along with the calculated rate constants for three neutral amino acids glycine, L-alanine and valine as well as the synthetic, non-metabolizable amino acid cycloeucine. A modification introduced in this work is the separation of the aqueous humor into anterior and posterior sections. Our results indicate that in all cases the test amino acids enter the anterior aqueous humor most quickly. Moreover, they readily enter the peripheral vitreous (nearest the retina) thereby suggesting that the retina is supplied with amino acids from choroidal blood through the pigment epithelium and not from the ciliary process of the anterior chamber. The blood vitreous transport constant Ki for all the amino acids studied was significantly higher than that previously determined for L-glucose in the peripheral vitreous. The transport of the amino-acid molecules could be aided by being smaller than L-glucose and being somewhat more lipid soluble, (Lev, A. et al., 1977, Chem. Rev., V. 71). However, it is unlikely that glycine would cross the blood vitreous barrier nine times faster than L-glucose without some transport facilitating mechanism.

Transport of the amino acids into the aqueous and vitreous compartments follows a pattern dependent on the size of the R group with the exception of cycloleucine which is similar in size to valine but crosses the ocular barriers significantly slower. This further supports the notion that amino acid transport is not passive. It is noteworthy that we observe a decrease in transport rate with increasing size (i.e., glycine > L-alanine > valine), whereas the octanol partition coefficients are in the reverse order indicating that lipid solubility is not the deciding factor and that transport is facilitated. The octanol/water partition coefficients for the substances studied are: glycine- .0093; alanine- .00182; valine- .00894; cycloleucine- .0282; urea- .00179; glucose- .00051.

Previous work with the rabbit has clearly indicated an active transport of amino acids into the aqueous humor. Our results in the dogfish support this notion in that transport of the tested amino acids is much faster (approx. 7 times) than L-glucose although not as fast as D-glucose or urea. This work was supported by NIH Research Grant No. PHS/EY-01340.

THE EFFECT OF PARDAXIN ON THE DOGFISH ISOLATED RECTAL GLAND

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The Red Sea flatfish Pardachirus marmoratus (Soleidae) has been shown to have 212-235 glands located along the dorsal and anal fins as described by Clark and Chao (Sea Fish Res. Stn. Haifa Bull. 60: 53-56, 1973). The secretion was found to be toxic to different teleosts (Clark and George, Env Biol. Fish. 4: 103-123, 1979). The toxic factor (Pardaxin) was identified as a protein of 15,000 daltons of a single chain and four disulfide bridges (Primor and Tu, Biochim. Biophys. Acta., in press). Pardaxin (PX) toxicity and its effect on teleost gill suggests a possible involvement in osmoregulating processes (Primor et al., J. Exp. Zool., 211: 33 43, 1980).

The in vitro perfused rectal gland of the dogfish (Squalus acanthias) was shown to secrete sodium chloride against its electrochemical gradient (Stoff et al., J. Exp. Zool., 199: 493-498, 1977) and be useful in studying the action of different drugs (Forrest, this Bulletin). The effect of the drug on the rate of secretion could be studied in a perfused rectal gland preparation. However, the effect on the electrical parameters could be more readily studied as a flat tissue in Ussing type chambers (Ussing-Zheran, 1951). The effect of PX on the electrical parameters of a short-circuited Db cyclic-AMP and thyophylline stimulated rectal gland is shown in Figure 1. PX at 200 μg/ml was observed to increase the short-circuit current (SCC) from 24 to 30 $\mu A/cm^2$ and potential difference (PD) from 4.5 to 6.3.

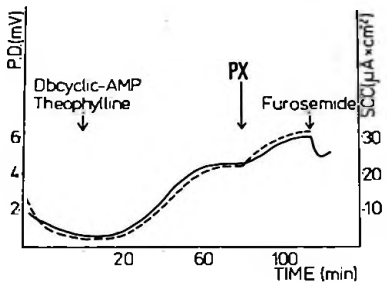

Figure 1.--The effect of pardaxin (PX) on dogfish short-circuited rectal gland preparation. Prior to PX administration the rectal gland was stimulated with Db-cyclic-AMP (0.05 mM) and theophylline (0.25 mM). PX at 200 μg/ml and furosemide (1.0 mM) were added to both sides of the rectal gland. (——), tracing of continuous short-circuit current recording (SCC); (---), transepithelial potential difference (PD).

In the perfused rectal gland preparation the urea and chloride concentrations in the secretion fluid were found to be in a range of 8-20 and 490-500 mM respectively. PX at 400 μg injected into the perfused artery causes an increase in urea concentrations in the rectal gland secretion fluid reaching a value of 300 mM (Figure 2), which is close to that in the perfusate (350 mM). At the same dose, the chloride gradient between the rectal gland secretion fluid and the perfusate was found to be abolished, reaching a value of 315 mM (Fig. 3) when the chloride in the perfusate is 290 mM. The effect of different doses of PX on urea and chloride rectal gland secretion fluid contents is given in Figure 4.

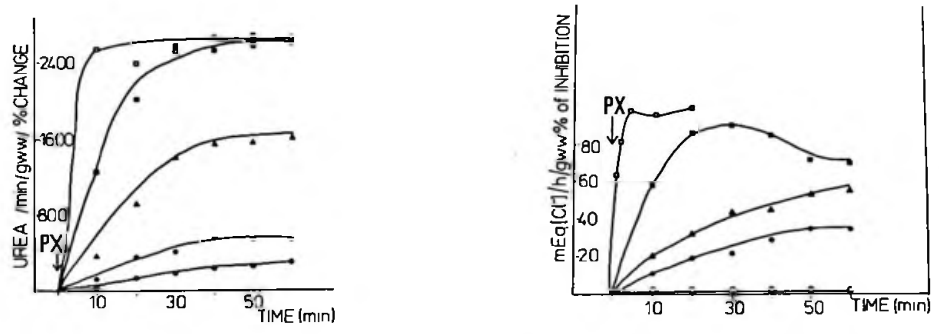

Figure 2.--Pardaxin (PX) effect at different doses on urea content in the perfused dogfish rectal gland fluid. The rectal gland was stimulated with Db-cyclic AMP (0.05 mM) and theophylline (0.25 mM). After reaching a constant rate of secreted chloride, a single dose of PX was injected by the canulated artery. The urea content in the rectal gland fluid was determined by the phenolhypochloride method and expressed as clearance of urea per min. flow and normalized to 1 g gland wet weight. For doses of PX (μg): (0——0), 50. (●——●), 100. (Δ——Δ), 200. (■——■), 400, and (□——□), 1000. The data are means of two experiments.

Figure 3.--Pardaxin (PX) effect at different doses on chloride concentration in the perfused rectal gland fluid. The rectal gland was stimulated with Db-cyclic AMP (0.05 mM) and theophylline (0.25 mM). After reaching a constant rate of secreted chloride, a single dose of PX was injected by the canulated artery. The chloride concentration in the rectal gland fluid was determined and expressed in μ equivalents Cl^- secreted per hour and normalized for 1 g gland wet weight. The rate of secreted chloride following PX injection is compared to the difference between the chloride concentration in the rectal gland fluid (490-500 mM) and the perfusate (290 mM) and expressed as % of inhibition. Symbols as Figure 2. The data are means of two experiments.

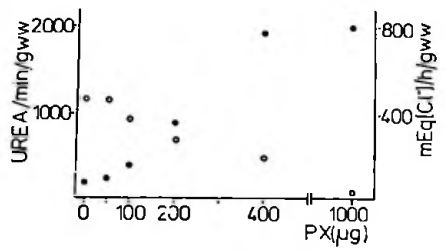

Figure 4.--Chloride concentration and urea content in perfused dogfish rectal gland fluid, 10 minutes after injection of different Pardaxin (PX) doses. The data are taken from Figures 1 and 2 for a value of 10 min. (•) for urea. (0) for chloride.

The short-circuited rectal gland was shown to secrete chloride at a rate equivalent to the applied SCC (Zadunaisky and Garretson, Bulletin, 17: 1977). Therefore, the increase in SCC could be due to an increase of net chloride secretion. The ability of furosemide to reduce the SCC following PX stimulation (Fig. 1) supports this hypothesis. An increase of SCC and PD were also observed in the elasmobranch Rhizoprionodon terraenovae rectal gland (Primor, recent observation). The increase of urea in the perfused rectal gland (Fig. 2) strongly suggests that PX causes leakage of the epithelium. The decrease in chloride gradient (Fig. 3) supports this idea. The PX stimulation of SCC in the presence of Db cyclic-AMP could also be explained as a PX induced increase in permeability allowing more c-AMP to enter the cells. Additional study is required for understanding PX mode of action in the rectal gland. This study was supported by the Office of Naval Research, Research Grant No. N00014-80-C-0757, NIH Research Grant No. GM25002, and NIH Research Grant No. PHS EY-01340.

A STUDY OF PARDAXIN TOXICITY IN THE DOGFISH

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The secretion of the Red Sea flatfish Pardachirus marmoratus was shown by Clark (Nat. Geogr. Mag. 146: 718-728, 1974), to be repellent to sharks and to be toxic to different teleosts (Clark and George, Env. Biol. Fish. 4: 103-123, 1979). It was suggested that the mechanism of the toxicity to fish is caused by an inability to osmoregulate adequately (Primor et al., J. Exp. Zool., 211: 33-43, 1980). The principle toxic component (Pardaxin) of the secretion was isolated and characterized as a single chain protein of 15,000 daltons (Primor, Parness and Zlotkin, In: Toxins: Animal, Plant and Microbiol. Ed. P. Rosenberg, Pergamon Press, 539 547, 1978). The purpose of this work is to study the toxicity of Pardaxin (PX) to elasmobranchs.

The toxicity of Pardachirus secretion (PMC) and PX were determined using a group of 40 dogfish (Squalus acanthias) fetuses (pups) weighing 40-46 g each. The lethal concentration (LC_{50}) of PMC and PX was found to be 8.0 and 5.1 µg/ml/g body weight respectively as determined one hour after administration into 250 ml of sea water. The LC_{50} (determined after 1 hr. exposure) of PMC and PX injected into a dorsal artery was found to be 90.0 and 54.0 µg/g body weight respectively. PX at 10 µg/ml/l g body weight administered into sea water caused a severe struggling response and a marked decrease in the operculum rate from 50-55/min. to 3-5/min during the first 4 min., then increasing to 20-25 min after 10 min. The effect of PMC on teleosts respiration rate was noted by Clark and George (1979). In order to determine the target of toxicity, a chamber partitioned with a diaphragm was constructed so that the sea water volumes were 40 ml (head part) and 200 ml (rear part) the diaphragm was positioned