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THE EFFECT OF DICHLOROACETATE ON THE ISOLATED NO FLOW ARRESTED RAT HEART

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Summary

Ischemic dysfunction, including contracture, has been attributed to lack of ATP, although previous work has not been consistent with this concept. We describe here a model of no flow ischemic arrest, characterized by depressed levels of mechanical function upon reperfusion and high energy phosphate stores within normal limits. The decreased mechanical function bears an inverse relationship to myocardial lactate levels after twenty-minutes of reperfusion in the absence or presence of dichloroacetic acid (DCA). Post-ischemic non-DCA treated hearts attained peak work of only 25% of that of controls, while those treated with DCA following ischemia performed almost as well as controls. ATP and CP levels remained high in both DCA treated and non-DCA treated hearts. Lactate levels were high in hearts immediately following ischemia, but were reduced to control levels in post-ischemic hearts perfused with DCA within twenty minutes, whereas those not treated with DCA had lactate levels two to three times that of controls within the same time period. Pyruvate dehydrogenase (PDH) activity was reduced in non-DCA treated post ischemic hearts after twenty minutes reperfusion but was elevated above controls in hearts reperfused with DCA. The data indicates that DCA increases mechanical performance of the isolated post-ischemic rat heart and the proposed mechanism for this increase is the oxidative removal of lactate resulting from an increase in PDH activity.

Introduction

Mechanical dysfunction of the heart resulting from low blood flow is a serious medical problem that arises both as a consequence of myocardial infarctions and therapeutically initiated low flow states necessitated by coronary bypass procedures. Depending upon the extent and length of the low perfusion state the dysfunction can be either transient or irreversible (the so called "stone heart") (1). Hearse et al. have found that the time of onset of ischemic contracture can be correlated with a decrease in myocardial ATP and suggested that the irreversible contraction results from the accumulation of rigor complexes arising from ATP deficiency (2). Similarly, it has been postulated that the persistent dysfunction that follows brief periods of transitory ischemia may be due to depletion of high energy phosphates (3). It is known however, that the onset of pump failure early in ischemia occurs with only a modest decrease on total myocardial ATP stores (4). Neely et al. have found that the increase in lactate levels in ischemic hearts corresponded to the inhibition of glycolysis and mechanical activity when there was little change in ATP and suggested that damage resulted from a build up of metabolic end products before adenine nucleotide stores are significantly reduced (5).

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Furthermore, there is evidence to suggest that lactate is toxic even in well oxygenated preparations of cardiac muscle (6).

Dichloroacetic acid (DCA) a stimulant of pyruvate dehydrogenase has been used to relieve lactic acidosis, improve the electrical activity of experimentally derived ischemic hearts and to increase the cardiac index of human patients suffering from lactic acidosis (7,8,9). Similarly, we have shown that DCA exerts a positive inotropic effect on hearts derived from animals in endotoxin shock and augments the inotropic effect of ouabain and amrinone in the same model (10,11). DCA exerts its effect by the inhibition of the kinase responsible for the conversion of pyruvate dehydrogenase (PDH) to its' inactive (phosphorylated) form (12). As a consequence DCA's effect is to stimulate PDH activity which is the major rate limiting step governing the entry of acetyl Co-A derived from carbohydrates into the Krebs cycle.

It was of interest therefore, to study the effect of DCA in vitro on the mechanical activity of the ischemic arrested heart and attempt to correlate alterations in pumping ability with changes in high energy phosphate stores, lactate or PDH activity levels. We have found that hearts reperfused with DCA containing buffer following twenty minutes of normothermic ischemia resulted in a significant improvement in pumping capacity. There were no significant changes in high energy phosphate concentrations, but PDH activity was increased and lactate content decreased in hearts treated with DCA. The results are consistent with the concept that elevated post-ischemic mechanical performance occurs as a result of lactate removal via DCA stimulation of PDH activity.

Materials and Methods

Adult male Sprague-Dawley rats weighing 300-350 grams were obtained from Hilltop Farms. They were anesthetized with intraperitoneal sodium pentobarbital. The hearts excised and immediately placed in ice cold saline. When spontaneous contractions stopped, the hearts was mounted by the aortic stump on a steel cannula and retrograde aortic perfusion initiated. A second cannula was placed in the left atrial appendage and antegrade perfusion initiated in the working configuration of Neely (13). In all experiments, the perfusate was Kreb-Ringer Henseleit Bicarbonate buffer pH 7.4 with 5 mM glucose present as substrate containing 2.5 mM free calcium and maintained at 37°C. The perfusate was gassed with 95% O_2, 5% CO_2 which maintained an O_2 content of 22.8 ul/ml at approximately 730 mmHg. A five minute equilibration period was allowed before each experiment commenced.

Before a heart was made ischemic, cardiodynamic measurements were made at left atrial filling pressures (preloads) of 7.5, 10.0 and 12.5 cm of water. Peak systolic pressure ranged from 115 mmHg (7.5 cm) to 160 mmHg (12.5 cm). Following these, preload was returned to 7.5 cm of water and the left atrial inflow and aortic outflow cannulae simultaneously occluded which stopped all perfusate flow into the heart. After the twenty minute ischemic period both the inflow and outflow tracts were opened and buffer with or without 1 mM DCA was allowed to flow into the heart via the left atrial cannula. In this configuration, aortic pressure upon reflow started out at 60 mmHg and then fell to approximately 40 mmHg before spontaneous contractions commenced with sufficient force to reverse the decline in aortic pressure. Five minutes later a series of measurements were made at left atrial filling pressure of 7.5, 10.0, 12.5 and 15 cm water. Temperature inside the organ jacket was 36-37.5° throughout the experimental procedure. The hearts were then quick