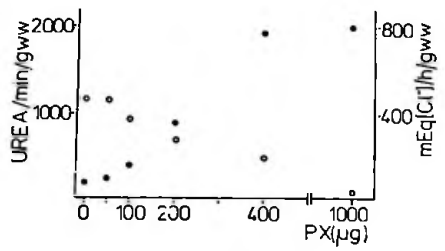

Figure 4.--Chloride concentration and urea content in perfused dogfish rectal gland fluid, 10 minutes after injection of different Pardaxin (PX) doses. The data are taken from Figures 1 and 2 for a value of 10 min. (•) for urea. (0) for chloride.

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

behind the gills. PX administered in the head part of the chamber was found to have a toxicity similar to that without the diaphragm. In addition, struggling and a diminished opercular rate were observed. However, 20 μg/ml/g body weight administered to the rear part of the diaphragm separated pups does not cause toxicity or other response.

The PX effect on plasma solutes was studied in adult dogfish. Female dogfish weighting 4.5-5.0 kgs were inserted into a holding box separating the head and the gills by a diaphragm from the rest of the body; front and rear volumes were 8 and 12 liters respectively. Injections and blood sampling were via a canulated dorsal artery. The urine papillae was canulated and the urine collected into a balloon. It was demonstrated that 25 μg/ml of PX administered to the head chamber induced struggling movements and a diminished opercular rate. No effect was observed when PX was introduced in the sea water bathing the rear part of the dogfish. The effect on opercular rate, blood pH and partial CO_2 pressure (pCO_2) are shown in Figure 1. PX was shown to have a cytolytic effect on the

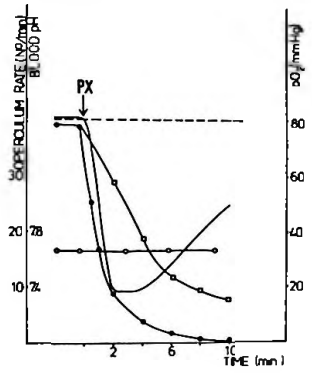

Figure 1.--The effect of Pardaxin (PX) on dogfish operculum rate movement, blood pH and the partial oxygen pressure (pO_2). The shark was inserted into the diaphragm holding box. The experiment began with stop sea water flow and administration of 25 μg/ml . PX into the head part of the chamger. Every 2 min. for a period of 10 min. blood samples were collected to determine pH and pO_2 and operculum movements were counted. (——), number of operculum movements per min. in the presence of PX. (---), number of operculum movements in stop flow without PX. (o——o), pO_2 in the blood samples in the presence of PX. (□——□), pO_2 in the blood samples in stop flow without PX. (0——0), pH in the blood samples.

teleost gill epithelium (Primor et al., 1980). Therefore, it was of interest to study if the PX effects in dogfish are associated with changes in the concentrations of its plasma solutes. The dogfish plasma sodium concentration (250 mM) is known to be markedly lower than that of sea water (440 mM). Plasma urea however, is 350 mM compared to an immeasurably small amount in sea water (measured by the phenol-hypochloride method). No measurable ^{14}C-urea was detected in the sea water of a control dogfish injected with 200 μCi ^{14}C-urea. PX causes a marked leakage of ^{14}C-urea from the plasma to sea water and in the opposite direction. In addition, a leakage of ^{22}Na from plasma to sea water was shown. After reaching a maximum this leakage tends to become reduced (Fig. 2). Introduction of 40 mg PX with 200 μCi ^{14}C-urea resulted in no measurable leakage of radioactivity into the holding box and no measurable leakage of radioactivity into the holding box and no changes in operculum rate.

It was shown in this study that the head part of the dogfish is the target of PX toxicity. PX was shown to induce a decrease in opercular rate, significant leakage between the fish plasma and the surrounding sea water. One may calculate that during 10 minutes of exposure to a sub-lethal PX dose 50 μ equivalents of urea had leaked from the fish to the sea water. The abolition of a chloride gradient and a leakage of urea in the perfused rectal gland (this Bulletin) supports this mechanism. The loss of the ability to maintain homeostasis as regards to solutes composition could be a reason for PX toxicity. This hypothesis demands further study. This study was supported by the

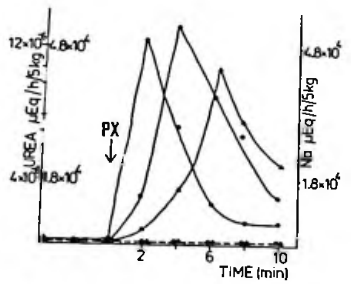

Figure 2.--The effect of Pardaxin (PX) on ^{14}C-urea and ^{22}Na rates of leakage between the blood and sea water. The shark was inserted into the diaphragm holding box and injected with 200 μCi of ^{14}C-urea or ^{22}Na by the calculated artery and allowed to stay for 3 h in a circulating water flow. The experiment began with stop water flow and the addition of 25 μg/ml PX into the head part of the chamber. Every 2 min. for a period of 10 min. water samples were collected from the head and the rear part of the diaphragm. (0——0), ^{14}C-urea in the head part. (0---0), ^{14}C-urea in the rear part. (Δ——Δ), ^{22}Na in the head part. (Δ---Δ), in the rear part. (•——•), 25 μg/ml PX and 250 μCi of ^{14}C-urea were administered into the head part of the diaphragm. Every 2 min. for a period of 10 min. blood samples were taken from a canulated artery. The rate is expressed in units of plasma μ equivalents of urea and sodium per hour and normalized to 5 Kg body weight. The data are means of five experiments.

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IONIC AND PHARMACOLOGICAL CHARACTERIZATION OF EXCITATION AND CONTRACTION IN DOGFISH HEART (SQUALUS ACANTHIAS)

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Ultrastructural study of dogfish heart suggests that the heart is composed of cells of 2-5 μm in diameter with considerable sarcoplasmic reticulum and peripheral couplings but lack T-tubular system (Maylie, Nunzi and Morad, MDIBL Bull. 19: 84, 1979). Electromechanical experiments in this preparation support the hypothesis that the development of tension is under strict membrane potential control and that calcium for contraction is provided from an extracellular compartment. In this report we explore the dependence of action potential on the ionic and pharmacological interventions. The results suggest that the upstroke of action potential is mediated by the influx of sodium and calcium. The effect of Ca^{2+} on the action potential plateau is mediated in part through control of K^{+} conductance. Epinephrine enhances and prolongs the plateau of the action potential in a manner consistent with potentiation of contraction. On the other hand potentiation of contraction by cardiac glyosides seems to be independent of the effect of the drug on the membrane potential.

Figure 1 shows alteration in action potential and contraction when a ventricular strip (0.3 mm in diameter) was exposed to a solution containing tetrodotoxin (TTX, 10^{-6}M). Note that although the rate of rise of action potential is considerably suppressed in the presence of TTX there is little or no change in either the overshoot or duration of the action potential (panel B). Panel C shows that the decrease of $[Ca]_0$ to 0.5 mM in the presence of TTX markedly suppresses the rate of rise and the magnitude of the overshoot potential. Figure 1 also shows that development of tension although unaltered by addition of TTX is strongly suppressed in calcium