The Role of Glucose in the Survival and 'Recovery' of the Anoxic Isolated Perfused Rat Heart

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Studies with the isolated perfused working rat heart were carried out to investigate factors that may enable the heart to recover after periods of anoxia. It was found that the presence of glucose in the perfusion fluid during anoxia was essential for complete post-anoxic recovery and the presence of a high concentration of K^+ increased not only the rate of recovery but also the final extent of recovery. In an attempt to clarify the roles played by glucose and K^+ in aiding the survival and recovery of the anoxic myocardium the concentrations of parameters associated with energy liberation and anaerobic glycolysis (ATP, ADP, AMP, P_i , creatine phosphate, glycogen and lactate) were measured in the presence and absence of glucose during the anoxic phase. Determinations of these parameters were carried out during the working aerobic control period, the anoxic period (K^+ arrest) and the recovery period. The results demonstrated that glucose acted as an energy source during anoxia and thus maintained myocardial concentrations of high-energy phosphates, particularly ATP. These studies have also shown a direct relationship between the ability of the heart to recover and the concentration of myocardial ATP at the time of reoxygenation.

During a previous investigation (Chain *et al.*, 1969), it was observed (K. R. L. Mansford, L. H. Opie & E. B. Chain, unpublished work) that the isolated perfused working rat heart recovered from anoxia far more readily if glucose was present in the perfusion fluid during the anoxic phase. In studies with Langendorff's (1895) perfusion method, Weissler *et al.* (1968) had also observed that glucose contributed to the preservation of the functional capacity and structure of the anoxic rat myocardium. Under aerobic conditions with optimum substrate availability, fatty acids provide the major source of energy and are extracted from the coronary supply in preference to carbohydrates. Under anaerobic conditions, however, it appeared that glucose played a major role in the survival and recovery of the myocardium. The present study was therefore concerned with the analysis of metabolic parameters affected by glucose in the anoxic isolated perfused rat heart.

Experimental

Animals

Male rats (280–320 g) of the Sprague–Dawley strain, maintained on a standard diet, were used throughout.

Chemicals

All substrates and enzymes used for the analysis of tissue extracts and perfusion fluids were obtained

from Boehringer Corp. (London) Ltd., London W5 2TZ, U.K. D-Glucose and general reagents were obtained from BDH Chemicals Ltd., Poole, Dorset, U.K. Heparin (B.P.) was obtained from Evans Medical Ltd., Speke, Liverpool, U.K.

Perfusion techniques

A rat was lightly anaesthetized with diethyl ether, the left femoral vein was exposed and heparin (200 i.u.) was administered intravenously. At 1 min after administration of heparin, the heart was excised as described by Neely *et al.* (1967) and was placed in ice-cold perfusion medium until contraction had ceased. The heart was then mounted on the perfusion apparatus. The mounting procedure and apparatus were based on modifications of that described by Neely *et al.* (1967) and that described by Chain *et al.* (1969). There were four modifications. First, a flow meter calibrated for flow (5–70 ml/min) at 37°C (type G-G/300/GS; Rotameter Manufacturing Co. Ltd., Croydon, Surrey, U.K.) was introduced to measure aortic flow rates. Secondly, constant-head devices were added to all perfusion-fluid reservoirs. Thirdly, retrograde perfusion down the aorta was always carried out so that the aortal pressure was 100 cm of water. Fourthly, in working heart preparations the atrial filling pressure was 20 cm of water and ventricular ejection of perfusate was against a hydrostatic pressure of 100 cm of water. The perfusion apparatus was designed so that it could be used for

two modes of perfusion and could be readily inter-converted from one to the other.

(i) *Non-working Langendorff-type system.* In all experiments, after being mounted on the aortic cannula, the arrested heart was restarted by perfusion as described by Langendorff (1895). In this preparation the perfusion fluid entered the heart via the aorta and, although beating, the heart was not capable of external work *in vitro*. The coronary effluent could be collected or recirculated.

(ii) *Working system.* In this preparation hearts were perfused via the left atrium as described by Neely *et al.* (1967) at an atrial perfusion pressure of 20 cm of water. The left ventricle spontaneously ejected 45–60 ml of perfusate/min against a hydrostatic pressure of 100 cm of water. The aortic output and coronary flow could be pooled and recirculated.

Perfusion medium

Krebs–Henseleit bicarbonate buffer (Krebs & Henseleit, 1932), pH 7.4, was the standard perfusion fluid. In some experiments glucose (11.1 mM) was included in the buffer. In cardiac-arrest studies with high K^+ concentrations in the perfusion fluid, the concentration of K^+ in the buffer was 16.0 mM and the concentration of Na^+ was correspondingly decreased. The perfusion fluid was maintained at 37°C, and in aerobic studies the fluid was equilibrated with $O_2 + CO_2$ (95:5, aortic O_2 partial pressure over $80.00 \times 10^3 \text{ N} \cdot \text{m}^{-2}$) and in anoxic studies the fluid was equilibrated with $N_2 + CO_2$ (95:5; aortic O_2 partial pressure less than $0.67 \times 10^3 \text{ N} \cdot \text{m}^{-2}$). The precautions suggested by Umbreit *et al.* (1964) were followed to prevent the precipitation of Ca^{2+} . Before use the perfusion fluid was filtered through a cellulose acetate filter (pore size $5.0 \mu\text{m}$) (Millipore Ltd., Wembley, Middx., U.K.).

Parameters monitored during perfusion period

(a) *O_2 partial pressure.* This was measured in the perfusion fluid before and after (coronary effluent) passage through the heart by using O_2 electrodes (type E5046; Radiometer, Copenhagen, Denmark). Continuous monitoring of both electrodes simultaneously was possible by using a digital acid-base analyser (type PHM 72; Radiometer) modified to take two PO_2 modules (type PHA 932; Radiometer).

(b) *Mechanical measurements.* Coronary flow rate and aortic flow rate (by flow meter) were monitored periodically. Aortic pressure was registered by means of a pressure transducer (type 4-82; S.E. Laboratories Ltd., Feltham, Middx., U.K.) attached to a side-arm of the aortic cannula. Heart rate was derived directly from the pressure recording and was visually displayed (instantaneous-rate meter; Devices Instruments Ltd., Welwyn Garden City, Herts., U.K.).

(c) *Temperature.* The apparatus was water-jacketed and maintained thermostatically at 37°C. The temperature of the perfusion fluid in the aortal cannula was continuously monitored (500 series microthermistor and telethermometer; Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.).

(d) *Recording.* All monitored parameters could be selectively recorded (M19 direct-writing multichannel recording system; Devices Instruments Ltd.).

Perfusion fluid analysis

The perfusion was terminated by freezing the heart between stainless-steel tongs cooled to the temperature of liquid N_2 (Wollenberger *et al.*, 1960). To remove frozen perfusate around the edges of the heart and also to remove the atrium, the heart was placed on a block of solid CO_2 and the unwanted parts were chipped away with the tip of a sharpened spatula, which had been chilled in liquid N_2 . The remaining frozen muscle was then placed in liquid N_2 and powdered in a percussion mortar. A small portion of the powder was transferred to a weighed container for dry-weight determination. A second portion was used for the determination of glycogen and the remaining powder was used for the determination of metabolites.

(a) *Dry-weight determinations.* The weighed tissue sample was dried for 12 h at 105°C, cooled *in vacuo* and reweighed for the determination of water loss. Owing to the possibility of oedema and residual perfusion fluid in the tissue preparation all results were expressed as per g dry wt.

(b) *Glycogen determinations.* The frozen powder (approx. 200 mg) was added to a preweighed tube containing 0.4 ml of 30% (w/v) KOH. The tube was reweighed, sealed, heated to 100°C for 30 min and cooled. Then 0.2 ml of 2% (w/v) Na_2SO_4 was added, followed by ethanol to a final concentration of 75% (v/v). After mixing, the resulting solution was stored at 4°C for 12 h and then centrifuged at 40000g for 10 min. The supernatant was discarded, the pellet was resuspended and washed in 0.5 ml of 85% (v/v) ethanol and centrifuged at 40000g for 10 min. The supernatant was discarded and the pellet was allowed to dry in air. The pellet was digested at 37°C for 60 min in a solution composed of 0.1 ml (500 $\mu\text{g/ml}$) of amylo- α -1,4- α -1,6-glucosidase (EC 3.2.1.-; Boehringer Corp.), 0.5 ml of 1 M-sodium acetate buffer, pH 5.0, and 1.5 ml of water. The resulting solution was made up to 2.5 ml with water and assayed for glucose as described by Crowne & Mansford (1962).

(c) *Metabolic determinations.* The frozen tissue powder was placed in a preweighed tube containing 4.0 ml of ice-cold 6% (w/v) $HClO_4$, rapidly homogenized and reweighed. The solution was then centrifuged (10 min, 2000g) and the supernatant was neutralized to pH 7.0 by the addition of 30% (w/v)

KOH saturated with KCl. The solution was centrifuged (10 min, 2000g), the supernatant was collected and its volume was recorded. The resulting solution was then immediately used for the analysis of ATP, ADP, AMP, creatine phosphate and lactate as described by Opie *et al.* (1971). P_i was determined by the method of Wahler & Wollenberger (1958).

Time-sequence of aerobic and anoxic perfusion periods

To eliminate errors resulting from variation between individual hearts, a control aerobic perfusion period preceded the anoxic experimental period. Immediately after mounting, the heart was perfused (with standard perfusion medium plus glucose) for a 5 min washout period by a non-recirculating Langendorff-type system; during this period no external work was done. The preparation was then converted into a working heart system for a 15 min period (standard perfusion medium plus glucose). During this time the stability of the preparation could be ascertained and also control values for the working heart could be established. For example, as an index

of external work, aortic flow rate against a hydrostatic pressure of 100 cm of water could be monitored. At the end of the control period the preparation was converted into a totally anoxic non-recirculating Langendorff-type system and perfused with the specified buffer for a predetermined time (usually 30 min). During the anoxic period, the heart chamber was maintained in a $N_2 + CO_2$ (95:5) environment. At the end of the anoxic period the heart was simultaneously reoxygenated and converted into a working system perfused with standard perfusion medium plus glucose. Recovery of aortic flow was monitored over a 30 min period and was expressed as a percentage of the control value for the pre-anoxic working heart.

Results and Discussion

Effect of glucose and K^+ on the recovery of the heart from anoxia

Fig. 1 shows the results obtained when rat hearts were perfused in the presence or absence of glucose during the anoxic period. Six rat hearts were used for each of the conditions studied. In the absence of

Fig. 1. *Effect of the presence or absence of glucose and the concentration of K^+ in the anoxic perfusion fluid on the post-anoxic recovery of the heart*

Recovery of aortic flow is expressed as a percentage of the pre-anoxic working control value. All pre-anoxic control and post-anoxic recovery perfusions were carried out with standard perfusion fluid plus glucose (11.1 mM). ▲ (12 rats), recovery profile after anoxic perfusion with high- K^+ buffer containing no glucose showing either no recovery or a delayed and poor recovery; ● (six rats), recovery profile after anoxic perfusion with standard buffer containing glucose (11.1 mM); ■ (six rats), recovery profile after anoxic perfusion with high- K^+ buffer containing glucose (11.1 mM). Each point represents the mean for six rats and the bars represent the S.E.M.

glucose ($\blacktriangle$), after reoxygenation, there was either no recovery or a delayed and poor recovery. If glucose was present during anoxia ($\bullet$) there was a rapid and progressive recovery such that after 20–30 min the hearts had recovered to 75–85% of their pre-anoxic aortic flow-rate control values. When high K^+ concentrations were present in the glucose-supplemented buffer ($\blacksquare$), the rate of recovery increased, as did the final extent of recovery, reaching 90–97% of the pre-anoxic control value. Because of the observation of this considerably improved recovery, in all subsequent experiments a high K^+ concentration (16 mM) was used throughout the anoxic period. In this connection, there are two points that should be considered. First, it is well known that there is a substantial loss of K^+ from the heart during anoxia (Russell *et al.*, 1961) and this causes severe cardiac impairment. It is possible that the high concentration of K^+ during anoxia may minimize the effect of these ion losses. Secondly, high K^+ concentrations may aid recovery indirectly by conserving cellular supplies of high-energy phosphates. With the normal K^+ concentration of standard buffer, occasional irregular contractions occurred during anoxia. High K^+ concentrations, however, cause complete cardiac arrest and as such may prevent considerable energy utilization during anoxia.

Effect of glucose on metabolic parameters associated with energy liberation and glycolysis

The above experiments confirmed that glucose plays a vital role in the survival and recovery of the

anoxic heart. It was therefore decided to investigate the biochemical parameters that could potentially be effected by the presence of glucose during anoxia. The only reaction of glucose known to occur under anaerobic conditions is glycolysis, and consequently it was decided to investigate biochemical intermediates associated with this pathway, with particular emphasis on those involved in processes of energy liberation. The following myocardial parameters were studied throughout the entire experimental time-course: ATP, ADP, AMP, creatine phosphate, P_i , glycogen and lactate. Lactate released into the perfusion medium was also determined.

ATP (Fig. 2) was maintained at a fairly constant concentration of approx. $27 \mu\text{mol/g}$ dry wt. for the duration of the working control period. After the onset of anoxia, in the absence of glucose, the concentration of myocardial ATP decreased rapidly and linearly to almost zero over a period of approx. 20 min. In contrast, in the presence of glucose, after an initial decrease, the myocardial ATP concentration was maintained at high values (approx. $23 \mu\text{mol/g}$ dry wt.) which, on reoxygenation, rapidly returned to near control values. As shown in Fig. 1, in the absence of glucose during anoxia there was no post-anoxic recovery.

The profiles for creatine phosphate concentration (Fig. 3) demonstrated that this compound was considerably more labile than ATP, the concentration decreasing during the later phases of the working aerobic control period. Despite this, after the onset of anoxia, in the absence of glucose, the rate of loss of creatine phosphate was considerably accelerated.

Fig. 2. Myocardial ATP concentration ($\mu\text{mol/g}$ dry wt.) during the experimental time-course

For experimental details see the text. $\blacktriangle$, concentration during the working control and post-anoxic recovery periods when the perfusion fluid was composed of standard buffer plus glucose (11.1 mM); $\bullet$, concentration during the anoxic period when the perfusion fluid contains high K^+ and no glucose; $\blacksquare$, concentration during the anoxic period when the perfusion fluid contains high K^+ and glucose (11.1 mM). Each point represents one heart. $\cdots\cdots$ represents the onset (at 20 min) and the termination (at 50 min) of the anoxic period.

Fig. 3. Myocardial creatine phosphate concentration ($\mu\text{mol/g dry wt.}$) during the experimental time-course
The conditions and symbols are as described in the legend to Fig. 2.

Fig. 4. Myocardial ADP and AMP concentrations ($\mu\text{mol/g dry wt.}$) during the experimental time-course

The conditions and symbols are as described in the legend to Fig. 2. ▲, ● and ■ are the values for ADP, and △, ○ and □ are for AMP.

Within 10 min most of the creatine phosphate had been depleted. In the presence of glucose, however, a protective effect was observed and both the rate and extent of creatine phosphate disappearance were considerably decreased. As with ATP, after reoxygenation, there was a rapid increase in the myocardial concentration of creatine phosphate.

The concentration of ADP (Fig. 4) during anoxia exhibited the expected changes. In the absence of glucose, after an initial increase at the onset of anoxia (resulting from ATP degradation), the concentration of myocardial ADP declined. In the presence of glucose the fluctuation of ADP concentration was less marked. In the presence of glucose the myocardial AMP concentration (Fig. 4) remained relatively constant during anoxia, but in the absence of glucose it showed some increase.

Cellular P_i concentration (Fig. 5) was maintained

at a very constant value during the working control period, but after the onset of anoxia in the absence of glucose the cellular concentration increased very rapidly as a result of the extensive breakdown of high-energy phosphates. The increase in P_i during anoxia in the presence of glucose was very much less; this was consistent with the stabilization of high-energy phosphates. Total lactate production (Fig. 6), i.e. cellular lactate plus lactate discharged to the perfusion medium, was negligible during the working control period. During anoxia in the presence of glucose there was an extensive and almost linear production of lactate (approx. $22 \mu\text{mol/min per g dry wt.}$), indicating an active and continuing anaerobic glycolysis. This production of lactate decreased rapidly to zero on reoxygenation and recovery. In the absence of glucose during anoxia considerable but less-extensive lactate production occurred. The amount and the

tailing-off of this lactate production was found to be consistent with the utilization and depletion of endogenous glycogen. In the absence of glucose, glycogen concentration (Fig. 7) was found to decrease rapidly to zero during the first 20 min of anoxia. In contrast, in the presence of glucose, the glycogen concentrations were maintained at working control values for the duration of anoxia.

The findings thus far would seem to support the premise that exogenous glucose supports the survival and recovery of the anoxic myocardium by promoting glycolytic ATP production. Glycogen, the alternative source of substrate for anaerobic ATP production, is rapidly depleted and is therefore present in insufficient

quantities to support the heart for the duration of the anoxic period.

Alternative energy sources to glucose

In support of the assumption that glucose aids the survival and recovery of the anoxic myocardium by supporting anaerobic glycolysis, it would be expected that glucose could not be replaced by pyruvate or lactate, but it should be possible to replace it by one or more of the early glycolytic intermediates. In experiments (six rats each) in which hearts were perfused with buffer containing pyruvate (22.2 mM) or lactate (22.2 mM), all preparations failed to recover

Fig. 5. Myocardial P_i concentration ($\mu\text{mol/g dry wt.}$) during the experimental time-course

The conditions and symbols are as described in the legend to Fig. 2.

Fig. 6. Total lactate (myocardial lactate plus lactate released to the perfusion fluid) production ($\mu\text{mol/g dry wt.}$) during the experimental time-course

The conditions and symbols are as described in the legend to Fig. 2.

Fig. 7. Myocardial glycogen concentration (μmol of glucose equivalents/g dry wt.) during the experimental time-course

The conditions and symbols are as described in the legend to Fig. 2.

from 30 min anoxia. Hexose phosphates are poorly transported across the cell membrane, but in studies with the rat diaphragm Beloff-Chain *et al.* (1953) demonstrated that at sufficiently high extracellular concentrations hexose phosphates enter the cell. Consequently recovery experiments (six rats) were done in which glucose was replaced during anoxia by glucose 1-phosphate (2%, w/v). Under these conditions, glucose 1-phosphate was found to support the post-anoxic recovery of the heart as efficiently as did glucose.

Relationship between recovery, duration of anoxia and concentration of ATP at the time of reoxygenation

Fig. 8(a) (17 rats) shows the close relationship between ATP concentration and the duration of anoxia when hearts are perfused in the absence of glucose. Experiments (15 rats) were done to determine the relationship between the duration of anoxia and the ability of the heart to recover when glucose was absent from the anoxic perfusion medium. For these studies the index of cardiac recovery was taken as the percentage recovery (relative to the pre-anoxic working control) of the aortic flow rate 60 s after reoxygenation. The results (Fig. 8b) revealed a linear relationship between duration of anoxia and ability to recover. The resultant curve was strikingly similar to that of Fig. 8(a), and a cross-plot (Fig. 8c) of myocardial ATP concentration at the time of reoxygenation against the ability of the heart to recover pro-

duced a linear relationship. It also indicated a minimal concentration for recovery of approx. $2 \mu\text{mol}$ of ATP/g dry wt. of heart.

Relevance of the findings to the clinical situation

Some possible ways in which glucose may help in maintaining the integrity of the anoxic heart have been reviewed by Opie (1970). Under the conditions of the study described in the present paper, exogenous glucose has been shown to support the survival and recovery of the anoxic myocardium to a great extent. This protective effect appeared to be achieved by the promotion of ATP production through anaerobic glycolysis. These findings do not allow extrapolation to the clinical condition in the ischaemic myocardium, nor do they allow the prediction of a therapeutic effect resulting from the administration of glucose to the ischaemic myocardium after infarction. There are several major differences between the animal model system used in this study and the clinical condition. For example, complete myocardial anoxia with maintained or increased coronary flow rates is a totally different condition to an ischaemic myocardial segment with decreased coronary flow as occurs in infarction. The latter condition is more likely to be hypoxic than anoxic and as such may exhibit some oxidative metabolism. Clearly, markedly different metabolic and haemodynamic consequences may arise. The time-scale of events is also significantly different, and whereas glucose may appear

Fig. 8. Relationship between recovery, duration of anoxia and the concentration of ATP at the time of reoxygenation

(a) Decrease in myocardial ATP ($\mu\text{mol/g dry wt.}$) during the anoxic period (30 min). The anoxic perfusion fluid contained high K^+ and no glucose. (b) Relationship between post-anoxic recovery (expressed as a percentage of the pre-anoxic working control) and the duration of anoxia. The composition of the perfusion fluids used were: for the working pre-anoxic control period and the post-anoxic recovery period, standard buffer plus glucose (11.1 mM); for the anoxic period, high K^+ with no glucose. (c) A cross-plot between Figs. 8(a) and 8(b) showing the relationship between the ability of the heart to recover and the concentration of ATP at the end of the anoxic period (time of reoxygenation).

beneficial over the course of a few hours, it has been proposed, from foetal-heart-culture studies, that longer exposure times may lead to different and possibly detrimental effects (Wildenthal, 1971). In addition, there are of course the obvious physiological differences between the heart *in vivo* and the isolated perfused heart. The adequacy of oxygenation

of the working aerobic preparation is often questioned, but there is now strong evidence (Opie *et al.*, 1971) that it is sufficient in these preparations.

Despite the inadequacies in the systems used, it is considered that the results described in the present paper do form a baseline for further studies, which it is hoped will move closer towards the clinical situation.

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