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Protective Effect of Glucagon on the Isolated Perfused Rat Heart Following Severe Hypoxia[1,2] (38380)

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It is well known that the heart loses its ability to maintain its strength of contraction during periods of hypoxia (1–3). Even upon reoxygenation, the heart frequently fails to recover to its prehypoxic contractile state. It has been suggested that this impaired capacity of the heart to maintain contractility during hypoxia and its inability to regain it after reoxygenation is related to both a depletion of energy substrates and a decreased rate of substrate utilization (4, 5). Despite the acceleration of glycogenolysis and glycolysis that occurs when the heart is exposed to hypoxic conditions, it has been calculated that only one-tenth to one-third of the energy needs of the mammalian heart can be met by these anaerobic metabolic pathways (6). In an attempt to prevent the depressant effects of hypoxia on cardiac contraction, several investigators have increased the amount of energy substrate available to the heart during hypoxia. Scheuer and Stezoski (7) using an isolated rat heart preparation, demonstrated that hearts from animals pretreated with reserpine contained increased levels of cardiac glycogen. Furthermore, these hearts exhibited greater glycolytic production of ATP and improved cardiac function during hypoxia. Hewitt *et al.* (8) have shown that dogs placed on a high fat diet have higher levels of myocardial glycogen which is associated with improved left ventricular performance after 30 min of ischemic anoxia.

Alternatively, the rate of glycogen breakdown may not depend on a simple maintenance of glycogen levels in the hypoxic heart. It has been shown that in the hypoxic rat heart the rate of anaerobic glycogenolysis declines long before glycogen stores are depleted (9). Thus, based on this observation any treatment that could enhance glycogen utilization could potentially prevent some of the deleterious effects normally observed during hypoxia. One such treatment might be the use of glucagon which has been shown to exert an effect on both glycogen metabolism and cardiac contractility. The effects produced by this hormone have been related to increased intracellular concentrations of the nucleotide, adenosine 3′,5′- monophosphate (cyclic AMP). Glucagon enhances glycogen metabolism via an activation of the enzyme phosphorylase which catalyzes glycogen breakdown to glucose-1-phosphate. This activation is linked to a cyclic AMP mechanism that results in the transformation of phosphorylase from the inactive form (phosphorylase$_b$) to the active form (phosphorylase$_a$). Furthermore, this nucleotide is thought to exert an effect on contractility by stimulating phosphoprotein formation via the activation of a proteinkinase (10).

In the present studies, isolated rat hearts were perfused with glucagon during exposure to severe hypoxia to determine if glucagon might improve cardiac recovery during reoxygenation.

Materials and Methods. Male Sprague–Dawley albino rats (200–250 gm) were fed *ad libitum* with Purina Lab Chow. The animals were injected with aqueous heparin (40 units ip) and were sacrificed by decapitation 15 min later. Following decapitation, the hearts were quickly removed and arranged on an Anderson perfusion apparatus. Coronary perfusion was conducted with modified Tyrode's solution (37°, pH 7.4) containing heparin 2500 units/l and equilibrated with 95% O_2 and 5% CO_2 (PO_2

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450–500 mm Hg). The ionic composition of the Tyrode's solution in millimoles per liter was: NaCl, 120; KCl, 5; $CaCl_2$, 4; $MgSO_4$, 0.6; $NaHCO_3$, 30; and KH_2PO_4, 0.6. Hypoxic perfusion medium (PO_2 10–20 mm Hg, pH 7.4) was produced by gassing a similar Tyrode's solution with 95% N_2 and 5% CO_2 for 30 min prior to perfusion. These two perfusion media were set in parallel systems so that the outflow tracts were just above the aortic cannula. With a turn of a stopcock, either medium could be selectively used for immediate cardiac perfusion. All hearts were perfused with an open end system such that none of the perfusate was recirculated. Rate of perfusion was maintained constant at 7 ml/min throughout the experiments.

Force of contraction was measured with a calibrated Grass FT 0.03 force displacement transducer connected to the heart by a silk suture attached to a hook placed into the apex. The transducer was mounted on a movable assembly in such a way that various degrees of tension could be placed on the hearts. In this manner each heart was equilibrated with sufficient tension to place the contraction at the peak of its length-tension curve. Recordings of contractile force were made on a Grass Model 7 polygraph.

In order to obviate the effects of changes in heart rate on coronary flow and contractile force, the heart rates were maintained constant at a rate of 20% above the intrinsic rate by square wave stimuli (4–8V, 4 msec duration) delivered from an E & M stimulator through hook electrodes placed on the apex and right atrium.

Hearts were divided into four experimental groups. In the first group, rat hearts were equilibrated for 10 min with oxygenated Tyrode's solution and then perfused with the hypoxic medium for 2–7 min to determine the time necessary for diminution of contractile force to approximately 5–10% of control. In the second group, hearts were equilibrated for 10 min and then perfused for 5 min with oxygenated Tyrode's containing various concentrations of glucagon ($8.7 \times 10^{-9} M$–$8.7 \times 10^{-7} M$) to determine the positive inotropic dose response of normal hearts to this hormone. In the third group, hearts were equilibrated with oxygenated Tyrode's, then perfused with hypoxic medium for 3 min; and finally reperfused for 5 min with oxygenated Tyrode's. In the fourth group, hearts were treated as described for Group III except that the hypoxic medium contained glucagon in concentrations ranging from $8.7 \times 10^{-9} M$ to $8.7 \times 10^{-7} M$. Recovery of the force of contraction during 5 min of reoxygenation was determined for both groups III and IV and the significance of differences was determined by the Student's t test. In all experiments each heart was treated with only one episode of hypoxia and one dose of glucagon.

In an additional series of experiments, the recovery of contractile force during 30 min of reoxygenation was determined in both the control (Group III) and glucagon perfused ($8.7 \times 10^{-9} M$) hearts (Group IV) after 3 min of hypoxia to evaluate the effectiveness of recovery over a longer period of time.

Results. As shown in Fig. 1, the perfusion of rat hearts with hypoxic Tyrode's solution produces a rapid decline in force of contraction to approximately 10% of control within 3 min. The most rapid decline occurs within 30 sec with a less marked but yet progressive decrement from 30 sec to 3 min. This pattern of cardiac failure was seen in both control and glucagon-perfused hearts. There was no statistically significant dif-

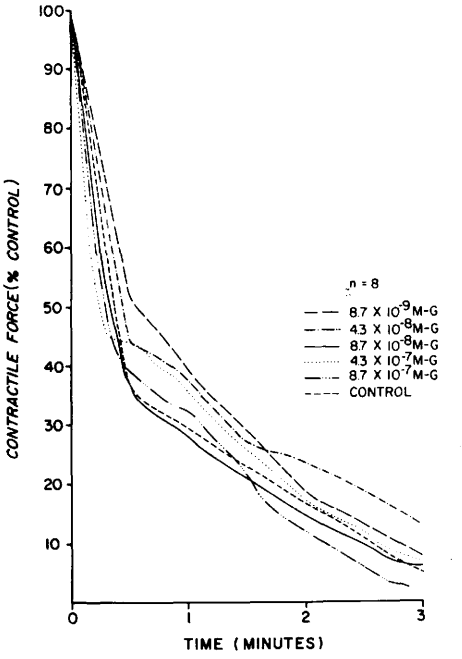

FIG. 1. Rate of decline in contractile force during exposure to hypoxia in control and glucagon perfused isolated rat hearts. The abcissa represents the time during which the hearts were perfused with hypoxic medium as described in the methods. Glucagon (G) was included in the hypoxic perfusion medium at various concentrations as represented.

ference ($P > .05$) in the degree or rapidity of heart failure in control as well as in all glucagon perfused hearts.

Figure 2 depicts recovery of contraction during a 5 min period of reoxygenation that followed a 3 min period of hypoxia. In concentrations of 8.7×10^{-9} M, 8.7×10^{-8} M, and 4.3×10^{-7} M glucagon significantly enhanced recovery of force of contraction at all time intervals measured ($P < .05$).

The maximal effect on recovery was generally seen at 2 min of reoxygenation. Between 2 and 5 min of reoxygenation there was a gradual decline in cardiac function, however, force of contraction was significantly greater than that in control hearts ($P < .05$). In a concentration of 8.7×10^{-7} M, glucagon did not improve recovery and in fact appeared to depress contractility with a reduction in developed tension although this difference was not statistically significant ($P > .05$) at any time interval.

The maximal force of contraction attained during reoxygenation of hypoxic hearts is illustrated in Fig. 3. In these experiments the effects of glucagon were evaluated at five different con-

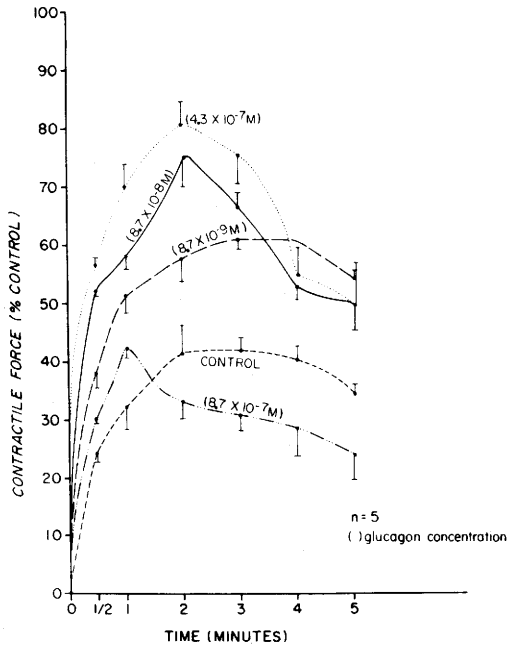

FIG. 2. Recovery of contractile force after hypoxia. The abcissa represents the time of reoxygenation of rat hearts following 3 min perfusion with hypoxic medium. When included in the hypoxic medium, glucagon, at all concentrations less than 8.7×10^{-7} M afforded a significant increase ($P<0.05$) in recovery of contractile force at each interval.

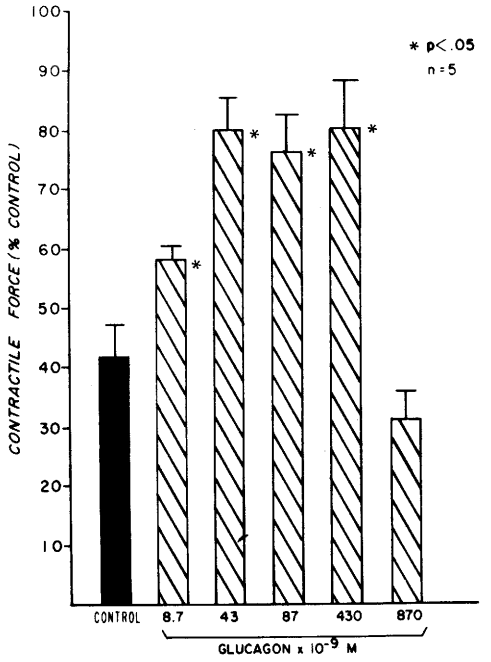

FIG. 3. Effects of glucagon on cardiac recovery at 2 min of reoxygenation. Each value is represented as the mean of five experiments ± SEM. Glucagon at the various concentrations indicated was included in the perfusions only during the 3 min of hypoxia as described in methods.

* Represents contractile force significantly different from control hearts not perfused with glucagon during hypoxia ($P < 0.05$).

centrations. In all cases, except that in which glucagon was perfused at a concentration of 8.7×10^{-9} M, the maximal force of contraction was seen at 2 min of reoxygenation. The force of contraction for all the glucagon treated hearts except those receiving the highest concentration (870×10^{-9} M) was significantly greater than that of the controls. Furthermore, the three intermediate concentrations of glucagon (43×10^{-9}, 87×10^{-9}, and 430×10^{-9} M) while producing the greatest inotropic response were not different from one another.

In another series of experiments, recovery of contractile force was observed during 30 min of reoxygenation following the 3 min exposure to hypoxia. Experimental hearts were perfused during the hypoxic period with 8.7×10^{-9} M glucagon. The experimental hearts demonstrated a decline in cardiac function after maximal recovery at 3–5 min (Fig. 4). However, glucagon perfused hearts maintained a greater force of contraction than did the controls at all time intervals. At the end of this 30 min period,

the contractile force of untreated hearts was 12.5% of control while that of the glucagon perfused hearts was nearly three times greater (34% of control).

When normal oxygenated hearts were perfused with $8.7 \times 10^{-9} M$ glucagon for 5 min, no positive inotropic effect was seen (Fig. 5), however, this dose of glucagon produced a significant effect on recovery after hypoxia (Fig. 4). When control hearts were perfused with glucagon at ten times this concentration, an 18% increase in contractile force was observed whereas a 100-fold increase in the glucagon concentration to $870 \times 10^{-9} M$ resulted in a decrease in contractility. Although not shown in this figure a dose of $43 \times 10^{-9} M$ was also sufficient to increase force of contraction approximately 20%; however, larger doses (including $430 \times 10^{-9} M$) which enhanced recovery after hypoxia, depressed contractility.

Discussion. In the present experiments, perfusion of isolated rat hearts with glucagon during hypoxia enhanced the recovery of contractile force during reoxygenation. During all perfusions, glucose was omitted from the medium to preclude the effects of an exogenous energy source. Though it is unclear how glucagon improves cardiac recovery after hypoxia, our studies suggest that the mechanism is unrelated to the direct positive inotropic effect of this hormone. Enhanced recovery after hypoxia was observed with several concentrations of glucagon, however, it was more sustained at a subinotropic dose. Inotropic doses of glucagon produced a higher maximal force contraction at two minutes after reoxygenation than did subinotropic doses. Hearts treated with inotropic concentrations also exhibited a more rapid decline in performance. This rapid decline in force of contraction seen with the higher doses could be explained by greater oxygen consumption and substrate depletion which results from the inotropic response. It is interesting to note that by increasing the glucagon concentration from 4.3×10^{-7} to $8.7 \times 10^{-7} M$ a marked decline in recovery from hypoxia was observed. Moreover, this high dose of glucagon failed to produce positive inotropic effects in the control hearts not exposed to hypoxia. Mayer *et al.* (11) have previously shown that high doses of glucagon depress cardiac contractility.

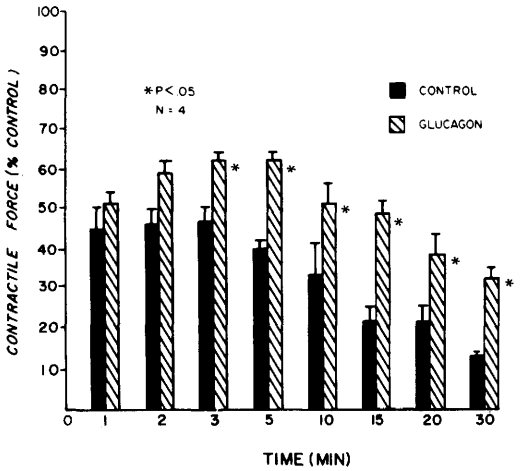

FIG. 4. Effects of glucagon on contractile force during reoxygenation of isolated perfused rat hearts. Glucagon was included in the hypoxic medium at a subinotropic concentration of $8.7 \times 10^{-9} M$ but was omitted from the reoxygenation medium. Each value represents the mean of four experiments ± SEM. Time refers to the interval of reoxygenation following the hypoxic perfusion.

* Denotes significant difference from the respective control at the same time without glucagon treatment ($P < 0.05$).

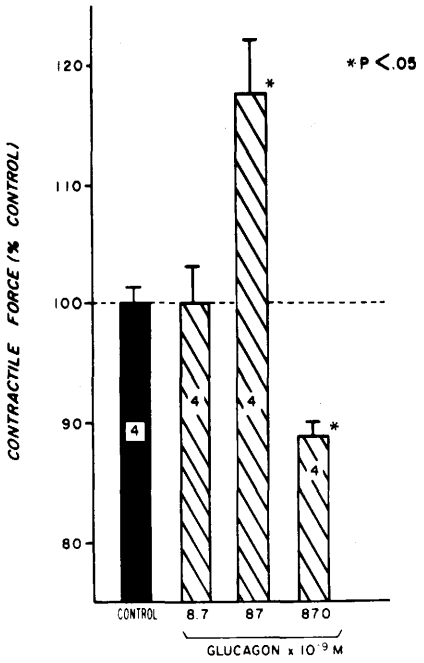

FIG. 5. Comparison of effects of several doses of glucagon on contractile force in non-hypoxic rat hearts. Contractile force was determined at 5 min following initiation of glucagon perfusion. Each value represents the mean of four experiments + SEM. Control hearts were not perfused with glucagon.

* Denotes significant difference from control ($P < 0.05$).

Our findings are consonant with those of Sheuer and Stezoski (12) who have reported that cardiac perfusion with glucagon during hypoxia does not alter cardiac performance during this period. However, these investigators reported that recovery could be enhanced upon reoxygenation only with concomitant administration of glucagon during the recovery period. This differs from our studies in which hearts were perfused with glucagon only during the hypoxic period. Our results indicate that, although glucagon was omitted from the perfusion medium during reoxygenation, improved recovery occurred.

There are several possible mechanisms by which glucagon could exert its protective effect on the hypoxic myocardium. After the heart is exposed to hypoxia there occurs an acceleration of glycolysis, increased utilization of glycogen, and an inhibition of the oxidation of fatty acids (2). The acceleration of both glycolysis and glycogenolysis has been related to the decreased cardiac levels of ATP, and to increased levels of both ADP and inorganic phosphate. These decreased ATP levels result in the activation of phosphofructokinase, the rate limiting enzyme in glycolysis, as well as enhanced glycogenolysis by increasing the conversion of phosphorylase$_b$ to phosphorylase$_a$ (9). In hypoxic rat hearts, rate of glycogenolysis declines prior to depletion of glycogen stores (9). Administration of glucagon during hypoxia might prevent this decline in glycogenolysis thus enabling the heart to maintain necessary levels of utilizable energy substrates.

In addition to the cyclic AMP-dependent metabolic events produced by glucagon, the positive inotropism action of this hormone has been directly related to increased levels of this nucleotide (10). Therefore, higher levels of cyclic AMP could be directly associated with the enhancement in contractility observed in those hearts perfused with glucagon during hypoxia.

On the other hand, glucagon might exert its effect independently of the changes in cyclic AMP concentration. Mayer and Namm (11) have shown that inotropism associated with glucagon can occur prior to elevation of cyclic AMP concentration. It has also been shown that there is a decline in contractility in the anoxic working heart at a time when cyclic AMP levels were increased (13), and this was explained by the supposition that during anoxia the control of the contractile event by cyclic AMP is disrupted.

A third and perhaps more attractive possibility is that the cardiac contractile response following exposure to hypoxia is dependent on the levels of cardiac cyclic AMP and a second cyclic nucleotide (guanosine 3′,5′-monophosphate (cyclic GMP)). Katz and coworkers (14) have reported that a cyclic AMP-dependent protein kinase may phosphorylate phospholamban, a protein in the sarcoplasmic reticulum which could increase inotropism via enhanced calcium transport. Cyclic GMP has been proposed to produce an opposite effect on this accumulation of calcium (16). It has been shown in a previous report that the levels of cardiac cyclic GMP in spontaneously beating isolated perfused rat hearts are increased during induced negative inotropism (15) and that the levels of this nucleotide are decreased during states of positive inotropism (16). Furthermore, it was reported that cardiac cyclic AMP levels are decreased when cyclic GMP concentrations are elevated. An antagonist effect of cyclic GMP on calcium accumulation could occur by the inhibition of protein kinase activity or by the stimulation of a phosphatase which would dephosphorylate phospholamban. Thus, it is conceivable that glucagon enhances cardiac recovery from hypoxia by regulating the ratio of cyclic GMP to cyclic AMP in this tissue. However, further work is necessary to clarify the precise biochemical mechanism by which glucagon exerts this salutary effect.

Summary. It has been demonstrated that exposure of isolated perfused rat hearts to glucose-free, hypoxic medium produces a marked decline in contractile force within 3 min. Hearts which were perfused with various concentrations of glucagon during the hypoxic period recovered better during reoxygenation than hearts not perfused with glucagon. This salutary effect of glucagon was apparently unrelated to this hormone's inotropic properties since the most sustained recovery was seen when the hearts were perfused with a subinotropic dose.

This evidence suggests that glucagon may exert a protective effect on the hypoxic myocardium.

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