potentiated contracture tension (lower middle panel). Washout of theophylline for long periods (30 min or longer), however, results in suppression of contracture tension. Often 1-2 hours of continuous perfusion were required to counteract this suppressant effect of theophylline.

The experiments seem to suggest that epinephrine produces its positive inotropic action by altering the membrane permeability to Ca^{2+}. The relaxant effect, on the other hand, may be mediated by stimulation of the relaxing system. The paradoxical effects of theophylline at high and low concentrations of the drug may be in part due to the independent sites of the action of the drug on the membrane and the sarcoplasmic reticulum.

SOME ASPECTS OF CONTRACTILITY IN THE SEA POTATO HEART

Lars Cleemann and Martin Morad, Departments of Physiology, Schools of Medicine and Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania

The experiments to be described below were directed primarily towards gaining an understanding of the functioning of the entire heart of the sea potato (Boltenia ovifera).

Experiments with a short segment of the sea potato heart. A new setup was used in order to increase viability and reduce the complexity of the experimental procedures. A segment of the tubular heart (about 5 mm in length) was mounted around two horizontal rods (0.6 mm in diameter) in a shallow rectangular channel (1.6 mm x 8 mm) as shown in Figure 1A. The two rods passed through the lumen of the heart. One of the rods was attached to a tension transducer.

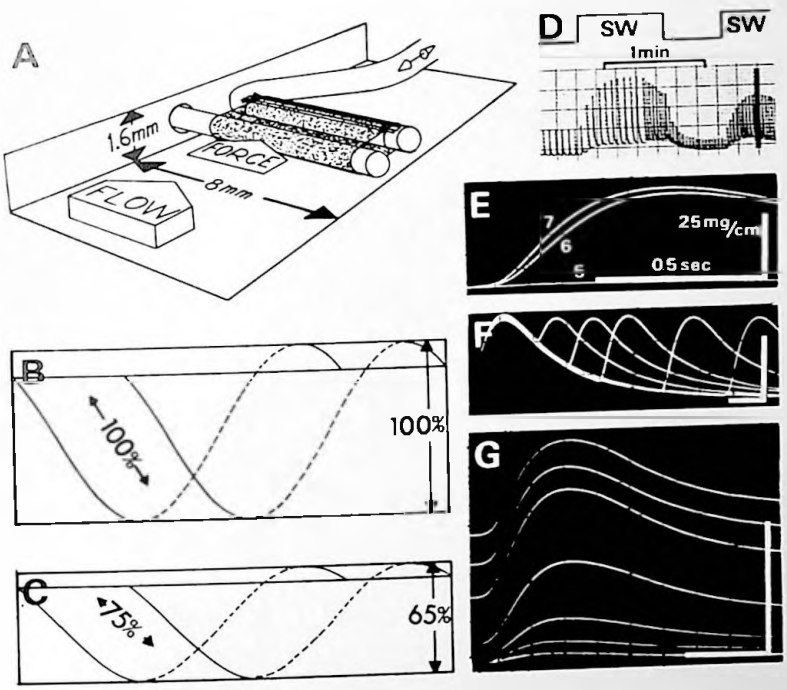

Figure 1. Results obtained with the new setup in panel A are shown in panels D, E, F, and G. Panels B and C show the orientation of the fibers in a short tubular section of the heart and demonstrate that the relative constriction of the heart may be larger than the relative shortening of the fibers. Panel D shows the response to a change of solution. Threshold is demonstrated in panel E. Panel F shows measurement of restitution and panel G shows measurement of length-tension relations. Vertical bars are 25 mg/cm and horizontal bars are 0.5 sec.

The other rod was attached to a motor which made it possible to stretch the preparation. Rapid exchange of the perfusate was accomplished by turning a valve which directs fluid through the channel. The response to a change of perfusate from sea water (SW) to artificial sea water is shown in Figure 1D. The response was complete in 10-20 sec. Myocardial preparations placed in this chamber had a well defined electrical threshold (5-6 volts, Fig. 1E). The

restitution curve in panel F shows that the final twitch tension was constant during paired stimulation independent of the interbeat time. The constancy of twitch tension may reflect maximal activation of contractile elements independent of small variations in the action potential. Figure 1G shows recordings of twitch tension as the initial distance between he rods was increased. These traces qualitatively resemble previous results obtained with the bulge-clamped preparation (Cleemann, Dillon & Morad, MDBIL Bull. 16, 8-13, 1976). Compared to the bulge-clamped preparation the new setup was much easier to use and it gave similar results but the recorded active wall tension was about 10%. The tendency of the heart to spiral around the two rods may be in part responsible for the lower tension. Microscopic examination of fixed whole hearts revealed that the orientation of the muscle fibers was helical with pitch angle of 30-40°. Near the center the heart had a narrower section where the helical orientation changed from right handed to left handed. Figure 1B and C schematically show a segment of the tubular heart and the orientation of the fibers for various degrees of contraction. These panels also suggest that only a minority of the fibers in a cut segment may generate a force which is directly transmitted to the transducer.

Figure 2 shows that twitch tension is decreased when the beat interval is decreased; that is, negative "staircase." The plotted tension is the steady state tension recorded about one-half min after a change in stimulus frequency. The patterns of excitation-contraction coupling demonstrated in Figures 2 and 1F indicate that there is no long term contractile history in development of tension.

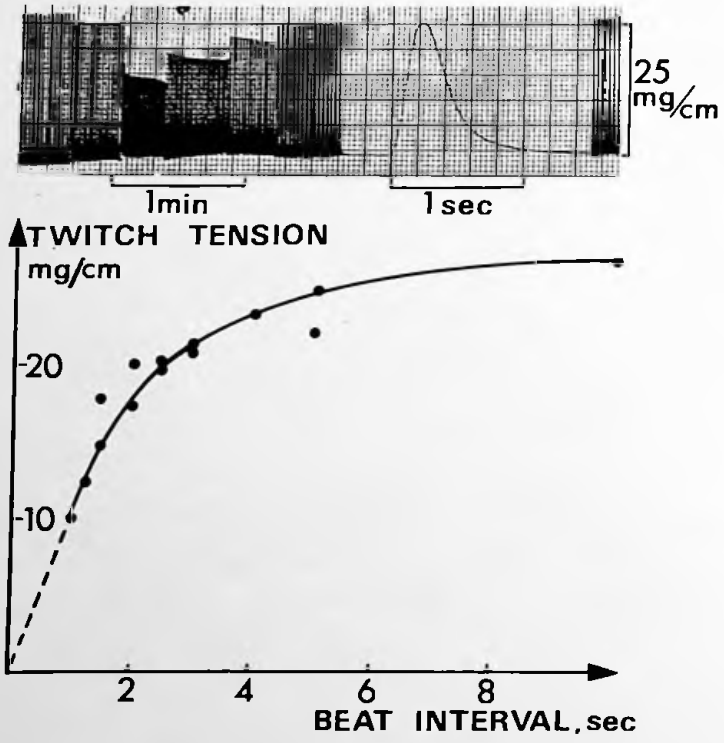

Figure 2. The top recording shows the twitch tension at various stimulus rates. Negative staircase is shown in the lower graph where the steady state twitch tension is plotted versus the beat interval.

Whole heart experiments. In some experiments the heart was cannulated at both ends as shown in the top panel of Figure 3. Stimulation of one end of the preparation results in a visible peristaltic wave which was recorded on an 8 mm movie film. With a pressure transducer attached to either end the pressure at the left, P_l, and right-end, P_r, could be recorded simultaneously. Frame by frame analysis of the movies showed that the speed of propagation of the contractile wave was about 12 mm/sec and it was confirmed that the total volume of the heart remained constant during single contraction. At each point along the heart the decrease in the diameter was typically 40% and the

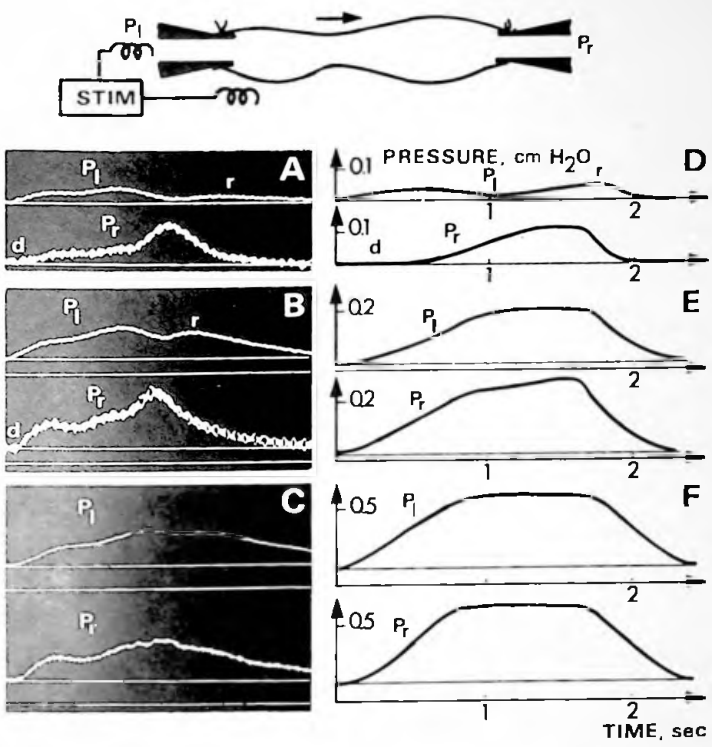

Figure 3. Pressures measured at the stimulated left end, P_l, and at the right end, P_r, of an entire heart are shown in panels A, B, and C for increasing filling of the heart. Panels D, E, and F show corresponding results obtained from a computer simulation. Delay and reflected waves are marked d and r, respectively. The scales of the graphs (D, E, and F) match those used during the corresponding recording (A, B, and C, respectively).

duration of the constriction was 1 to 1.5 sec. Consistent with the spiraling of the fibers the constriction was somewhat larger than the shortening of the fibers measured in sarcomere length-tension relationship (Fig. 1, panels B and C). Figure 3 panels A, B, and C shows the pressure at the left and right end when the initial volume of the heart is varied. The increase in resting pressure is indicated by horizontal lines in panels B and C. In panel A there is clearly a difference between the right and left-end pressures. In panel B the filling and baseline pressure were increased. Right and left-end pressures were increased but the difference between the two end pressures was less prominent. Further filling in panel C resulted in larger but virtually indistinguishable left and right-end pressures. Thus increasing the initial volume increases both left and right-end pressures but the pressure difference remains fairly small ($\simeq 0.1$ cm of H_2O).

A computer simulation was performed to gain insight into these results and to test whether they are compatible with the results from the bulge-clamped preparation. The basic equations for fluid motion in an elastic tube have the form of transmission line equations. The velocity, v, the radius, r, and the pressure, P, are calculated as functions of time, t, and distance along the axis of the heart, x. Conservation of energy yields:

$$\rho \frac{\delta v}{\delta t} = -v \frac{8\mu}{r^2} - \frac{\delta P}{\delta x}$$

where $\rho = 1$ g/cm^3 is the density of the fluid, and $\mu = 0.015$ poise and is the viscosity. Only the velocity in the longitudinal direction is considered. The viscosity term is estimated using Poiseuilles law. Conservation of the fluid volume inside the heart yields:

$$\frac{\delta}{\delta x}(vr^2) = \frac{\delta}{\delta t}(r^2)$$

The pressure is derived from the passive and active wall tension, T_p and T_a, respectively, using Laplace's law:

$$P = (T_p + T_a)/r$$

The passive tension is only a function of the local radius. The active tension was assumed to be the product of two factors of which one depends on the radius and the other on the time after the onset of excitation. This assumption is justified by previously reported experiments (Cleemann, Dillon & Morad, MDIBL Bull. 18:54-56, 1978). The expressions for length-tension relations and for the time course of the activation are approximations of previously measured curves (Cleemann, Morad & Dillon, MDIBL Bull. 16:8-13, 1976). The curves in panels D, E, and F of Figure 3 are obtained by step-wise integration of these nonlinear partial differential equations. The initial volume of the heart in the three panels was chosen to match approximately the magnitude of the resting and active pressures in panels A, B, and C. The agreement between measured and calculated pressures is satisfactory. The computer simulation shows that transmission delay is most prominent when the heart is least distended. The first contraction at the stimulated end is transmitted with some delay (d) to the other end and the contraction here is to some degree reflected back (r) to the stimulated end. As transmission velocity increases with increasing filling pressure the reflected wave fuses with the earlier pressure peak. The pressure difference between the two ends of the heart is related to the viscous resistance to backflow through the traveling constriction of the heart. The calculations assume a uniform radius of the relaxed heart. The narrow section present near the center of the heart may account for some of the differences between measured and calculated pressures. However, the calculations are sufficiently realistic to confirm that the pressure difference between the two ends of the heart remains of the order of 0.1 cm of water even when the increased filling pressures produce much larger total pressures.

Experiments with the whole heart were also performed under conditions where the lumen and the outside were perfused with different solution. Epinephrine was found to be effective only when added to the luminal perfusate. Epinephrine increased the strength of contraction and reversed the conduction block which often developed at the narrow central section of the heart at the end of the experiment.

While applying a subthreshold pulse through the cannula at one end of the heart and advancing an electrode through the cannula at the other end of the heart it was possible to measure the electrical space constant of the whole heart. The resistivity of the myocardial wall calculated from these measurements was not larger than that obtained from the bulge clamped preparation. This finding may suggest that damaged edges of the bulge-clamped preparation do not compromise electrical resistance measurements.

EFFECT OF "LOOP DIURETICS" ON SODIUM TRANSPORT BY PLASMA MEMBRANE VESICLES ISOLATED FROM THE RECTAL GLAND OF SQUALUS ACANTHIAS

R. Kinne, E. Kinne-Saffran, J. Epstein, P. Silva, F.H. Epstein, D. Miller and J. Eveloff, Max-Planck-Institut für Biophysik, Frankfurt (Am Main), Germany, Beth Israel Hospital, Boston, Massachusetts and Mt. Desert Island Biological Laboratory, Salsbury Cove, Maine.

Chloride reabsorption in the renal thick ascending limb of Henle's loop (TALH) in mammals and chloride secretion in the rectal gland of the shark have many similarities. Both chloride transport mechanisms are active, sodium dependent and inhibited by ouabain (1,2). Additionally, it was found that furosemide, a potent diuretic, inhibits salt transport in the rectal gland of Squalus acanthias as well as Scyliorhinus canicula (2,3). In the latter animals the diuretics bumetanide and piretanide also decrease the rate of chloride secretion in the perfused in situ rectal gland (3).

Recently it has been demonstrated that plasma membranes isolated from the rectal gland of Squalus acanthias contain a sodium-chloride cotransport system as indicated by the chloride dependence of sodium transport in these vesicles (4). The chloride-dependent sodium transport in the vesicles was also inhibited by furosemide (4). The