EFFECT OF GLUCOSE IN ISOPROTERENOL-INDUCED NECROTIC HEART UNDER ANOXIC PERFUSION

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Abstract—Studies of the isoproterenol-induced necrotic heart perfused under an anoxic condition were performed to determine the changes of metabolism and mechanical function. It was found that the increased concentration of glucose in a perfusion medium enabled the necrotic heart to preserve mechanical functions during anoxia. However, in the normal heart the protective effect of high glucose concentration was not significant. High glucose concentration enhanced anaerobic glycolysis in the necrotic heart during anoxia but had no significant effect on the levels of myocardial high energy phosphates.

Under an anoxic state, the anaerobic glycolytic pathway of the heart becomes the major source of metabolic energy (1). It was reported by Scheuer et al. (2) that the elevation in cardiac glycogen improved mechanical performance during anoxia and Hearse et al. (3) demonstrated that the presence of glucose in the perfusion fluid during anoxia was essential for complete post-anoxic recovery. Weissler et al. (4) also reported that glucose contributed to the preservation of the functional capacity and structure of the anoxic heart tissue. On the other hand, it was observed in our previous investigation (5) that metabolic shifts were present in the necrotic heart following a s.c. injection of isoproterenol. It was reported by Bester et al. (6) that coronary artery occlusion of perfused rat heart caused a depression in myocardial ATP and creatine phosphate contents.

The present study deals with the effect of glucose on metabolisms and mechanical functions in the necrotic heart perfused under an anoxic condition.

MATERIALS AND METHODS

Animals
Male Wister strain rats, 200-250 g, maintained on a standard diet, were used. Each experimental group consisted of 5 animals.

Materials
Substrates and enzymes used for assay of metabolites in myocardium and perfusion fluids were purchased from Sigma Chemical Company, St. Louis, Mo., USA. d-Glucose and reagents used for perfusion medium were purchased from Wako Pure Chemicals Ltd., Osaka, Japan. Isoproterenol hydrochloride was procured from Boehringer Ingelheim GmbH, Germany.
Preparation of necrotic heart

The necrotic heart was produced by a s.c. injection of isoproterenol hydrochloride (5 mg/kg) 24 hr before the perfusion.

Perfusion

Immediately after the rat was stunned by a blow of head, the heart was rapidly excised, mounted on an aortic cannula and transferred to a Langendorff apparatus (7). Gravity-flow reservoirs of the perfusion apparatus were fixed 40 cm above the heart level. Two parallel perfusion systems were employed in which the perfusion lines were switched alternately by a stopcock just above the aortic perfusion cannula. The apparatus was maintained thermostatically at 37°C. The perfusion medium, Krebs-Ringer’s bicarbonate buffer (pH 7.6) modified to contain chloride instead of phosphate was used. Glucose, 5.5 or 21.0 mM, was included in the medium. All hearts were perfused aerobically (bubbled with 95% O2-5% CO2) for 25 min and then subjected to anaerobic perfusion (bubbled with 95% N2-5% CO2) for an additional 10 min. The effluent was not recirculated and was allowed to flow into graduated cylinders for lactate determination.

Mechanical parameters monitored

Contractile force was monitored with a force-displacement transducer (Nihon Koden Kogyo Co., Ltd., SB-IT) and recorded on a polygraph (Nihon Koden Kogyo Co., Ltd., RM-5). Heart rate was recorded using a pulse rate tachometer (Nihon Koden Kogyo Co., Ltd., RT-5). Coronary flow rate was monitored periodically with a drop counter (Natsume Seisakusho Co., Ltd., KN-85).

Extraction from heart muscle

The perfusion was terminated by dropping and freezing the hearts into acetone precooled with dry ice. The freezing with liquid nitrogen gave the same results on myocardial metabolite contents as with the dry ice-acetone solution.

The frozen heart was homogenized with 5 volumes of 6% cold perchloric acid in a Potter-Elvehjem glass homogenizer which had been cooled with ice water containing NaCl. All treatments for the homogenization were performed in a room maintained at 5°C. A portion of the homogenate was used to measure glycogen. The remaining homogenate was centrifuged for 10 min at 10^4 g at 0°C. The clear supernatant fluid was decanted into a pre-cooled test tube and neutralized to pH 7 by the addition of 2M K2CO3 solution at 0°C. An aliquot of the neutralized extract was used for the determination of lactate, ATP and creatine phosphate.

For the determination of glycogen, 0.1 ml of saturated Na2SO4 solution was added to 0.5 ml of the homogenate, followed by 5 ml of ethanol saturated with Na2SO4. The mixed solution was centrifuged at 10^4 g for 10 min. The precipitate was resuspended with 0.5 ml of 1N HCl and boiled at 100°C for 30 min. The boiled suspension reached 10 ml with the addition of water and was then centrifuged to procure clear supernatant for assay.

The precipitate from the homogenate was dried for 48 hr at 105°C to obtain the dry heart weight. The data related to the hearts were expressed per g dry tissue weight.
Chemical analysis

Glycogen was assayed by the anthrone method (8). Lactate was determined by the method of Hohorst (9). Creatine phosphate and ATP were measured by the method of Lamprecht et al. (10, 11).

RESULTS

Effect of glucose on myocardial metabolism

The effect of glucose on myocardial energy metabolism under anoxic state was studied in the normal and necrotic heart. As shown in Table 1, the glycogen contents of both the normal and necrotic hearts decreased equally during 10 min of anoxic perfusion. In the necrotic heart, the concentration of myocardial lactate was maintained significantly higher than in the normal at the onset of anoxia. Ten min after the anoxia, cardiac lactate markedly increased in both the normal and necrotic hearts, though the level of lactate in the necrotic heart was maintained at a higher value than in the normal. There was no influence on myocardial glycogen and lactate levels with the varying concentrations of glucose (5.5 and 21.0 mM) in the perfusion medium.

| Treatment | Anoxic perfusion (min) | Glucose conc. in perfusion medium (mM) | μmoles/g dry weight ± S.E. |
|-----------|------------------------|----------------------------------------|---------------------------|
|           |                        | Glycogen                               | Lactate                   | CrP                       | ATP                       |
| Normal    | 0                      | 75.3 ± 4.6                             | 1.37 ± 0.73               | 23.2 ± 1.27               | 16.6 ± 0.49               |
|           | 10                     | 51.3 ± 7.9*                            | 7.37 ± 1.47**             | 6.02 ± 0.66**             | 12.1 ± 0.39**             |
|           | 10                     | 57.6 ± 3.6*                            | 6.26 ± 0.33**             | 5.44 ± 0.38**             | 10.8 ± 0.56**             |
| ISP-treated | 0                      | 76.2 ± 1.6                             | 3.94 ± 0.97§§             | 17.5 ± 1.89§§             | 11.3 ± 0.95§§             |
|           | 10                     | 56.6 ± 7.1**                           | 9.58 ± 0.76**             | 5.12 ± 0.22**             | 7.88 ± 0.11§§             |
|           | 10                     | 63.3 ± 5.6*                            | 9.60 ± 0.81**             | 4.04 ± 0.66**             | 8.00 ± 0.71§§             |

* and **: Significantly different from each 0 min value with P<0.05 and P<0.01 respectively.
§ and §§: Significantly different from the corresponding values of normal hearts with P<0.05 and P<0.01 respectively.

At the onset of anoxia, the concentrations of creatine phosphate and ATP in the necrotic heart were lower than in the normal. Ten min after anoxia, creatine phosphate content decreased remarkably in both the necrotic and normal hearts. On the other hand, ATP in the normal heart remained at a higher level than in the necrotic one after 10 min of anoxia, though ATP levels of both the hearts decreased markedly during anoxia. There was no noticeable influence on the levels of ATP and creatine phosphate under the different concentrations (5.5 and 21.0 mM) of medium glucose.

Fig. 1 indicates the lactate output to the effluent from the normal and necrotic hearts treated with 5.5 or 21.0 mM glucose during anaerobic perfusion. In the normal heart, lactate production increased rapidly to about 2 μmoles/min after 2 min of anoxia and
FIG. 1. Effect of glucose on lactate output of normal and necrotic hearts perfused under an anaerobic condition.

Normal (○•○) or necrotic (●●●) hearts were perfused with 21.0 mM glucose; (a) or 5.5 mM glucose; (b). * and **, Significantly different from the corresponding values in normal hearts with P<0.05 and P<0.01 respectively. Vertical bars represent standard errors of the means.

FIG. 2. Effect of glucose on mechanical functions of normal and necrotic hearts perfused under an anaerobic condition.

Perfusions of normal hearts: (a) and (c), or necrotic hearts: (b) and (d) were carried out with medium containing 21.0 mM glucose (○•○) or 5.5 mM glucose (○--○). * and **, Significantly different from the corresponding values in normal hearts with P<0.05 and P<0.01 respectively. Vertical bars represent standard errors of the means.
maintained the increased level thereafter. Lactate output in the necrotic heart increased about twice as much as in the normal after 2 min of anoxia, and with 21.0 mM glucose, maintained the highest level until 8 min, but with 5.5 mM glucose, declined thereafter.

**Effect of glucose on mechanical function**

The tension of contractile force at the beginning of anoxia was $2.19 \pm 0.09$ g in the normal heart versus $0.79 \pm 0.16$ g in the necrotic heart. After 8 min of anoxia, contractile force and heart rate of the normal heart fell to about 50% and 40% of those at the onset of anoxia respectively, as shown in Fig. 2. In the necrotic heart, the decreases of contractile force and heart rate during anoxia were significantly recovered with 21.0 mM glucose in comparison with 5.5 mM glucose in the medium. The high concentration of glucose (21.0 mM) did not, however, influence the mechanical functions of the normal heart during the anoxic perfusion.

**DISCUSSION**

Myocardium utilizes a variety of substances for generating energy under aerobic conditions. When the oxygenation of myocardial cell is compromised, the formation of ATP by oxidative phosphorylation is inhibited and the anaerobic glycolytic pathway becomes the major source of metabolic energy (1). It was reported that myocardial contractility was only briefly maintained during a period of relative oxygen deprivation and the abrupt onset of myocardial anoxia caused a loss of contractility within a few beats (12). It was shown from the present study that, despite increased glycolysis, high energy phosphates and mechanical functions declined during 10 min of anoxia.

Weissler and co-workers (13) demonstrated that myocardial glycolytic flux and left ventricular performance were increased with increasing concentration of perfusate glucose, and insulin significantly enhanced mechanical performance and lactate production, suggesting direct stimulation of glucose transport by insulin. In our experimental condition, however, increased concentration of glucose in the perfusion medium did not improve the decrease of mechanical performance and did not enhance anaerobic glycolysis in the normal rat heart during anoxia, suggesting that glucose transport into heart tissue was a rate-limiting factor of glycolytic flux.

Rona and his associates (14) found that isoproterenol produced subendocardial necrosis of uniform severity. Histological and electron microscopical studies were made on the heart of rats treated with isoproterenol and the general morphological features of the infarction in the rat heart were similar to those produced by ischemia (15).

In our study, isoproterenol was used for the induction of necrotic heart in a rat. In the necrotic heart, myocardial high energy phosphates decreased and lactate increased even in the state of aerobic perfusion. Lactate increase may be generated from glucose as myocardial glycogen was not decreased (Table 1). After the onset of anoxia, lactate production was clearly greater in the necrotic heart than in the normal. Increasing concentration of glucose in the medium enhanced the lactate formation in the necrotic heart but not in the normal heart.
The rate of glucose transport into the necrotic heart tissue from perfusion medium might be augmented by an increasing concentration of glucose, resulting in an increasing utilization to glycolytic flux. Glycolytic flux is controlled by the enzymes which catalize the rate-limiting steps in glycolysis and ATP is a potent allosteric inhibitor of the enzymes (16, 17). Therefore, it is suggested that the decreased level of ATP in the necrotic heart caused the increase of glycolytic flux. In addition to the increase of glycolytic flux, the decreased tension of contractile force in the necrotic heart may contribute to limit the consumption of energy with contraction.

In this report, mechanical performances of the necrotic heart were compared with those of the normal heart. Tension of contractile force in the necrotic heart was less than half of the normal control in aerobic perfusion period. The onset of anoxia resulted in a significant reduction in the mechanical performances of both the necrotic and normal hearts. By an increasing concentration of glucose, however, the mechanical function in the necrotic heart was partly protected from corresponding reduction by anoxia. This phenomenon was not observed in the normal heart. Inhibition of anoxia-induced reduction of mechanical performances only in the necrotic heart may be due to the greater supply of high energy phosphates by stimulated anaerobic glycolysis.

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