55:113-119, 1978). The above results are also in accordance with the hagfish kidney working as a filtering as well as a non-filtering kidney depending on its blood pressure in relation to volume regulation. Supported by DFG and DAAD.

TRANSPORT OF D-GLUCOSE IN THE ARCHINEPHRIC DUCT OF THE ATLANTIC HAGFISH (Myxine glutinosa)

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After in vivo experiments had shown that D-glucose is reabsorbed despite zero net fluid reabsorption (Alt, J.M. et al., J. Exp. Biol. 91:323-330, 1981), we were able to demonstrate that a sodium-dependent D-glucose transport system is present in the archinephric duct of the early vertebrate Myxine glutinosa (J. Floege et al., Bull. MDIBL 21:86-88, 1981). We now were interested to see whether this transport mechanism shows features similar to those detected in higher vertebrates, i.e., inhibition by phloridzin, electrogenicity and stimulation of the sodium-influx by D-glucose.

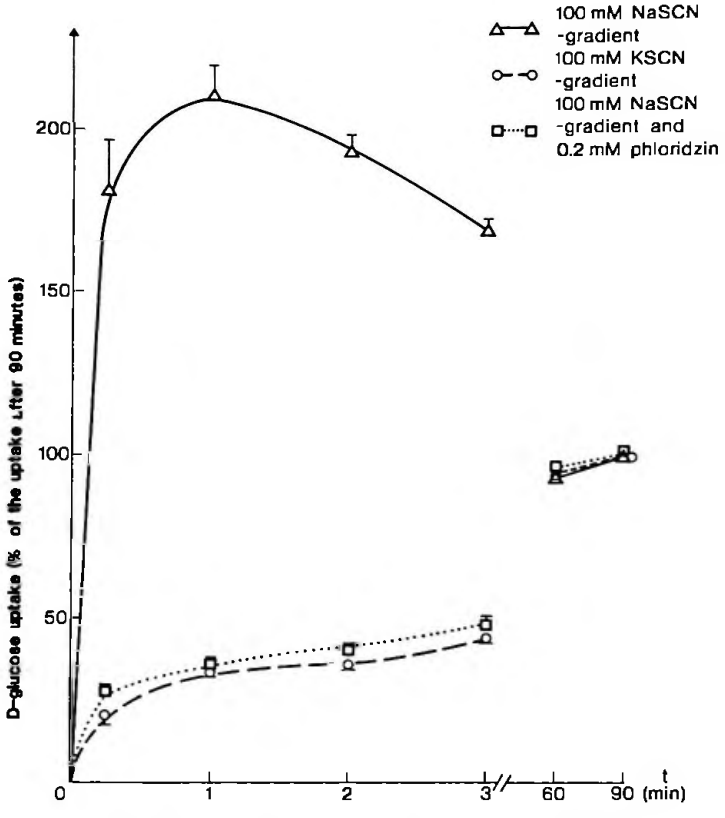

Figure 1: Sodiumdependency and inhibition by phloridzin of the D-glucose uptake at 25°C. Mean values ± SEM of 4 experiments. Membranes prepared by method 2.

Atlantic hagfish were caught near St. Andrews, Canada, and maintained in running seawater tanks at a temperature of 4° C until used. After killing the fish by decapitation the archinephric ducts were excised and placed on dry ice until the membrane preparation. Brush border membrane vesicles were prepared at 4°C by a modified calcium precipitation method (J. Floege, H. Stolte and R. Kinne: unpublished). Protein was determined by the method of Lowry et al. (J. Biol. Chem. 193:265-275, 1981), using bovine serum albumin as standard. Enrichment of brush border membranes and contamination by basolateral membranes was controlled by measuring the specific activity of the marker enzymes (J. Floege et al., Bull. MDIBL 21:86-88, 1981) alkaline phosphatase (brush border membranes) and Na^+K^+ATPase (basolateral membranes).

Uptake of ^{3}H-D-glucose and ^{22}Na were followed by the rapid filtration technique originally described by Hopfer et al. (J. Biol. Chem. 248:25-32, 1973). All reactions were carried out at 20°C. Composition of the incubation media are given in the figure legends. All values presented below were corrected for a filter blank that was obtained by adding vesicle buffer instead of membranes to the incubation medium. Results are expressed as pmoles/mg of protein using the specific activity determined in each sample by pipetting 5 µl of the incubation medium directly into the scintillation vials.

Results

The isolated brush border membranes showed a 7.0-fold enrichment in alkaline phosphatase and a 1.5-fold enrichment in Na^+K^+ATPase.

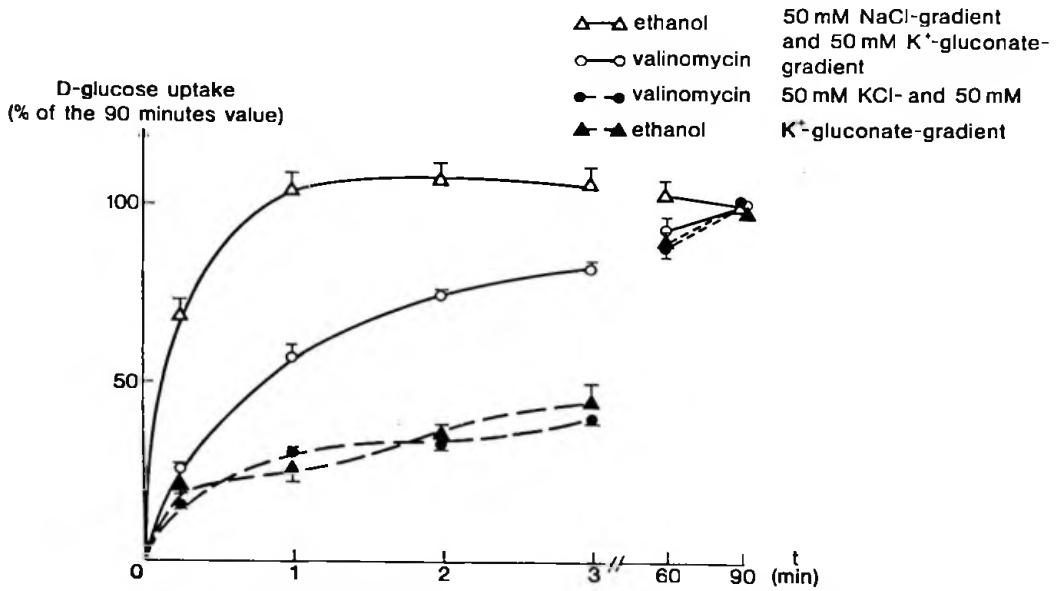

Figure 2: Effect of valinomycin on the uptake of D-glucose (concentrations of valinomycin and ethanol - see text). Mean values ± SEM of 3 experiments.

As shown in Figure 1 the uptake of D-glucose into the brush border membrane vesicles was enhanced about 7-fold by an outside to inside 100 mM sodium thiocyanate gradient as compared to a 100 mM potassium thiocyanate gradient. If a sodium gradient existed, but 0.2 mM phloridzin was added to the incubation medium, the D-glucose uptake did not show an overshoot phenomenon and the values are only slightly higher than the values in the presence of a potassium gradient.

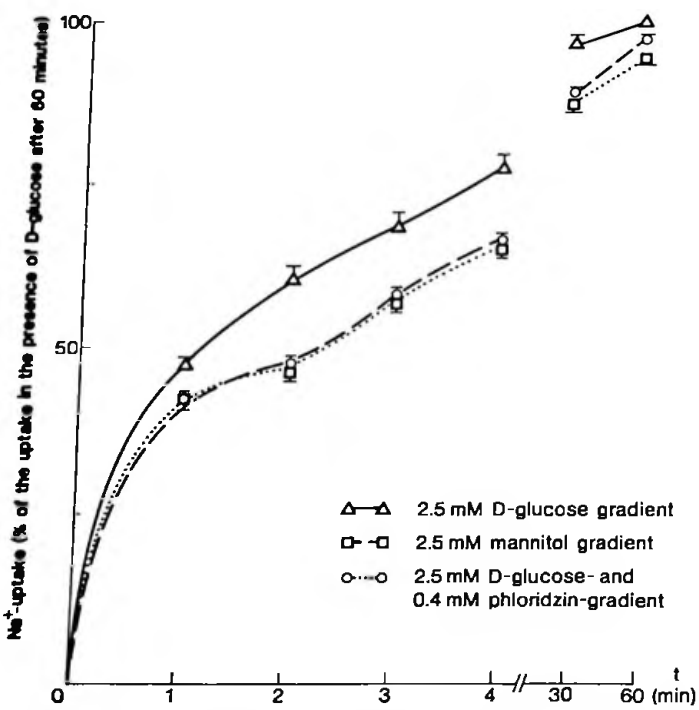

Figure 3: Stimulation of sodium uptake by a 2.5 mM gradient of D-glucose. Mean values ± SEM derived from 4 experiments.

The electrogenicity of the D-glucose sodium cotransport mechanism was examined by use of valinomycin: to 0.45 µl membrane suspension either 0.3 µl valinomycin (10 mg/ml ethanol) or 0.3 µl ethanol were added about 10 minutes prior to the start of the experiment. As shown in Figure 2 the D-glucose uptake decreased markedly in the presence of valinomycin as compared to ethanol. No such effect was observed if the incubation medium did not contain sodium.

Figure 3 compares the uptake of sodium into the vesicles in the presence of D-glucose, mannitol or D-glucose and phloridzin. A significant enhancement of the sodium influx into the vesicles by D-glucose can be detected, whereas no statistically significant difference was observed between the sodium uptake in the presence of a D-glucose-phloridzin-or mannitol-gradient.

The results obtained in our studies show that: -the uptake of D-glucose into the brush border membrane vesicles strongly depends on the presence of sodium in the extravesicular medium; -the effect of sodium can be nearly completely abolished by 0.2 mM phloridzin in the extravesicular medium; -the uptake of D-glucose in the presence of sodium decreases if valinomycin is added. This suggests that the ionophore valinomycin rapidly creates an inside positive potential by providing an electrogenic shunt for potassium and thus decreases the sodium dependent influx of D-glucose. These results strongly suggest that the sodium dependent D-glucose transport is an electrogenic process; -the stimulation of D-glucose by sodium is reciprocal, i.e., it is also possible to demonstrate enhancement of the sodium uptake by a D-glucose gradient.

In addition to these results the presence of a sodium-potassium ATPase only in the basolateral membranes suggests that the mechanism of transepithelial D-glucose transport in the Atlantic hagfish includes the basic mechanisms found in higher vertebrates. Thus, D-glucose would enter the cell through the luminal membrane by a sodium co-transport system with the driving force being a transmembranal sodium gradient [in vivo concentrations amount to an extracellular sodium of 450 mM/l and 150 mM/l intracellular, (A. Brodal and R. Faenge, The Biology of Myxine, Universitetsforlaget, Oslo, 1963)], which is maintained by the Na^+K^+ATPase, i.e., D-glucose transport in the Atlantic hagfish archinephric duct is a secondary active transport. Supported by DFG and NIH.

THE EFFECT OF ESTRADIOL ON THE VERSCHLUSSVORRICHTUNG OF SQUALUS acanthias

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Introduction

The dogfish oviduct posterior to the shell gland coils before inserting in the wall of the uterus (Fig. 1). In 1907 Widakowich described this structure as a closing mechanism (Verschlussvorrichtung) and speculated that its function during pregnancy was to prevent uterine fluid from flowing through the oviduct and into the peritoneal cavity (Widakowich, V., Zool. Anz. 31, 636-643, 1907). He also pointed out the size discrepancy between the Verschlussvorrichtung of pregnancy and the maturing ova and further recognized that in order for eggs to traverse that portion of the oviduct a means of opening the oviductal lumen would be necessary. This report examines the possibility that hormones are involved in regulating the size of the Verschlussvorrichtung.

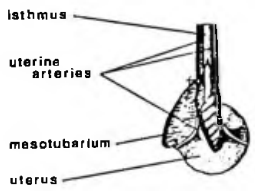

Figure 1.--The Verschlussvorrichtung in the oviduct of Squalus acanthias (after V. Widakowich, 1907).

Methods and Results

Pregnant state A (embryos < 4 cm) and stage C (fetuses > 19 cm) Squalus acanthias were randomly assigned to one of the following treatment groups: control, 17β-estradiol, relaxin, insulin, estradiol plus relaxin or estradiol plus insulin. 1 mg 17β-estradiol was adminstered in sesame or vegetable oil on days one and three of the treatment protocol. 500 μg porcine relaxin (N.I.H.) or 100 IU/kg bovine insulin (Sigma Chem. Co.) dissolved in 0.9% NaCl with 0.1% Benzopurpurine-4B were administered in a single dose on day five. All hormones were given via intraperitoneal injection. Animals were sacrificed on day six, three days after the second estradiol treatment or twenty-four hours after peptide hormone administration. The maximum circumference - that circumference at which the tissue ruptures - of the Verschlussvorrichtung was measured on 1 cm wide loops of tissue as previously described (Koob, et al., The Bulletin 21, 46, 1981). Cross-sectional areas were calculated using these data and assuming a circular shape. The area data are presented in Figure 2.