Calcium Fluxes in Frog Heart Ventricles

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Summary. Calcium fluxes in frog heart ventricles are determined both during quiescence and during periods of activity. The two main results: (a) calcium influx is much increased by activity; (b) a net release of calcium from the cells occurs immediately after activity, in parallel with, and probably related to, the decline in tension of the downward "staircase".

Key-Words: Calcium Fluxes — Heart Muscle.
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When heart ventricles which have been kept at rest are subjected to a period of continuous stimulation, heart beats build up in strength during the first 5 to 20 min. When stimulation is ended, the strength of heart beats elicited by infrequent test shocks gradually subsides. This "staircase" phenomenon has been attributed to the accumulation of calcium by heart cells during activity followed by release of the ion during the subsequent period of rest (Moulin and Wilbrandt, 1955; Niedergerke, 1956; Niedergerke and Orkand, 1966). This hypothesis has now been tested by direct determination of calcium fluxes, using specially developed procedures which have been described in the preceding communication (Niedergerke and Page, 1969).

In all experiments 45Ca-labelled Ringer's fluid (1.5 mM[Ca]) was used, approximately two hours after dissection, to perfuse heart ventricles at rest (controls) or during periods of activity (at 20 beats/min). The resting state of ventricles was maintained by the drug tetrodotoxin, TTX, in a concentration of $2.5 \times 10^{-7}$ g/ml, which served to block action potentials which might otherwise be set up by pacemaker tissue in the ventricle (for the action of TTX in the frog heart cf. Hagiwara and Nakajima, 1965). A small proportion of hearts, not sufficiently responsive to TTX, was discarded. TTX, by itself, did not appear to influence 46calcium fluxes of resting ventricles.

Fig. 1 a shows the time course of 45Ca uptake in a ventricle at rest. The effect of a period of activity is illustrated in Fig. 1 b. In both experiments, the quantities of labelled calcium taken up, or released, by the tissue in each successive perfusion period have been calculated from the measured concentrations and volumes of perfusion fluid by means of
equation (1) of the preceding communication (Niedergärke and Page, 1969) and the cumulative amounts of $^{45}$calcium in the tissue so obtained have been plotted against time of perfusion. Resting uptake of $^{45}$calcium showed an initial rapid rise which was followed by a slow phase to eventual saturation of the tracer in the tissue (the saturation point is not shown in Fig. 1a). While the initial phase, of magnitude approximately 0.2 $\mu$-mole/ml heart cells (as obtained by extrapolation of the linear portion of the slow phase to time zero), is probably mainly due to adsorption of calcium to tissue surfaces, the later phase, of approximately 0.6 $\mu$-mole/ml, is considered to occur as the result of $^{45}$Ca influx into heart cells.

The effects of activity were examined in ventricles which were stimulated after the first 40 to 60 min of tracer loading, i.e. when the fast phase of $^{45}$calcium uptake was over. As is seen in Fig. 1b, $^{45}$calcium uptake was greatly enhanced during activity, the rate of build up becoming up to eight times greater compared with that at rest. The magnitude of resting and extra $^{45}$calcium influx was obtained from the slopes of the tangents drawn to the appropriate sections of the curves, as shown in Fig. 1a and b, and by use of the scaling factor S/V, of $1 \times 10^4$ cm$^{-1}$ (the appropriate value of the ratio of the surface over the volume of heart fibres, Niedergärke, 1963, page 566). $^{45}$Calcium influx came to $4.1 \pm 0.3 \times 10^{-15}$ mole/cm$^2$sec (mean $\pm$ s.d., $n = 8$) for the resting influx and to $130 \pm 4 \times 10^{-15}$ mole/cm$^2$ (mean $\pm$ s.d., $n = 8$) for the extra influx per heart beat, respectively. These results are very similar to those previously obtained with a different method and under somewhat different conditions (Niedergärke, 1963) (10 shocks/min at 6--8°C, as compared with 20 shocks/min at 20°C in the present work).

Results of particular importance in the present context were obtained during the period of rest following stimulation. Fig. 1b shows that a proportion of $^{45}$calcium which had previously been taken up was released from the tissue during this time. The release consisted of an early phase, of $5.7 \pm 0.9 \times 10^{-6}$ mole/ml heart cells (mean $\pm$ s.d., $n = 7$) during the initial 7 to 8 min, and of a slow, rather variable phase, of $5.0 \pm 3.7 \times 10^{-6}$ mole/ml (mean $\pm$ s.d., $n = 6$) during the subsequent 100 min of rest. Since the concentration of external $^{45}$calcium rose under these conditions to values above that which was present in the perfusion fluid added to the ventricle, the release cannot have occurred in exchange with external label but must have been due to net efflux of the ion from the cells. The inference from this is that a net uptake of calcium by heart cells had taken place during the preceding period of activity. It may be mentioned that the net calcium influx responsible for this effect was in addition to the extra influx which resulted from the known increase of calcium exchange in heart cells during stimulation (Niedergärke, 1963).