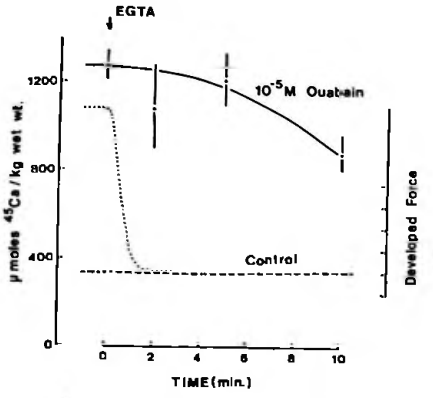

Figure 4.--Relaxation and exchangeable Ca^{2+} content (See Fig. 2) are measured when Ca^{2+} is removed (0 Ca^{2+} + 2 mM EGTA) after shark atrial pieces have been exposed to 10^{-5} M ouabain for 1 hr.

We thus observed a consistent pattern of Ca^{2+} gain and contraction during reduction of the transmembrane Na^+ gradient. Procedures which caused subsequent relaxation did not cause a reversal of the net Ca^{2+} movements. These results are consistent with the view that either a decrease of $[Na^+]_e$ or an increase in $[Na^+]_i$ stimulate Ca^{2+} influx. Inhibition of this stimulated Ca^{2+} influx allows intracellular Ca^{2+} accumulation by SR to induce relaxation.

POSSIBLE MECHANISMS FOR Ca^{2+} TRANSPORT AND DEVELOPMENT OF TENSION IN DOGFISH HEART

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Development of tension in shark myocardium depends directly on external $[Ca^{2+}]$ (Maylie, Nunzi and Morad, 1979, MDIBL Bull. 19, 1979). Three major mechanisms have been proposed to account for the transport of Ca^{2+} into the myocardium. These mechanisms involve: 1) an electrogenic diffusion of Ca^{2+} through a voltage dependent Ca^{2+} channel (Model 1); 2) Ca^{2+} transport via a neutral carrier in a membrane where Ca^{2+} and Na^+ compete for a common site on the external surface of the membrane (Model 2), and 3) a counter exchange carrier in which the Na^+ gradient drives the transport of Ca^{2+} inward or outward depending on the direction and magnitude of Na^+-gradient (Model 3). Since shark myocardial cells are 2-5 μm in diameter and are highly sensitive to the extracellular $[Ca^{2+}]$ we attempted experiments to probe the nature of Ca^{2+} transport in this tissue.

Figure 1 shows the effect of replacement of NaCl with LiCl on the development of tension. Ca^{2+} concentration of the shark Ringer was reduced to 1 mM in order to prevent the damaging effect of higher Ca^{2+} on intracellular structures . 95% replacement of Na^+ with Li^+ produces maintained contracture which rapidly relaxed upon replacement with normal Na^+ (upper panel first contracture). The Li^+ induced contracture could also be relaxed upon addition of 2 mM EGTA. Although the rate of relaxation is considerably slower, the fact that relaxation does occur suggests that 1) the intracellular Ca^{2+} stores play a minor role for providing Ca^{2+} for contraction and 2) the influx of Ca^{2+} during exposure to Li^+ is primarily responsible for development of tension. Similar findings are observed when choline chloride or sucrose were used as substitutes for NaCl. The finding of Figure 1 does not distinguish between Models 2 and 3 for transport of Ca^{2+} but it does suggest that a voltage dependent Ca^{2+}-channel (Model 1) is not primarily responsible for generation of low Na^+-contractures.

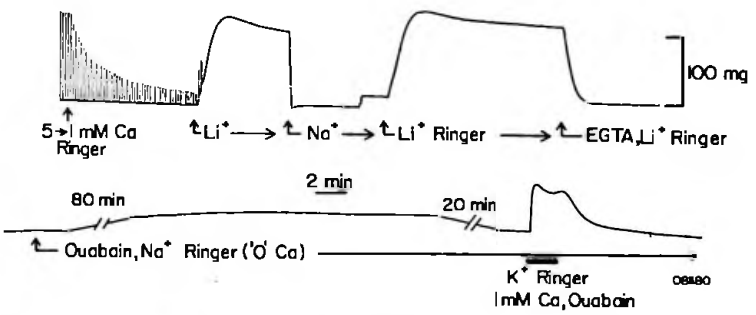

Figure 1.--Comparison of low Na^+-contractures and Ouabain induced contractures in shark atria. The top panel shows the decrease in twitch tension when the $[Ca^{2+}]$ in normal Ringers is decreased from 5 to 1 mM. When the $[Na^+]$ is reduced from 290 to 10 mM using Li^+ as a substitute (1 mM Ca^{2+} Ringer) the preparation immediately develops a sustained maximal contracture. Relaxation of the contracture can be induced by either readdition of the normal Na^+ Ringer or 2 mM EGTA to reduce the zero Ca^{2+} solution (right contracture, top panel). Note that the rate of relaxation induced by Na^+ Ringer is much faster than that induced by a zero Ca^{2+}, EGTA containing Li^+ Ringer. The composition of normal Ringer in mM is: NaCl, 280, KCl 6, $CaCl_2$ 5, $MgCl_2$ 3, Na_2SO_4 0.5, NaH_2PO_4 1, $NaHCO_3$ 8, Urea 350, glucose 5. Low Na^+ soltuions were obtained by replacing all the NaCl with LiCl leaving a final $[Na^+]$ of 10 mM. The lower panel shows that 10^{-5} Ouabain slowly induces a contracture in Na^+ containing solutions. The contracture is submaximal and is not maintained because Ca^{2+} is omitted from the Ouabain Ringer. Replacing Na^+ with K^+ depolarizes the membrane potential and produces a large contracture in 1 mM Ca^{2+} with Ouabain (lower panel right contracture). The preparation immediately relaxes upon return to zero Ca^{2+}, Ouabain Ringer.

An attempt to alter the internal $[Na^+]$ was made by exposure of the ventricle to high concentration of Ouabain (10^{-5}M). In absence of added Ca^{2+} to the Ringer solution the preparation, within 80 to 90 min of drug addition, generated a submaxinal contracture (compare lower and upper panels, Figure 1). Although contracture tension decreases spontaneously after some time in the absence of Ca^{2+}, addition of high KCl solution in the presence Ouabain often resulted in maintained contracture (Fig. 1, lower left panel). Since 1mM Ca^{2+} was included in the depolarizing solution it is safe to assume that activation of a voltage dependent Ca^{2+} channel was primarily responsible for the KCl contracture. The development of Ouabain induced contracture on the other hand is consistent with the idea that increases in the intracellular $[Na^+]$ could generate tension through a counter transport system similar to that of model 3. The Ouabain induced contracture, however, is incompatible with model 2 which relies upon a competition between Na^+ and Ca^{2+} at the extracellular surface of the membrane.

Consistent with the hypothesis that accumulation of internal Na^+ in the presence of Ouabain is responsible for steady state maintainence of tension is the observation shown in Figure 2. In this experiment the time course of development of contracture during the initial exposure to 10^{-5}M Ouabain in 0.1 mM Ca^{2+} Ringer is much slower than that observed during a Li^+ induced contracture (compare top and middle contracture). The Ouabain-induced contracture could be relaxed upon exposure to 2 mM EGTA, suggesting that Ca^{2+} influx plays a crucial role in the development of contracture in the presence of Ouabain. Removal of EGTA re-establishes contracture tension but with a much faster time course. The faster rate of development of contracture implies that in preparations in which intracellular $[Na^+]$ has been increased by previous exposure to Ouabain (1-2 hrs) the rate of transport of calcium is highly accelerated (bottom strip chart Figure 2). This observation is only consistent with the counter transport of calcium depicted by model 3. Inhibition of the Na^+ pump by "zero" K^+ Ringers produces qualitatively similar results although there are some differences with respect to replacement of Na^+ with Li^+.

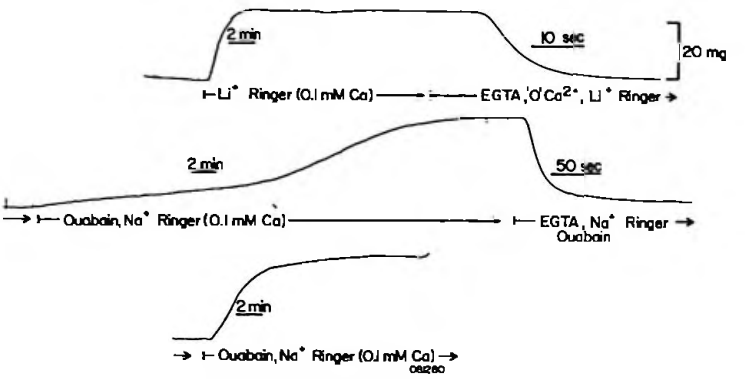

Figure 2.--Comparison of time course of the development of contracture in shark atria in Li^+ Ringer or 10^{-5} molar Ouabain Ringer. The top panel shows a Li^+ induced contracture that is maximal in 10^{-4} molar Ca^{2+}. The chart speed is increased to show the rate of relaxation induced by removal of Ca^{2+} from the Li^+ Ringer to which 2 mM EGTA had been added. The middle panel shows the slow rate at which contracture in the presence of 10^{-5} molar Ouabain Ringer containing 10^{-4} molar Ca^{2+}. The contracture was relaxed by zero Ca^{2+} Ringer containing 2 mM EGTA and Ouabain. The bottom panel shows the redevelopment of contracture tension when the EGTA is removed and 10^{-4}M Ca^{2+} is added back to the Ouabain Ringer.

The Li^+ induced contractures are dependent on the extracellular [Ca^{2+}] as demonstrated in Figure 3.

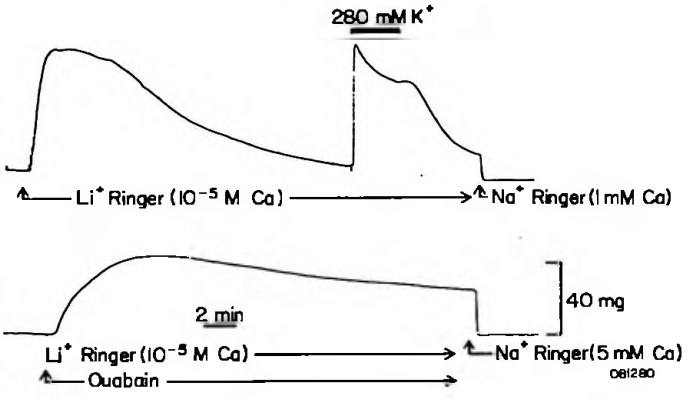

Figure 3.--Demonstration that Ouabain blocks the spontaneous relaxation during a low Na^+, low Ca^{2+} contracture in a shark atrial strip. Top panel shows that the contracture in 10^{-5} molar Ca^{2+}, Li Ringer is not maintained and relaxes. Addition of 280 mM KCl to the solution produces a large contracture (top panel, right contracture). The bottom panel shows that 10^{-5} molar Ouabain produces a sustained contracture in 10^{-5} molar Ca^{2+}, Li-Ringer. Return to normal Na^+-Ringer with 5 mM Ca^{2+} results in an immediate relaxation of the contracture.

The top panel shows that maximal contracture develops rapidly upon exposure to Li^+-Ringer with 10^{-5} molar $CaCl^2$. However, unlike the Li^+ contractures with Ca^{2+} greater than 10^{-4} molar (Figures 1 and 2) the contracture is not maintained but slowly relaxes. A short exposure to high KCl immediately produced a maximal contracture showing that the preparation is still viable and able to produce tension via a voltage dependent Ca^{2+} channel (top panel Fig. 3). The requirement that the [Ca^{2+}] needs to be 10^{-4} molar or greater to produce maintained contractures is also observed with Ouabain induced contractures (compare bottom panels of Figures 1 and 2). The slow relaxation of the contracture in low Ca^{2+} Ringer is believed to be mediated by a partial re-establishment of the Na^+-gradient. This would occur because the Li^+-Ringer contains 10 mM of Na^+ and the Na-pump would actively deplete internal Na^+. Evidence for this argument is demonstrated in the lower panel of Figure 3 in which Ouabain (added to the low Ca^{2+}, Li-Ringer to block the Na^+ pump) reduced the rate of spontaneous relaxation and produced a larger

final contracture in Li^+ Ringer with 10^{-5} molar Ca^{2+}. This experiment also shows that an internal accumulation of Na^+ is responsible for the Ouabain-induced contracture. This is demonstrated by the immediate relaxation of the contracture (produced by the Ouabain containing Li^+ Ringer) when the preparation is exposed to normal Na^+-Ringers. Note that the Ouabain-induced contractures can only be relaxed by zero Ca^{2+} Ringer containing EGTA (middle panel, Figure 2).

Figure 4 shows similar experiments in which caffeine is used in an attempt to block internal sequestration of

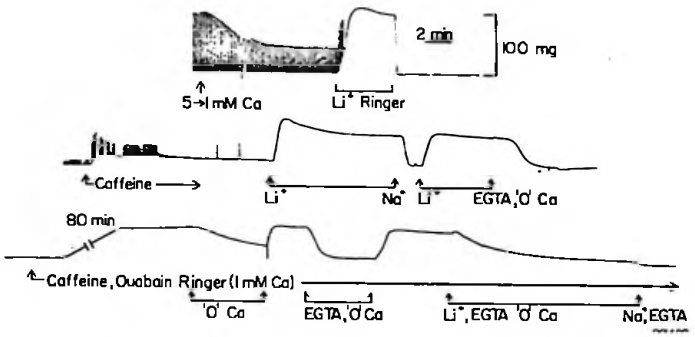

Figure 4.--Effect of caffeine on low Na^+ and Ouabain induced contractures in a shark ventricular strip. The top panel shows the reduction in twitch tension when the $[Ca^{2+}]$ was reduced from 5 to 1 mM in normal shark Ringer. Switching to Li^+ Ringer with 1 mM Ca^{2+} resulted in a maximal sustained contracture that relaxed completely only upon readdition of normal Na^+ Ringer. In the middle panel 50 mM caffeine added to the Na^+-Ringer produced a slight increase in baseline tension and twitch contractions. The stimulus threshold became so large in caffeine that the stimulator was turned off. Contractures produced by Li-Ringer in the presence of caffeine were relaxed by addition of Na^+-Ringer or 'zero' Ca^{2+}, buffered with 2 mM EGTA (middle panel, 1st and 2nd contracture respectively). Bottom panel shows the development of maximal contracture by Ouabain (10^{-5} molar) in 50 mM caffeine Ringer (normal Na^+). The contracture slowly relaxed when the Ca^{2+} was removed. However, if 2 mM EGTA was added in addition the contracture completely relaxed much quicker. If however, Na^+ was replaced by Li^+ in zero Ca^{2+} with 2 mM EGTA the contracture relaxed slowly and only readdition of Na^+ completely relaxed the contracture.

Ca^{2+} by the SR. Exposure to 50 mM caffeine (middle panel) produces a slight increase in baseline tension and potentiates the twitches that were suppressed from the previous Li^+-contracture (top panel). Under these conditions relaxation of the Li^+-induced contracture during the perfusion of normal Na^+ Ringer was slower in the caffeine containing Ringer than in normal Ringer (compare top and middle panels Figure 4). The bottom panel shows a Ouabain induced contracture in the presence of caffeine and compares the rates of relaxation by 1) zero Ca^{2+}, 2) zero Ca^{2+} with EGTA, and 3) zero Ca^{2+}, EGTA, Li^+-Ringer, EGTA added to the zero Ca^{2+} solution increases the rate of relaxation but if Li^+ is substituted for Na^+ in the zero Ca^{2+}, EGTA solution the rate of relaxation is reduced. These findings are consistent with model 3 in which countertransport of Ca^{2+} is driven by the direction of the Na^+ gradient. They are not consistent with model 2 which predicts a dependence on external Na^+ or Ca^{2+} and not on the internal levels of Na^+ or Ca^{2+}.

The results of ionic and drug interventions on development of tension in shark atrium and ventricle are consistent with two mechanisms of Ca^{2+} transport. Activator Ca^{2+} may be either transported by activation of voltage-dependent Ca^{2+}-channel during depolarization, or via a counter transport system. The counter-transport for Ca^{2+} in this model uses Na^+-gradient across the cell membrane to determine the directionality of Ca^{2+} transport. While the physiological significance of a voltage-dependent Ca^{2+} transporting system is fairly obvious in this preparation,

it is not clear at present what rate the Na^+-dependent Ca^{2+} influx plays in activation of tension. Therefore, if the counter-transporter is to play a physiological role in Ca^{2+} homiostasis, it has to be in sequestration of Ca^{2+}. However, radio isotropic studies (C. Van Breemann et al., in this issue of Bull.) suggests that sequestration of Ca^{2+} is primarily handled by the SR rather than the Na-dependent counter transport of Ca^{2+} across the surface membrane.

CHANGES IN THE HEPATIC CYTOCHROME P-450 DEPENDENT MONOOXYGENASE SYSTEM OF WINTER FLOUNDER FOLLOWING TREATMENT WITH POLYCYCLIC AROMATIC HYDROCARBONS

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Marked variability in hepatic monooxygenase activities of winter flounder caught in the area of Mt. Desert Island was first reported by this lab (Bend et al., MDIBL Bull., 17: 47, 1977). We have now characterized 2 subpopulations of flounder based on individual hepatic levels of 7-ethoxyresorufin deethylase activity (7-ERF), aryl hydrocarbon hydroxylase activity (AHH), and response of hepatic AHH to in vitro 7,8-benzoflavone (ANF). One subpopulation has high hepatic 7-ERF and AHH activities, the latter which are inhibited by ANF; the other subpopulation has low hepatic 7-ERF and AHH activities, their AHH activity being stimulated by ANF. In mammals, studies have shown that these elevated activities (AHH and 7-ERF) are correlated with the presence of a certain cytochrome species (cytochrome $P\text{-}450_1$ or cytochrome P-448) whose formation is a response to exposure to a specific class of inducing compounds which includes polycyclic aromatic hydrocarbons (PAH), dioxin derivatives and certain PCBs and PBBs.

The objective of this report is to investigate the possibility that these high cytochrome $P\text{-}450_1$ dependent activities found in native flounder could also be the result of exposure to pollutants of the PAH class. To accomplish this, comparisons were made between untreated flounder and flounder which were treated with the PAH-type inducers 1,2,3,4-dibenzanthracene (DBA) and 5,6-benzoflavone (BNF).

METHODS

Animal capture, storage conditions, and tissue preparation (liver) were as described earlier (Bend et al., MDIBL Bull., 19, 11, 1979) as were the assays for 7-ERF activity and AHH activity in the presence and absence of ANF. Glutathione transferase (G-T) activity was assayed according to Booth et al., Biochem. J., 79: 516, 1961, at 30°C with 1 mM of 2,4-dinitrochlorobenzene (DNCB) as substrate and 5 mM glutathione as co-substrate.

That portion of flounder gut from just distal to the pyloric sphincter to proximal to the anal sphincter was removed and washed with at least 20 ml of cold isotonic KCl buffer (1.15% KCl, 1.25 mM HEPES buffer at pH 7 6). The washed intestines were then cut open lengthwise, the mucosa scraped off and homogenized (33% w/v) in the isotonic KCl buffer. Whole homogenate was used as the enzyme source for all assays except the cytosolic G-T where 100,000 g supernatant fractions were used.

Sodium dodecyl sulfate (SDS) gel electrophoresis was done according to Laemmli (Nature, 227: 680, 1970), using 7.5% polyacrylamide gels (PAG) with a 3% stacking gel. Samples were stained in 0.115% Commassie Brilliant Blue for at least 45 minutes prior to destaining in 25% ethanol/8% acetic acid.

Treatment of fish was by IP injection of either a single dose of BNF at 50 mg/kg in corn oil (13 mg/ml) or 3 doses, 24 hours apart, of DBA at 10 mg/kg also in corn oil (8 mg/ml). The animals were sacrificed either 9 or 11 days after the initial injection.