Effects of ischemia on function and metabolism of the isolated working rat heart

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The acute effects of ischemia on myocardial function and metabolism are poorly understood (10, 11, 14, 18). The primary defect is sluggish blood flow to the tissue, which results in decreased supply of oxygen and perhaps other metabolic substrates, reduced rates of energy production, and accumulation of metabolic products. Not only is oxidative metabolism reduced, but also anaerobic production of ATP proceeds at less than maximum capacity (14, 22). Contractile activity of the muscle rapidly deteriorates, and this deterioration appears to result from factors other than reduced levels of high-energy phosphates (8, 10, 11). The mechanisms of these alterations in metabolic and mechanical functions are not known.

Several techniques are presently used to experimentally induce myocardial ischemia. One of the most widely publicized methods involves occlusion of a branch or branches of the coronary arteries of in situ hearts. This technique has been used for studies concerned with general and regional cardiac performance (1, 5, 9, 11, 15), long-term effects of regional ischemia (4, 9), the therapeutic efficacy of various pretreatments (6, 7, 20), and estimation of infarct size (13). However, for studies of myocardial metabolism and function during the acute phase of ischemia, this model possesses technical problems (13, 19). Mainly it is difficult to assess the metabolic and contractile activity of ischemic tissue, since it is surrounded by normal tissue.

In the present study, the isolated, working rat heart preparation that was described previously (16) has been modified to allow control of coronary flow independent of cardiac output and ventricular pressure development. The perfusion techniques that are described result in whole-heart ischemia under conditions where ventricular function and metabolism can be followed. Ischemia produced by these techniques resulted in changes in tissue high-energy phosphates and lactate that were similar to those found in other studies (3, 8, 21, 22).

METHODS

Male Sprague-Dawley rats (250–500 g) were employed throughout the study. Removal of the heart, initiation of perfusion, and preparation of perfusate were as previously described (16). All perfusates except those for study of lactate production involved recirculation of about 100 ml of buffer. Aortic pressures were recorded on a Beckman P23GB pressure transducer. Oxygen consumption was calculated from the arterial-venous difference in PO₂ and the rate of coronary flow. Venous oxygen tensions were determined on samples of perfusate that were withdrawn directly from the cannulated pulmonary artery. Arterial samples were taken from the left atrial cannula. PO₂ was determined with a Radiometer blood gas monitor (type P11A927).

At the end of perfusion, hearts used for biochemical analysis were quickly frozen by clamping with a Wollenberger clamp cooled in liquid nitrogen. Adenine nucleotides, creatine phosphate (CP), and lactate were analyzed in 6% perchloric acid extracts of the frozen tissue by the enzymatic methods outlined in ref. 3. ATP and CP were analyzed spectrophotometrically in 50 mM Tris buffer, pH 7.5, using hexokinase, glucose-6-phosphate dehydrogenase, and creatine phosphokinase. AMP and ADP were analyzed in 50 mM Tris buffer, pH 7.0, using pyruvate kinase, lactate dehydrogenase, and myokinase.

Perfusion technique. Preischemic perfusion and perfusion of control hearts were as described for the isolated working heart preparation (16). In this preparation, the aorta and left atrial appendage were cannulated. Perfusate was in-
from this bubble trap into the left atrium. Ventricular con-
traction pumped the perfusate into a small pressure chamber located immediately above the heart. This chamber con-
tained a measured volume of air which provided compliance in the aortic outflow tract. From the pressure chamber, per-
husate was pumped back into the oxygenating reservoir against an afterload of 60 mm Hg hydrostatic pressure. Cardiac output and ventricular pressure development were varied by changing the left atrial filling pressure and/or the resistance of the aortic outflow tract. Since the right atrium and pulmonary artery were not cannulated, coronary effluent dripped off the heart into a small chamber and was returned to the reoxygenating reservoir. Coronary flow in this isolated preparation was a function of the aortic perfu-
sion pressure and increased directly with ventricular pressure development. For the study of myocardial metabolism during ischemia, this working heart preparation has been modified to allow the rate of coronary flow to be controlled.

Ischemic heart model. Two modifications of the working heart apparatus have been used to induce ischemia. 1) Coronary flow was reduced by restricting cardiac output, whereas ventricular pressure development was maintained by increasing aortic resistance. 2) Coronary flow was re-
duced while maintaining cardiac output and ventricular pressure development by placing a one-way valve in the aortic outflow tract in such a way that it prevented retro-
grade perfusion of the coronary arteries during diastole. Fig. 1 illustrates the portion of the working heart appa-
ratus that was modified for these two procedures.

1) The cannula assembly and arrangement of bubble traps that were used to induce ischemia by the low cardiac output, high-resistance technique are shown in Fig. 1, A and B. This is essentially the same cannula assembly used for the working heart preparation. The arrows indicate direction of flow of the perfusate. The aorta and the left atrial appendage were cannulated as described above. Perfusate was pumped from the main oxygenating chamber to the atrial bubble trap by a variable-speed, Masterflex rotary pump (model 7014). The atrial bubble trap was provided with an overflow sidearm, which was 10 cm above the left atrium.

For control perfusions, the speed of the mechanical pump was adjusted so that perfusate overflowed from the atrial bubble trap at all times, thus providing a constant left atrial filling pressure of 10 cm H2O. Perfusate flowed from this bubble trap into the left atrium. Ventricular con-
traction pumped the fluid into the pressure chamber and out the aortic outflow tube to the top of the oxygenating chamber (75 cm above the heart). Aortic pressure was monitored via a sidearm on the aortic cannula. This sidearm also was used for a 10-min preliminary Langendorff perfusion during which time the pulmonary vein was cannulated. At the end of this period, flow from the pre-
perfusion reservoir was stopped, and the tube leading from the left atrial bubble trap was opened. This allowed the

ventricle to start pumping fluid through the aortic pressure chamber. The hearts were electrically paced at approxi-
ately 300 beats/min, and perfusion as a working heart was continued for an additional 10 min. At the end of this control working period (referred to as zero time in the figures below), perfusion was either continued under the same conditions (controls) or coronary flow was reduced (ischemia).

Ischemia was induced by closing the aortic outflow tract and by reducing cardiac output to equal the desired rate of coronary flow. Cardiac output was reduced by adjusting the speed of the mechanical pump that supplied perfusate to the left atrial bubble trap. Decreasing the speed of this pump prevented overflow of perfusate from the side-
arm of the atrial bubble trap and caused the filling pressure to decrease. Under this condition, the adjusted flow from the pump was equal to left atrial input, to cardiac output, and, therefore to coronary flow as long as left atrial pres-

ure remained below 10 cm H2O. A reasonable level of ventricular pressure development was maintained at the reduced levels of cardiac output by clamping off the aortic outflow tube distal to the pressure chamber and by reducing the volume of air in the pressure chamber from 2.5 to 0.3 ml. In this arrangement, atrial pressure was equal to the resistance of the coronary vascular bed, and ventricular pressure development was a function of left atrial pressure, coronary resistance, and the small residual compliance in the atrial pressure chamber.

With these manipulations, the rate of cardiac output and therefore coronary flow could be controlled by adjusting the speed of the mechanical pump. Since ventricular pressure development was dependent on left atrial input, lowering the rate of coronary flow in this manner also lowered peak systolic pressure. Likewise, as ventricular failure occurred during ischemia, the rate of coronary flow decreased from its initially adjusted level. A minimum rate of coronary flow could be maintained, however, by providing a minimum aortic perfusion pressure as ventricular performance de-

teriorated. This was accomplished by use of a second over-
flow bubble trap (left-hand side of Fig. 1, A and B), which supplied perfusate to the heart via the sidearm on the atrial cannula. The tube leading from this bubble trap to the aortic cannula was fitted with a one-way ball valve, which allowed perfusate to flow into the aorta when the aortic pressure decreased below a preselected level. The one-way valve was arranged to prevent flow of perfusate out this route when ventricular pressure development was sufficient to maintain aortic pressure at a level greater than the adjusted hydro-

static pressure from the bubble trap. Therefore, by adjusting the height of this bubble trap, it was possible to preselect a minimum aortic perfusion pressure and coronary flow during ventricular failure. Both this ischemic heart apparatus and the one described below were equipped with this mini-

mum flow arrangement. Data obtained utilizing this feature are not presented in the present report and will be pub-

lished separately at a later date.

This perfusion technique appeared to result in myocardial ischemia, as indicated by the data to be presented later. It would appear to be suitable for studies concerned with the correlation of metabolic events and the duration of ischemia or for testing the ability of various metabolic
MYOCARDIAL ISCHEMIA IN ISOLATED RAT HEART

INTRODUCTION

Myocardial ischemia in isolated rat heart can be induced using various methods. These methods allow for the examination of the effects of ischemia on cardiac function. The perfusion apparatus used in this study was designed to facilitate the induction of ischemia in isolated hearts.

METHODS

The perfusion apparatus consisted of several components including bubble traps and cannula assemblies. FIG. 1A shows the arrangement of bubble traps and cannula assembly used for low cardiac output, high aortic resistance method of inducing ischemia. This apparatus was the same as that described earlier for isolated working rat heart (1b), except that a minimum coronary flow assembly was added. A one-way valve was used in this assembly to allow ventricle to eject fluid but prevent retrograde perfusion of coronaries during diastole.

2) Fig. 1B illustrates the placement of a one-way valve in the aortic cannula to prevent perfusion of the coronaries during ventricular diastole. Since the largest fraction of coronary flow occurs during diastole, this one-way valve severely restricted coronary perfusion, but did not influence aortic output or ventricular afterload.

RESULTS

Mechanical performance of ischemic hearts. Figures 2 and 3 illustrate the hemodynamic status of hearts in which ischemia was induced by these two procedures. Fig. 2A shows a typical aortic pressure tracing from a heart in which ischemia was induced by the low-output, high-resistance method. At zero time on the tracing, ischemia was induced by clamping the aortic outflow tract, reducing the volume of air in the pressure chamber from 2.5 to 0.3 ml. The presence of the one-way valve prevented retrograde flow of perfusate into the aorta during diastole and allowed the diastolic aortic pressure to decrease. This decrease in diastolic coronary perfusion pressure resulted in decreased coronary flow and in ventricular failure. Since coronary flow now occurred primarily during systole, the rate of flow decreased as ventricular performance deteriorated.

Ischemia was induced in this preparation by simply clamping the bypass tube at the point indicated in the figure. With this maneuver, the ventricle ejected perfusate via the one-way valve but against the same hydrostatic pressure as prior to clamping the bypass tube. The presence of the one-way valve prevented retrograde flow of perfusate into the aorta during diastole and allowed the diastolic aortic pressure to decrease. This decrease in diastolic coronary perfusion pressure resulted in decreased coronary flow and in ventricular failure. Since coronary flow now occurred primarily during systole, the rate of flow decreased as ventricular performance deteriorated.
and adjusting the rate of the atrial input pump to 7 ml/min. As a result, cardiac output and coronary flow decreased to 7 ml/min, and peak systolic pressure decreased from 90 to 60 mm Hg. These reduced levels of coronary flow and ventricular pressure were maintained for only about 7 min. Ventricular function decreased rapidly between 7 and 12 min of ischemic perfusion.

At zero time (Fig. 2B), ischemia was induced by clamping the bypass tube around the one-way aortic valve. Peak systolic pressure increased only slightly by diverting flow through the one-way valve. Diastolic pressure below the valve, however, decreased from 40 to 15 mm Hg. Under this condition, ventricular pressure development was maintained for only about 5 min and decreased rapidly between 5 and 8 min.

Figure 3 illustrates the average performance of eight hearts perfused by each of these techniques. Fig. 3A shows the performance of hearts perfused by the low-output, high-resistance technique. Since the aortic outflow tract was open during the control perfusion, 30 ml of the total cardiac output were pumped through the aortic pressure chamber and 13 ml flowed through the coronary vessels. At zero time in the figure, coronary flow was reduced as described above. This resulted in an immediate decrease in left atrial filling pressure and peak systolic pressure. Ventricular failure was characterized by a rise in left atrial pressure from the adjusted low of 2–10 cm H}_2O (the maximum allowed in this apparatus) and by decreased peak systolic pressure and cardiac output. In contrast to ischemic hearts, control hearts with unrestricted coronary flow continued to develop about 90 mm Hg peak systolic pressure throughout the 30-min perfusion.

Fig. 3B illustrates the average performance of hearts in which ischemia was induced with the one-way aortic valve. With this procedure, left atrial filling pressure was maintained at 10 cm H}_2O throughout the perfusion. At zero time in the figure, the one-way valve bypass tube was closed. Since the hydrostatic pressure above the valve was maintained at 60 mm Hg and left atrial pressure was not changed,
peak systolic pressure was essentially unchanged by diverting aortic output through the one-way valve. Total cardiac output was unchanged during the first 30 sec of ischemic perfusion, since the fraction of the total output that was pumped out the aortic outflow tract was increased due to the presence of the one-way valve. The aortic pressure below the valve decreased from 40 to 13 mm Hg during diastole, and the fraction of total cardiac output that passed through the coronary vessels was reduced from 30 to 10%. In these hearts, reducing the rate of coronary flow resulted in rapid ventricular failure between 5 and 10 min of perfusion.

To further characterize the ischemic state induced by these two procedures, the rate of oxygen consumption, the tissue levels of high-energy phosphates, and the production of lactate were measured at various times during the perfusion. Inducing ischemia in the isolated rat heart by either of these procedures resulted in qualitatively and quantitatively similar changes in these metabolic parameters. Therefore, the metabolic data to be presented below represent a composite of the data collected for each of these perfusion techniques.

**Energy state of ischemic hearts.** Oxygen consumption was greatly restricted in ischemic hearts due to the reduced rate of coronary flow. Figure 4 illustrates the Po2 of arterial and venous perfusates in both control and ischemic hearts. Ischemic hearts extracted a significantly higher percentage of the arterial oxygen as indicated by the reduced venous Po2. In spite of the greater oxygen extraction, ischemic hearts consumed much less oxygen due to the reduced rate of coronary flow and oxygen delivery.

With these low rates of oxygen consumption, production of ATP was insufficient to maintain normal levels of high-energy phosphates. Figure 5 illustrates the tissue levels of creatine phosphate (CP) and adenine nucleotides in control and ischemic hearts during 30 min of perfusion. The levels of CP decreased rapidly in ischemic hearts. Within 2 min after inducing ischemia, the level of CP had fallen from about 23 to 8 μmoles/g dry wt and decreased slowly with continued perfusion. The decrease in ATP during the first 2 min of ischemia represented about 25% of the total ATP that was present. As perfusion was continued, the rate of ATP disappearance was much slower. The decrease in ATP was accompanied by a rapid rise in ADP and a slower rise in AMP. Ventricular performance rapidly declined after 5 min of ischemia (Fig. 3), even though the level of ATP remained at about 75% of the control value (Fig. 5). These data indicate that about 25% of the initial tissue ATP was immediately used to support mechanical per-
formance. The remaining 73% was utilized at a much slower rate, and the decrease in ATP did not parallel the deterioration in mechanical performance.

Associated with the decrease in oxygen supply, the rate of lactate production increased rapidly and was maintained at a high level for the first 15 min of ischemia (Fig. 6A). Between 15 and 30 min of perfusion, the rate of lactate release into the perfusate decreased as coronary flow was reduced to extremely low rates. Tissue lactate, on the other hand, accumulated slowly during the first 3 min of perfusion under ischemic conditions (Fig. 6B). During this time, coronary flow was about 6 ml/min. As ventricular failure progressed between 5 and 12 min, the rate of coronary flow decreased even further, and lactate rapidly accumulated in the tissue.

Recovery from ischemia. The ability of the heart to recover mechanical function was determined after various periods of ischemia. Ischemia was induced as described above for the one-way valve technique, and perfusion was continued for a preselected time. To test the ability of the heart to recover mechanical function, coronary perfusion pressure was restored to the control level by unclamping the one-way valve bypass tube. The left atrial input tube was clamped, and the hearts were perfused for 30 min retrograde through the aortic cannula (Langendorff preparation). At the end of this time, the left atrial input tube was unclamped and the hearts were perfused as control working hearts for 10 min with a left atrial filling pressure of 10 cm H$_2$O. Ventricular pressure development and cardiac output were monitored as an index of recovery of ventricular function. If a heart resumed rhythmic contraction, developed a peak systolic pressure greater than 60 mm Hg, and had a cardiac output greater than 15 ml/min, it was considered recovered.

The ability of the heart to recover from ischemia was found to correlate better with the duration of ischemia following the rapid decline in systolic pressure than with the total period of ischemia. This rapid decline in pressure development occurred when the peak pressure had decreased to approximately 60 mm Hg (Figs. 2 and 3), corresponding to 5–7 min of ischemia in the majority of hearts. However, there was considerable variation in the time required to reach this pressure. Systolic pressure decreased from 60 to 30 mm Hg in approximately 2 min (Fig. 2), and little variation in this time was found between hearts. A systolic pressure of 30 mm Hg was arbitrarily chosen to represent ventricular failure. Fig. 7A shows the percentage of hearts in which peak systolic pressure had decreased to 30 mm Hg after various periods of ischemia. Ventricular failure occurred in 68% of the hearts between 5 and 10 min of ischemia, and 100% had failed before 15 min. The average peak systolic pressure for these hearts is shown on the upper curve in panel A.

Fig. 7B illustrates the percentage of hearts that recovered ventricular function following various periods of ischemia. This curve relates recovery to the time of ischemia after ventricular pressure development had decreased to 30 mm Hg. Fifty percent of the hearts perfused for 13 min following ventricular failure did not recover, whereas 100% failed to

![Graph 6: Lactate production in control (solid lines) and ischemic hearts (dashed lines). Rate of lactate release into perfusate (A) and accumulation in tissue (B) were measured for times indicated. Each value represents mean ±SE for 12 hearts.](http://ajplegacy.physiology.org/doi/abs/10.1152/ajplegacy.00073.2016)
recover after 25 min of ischemia. Therefore, irreversible failure occurred in 100% of the hearts after a total of approximately 33 min of ischemic perfusion.

DISCUSSION

It would be possible to reduce coronary flow in the isolated, perfused heart by simply reducing the aortic perfusion pressure. However, in both the working heart preparation and in hearts perfused by the Langendorff technique, the level of ventricular pressure development was directly proportional to the aortic perfusion pressure or afterload (16). Since the rate of myocardial oxygen consumption (16) and glucose utilization (17) were directly related to peak systolic ventricular pressure, any decrease in aortic perfusion pressure would greatly influence the rate of ATP hydrolysis and substrate utilization. Therefore, inducing ischemia by lowering the aortic perfusion pressure would not allow a comparison of mechanical performance and metabolic rates between control and ischemic hearts. Obviously, if the ischemic condition is severe, ventricular performance will eventually deteriorate, but one would like to be able to follow the deterioration in function during the course of ischemia and to correlate this with concomitant metabolic events. For this reason, the working heart preparation was modified to allow coronary flow to be controlled in hearts that were initially developing reasonable levels of ventricular pressure.

The two perfusion techniques that are described in this paper resulted in myocardial ischemia as indicated by reduced rates of coronary flow and oxygen consumption, reduced tissue levels of ATP and creatine phosphate, high tissue levels of ADP and AMP, increased lactate production, and ventricular failure. Since the rate of coronary flow was reduced in the entire heart, it was assumed that the flow rate to all parts of the heart was reduced in equal proportion. Experiments to verify this point are currently in progress. Preliminary data for tissue distribution of sorbitol-3H and albumin-125I indicate that these perfusion techniques result in an equal reduction of flow to all parts of the myocardium.

A similar reduction in coronary flow resulted in essentially the same changes in mechanical performance and metabolism with either the low-output, high-resistance technique, or the one-way aortic valve method of inducing ischemia. The first technique has the advantage of affording a more exact control of coronary flow in the initial phase of ischemia but has less flexibility in selecting initial levels of cardiac output and ventricular pressure development. In addition, the mechanical and metabolic changes that resulted from the initial adjustments of cardiac output and ventricular pressure development could not be completely separated from the effects of reduced coronary flow. After these initial adjustments, however, further deterioration in mechanical performance and metabolism appeared to result directly from the lower rate of coronary flow.

The one-way valve procedure affords far more flexibility in selecting the initial levels of cardiac output and ventricular pressure development. Since inducing ischemia by this procedure does not change either the preload or the afterload, the initial rates of cardiac output and levels of pressure development were the same as the preischemic values. Thus, the effects of ischemia on mechanical performance could be correlated with metabolic events throughout the ischemic period. With this technique, however, the rate of coronary flow was dependent on the aortic pressure during systole, and the initial rate of coronary flow could not be controlled as accurately as in the low cardiac output procedure. In addition, it is difficult to maintain a competent one-way valve. The valves must be disassembled, cleaned, and polished frequently to prevent backflow of perfusate during diastole.

These major advantages and disadvantages of the two methods applied only during the first few minutes of ischemia. After ventricular failure had occurred, the rate of coronary flow decreased to the same extent in both cases. Therefore, the long-term effects of ischemia on metabolism, irreversible tissue damage, and on the ability of various agents to improve cellular stability can be determined equally well with either procedure. Although the effects of maintaining a minimum coronary flow after ventricular failure occurred are not reported in this paper, it was possible to select various rates of coronary flow by use of the minimum-flow assembly in either of the perfusion techniques.

The metabolic effects of producing ischemia in the isolated heart by these two procedures agree with data obtained from other methods of inducing ischemia (8, 21). The large decrease in the rate of oxygen delivery resulted in a greater oxygen extraction from the perfusate but lower total consumption. Mechanical performance was maintained initially at the expense of tissue levels of high-energy phosphates, principally creatine phosphate. The decrease in high-energy phosphates was very similar to that found in the ischemic regions of the in situ dog heart after coronary occlusion (5, 8). These authors found that nearly 90% of the normal ATP content remained in the ischemic myocardium at a time when the performance of this tissue was apparently severely depressed. The ATP content of the tissue in the present work had likewise decreased only 25% by the time the rapid decline in ventricular pressure began. Although tissue ATP was not depleted during the early phases of ischemia, ventricular failure may have resulted from the compartmentalization of the available ATP in an inaccessible pool or inability of the contractile proteins to utilize the available ATP (8, 12). Tissue ATP was depleted 70% by the time ischemia had resulted in irreversible damage, suggesting that loss of ATP may be associated with this phase of ischemia. However, it is not clear that depletion of ATP is directly responsible for failure of the heart to resume contracting upon restoring coronary flow to normal. A direct cause-and-effect relationship is not likely, since the function of nonischemic tissue at low ATP levels is not impaired in an irreversible manner (9). Thus, it is more likely that the depletion of ATP is indicative, rather than a cause, of irreversible tissue damage following ischemia. It is of interest to note that onset of irreversibility of ventricular function occurred after about 30 min of ischemia in the present study. This time corresponds to the period in which cell death occurred in ischemic areas of the in situ dog heart (10). In this study 50 and 100% of the severely ischemic cells died after 40 and 60 min, respectively.

The increase in ADP and AMP in the present study could
not account for the amount of ATP that had disappeared at any time during ischemia, suggesting that adenine nucleotides were lost from the tissue. This observation is in agreement with an earlier observation of Benson et al. (2) that oxygen deprivation leads to loss of tissue nucleotides.

The rate of lactate production in the present experiments declined following 12 min of ischemia and had returned to normal levels by 30 min. This reduced rate of lactate production by the severely ischemic myocardium is indicative of a very low level of anaerobic metabolism. It is difficult to infer anything about the initial ischemic rate of glycolysis from measurements of lactate production. Although lactate production was increased, flux of glucose through the glycolytic pathway was not necessarily increased. A shift of pyruvate from the citric acid cycle to production of lactate would appear to indicate a stimulation of glycolysis when in reality pyruvate production may have been decreased. Several authors (8, 12, 18) have indicated (based on production of lactate) that the rate of glycolysis was increased during ischemia. Initially the rate of lactate production was very high but quickly decreased to lower levels, indicating that a restriction of glycolytic flux developed during the course of ischemia (8).

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