probably K^+). While adrenaline alters action potential by activation of an inward sodium or calcium current, ouabain alters the action potential by suppression of an electrogenic Na^+-pump. It is the loss of internal [K^+] concomitant with gain of Na^+ that leads to depolarization of membrane potential and shortening of the action potential.

THE EFFECTS OF Na^+ SUBSTITUTION ON NET Ca^{2+} MOVEMENTS AND FORCE DEVELOPMENT IN SHARK ATRIUM

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Na ions have long been assigned a physiological role in regulating cardiac cell Ca^{2+} fluxes and contractility (Wilbrandt and Koller, Physiol. Pharmacol. Acta., 6: 208, 1948). Twenty years later, a mechanism was proposed which couples Na^+ and Ca^{2+} fluxes in opposite directions (Reuter and Seitz, J. Physiol., 195: 431, 1968). The carrier hypothesis predicts that Na^+ removal causes a contraction due to Ca^{2+} gain and that Na^+ replenishment relaxes the muscle due to net Ca^{2+} extrusion. Since the earlier theory of Na^+, Ca^{2+} competition also predicts contraction and Ca^{2+} gain during Na^+ removal, we were particularly interested in gaining direct evidence concerning the relationship between relaxation and net Ca^{2+} extrusion during Na^+ replenishment.

Cardiac tissue contains a high concentration of extracellular Ca^{2+} binding sites (Langer et al., Am. J. Physiol., 237: H239, 1979) which require desaturation in order to define Ca^{2+} movements across the proteo-lipid cell membrane. Figure 1 illustrates that no distinction between Ca^{2+} compartments can be made on the basis of

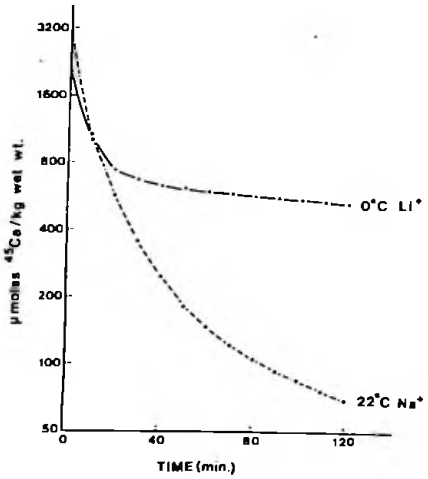

Figure 1.--Small pieces of shark atrium (about 8 mg) were equilibrated in ^{45}Ca labeled shark Ringer for 3 hrs. They were then washed out in a series of tubes containing non-labeled shark Ringer at 22°C or shark Ringer modified by substitution of LiCl for NaCl omission of Ca^{2+} addition of 2 mM EGTA and 10 mM $MgCl_2$ at 0°C. The tissue content of ^{45}Ca is plotted against time of washout; each curve is the average of three experiments.

^{45}Ca washout from previously labeled atrium into shark Ringer at room temperature. If efflux is effected into a cold washout medium (Ringer with LiCl substituted for NaCl, no added Ca^{2+}, 10 mM $MgCl_2$ at 0°C), then we see the emergence of a slow component ($t_{1/2}$ = 45 hrs.). Since cold will reduce rates of permeation across a lipid barrier more than rates of dissociation from binding sites in an aqueous environment, we assume the slow component to be cellular ^{45}Ca. We found that three hrs in ^{45}Ca labeled shark Ringer is sufficient for the ^{45}Ca-^{40}Ca exchange to reach equilibrium (Fig. 2). If, subsequently, the specific radioactivity is kept constant, a measurement of radioactivity then constitutes an indication of net exchangeable cellular Ca^{2+} (van Bremmen et al., Phil. Trans. Roy.

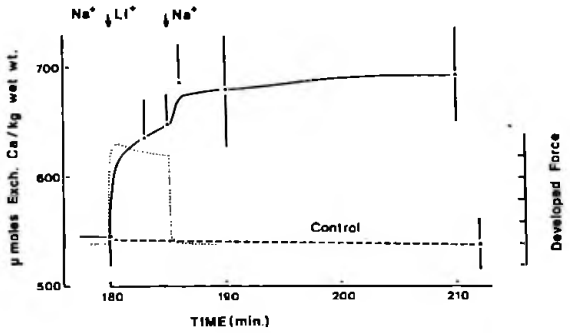

Figure 2.--Pieces of shark atrium were equilibrated in ^{45}Ca labeled shark Ringer for 3 hrs. They were then exposed to Li substituted shark Ringer followed by shark Ringer. All solutions were labeled to exactly the same specific radioactivity. Tissues were taken out at the times indicated and their cellular ^{45}Ca determined after a 40 min wash in ice-cold modified shark Ringer, the composition of which is given in Fig. 1. Contraction and relaxation in the same solutions were measured in a separate muscle bath.

Soc., 265: 57, 1973). Figure 2 shows that substitution of LiCl for NaCl in the bathing medium causes a rapid contraction accompanied by a net Ca^{2+} uptake. However, when the artria return to Na^+, cell Ca^{2+} stays high even though the muscle relaxes. When the ionic substitute is KCl a larger uptake is seen during a greater contraction. No significant loss of cell Ca^{2+} could be measured when the muscle relaxes in normal shark Ringer. Cell Ca^{2+} appears to return more slowly toward control levels.

Another way to reduce the transmembrane Na^+ gradient is to inhibit the Na^+, K^+ ATPase with ouabain or removal of external K^+. These procedures also cause slow gains in cell Ca^{2+} (Fig. 3), which are accompanied by delayed contractions. It is of interest that K^+ removal, increases cell Ca^{2+} more slowly and in an asymptotic fashion followed by a sudden secondary rise in Ca^{2+}. Ouabain causes a monotonic Ca^{2+} gain. As shown in Fig. 4, removal of extracellular Ca^{2+} with the addition of millimolar EGTA causes a rapid relaxation while the cell Ca^{2+} remains elevated for at least 5 min.

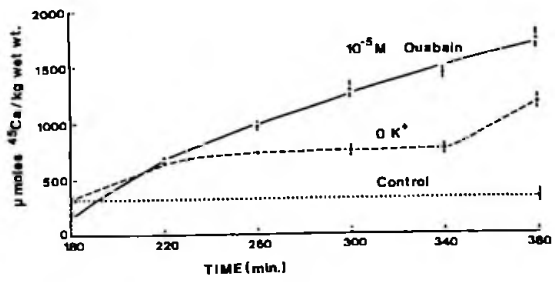

Figure 3.--Net exchangeable cellular Ca^{2+} was measured according to the method described under Fig. 2 when pieces of shark atrium were exposed to either 10^{-5} M ouabain or shark Ringer without KCl.

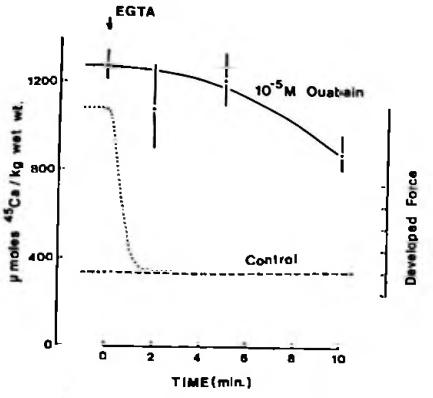

Figure 4.--Relaxation and exchangeable Ca^{2+} content (See Fig. 2) are measured when Ca^{2+} is removed (0 Ca^{2+} + 2 mM EGTA) after shark atrial pieces have been exposed to 10^{-5} M ouabain for 1 hr.

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.