INVESTIGATION OF THE ROLE OF MITOCHONDRIA IN THE CARDIAC CONTRACTION–RELAXATION CYCLE

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1. Introduction

The contraction–relaxation cycle of cardiac muscle, like that of skeletal muscle, is quantitatively controlled by the translocation of Ca^{2+} between intracellular structures [1–4]. However, unlike skeletal muscle, contraction of cardiac muscle is dependent on the concentration of extracellular Ca^{2+}. Entry of a small amount of Ca^{2+} during the plateau phase of the action potential is thought to provide a trigger for the release of a larger amount of bound calcium from intracellular storage sites [5, 6]. When the intracellular Ca^{2+} concentration rises above about 10^{-7} M, inhibition of actomyosin ATP-ase is relieved by Ca^{2+} binding to a subunit of troponin in the troponin–tropomyosin–actomyosin complex, and contraction is initiated [4]. Relaxation occurs by an energy-dependent removal of Ca^{2+} from its troponin binding sites. Isolated sarcoplasmic reticulum vesicles and mitochondria are both able to accumulate Ca^{2+} against a concentration gradient by energy-linked processes [7, 8], and a question has arisen recently concerning their relative roles for Ca^{2+} sequestration *in vivo* [9–11].

Experiments reported here were designed to elucidate the role of mitochondria in the calcium cycle, using intact working rat heart preparation perfused with medium containing glucose. Data obtained with the rat heart are compared with similar studies using frog hearts, since amphibian cardiac muscle is noted for its relative deficiency of sarcoplasmic reticulum [12]. Use is made of the energy transfer inhibitor oligomycin, which prevents ATP-supported but not respiration-supported Ca^{2+} uptake by isolated mitochondria [8]. Our conclusion is that mitochondria in the intact mammalian heart play only a minor role in the control of beat-to-beat calcium cycle, and appear not to be involved in producing an energy dependent decrease of intracellular Ca^{2+} during relaxation. On the other hand, in frog cardiac muscle, mitochondrial Ca^{2+} sequestration and release is of importance in the contraction–relaxation cycle, and part of the mitochondrial respiration is Ca^{2+}-linked.

2. Methods

Hearts from well fed male rats (220–250 g) of the Wistar strain were perfused at 35° in a modified version of the working heart apparatus described by Neely et al. [13]. The basic modification consisted in having a constantly filled 20 ml reservoir, fitted with an overflow, 80 cm above the aortic cannula. This ensured a constant perfusion of the coronary circulation when the aortic pressure failed to rise above the pressure head during systole. Left ventricular filling occurred via the cannulated pulmonary vein from a small reservoir placed about 10 cm above the heart. The perfusion fluid was modified Krebs–Henseleit buffer containing 118 mM NaCl, 25 mM $NaHCO_3$, 4.7 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM

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$MgSO_4$ and variable amounts of $CaCl_2$, which was equilibrated with O_2 or N_2 gas containing 5% CO_2.

Left ventricular pressure was measured with a Statham P23d pressure transducer by inserting a 22 gauge needle through the ventricle wall. Aortic pressure was measured directly by means of a similar transducer. Pressure changes were recorded on a Brush 440 recorder. Cardiac output was estimated from the sum of the left ventricular output and the coronary flow rate. Pressure work was calculated according to Neely et al. [13]. Oxygen consumption was calculated from the coronary flow rate measured at 2 min intervals and the A–V oxygen tension difference. The oxygen tension of the coronary effluent was measured continuously by means of a Clark oxygen electrode.

Frog hearts were perfused with Ringer's fluid through the aorta using air saturated medium and a pressure head of 25 cm. Ventricular pressure was measured as for rat hearts.

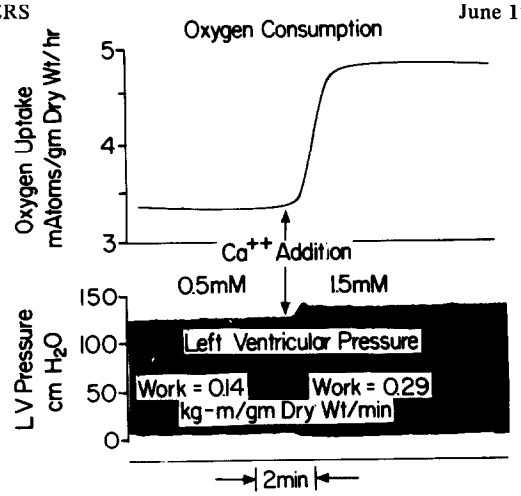

Fig. 1. Effect of Ca^{2+} addition on oxygen consumption, left ventricular pressure and work in the perfused rat heart. The solid area of the pressure trace represents the maximum systolic–diastolic excursion.

3. Results

Both heart work and oxygen consumption are critically dependent on the Ca^{2+} concentration of the perfusion medium. Fig. 1 illustrates the rapidity of the response to a stepwise increase of Ca^{2+} from 0.5 to 1.5 mM in a rat heart paced at 240 beats/min and perfused with 20 mM glucose. Oxygen uptake increased within 2 min from 3.4 to 4.8 matoms/g dry wt/hr, while pressure work doubled from a value of 0.14 kg-m/g dry wt/min. Although the left ventricular pressure change was small, the work of the heart doubled and was accounted for mainly by an increased stroke volume.

Addition of oligomycin (6 μg/ml) to a rat heart paced at 240 beats/min and perfused with 20 mM glucose and 0.5 mM Ca^{2+} produced a gradual fall of oxygen uptake from 3 to 1.3 matoms/g dry wt/hr and a 65% decrease of left ventricular pressure over a 20 min interval (fig. 2). Further additions of oligomycin caused no additional effects indicating that respiration was maximally inhibited. Subsequent increase of the external Ca^{2+} to 1.5 mM produced a marked increase of left ventricular pressure, but oxygen uptake remained unaffected. Confirmatory evidence that flux through the mitochondrial electron transport chain was not changed upon Ca^{2+} addition was also provided by surface fluorometry measurements. No effect was observed on the oxidation–reduction state of either the flavin or pyridine nucleotides after Ca^{2+} addition in the presence of oligomycin.

In a similar experiment, but with the heart perfused initially with 1.5 mM Ca^{2+}, oligomycin decreased oxygen uptake from 5 to 1.4 matoms/g dry wt/hr (fig. 3). Left ventricular pressure fell to 30% of its initial value, and the heart ceased pumping fluid against the aortic pressure head. Addition of 0.1 μg/ml of norepinephrine doubled the left ventricular pressure, but again no effect on oxygen uptake was seen. A similar response to norepinephrine in the presence of oligomycin was observed at a perfusate Ca^{2+} concentration of 0.5 mM.

These data illustrate two points. First, the large decrease of oxygen uptake obtained upon inhibiting mitochondrial ATP production with oligomycin shows that in the working rat heart more than 70% of the respiration must be ADP-linked. The low rates of respiration observed here with oligomycin-inhibited hearts are similar to those reported by Challoner [14, 15] for Langendorff-perfused rat hearts after oligomycin addition or K^+ arrest. Thus, the residual, oligomycin-insensitive respiration appears unrelated to the mechanical activity of the heart. The low phosphate potential induced by oligomycin [14] causes a stimulation of phosphofructokinase. Enhanced ATP production by glycolysis is able to support cardiac

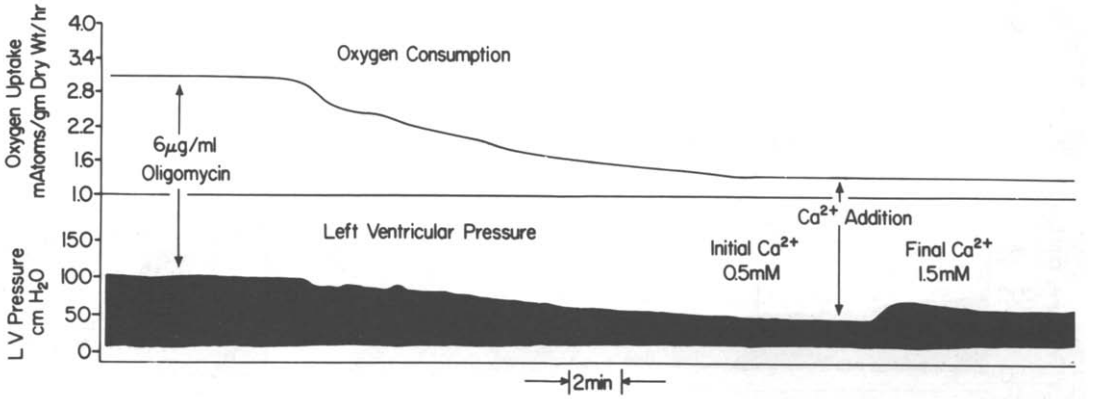

Fig. 2. Effect of Ca^{2+} addition on oxygen consumption and left ventricular pressure in rat hearts perfused in the presence of oligomycin.

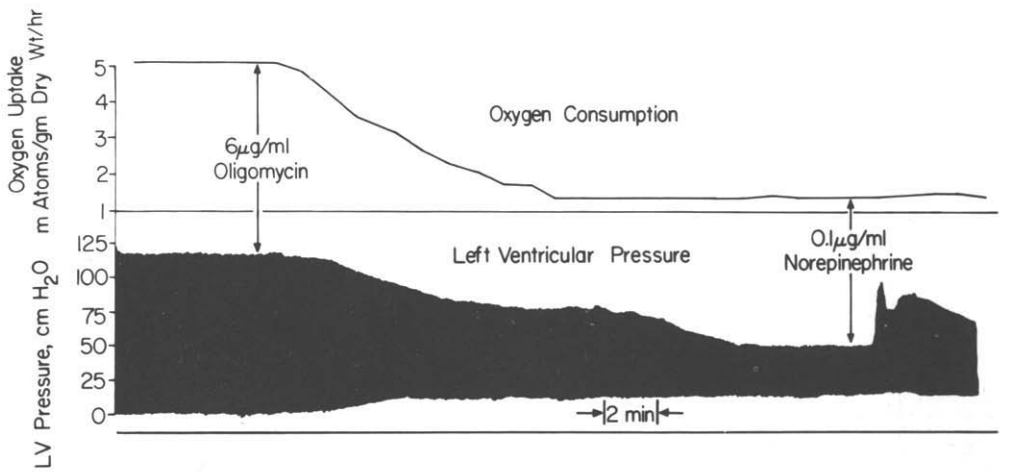

Fig. 3. Effect of norepinephrine addition on oxygen consumption and left ventricular pressure in rat hearts perfused in the presence of oligomycin with 1.5 mM Ca^{2+}.

contractile activity, although with diminished performance. Secondly, the lack of effect of an increased availability of Ca^{2+} to the actomyosin complex on respiratory activity indicates that mitochondria in the rat heart are not involved in the energy-linked Ca^{2+} uptake required for relaxation.

Further support for this conclusion was provided by the experiments shown in fig. 4 in which rat hearts were perfused under anaerobic conditions in the presence of 20 mM glucose. Intraventricular pressure development was well maintained when the mechanical activity was supported by ATP produced from glycolysis, although the heart rate fell to 60–80 beats/min. Addition of oligomycin to prevent any ATP-supported Ca^{2+} uptake by mitochondria caused only a transient decrease of left ventricular pressure, and contractility recovered after a few minutes. Left ventricular pressure development was increased either by raising the external Ca^{2+} concentration from 0.5 to 1.5 mM (fig. 4A) or by the addition of norepinephrine (fig. 4B). Perfusion of the hearts beyond the times shown on the traces gave no evidence that the ability of the heart to relax was impaired. These data indicate that non-mitochondrial Ca^{2+}-sequestering systems (presum-

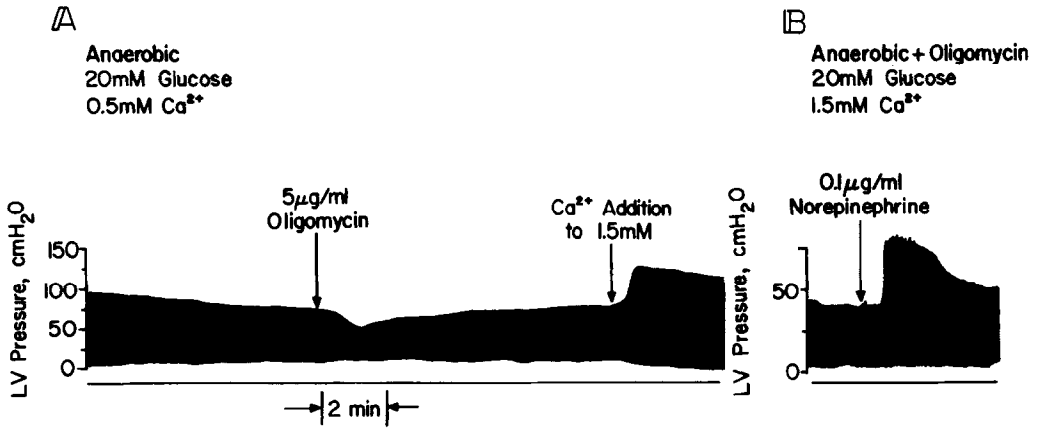

Fig. 4. Effects of Ca^{2+} addition and norepinephrine on left ventricular pressure in rat hearts perfused anaerobically in the presence of oligomycin.

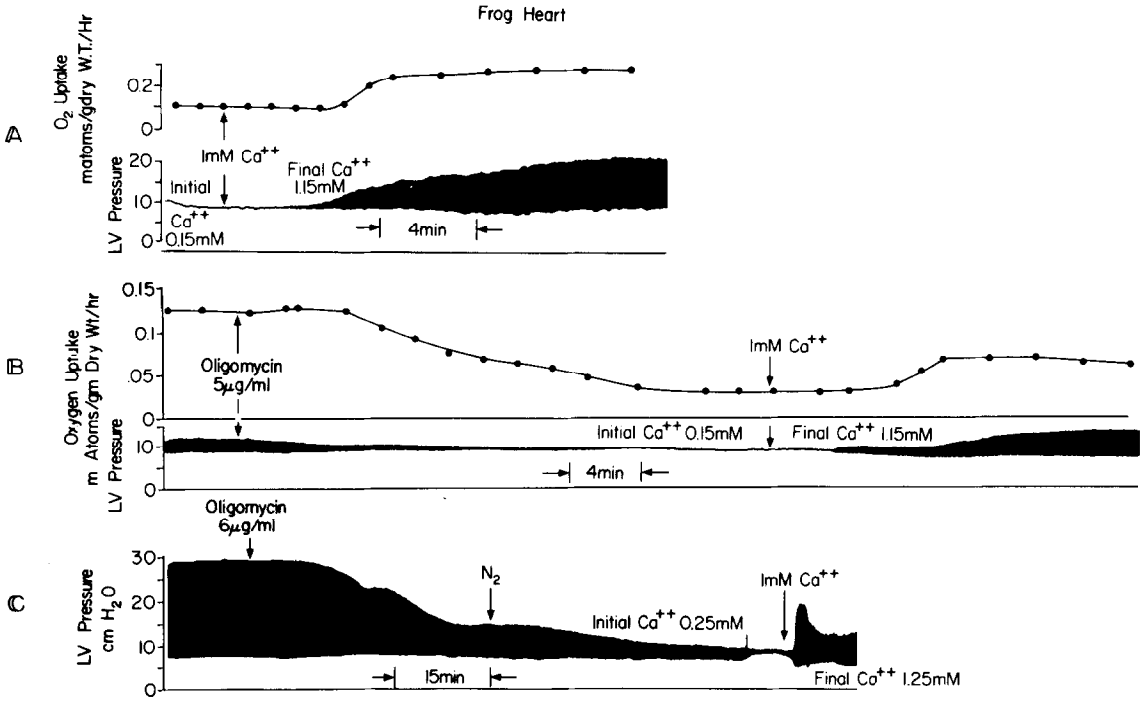

Fig. 5. Effect of Ca^{2+} addition on ventricular pressure in frog hearts perfused aerobically in: A) the absence, and B) the presence of oligomycin. Trace C) shows the effect of Ca^{2+} addition in anaerobic frog hearts perfused in the presence of oligomycin.

ably sarcoplasmic reticulum) are sufficiently active to support complete relaxation when the heart is stressed under conditions in which possible Ca^{2+} uptake by mitochondria is prevented.

In contrast to the above studies with the rat heart, similar experiments with perfused frog hearts gave markedly different results. Figs. 5A and 5B show the

effect of oligomycin on the response of the aerobic, glucose perfused frog heart to an increase of perfusate Ca^{2+} from 0.15 to 1.15 mM. With frog heart, unlike rat heart, addition of Ca^{2+} in the presence of 5 μg/ml oligomycin not only augmented contractility but also more than doubled the rate of oxygen uptake, indicating a direct involvement of mitochondria in the energy-linked removal of Ca^{2+} from troponin during relaxation. Fig. 5C shows that ventricular pressure development by the frog heart was essentially suppressed when the heart perfused initially with 0.25 mM Ca^{2+} was subjected first to oligomycin and then to anoxia in order to prevent both ATP-linked and respiratory-linked Ca^{2+} uptake by the mitochondria. However, subsequent Ca^{2+} addition to 1.25 mM enhanced contractility with no development of contracture, indicating that Ca^{2+} sequestering systems other than mitochondria are also involved in the calcium cycle of frog heart.

4. Discussion

Previous studies by Horn et al. [16] using rat hearts perfused with 1 mM iodoacetate, 5 mM pyruvate and 1 μg/ml oligomycin showed that upon subsequent epinephrine addition or electrical stimulation, contraction of the muscle developed. These results were interpreted on the basis of a failure of mitochondria to support Ca^{2+} uptake. Since in our experiments rat hearts perfused anaerobically in the presence of oligomycin showed no impairment of relaxation, the data of Horn et al. [16] may be interpreted on the basis of a non-specific toxic action of iodoacetate or as a consequence of ATP deficiency. Other evidence in favor of mitochondria being involved in Ca^{2+} uptake during muscular relaxation *in vivo* is also very tenuous, being based on the distribution of $^{45}Ca^{2+}$ between mitochondria and sarcoplasmic reticulum vesicles isolated from cardiac or skeletal muscle after injection of $^{45}Ca^{2+}$ to the whole animal [9, 10] or to the perfused rat heart [11]. Although mitochondria contained the bulk of the $^{45}Ca^{2+}$ found in the homogenate, it is probable that this can be accounted for by the isotope becoming bound to the mitochondrial membranes during fractionation, followed by release of $^{45}Ca^{2+}$ upon discruption of the sarcoplasmic reticulum tubules and other membranes. Three other points based on experiments with isolated mammalian mitochondria argue against their having more than a minor role for Ca^{2+} uptake during muscle relaxation: i) the rate of Ca^{2+} uptake is too slow at Ca^{2+} concentrations between 10^{-5} and 10^{-7} M [17, 18]; ii) the affinity of mitochondria for active Ca^{2+} uptake is much lower than that for sarcoplasmic reticulum [7, 19]; and iii) the only known mechanisms for discharging Ca^{2+} from the mitochondria provide for a relatively slow Ca^{2+} release and are associated with complete energy depletion [8, 17]; an event unlikely to occur *in vivo*. Non-energy-linked binding of Ca^{2+} to mitochondria during relaxation also appears unlikely because of the relatively low affinity of the bulk of the cation binding sites for Ca^{2+} (10^{-4} M) and the small number of high affinity (10^{-6} M) Ca^{2+} binding sites [8, 20].

It is of particular interest that in frog hearts, which are characterized by an almost nonexistent sarcoplasmic reticulum, the mitochondria are shown to be involved in an energy-dependent Ca^{2+} uptake during the contraction–relaxation cycle. These data contrast with results obtained with the rat heart, and suggest the possibility that amphibian cardiac mitochondria may have a higher affinity for Ca^{2+} than mammalian mitochondria in order to effect a release of Ca^{2+} from the troponin binding sites. Since evidence is at hand that Ca^{2+} release occurs with frog cardiac mitochondria *in vivo*, studies with these mitochondria may provide a more suitable preparation than mammalian mitochondria for elucidation of the physiological mechanism of Ca^{2+} release. Our data suggest that significant differences may exist between the two types of mitochondria in relation to the physiological mechanisms of Ca^{2+} uptake and release.

Acknowledgements

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