Effect of Insulin on the Performance and Metabolism of the Anoxic Isolated Perfused Rat Heart

By Arnold M. Weisler, Ruth A. Altschuld, Lorrense E. Gibb, Mary Ellen Pollock, and Fred A. Kruger

ABSTRACT

When the oxygen supply to the myocardium is compromised, glycolysis may provide the critical energy necessary for the heart's survival. We investigated whether insulin, through its effects on myocardial glycolysis, influences the performance and the metabolism of the perfused rat heart during anoxia. Hearts in a modified Langendorff apparatus were perfused for 30 minutes with anoxic media containing glucose (50-500 mg/100 ml) with and without insulin (100 munits/ml) or with anoxic media containing 200 mg glucose/100 ml and varying insulin concentrations (0.1-100 munits/ml). Hearts were paced, and left ventricular pressure, maximum rate of rise of left ventricular pressure, and lactate production were monitored. Increasing glucose concentration alone progressively enhanced performance and lactate generation in the beating anoxic heart. Addition of insulin resulted in significant increases in left ventricular performance and lactate production at all levels of glucose concentration. The myocardial content of high energy intermediates (creatine phosphate, adenosine triphosphate, adenosine diphosphate, and adenosine monophosphate) after 30 minutes of anoxia was not altered by varying concentrations of glucose or insulin. To assess the effects of insulin and glucose in the absence of contraction, hearts were arrested with media containing a high potassium concentration (26 mEq/liter). Although lactate production was lower in arrested hearts than it was in beating hearts, it was enhanced by insulin. Insulin also produced significant increases in high-energy intermediates in arrested hearts. It was concluded that insulin increases the utilization of glucose and thus causes the enhancement of ventricular performance in the anoxic heart.

KEY WORDS glucose left ventricular performance lactate production potassium arrest high-energy phosphates Langendorff perfusion

The ability of the myocardium to shift, under conditions of reduced oxygen tension, from oxidative to glycolytic energy sources is an important survival mechanism. Glycogen stores, though relatively limited, can provide the necessary energy to support contractile performance during the first few minutes of anoxia (1, 2). However, a more abundant, constant supply of glycolytic substrate along with metabolic interventions to facilitate its utilization might enable the myocardium to better resist prolonged periods of anoxia. Studies (3, 4) have demonstrated that the availability of the anaerobic substrate, glucose, is a major determinant of pacemaker and contractile performance of the anoxic heart. Morgan and co-workers (5) have demonstrated that insulin increases glucose transport into both the anaerobic and the aerobic myocardium. In view of these observations, the present study was undertaken to ascertain whether insulin, through its influence on glucose transport, favorably influences the anaerobic performance and metabolism of the isolated perfused rat heart.

METHODS

Male Wistar rats with a mean body weight of 238 ± 16 g were fed ad libitum with standard Purina laboratory chow until they were killed by decapitation. The heart was rapidly excised, mounted on an aortic cannula, and transferred to a modified recirculating Langendorff apparatus. The perfusion system permitted constant monitoring of the electrocardiogram, the left ventricular pressure, the first derivative (the rate of rise) of the left ventricular pressure (dP/dt), the perfusion pressure, and the oxygen consumption. The hearts performed isovolumically. Coronary flow was maintained at 10 ml/min and temperature at 32°C. The heart preparation and the perfusion system have been described in detail previously (3).

The perfusion fluid was 5% bovine serum albumin (Armour Pharmaceutical Company) which had been dialyzed against and diluted with Krebs-Ringer's bicarbonate buffer (pH 7.4) modified to contain 2.5

From the Department of Medicine, The Ohio State University, Columbus, Ohio 43210.

This investigation was supported by U. S. Public Health Service Grant HE09884 from the National Heart and Lung Institute and by the Central Ohio Heart Association.

Received July 18, 1972. Accepted for publication November 9, 1972.
mixed and centrifuged at 5,000 g for 10 minutes. Equal volumes of the perchloric acid extract and 0.4M triethanolamine buffer (pH 7.6, 0.55M KCO₃) were combined, mixed, allowed to stand in the cold for 10 minutes, and centrifuged at 5,000 g for 10 minutes.

In some experiments, hearts were perfused aerobically for 1 hour and then subjected to anaerobic perfusion (90% N₂, 10% CO₂) for an additional 20 minutes. Heart rate was controlled by pacing at 100 beats/min with bipolar electrodes on the left ventricular surface. The pacing stimuli were square-wave pulses of 3-msec duration delivered at voltages approximately 10% greater than the threshold for capturing the anoxic heart. The rate of 100 beats/min was selected after preliminary experiments demonstrated that anoxic hearts follow a 1:1 pacing response at this level when glucose is present in the perfusate although spontaneously beating hearts have a varying endogenous rate. Ventricular capture was verified by the presence of a ventricular wave (QRS complex) coincident with the pacing spike. The anoxic media contained either 200 mg glucose/100 ml perfuse in the presence or the absence of varying concentrations of insulin (0.1-100 munits/ml) or varying concentrations of glucose (50-500 mg/100 ml) in the presence or the absence of insulin (100 munits/ml). Boron zinc crystalline insulin, which was glucagon free (content less than 0.0003%), was used.¹

In some experiments, hearts were perfused aerobically for 1 hour and then arrested during a 30-minute period of anoxia by the addition of KCl to the perfusion media (final concentration 26 mEq/liter). The perfusate was maintained with medium containing 200 mg glucose/100 ml in the presence or the absence of insulin (100 munits/ml).

Samples of the perfusion media were collected throughout the experimental period for the determination of lactate and lactic acid (6). The samples were deproteinized immediately by the addition of an equal volume of 6N perchloric acid.

Immediately following perfusion, all hearts were rinsed by perfusing them with albumin-free buffer for 5 minutes under conditions identical to those of the previous experimental period. This procedure eliminated protein contamination by perfusate albumin. The hearts were then clamped between liquid nitrogen-cooled Wollenberger tongs. All frozen hearts were stored in liquid nitrogen until analysis of metabolic intermediates.

Each frozen heart was ground to a fine powder under liquid nitrogen in a nitrogen-cooled mortar and pestle. The frozen powder was gradually added, with mixing, to 6N perchloric acid. The final ratio of volume to tissue weight was 4:1. The samples were well mixed and centrifuged at 5,000 g for 10 minutes. Equal volumes of the perchloric acid extract and 0.4M triethanolamine buffer (pH 7.8, 0.55M K₂CO₃) were combined, mixed, allowed to stand in the cold for 10 minutes, and centrifuged at 5,000 g for 10 minutes.

Samples of the clear supernatant fluid were used immediately for the determination of metabolic intermediates.

Adenosine triphosphate (ATP) was measured enzymatically using ATP-quinhydrone (Calbiochem Corp.) and the values were corrected for glucose triphosphate. Creatine phosphate (CP), adenosine diphosphate (ADP), and glucose 6-phosphate were determined enzymically (7). Total available high-energy phosphates = ATP + 2ATP + ADP. Protein content of the perfused hearts was determined by a modification of the biuret method (7).

To establish a control for the effects of anoxia, two groups of six hearts each were perfused aerobically for 60 and 90 minutes, respectively. No significant differences in physiological performance or in myocardial metabolite content were observed between the two groups. The 60-minute aerobic data for physiological performance and metabolic content were therefore employed as the reference for anoxia-induced changes during the subsequent 30-minute perfusion period.

Mean left ventricular pressure, left ventricular dP/dt max, and left ventricular end-diastolic pressure for 30 minutes of anoxia were calculated from measurements made at 5-minute intervals for each heart. All metabolic data are expressed per gram of heart protein. Statistical methods were used according to Snedecor (8).

Results

Effect of Insulin on the Performance and Lactic Acid Production of the Paced Anoxic Heart.—The effect of insulin (100 munits/ml) on the anaerobic performance characteristics of rat hearts perfused with glucose-containing media (200 mg/100 ml) is illustrated in Figure 1. Zero-time data represent the mean performance at 60 minutes of control aerobic perfusion. Exposure to anoxia was accompanied by a prompt decrease in peak left ventricular pressure and left ventricular dP/dt max in all hearts. Performance stabilized within 10 minutes and was constant throughout the remaining period of anoxia. Insulin in the perfusion media resulted in significantly increased left ventricular pressure and left ventricular dP/dt max throughout anoxia, although left ventricular end-diastolic pressure remained unchanged and was essentially 0 mm Hg in all hearts. Lactic acid production remained relatively constant during the 30-minute period of anoxia and was increased in the presence of insulin.

The effects of varying concentrations of insulin (0.1-100 munits/ml) on anaerobic ventricular performance and lactate generation at constant glucose concentration (200 mg/100 ml) are presented in Table 1. Significant increases in left ventricular pressure, left ventricular dP/dt max, and lactate

¹Generously supplied by Eli Lilly and Company.
The effects of insulin (100 munits/ml) and of varying concentrations of glucose (50-500 mg/100 ml) on left ventricular performance of hearts perfused anaerobically are shown in Table 2. Of note is the increasing ventricular performance as reflected in left ventricular pressure and left ventricular dP/dt max with increasing perfusate glucose concentration in the absence of insulin. In the presence of insulin, maximum performance was attained at 100 mg glucose/100 ml, and further increases in perfusate glucose concentration evoked no further increase in myocardial performance. At glucose concentrations from 50 to 300 mg/100 ml, left ventricular pressure and left ventricular dP/dt max were significantly higher in the presence of insulin. These differences were most pronounced at the lower glucose levels.

Concomitant with the increased performance during anoxia, both the presence of insulin and the increasing glucose concentrations resulted in increased lactic acid production (Table 2). At each concentration of glucose studied, lactate production was significantly higher in the presence of insulin. Since heart rate was fixed by pacing and end-diastolic pressure remained virtually constant, the performance of these isoventricularly beating hearts was reflected by left ventricular pressure and left ventricular dP/dt max. As shown in Figure 2, there was a significant relationship between both left ventricular pressure (P < 0.001) and left ventricular dP/dt max (P < 0.001) and lactic acid production when glucose concentration was varied in the presence or the absence of insulin (100 munits/ml).

Although a general relationship between performance and lactate generation was observed, an apparent discontinuity occurred between the effects of increasing glucose concentration on lactate production and performance in the presence of insulin, an effect not observed in its absence (Table 2). The maximum increase in lactate production occurred when perfusate glucose concentration was increased from 100 to 200 mg/100 ml (P < 0.025) whereas the maximum increase in performance, as measured by both left ventricular pressure and left ventricular dP/dt max, occurred between 50 and 100 mg/100 ml (P < 0.001).

Effect of Insulin on Lactic Acid Production of the Potassium-Arrested Anoxic Heart—Since insulin might directly influence ventricular performance and thus secondarily increase lactic acid production, the effect of insulin on the metabolism of the potassium-arrested heart was investigated. As shown in Figure 3, although lactate production in the arrested heart was clearly less than that in the beating heart, inclusion of insulin in the perfusate significantly increased lactate generation at glucose concentrations ranging from 50 to 300 mg/100 ml. In the absence of perfusate glucose, there was still appreciable lactate production by the anoxic heart, presumably arising from myocardial glycogen. Insulin had no effect on lactate production from this source.
TABLE 1

Effect of Varying Concentrations of Insulin on Mean Left Ventricular Performance and Lactate Production

<table>
<thead>
<tr>
<th>Insulin (mU/ml)</th>
<th>0</th>
<th>10</th>
<th>100</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVP (mm Hg)</td>
<td>29.3 ± 0.8</td>
<td>24.6 ± 1.8*</td>
<td>26.0 ± 1.0*</td>
<td>31.7 ± 1.6*</td>
</tr>
<tr>
<td>LV dP/dt max (mm Hg/sec)</td>
<td>882 ± 57</td>
<td>832 ± 31*</td>
<td>60 ± 49*</td>
<td>71 ± 36*</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>3.0 ± 0.1</td>
<td>6.1 ± 0.1</td>
<td>0.0 ± 0.3</td>
<td>0.3 ± 0.5</td>
</tr>
<tr>
<td>Lactate (mmoles/g protein/30 min)</td>
<td>24.6 ± 5.3*</td>
<td>24.6 ± 5.3*</td>
<td>24.6 ± 5.3*</td>
<td>24.6 ± 5.3*</td>
</tr>
</tbody>
</table>

The perfusion media contained 200 mg glucose/100 ml. Performance values represent the mean data for the 30-minute anoxic period ± SE. N = 6 for each group. LVP = left ventricular pressure, LV dP/dt max = maximum rate of rise of left ventricular pressure, and LVEDP = left ventricular end-diastolic pressure.

*P < 0.05, comparing hearts perfused in the absence (-) or the presence (+) of insulin.

TABLE 2

Effect of Insulin and Varying Concentrations of Glucose on Mean Left Ventricular Performance and Lactate Production

<table>
<thead>
<tr>
<th>Insulin (mU/ml)</th>
<th>0</th>
<th>10</th>
<th>100</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>50</td>
<td>100</td>
<td>300</td>
<td>500</td>
</tr>
<tr>
<td>LVP (mm Hg)</td>
<td>21.8 ± 2.0</td>
<td>25.5 ± 1.5*</td>
<td>25.5 ± 1.5*</td>
<td>25.5 ± 1.5*</td>
</tr>
<tr>
<td>LV dP/dt max (mm Hg/sec)</td>
<td>484 ± 21</td>
<td>526 ± 16</td>
<td>526 ± 16</td>
<td>526 ± 16</td>
</tr>
<tr>
<td>(mm Hg/sec)</td>
<td>218 ± 21</td>
<td>255 ± 15*</td>
<td>255 ± 15*</td>
<td>255 ± 15*</td>
</tr>
<tr>
<td>LVEDP (mm Hg)</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.2</td>
</tr>
<tr>
<td>Lactate (mmoles/g protein/30 min)</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
</tr>
</tbody>
</table>

Values for performance represent the mean data for the 30-minute anoxic period ± SE. Abbreviations are the same as in Table 1. N = 8 for each group. All P values compare hearts perfused in the absence (-) or the presence (+) of insulin (100 mU/ml/ml) at a given concentration of glucose.

*P < 0.05.
higher CP contents when insulin was included in the media. Beyond perfusate glucose concentrations of 50 mg/100 ml, the presence of insulin in the perfusate did not effect any significant changes in CP, ATP, ADP, or AMP. Thus, although both glucose and insulin increased the ventricular performance of the anoxic heart; they did not alter the myocardial concentration of total adenine nucleotides or high-energy phosphates after 30 minutes of anoxic perfusion, with the exception of hearts perfused at 50 mg glucose/100 ml.

The effect of insulin (100 munits/ml) on the myocardial content of CP, adenine nucleotides, and high-energy phosphates in the potassium-arrested heart following 30 minutes of anoxic perfusion with varying concentrations of glucose (0-300 mg/100 ml) is illustrated in Table 4. There was increased preservation of CP and ATP with increasing perfusate glucose (0-300 mg/100 ml). Addition of insulin resulted in a further increase in high-energy phosphates at glucose concentrations ranging from 50 to 300 mg/100 ml. No effect of insulin was demonstrated in the absence of perfusate glucose.

In the paced anoxic heart there was no correlation between lactate generation and CP or adenine nucleotide content. In contrast, lactate production during 30 minutes of anoxia for all potassium-arrested, glucose-supported hearts correlated significantly with the myocardial content of CP ($r = 0.918$, $P < 0.001$), ATP ($r = 0.914$, $P < 0.001$), and LVP ($r = 0.925$, $P < 0.001$).
Effect of anoxic perfusion time on creating phosphate and adenosine nucleotides. Hearts were perfused with media containing 200 mg glucose/100 ml and either (a) subjected to ventricular pacing (100 beats/min) or (b) arrested by addition of KCl to the perfusate (final concentration 26 mEq/liter). The hearts were freeze-clamped at the times indicated and analyzed for creatine phosphate (CP), adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP). Total high-energy phosphate (\(\text{H}_P\)) = CP + 2ATP + ADP. Mean metabolite content for the 60-minute aerobic control period is given at zero time. N = 6 at 0 and 30 minutes, and N = 4 at 5 and 15 minutes.

Discussion

The availability of metabolic energy rarely limits ventricular performance under aerobic conditions. With the onset of anoxia however, oxidative phosphorylation ceases and the anaerobic glycolytic pathway becomes the major source of metabolic energy. Glycolytic flux increases severalfold consequent to anoxia-induced acceleration of glucose transport (5) and activation of phosphorylase (9) and the rate-limiting enzymes of glycolysis (10, 11).

It is apparent from the present study that, despite increased glycolytic flux, available high-energy phosphates and ventricular performance decline during the first 5-10 minutes of anoxia. Thereafter, in the presence of perfusate glucose, a new steady state is reached wherein energy utilization by the contracting myocardium is balanced by glycolytic energy generation and there is no further decline in high-energy stores. Under these conditions ventricular performance is apparently determined by the rate of glycolysis as evidenced by the close correlation between left ventricular performance and lactic acid production when the latter is varied by changing the perfusate glucose concentration in the presence or the absence of insulin.

Morgan and co-workers (5) have demonstrated that glucose transport in the isolated perfused rat heart is a function of the extracellular glucose concentration and is accelerated by insulin under both aerobic and anaerobic conditions. The present study demonstrates that myocardial glycolytic flux and left ventricular performance are increased with increasing concentrations of perfusate glucose. In addition, insulin significantly enhances ventricular performance and lactate production at all concentrations of glucose studied. It is reasonable to postulate that the increased lactic acid production observed in the presence of insulin is caused by a direct stimulation of glucose transport by the hormone. An alternative explanation which must be considered is that an insulin-induced increase in contractile performance indirectly results in the changes in glycolytic flux. In this regard, Neely and co-workers (12) have demonstrated that enhanced membrane transport of glucose occurs in the aerobic isolated rat heart when ventricular pressure...
Effects of Insulin and Varying Concentrations of Glucose on Creatine Phosphate and Adenine Nucleotide Contents of the Beating Heart

The effects of insulin and varying concentrations of glucose on creatine phosphate (CP) and adenine nucleotides (ATP, ADP, AMP) were studied. Glucose was provided in the perfusion medium, and the presence of insulin (100 units/ml) was varied. The results showed a significant increase in CP and AMP content with insulin and glucose, while ATP and ADP levels were not significantly affected by insulin alone. The presence of glucose in the perfusate facilitated the effect of insulin on CP and AMP levels.

Table 2: Effects of Insulin and Varying Concentrations of Glucose on Creatine Phosphate and Adenine Nucleotide Contents of the Beating Heart

<table>
<thead>
<tr>
<th>Glucose (mg/dl)</th>
<th>Insulin</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP (mole/g)</td>
<td></td>
<td>0.01 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>AMP (mole/g)</td>
<td></td>
<td>0.00 ± 0.00</td>
<td>3.08 ± 0.05</td>
<td>3.00 ± 0.05</td>
<td>3.00 ± 0.05</td>
<td>3.00 ± 0.05</td>
</tr>
<tr>
<td>ADP (mole/g)</td>
<td></td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>ATP (mole/g)</td>
<td></td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

*P < 0.05 comparing hearts perfused anaerobically for 30 minutes in the absence (−) or the presence (+) of insulin (100 units/ml) at a given concentration of glucose.

Abbreviations: CP = creatine phosphate, ATP = adenosine triphosphate, ADP = adenosine diphosphate, AMP = adenosine monophosphate, TAN = total adenine nucleotides, and ~P = total high-energy phosphate.

Effects of Insulin and Varying Concentrations of Glucose on Creatine Phosphate and Adenine Nucleotide Contents of the Arrested Heart

In an aerobic environment, the presence of glucose and insulin significantly increased CP and AMP levels. The presence of insulin and glucose in the perfusate facilitated the restoration of energy levels in the arrested heart. The combination of insulin and glucose promoted the maintenance of high-energy phosphate stores, facilitating the recovery process.
INSULIN AND THE ANOXIC HEART

When insulin is present in the perfusate of beating hearts, the levels of high-energy intermediates are not essentially different from those found in the absence of insulin at any given glucose concentration (Table 3). The increase in lactate production resulting from the action of insulin most likely results from increased glucose transport, and the additional energy generation is balanced by increased energy utilization. It should be noted, however, that in the presence of insulin, although lactate production increases as glucose concentration is increased from 50 to 200 mg/100 ml, ventricular performance does not increase beyond that observed at 100 mg glucose/100 ml. Other functions of insulin which have not been investigated in this study may be involved. For example, insulin is directly involved in the stimulation of glycogen synthesis (18), protein synthesis (19), and perhaps triglyceride synthesis, all energy-requiring processes. Thus the increased lactate production seen with insulin and glucose concentrations above 100 mg glucose/100 ml may be related to increased ATP demands for biosynthetic processes.

When insulin is present in the perfusate of arrested hearts, the levels of high-energy intermediates are generally higher at any given level of glucose than they are in the absence of insulin. This finding is most clearly evident in the total available high-energy phosphates (Table 4). The finding may appear to be anomalous with regard to the increased glycolytic flux indicated by the higher lactate production in the presence of insulin (Fig. 3). However, if the primary effect of insulin is to increase glucose transport, it follows that, under conditions where glucose availability is rate limiting, the presence of insulin would result in increased glycolytic flux and that, since energy utilization is minimal in the arrested heart, the presence of insulin would also result in increased available energy in the cell. It is of interest to note that in the absence of perfusate glucose there is no effect of insulin on the high-energy content (Table 4) or lactate production (Fig. 3) of the arrested heart.

References


Effect of Insulin on the Performance and Metabolism of the Anoxic Isolated Perfused Rat Heart

ARNOLD M. WEISSLER, RUTH A. ALTSCHULD, LORRAINE E. GIBB, MARY ELLEN POLLACK and FRED A. KRUGER

doi: 10.1161/01.RES.32.1.108

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1973 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/32/1/108

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org//subscriptions/