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EXCITATION-CONTRACTION UNCOUPLING DURING ISCHEMIA IN THE BLOOD PERFUSED DOG HEART

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The bioluminescent Ca^{2+}-indicator, aequorin, was loaded into the left ventricular apex of blood-perfused hearts from 13 dogs for simultaneous recording of left ventricular pressure and intracellular calcium levels. During a 2 minute period of ischemia, systolic and diastolic pressures significantly decreased. In contrast, these pressure changes were associated with an increase in both systolic and diastolic calcium reaching a maximum diastolic value of 0.59 μM and a systolic value of 1.11 μM. This apparent dissociation between pressure and $[Ca^{2+}]_i$ supports the hypothesis that changes in myofilament Ca^{2+} responsiveness are of major importance in modulating contractility during ischemia in large mammalian hearts.

In the isolated, buffer-perfused, isovolumic ferret heart, the bioluminescent Ca^{2+} indicator, aequorin (1) has been used to investigate the changes that occur in intracellular ionized calcium concentrations ($[Ca^{2+}]_i$) during hypoxia and ischemia. The light emitted by the interaction of Ca^{2+} with aequorin serves as a qualitative and quantitative index of cytosolic calcium levels (2-4). Results of these studies indicate that changes in myofilament Ca^{2+} responsiveness are of major importance during ischemia, an observation that has considerable significance with regard to therapeutics in these conditions. However, because of 1), the intrinsic differences in excitation-contraction coupling processes between small and large animal hearts, and 2), the use of saline buffer rather than blood as the coronary perfusate, the results of these studies must be extrapolated to man with great caution. The hypotonicity and

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reduced oxygen carrying capacity of saline perfusate are of particular concern in large heart studies of ischemia where metabolic compensation may be marginal and slight changes in supply or demand can cause major alterations in function. Accordingly, the present study was undertaken to develop a blood-perfused large heart model that would allow direct measurement of $[Ca^{2+}]_i$ handling during physiologic and pharmacologic interventions, and in particular, during ischemia.

METHODS

Isolated, blood-perfused hearts were prepared from 13 mongrel dogs as previously described (2,3). Each dog was anesthetized intravenously with a mixture of urethane, 500 mg/kg, and chloralose, 50 mg/kg, then intubated and maintained on mechanical ventilation until removal of the heart. The coronary arteries were perfused with blood obtained from the same animal after cardiac excision (pH=7.6, pO_2=600 mm Hg, pCO_2=45 mm Hg) and circulated by a roller pump through a bubble trap, flowmeter, and heat exchanger (with temperature maintained at 30°C). Coronary perfusion pressure was maintained at 80-100 mm Hg throughout the experiment. Coronary venous blood was drained from the right ventricle and an intramyocardial thermistor was placed in the right ventricle to monitor temperature. A left ventricular conduit was inserted in the apex to drain Thebesian flow. An inflatable latex balloon was inserted through the mitral valve into the left ventricle and secured just below the annulus. A balloon size was chosen that was greater than left ventricular diastolic volume in order to ensure that pressure increments would reflect left ventricular rather than balloon tension changes. Electrocardiographic leads and pacing electrodes were secured on the surface of the myocardium.

The entire isolated heart preparation was positioned in a light-tight box for collection of the aequorin light signal (2,3). Aequorin was loaded into a 5 mm diameter area of the left ventricular apex by "macroinjection", which we have previously utilized only for saline-perfused small animal hearts (2,3). Aequorin-light signals were recorded from the photomultiplier as anodal current that was filtered at a time constant of 0.01 sec. The light signal, left ventricular isovolumic pressure, coronary perfusion pressure, and electrocardiographic signals were recorded simultaneously. A calibration procedure similar to that described previously was used for conversion of light signals to quantitative Ca^{2+} concentrations. This procedure involves normalization of the aequorin light at each phase of the experiment by the amount of active aequorin in the preparation and then conversion of the normalized light signal to a $[Ca^{2+}]_i$ value by use of an *in vitro* calibration curve (2,3). Results were analyzed by Student's t-test. A p value <0.05 was considered significant. Data are expressed as mean ± S.E.

RESULTS

Aequorin Signal and Quantitative Ca^{2+}_i

Figure 1A shows individual, unaveraged tracings of the $[Ca^{2+}]_i$ transients and left ventricular pressure from a representative experiment. Each $[Ca^{2+}]_i$ transient shows a time course and temporal relation to the mechanical activity that is similar to those obtained from ferret ventricular papillary muscles and buffer perfused, isovolumic whole heart preparations. The ability to evaluate individual $[Ca^{2+}]_i$ transients in real time allowed us to study changes that occur with each cardiac cycle during short-term force-frequency responses (Figure 1A), and inotropic manipulation with increased extracellular calcium (Figure 1B) or isoproterenol (Figure 1C). Note that the positive inotropic effect of each of

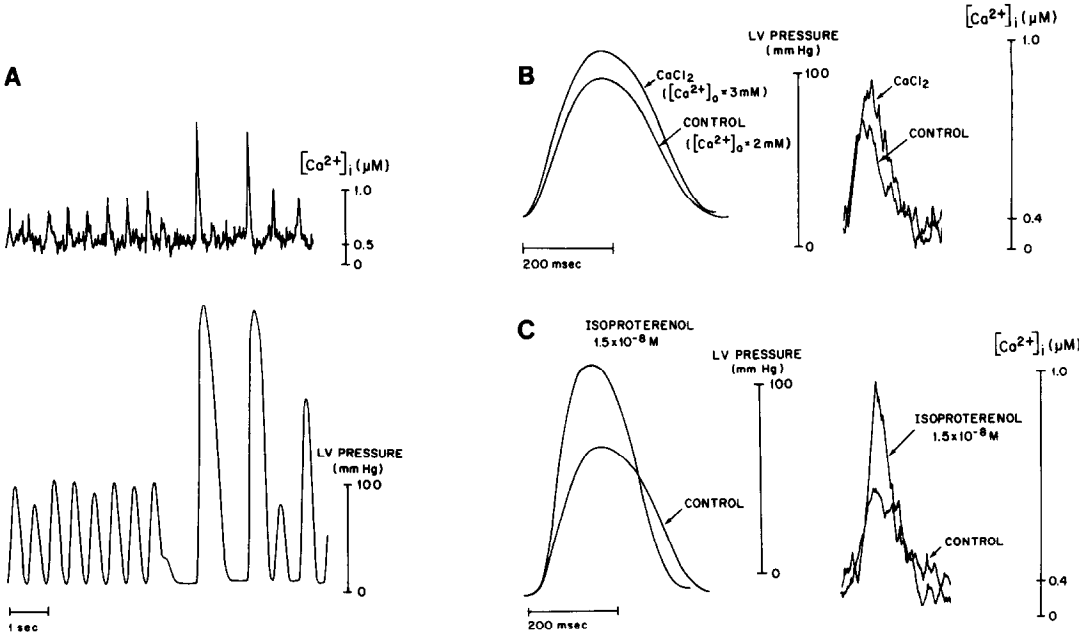

Figure 1A Intracellular calcium transients (upper trace) and isovolumic left ventricular pressure ($[Ca^{2+}]_i$) trace (LV, lower trace), in a blood-perfused dog heart showing spontaneous rhythm changes. Note the marked increase in Ca^{2+}_i and LV pressure with post-extrasystolic potentiation. Each trace represents an individual signal; peak aequorin light is proportional to the 2.5th power of peak $[Ca^{2+}]_i$ (1). **Figure 1B**, effects of elevating blood $[Ca^{2+}]_i$ from 2 to 3 mM; **Figure 1C**, effects of isoproterenol. Note that the amplitudes of the aequorin signals are proportional to those of left ventricular pressure.

these interventions was associated with enhanced $[Ca^{2+}]_i$ availability, as evidenced by the increased amplitude of the aequorin light signal. Under the control conditions of our experiments, peak systolic calcium was determined to be 0.72±0.11 μM (n=8), and end-diastolic calcium 0.27±0.09 μM (n=8).

Effects of Ischemia

Representative left ventricular pressure and aequorin signals during control blood perfusion, after 2 minutes of global ischemia, and after 5 minutes of reperfusion are shown in Figure 2. Similar effects of ischemia and reperfusion were observed in each of the 5 hearts studied. Systolic pressure development was rapidly and severely depressed to 36±13% of pre-ischemic values when ischemic conditions were produced by total occlusion of the perfusion line at the descending aorta. The diastolic pressure level also decreased by 25±14% when coronary perfusion was interrupted, and no increase in diastolic pressure was observed during the subsequent period of ischemia. In contrast, this depression of function was associated with a marked rise in cytosolic Ca^{2+}. End-diastolic levels rose from 0.22±0.09 to 0.39±0.14 μM and peak systolic levels from 0.84±0.07 to 1.04±0.07 μM, $p<.01$. End-diastolic $[Ca^{2+}]_i$ continued to rise throughout the period of ischemia reaching a maximum level of approximately 0.6

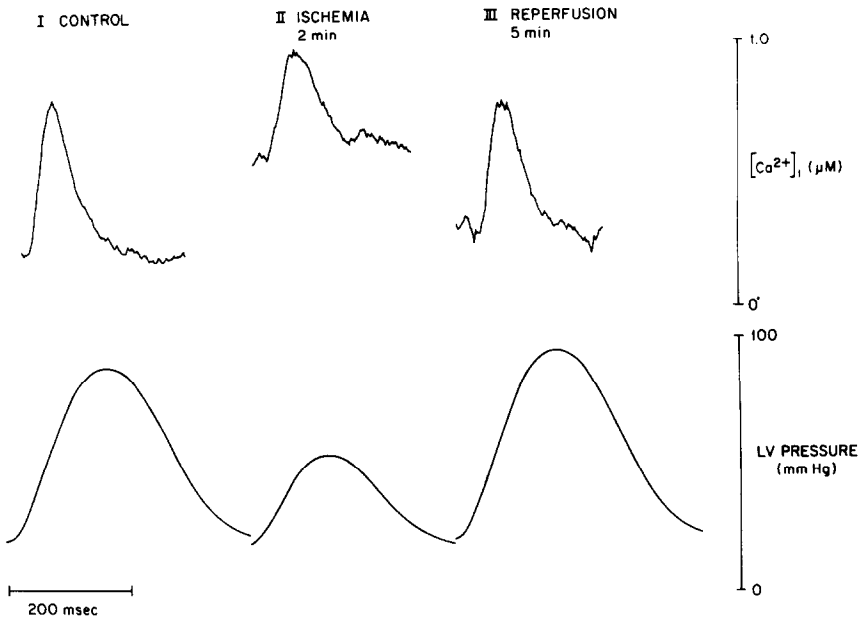

Figure 2 The effect of 2 minutes of total global ischemia on an aequorin-loaded, blood-perfused dog heart. The upper traces are aequorin signals; the lower traces the left ventricular (LV) pressure. To the right of the figure, intracellular calcium levels and left ventricular pressure are shown. Representative tracings are shown from individual contraction-relaxation cycles during control (I), after 2 minutes of ischemia (II) and after 5 minutes of reperfusion (III).

μM. All of these changes in $[Ca^{2+}]_i$ and mechanical function were reversible with reperfusion and returned to control (pre-ischemic) levels.

DISCUSSION

This is the first report of direct intracellular recording of $[Ca^{2+}]_i$ during blood perfusion of aequorin-loaded whole heart preparations and provides a significant advantage over the other currently available techniques that cannot be readily used in combination with blood perfusion, including nuclear magnetic resonance and fluorescent indicator approaches (4). The decline in left ventricular diastolic pressure during ischemia occurs rapidly after cessation of perfusion and is probably due to mechanical factors (i.e., loss of erectile effect of coronary vascular perfusion). The decrease in peak systolic pressure is more gradual and does not appear to be due to decreased availability of activator Ca^{2+}, since calcium levels rise during this period (Figure 2). These results are similar to those previously reported in the ischemic ferret heart (2,3). As was the case in that earlier study, we believe that this apparent dissociation between force and calcium may be due to the accumulation of inorganic phosphate and hydrogen ions during ischemia, both of which have been reported to decrease myofilament calcium responsiveness (2-5). This change could result from effects on Ca^{2+} binding to troponin C, the calcium receptor protein of the myofilaments, or to other direct effects on the contractile apparatus.

All of these changes occurring during a brief two minute period of ischemia were promptly and fully reversible after restarting blood perfusion.

Lorell et al. (5) have recently shown that perfusion of hearts with the fluorescent Ca^{2+} indicator indo-1 may result in significant vascular endothelial as well as myocardial cell loading, which complicates interpretation of the calcium signal. This does not appear to be a problem with our loading method for aequorin since 10^{-5} M bradykinin, which increases $[Ca^{2+}]$ in endothelial cells, produces no effect in our preparations (4). Mg^{2+} also modulates the interaction of aequorin with Ca^{2+} (1,2). In the present study, we assumed that the intracellular ionized $[Mg^{2+}]_i$ was 1 mM (2). During prolonged hypoxia or ischemia, $[Mg^{2+}]_i$ would rise as ATP is consumed, which could interfere with the ability to make quantitative measurements during this period. To minimize these problems, we limited ischemia to a short period, during which time a significant decline in $[ATP]_i$ would not be expected to occur (2). The systolic and diastolic $[Ca^{2+}]_i$ values in the present study, under control conditions and ischemia, are similar to those previously reported with aequorin in the buffer-perfused ferret heart (2,3). Of interest, values of $[Ca^{2+}]_i$ assessed by NMR techniques are somewhat lower, perhaps due to the Ca^{2+}-buffering properties of the indicator, 5F-BAPTA, used in those studies (4).

These results have several important ramifications. First, the macroinjection technique of loading aequorin into the blood perfused dog heart produces easily detectable light signals with reproducible and quantifiable alterations of $[Ca^{2+}]_i$ during a variety of pharmacological and physiological perturbations. Second, the behavior of $[Ca^{2+}]_i$ during global ischemia in this model is identical to that seen in the buffer perfused ferret heart (2,3). This appears to support the hypothesis that changes in myofilament Ca^{2+} responsiveness are of major importance in modulating contractility during ischemic states. Finally, this model, because of the use of a large animal heart, has the potential to allow assessment of clinically important regional differences in $[Ca^{2+}]_i$; for example, those that might occur during regional ischemia or ventricular fibrillation (3,4).

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