for 6 fish; this is indicated by a bar point. The remaining points represent ocular rate constant determinations in individual experiments in which various infusions resulted in elevated D-glucose levels. Up to 35 mM D-glucose, which is almost 10 times the resting level, the Ki remains essentially unchanged and D-glucose transport rate is proportional to the plasma-ocular concentration difference. Beyond 35 mM, the rate constant Ki begins to decrease with $\bar{c}$ indicating a loss of linearity and that transport is no longer solely dependent on concentration difference. This indicates that some maximum rate of

D-glucose transport will be reached since as $\bar{c}$ increased, Ki decreases. Thus, our work indicates that both the aqueous and vitreous humors, the D-glucose transport mechanism is saturable. Although time limited the number of experiments (we would have liked more points at the higher glucose concentration), our data suggest that the vitreous barrier is more easily saturable as indicated by a 53% drop in the vitreous humor rate constant from resting to 50 mM. D-glucose concentration, apposed to only a 28% drop in the aqueous humor rate constant for the same range of plasma D-glucose concentrations.

In conclusion, our work to date indicates that in the dogfish D-glucose is transported into the aqueous and vitreous humors by a mechanism of downhill facilitated diffusion which is stereospecific and saturable. These results are not inconsistent with a stereospecific membrane bound carrier molecule model and perhaps similar to that postulated to exist at the level of the blood-brain barrier. These investigations were supported by NIH Research Grant EY 1340 and Training Grant EY 07009.

INHIBITION OF CHLORIDE SECRETION BY PROLACTIN IN THE ISOLATED OPERCULAR EPITHELIUM OF *Fundulus heteroclitus*

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Fresh water adaptation in teleost fishes depends on the endocrine control of osmoregulation (Hirano and Mayer-Gostan, VIII Int. Symp. Comp. Endocrinol., Amsterdam, 1978). Prolactin appears to be the main hormone involved, since hypophysectomized *Fundulus heteroclitus* perish (Burden, Biol. Bull. 110:8-28, 1956), prolactin keeps them alive after hypophysectomy (Pickford and Philips, Science 130: 454, 1959; Pickford, Robertson and Sawyer, Gen. Comp. Endocrinol. 5:160, 1965) and it stops the excessive loss of salts through the gills (Mayer, Bull. Inf. Sci. Techn. CEA 146:45, 1970). Prolactin effects take many hours to become operative, and consists in a reduction of membrane permeability. The actions of prolactin are permissive and require the presence of other hormones.

Prolactin effects were tested on the chloride secretory process of the isolated opercular epithelium of *Fundulus heteroclitus* (Karnaky et al. Science 195:203, 1977). Electrical properties and chloride fluxes were compared in operculi obtained from 3 groups of *Fundulus heteroclitus*: (1) adapted to seawater, (2) adapted to seawater receiving prolactin, and (3) fresh water adapted fishes. Ovine prolactin NIH P.S. 9 was dissolved in Ringer's and injected for 2 to 3 days (5 µg/gr body weight), controls were injected with 2.5 µl/gr body weight of Ringer's. Blood samples were collected in some fishes for Na and Cl plasma determinations. Dissection, electrical and flux determinations followed published methods for the opercular epithelium (Degnan et al. J. Physiol. 271:155, 1977). In preparations in which there was no resting potential after treatment, electrical resistance was determined by a small imposed potential difference.

Prolactin induced known changes in plasma electrolytes. Blood Na and Cl were respectively 152.6 ± 7.4 and 156.6 ± 17.9 in controls while in the experimental fishes Na was 172.4 ± 14.3 and Cl 174.2 ± 9.85.

The changes in fluxes of chloride and electrical parameters are shown in Table 1. Prolactin induced a very significant decrease ($P < 0.01$) in the transepithelial potential associated with an increase in electrical resistance of the membrane ($P < 0.02$) after the prolactin treatment. The difference between seawater adapted fish and fresh water adapted was highly significant ($P < 0.001$) for both the transepithelial P.D. and resistance of the membrane.

The changes observed in the transepithelial potential difference are the result of a large decrease in chloride secretion (Table 1). The control chloride fluxes are comparable to those reported before for this preparation, but under treatment with prolactin the net chloride flux is abolished at