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# Effect of Endotoxin Administration on Heart Glycolytic Enzyme Activities in Normal and Diabetic Dogs[1] (41183)

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*Abstract.* The effects of *Escherichia coli* endotoxin administration on the activities of key glycolytic enzymes in normal and alloxan-diabetic dog hearts were studied and the results were correlated with changes in plasma glucose concentrations. Normal dogs had a fasting plasma glucose concentration of 100 mg%, which was increased to 130 mg% 1 hr postendotoxin, then followed by a progressive decrease at 2 hr and thereafter to hypoglycemic levels (68 mg% at 4 hr). Diabetic dogs had a fasting plasma glucose concentration of 360 mg%, which was decreased to and maintained at 250 mg% at 2 and 4 hr following endotoxin injection. Hexokinase activity was not significantly different between the normal and diabetic dogs ($P = 0.09$). After endotoxin injection hexokinase activity tended to increase at 2 to 4 hr ($P = 0.056$) but it did not respond differently between the two groups, normal versus diabetic ($P = 0.29$). Phosphofructokinase activity was not different between normal and diabetic dogs ($P = 0.49$). After endotoxin injection phosphofructokinase activity was depressed at 2 hr but returned nearly to the control value at 4 hr ($P = 0.48$); there was no indication that this response with time differed between normal and diabetic dogs ($P = 0.64$). In normal dogs, pyruvate kinase activity was increased by 152.8% at 2 hr but then returned to the control value 4 hr after endotoxin injection. In alloxan diabetes, pyruvate kinase activity was increased by 216.8% at 2 hr and then further increased to 425.4% at 4 hr following endotoxin injection. Postendotoxic pyruvate kinase activity in diabetic dogs was significantly higher than that in normal dogs ($P < 0.01$) and also the change in pyruvate kinase activity with time differed significantly between the two groups ($P < 0.01$). Since the transport of glucose across myocardial cells has been found to be unaltered by endotoxin, it is concluded that the increase in pyruvate kinase activity is responsible at least in part for the augmented glycolysis of the myocardium during endotoxic shock.

*Escherichia coli* endotoxin has been shown to affect myocardial oxidative metabolism by shifting from fatty acid to glucose and lactate utilization (1–3). Previous work from this laboratory indicated that the changes in substrate oxidation were not the result of an alteration in transport of substrates across the myocardial cells (3). In order to understand further the mechanism responsible for the increased glucose oxidation by the heart during endotoxemia, we have examined the activities of key enzymes in the glycolytic pathway. Since it has been suggested that the known changes in plasma insulin and glucose concentrations play an important role in hepatic glucose homeostasis during endotoxemia (4, 5), it is possible that endotoxin also affects cardiac glucose metabolism in a similar manner. Therefore, the effect of endotoxin administration on the activities of key glycolytic enzymes, hexokinase, phosphofructokinase, and pyruvate kinase, in the heart was studied using normoglycemic (normal) as well as hyperglycemic (alloxan-diabetic) dogs.

**Materials and Methods.** Experiments were carried out on mongrel dogs of either sex weighing from 7 to 15 kg. They were divided into four groups: (i) normal dogs, (ii) normal dogs given endotoxin, (iii) diabetic dogs, and (iv) diabetic-endotoxic dogs. Endotoxic shock in group 2 animals was induced by a single injection of 0.5 mg/kg *E. coli* endotoxin ($LD_{30}$ in 6 hr) via

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the saphenous vein. In group 3 experiments, diabetes was produced by a bolus iv injection of 80 mg/kg alloxan monohydrate. Only dogs with an overnight-fasted blood glucose concentration of 300 mg% or higher 6 to 8 days later were used. Group 4 experiments were performed on dogs which were diabetic as induced in group 3 and then subsequently treated with endotoxin in the same manner as in group 2. The number of animals used was 8 and 9 for groups 1 and 3, respectively; 8 and 6 for group 2 at 2 and 4 hr postendotoxin, respectively; and 9 and 8 for group 4 at 2 and 4 hr postendotoxin, respectively. Group 1 dogs received no treatment. It should be mentioned that all the endotoxin employed in this study was of the same lot. All animals (groups 1–4) were fasted overnight with free access to water prior to the experiments. After anesthetizing the animals with 2% chloralose in 20% urethane (4 ml/kg, iv), hearts were removed, washed, and then homogenized with 3 vol of 0.05 M Tris–HCl buffer containing 1 mM EDTA and 5 mM $\beta$-mercaptoethanol in a loose Teflon pestle homogenizer. The homogenization was standardized by three strokes up and down at 750 rpm. A portion of the homogenate was centrifuged at 2000*g* for 20 min and the resulting supernatant was used for the hexokinase assay. The remaining homogenate was centrifuged at 100,000*g* for 1 hr and the resulting supernatant was used for the assays of phosphofructokinase and pyruvate kinase. For the phosphofructokinase assay, the 100,000*g* supernatant was diluted 10 times with 20 mM Tris–HCl buffer (pH 8.0) containing 0.1 mM ATP, 10 mM fructose-1,6-diphosphate, 50 mM $\beta$-mercaptoethanol, and 0.1 mM EDTA. For pyruvate kinase measurement, the 100,000*g* supernatant was diluted 50 times with 0.5 M sucrose solution containing 10 mM Tris–HCl (pH 6.0), 10 mM maleate, 1 mM EDTA, 4 mM acetylcysteine, 1 mM $MgCl_2$, and 1% bovine albumin.

Hexokinase was assayed by the method of Sharma *et al.* (6). The reaction mixture in a final volume of 3 ml contained 3 mM ATP, 0.75 mM NADP, 44 mM sodium glycylglycinate (pH 7.5), 7.5 mM $MgCl_2$, 0.5 mM glucose, and 0.2 units of glucose-6-phosphate dehydrogenase. The reaction was started by the addition of freshly prepared enzyme (2000*g* supernatant) containing 1 to 1.8 mg protein. The formation of NADPH was recorded spectrophotometrically at 340 nm for 15 min at 23°.

Phosphofructokinase activity was determined according to Mendicino *et al.* (7). The reaction mixture in a final volume of 3 ml contained 35 mM Tris–HCl buffer (pH 7.5), 2 mM ATP, 0.2 mM NADH, 1 mM $MgCl_2$, 154 mM KCl, 6 units adolase, 6 units triose-phosphate isomerase, 6 units glycerophosphate dehydrogenase, and enzyme preparation (100,000*g* supernatant) containing 0.6 to 0.9 mg protein. The reaction was initiated by the addition of 3 mM fructose 6-phosphate. The rate of disappearance of NADH was measured spectrophotometrically at 340 nm at 23°.

The activity of pyruvate kinase was assayed according to the method of Farina *et al.* (8). The reaction mixture in a final volume of 3 ml contained 13 mM phosphoenolpyruvate, 0.75 units lactate dehydrogenase, 100 mM KCl, 10 mM $MgCl_2$, 0.15 mM NADH, 50 mM glycylglycine buffer (pH 7.5), and freshly prepared enzyme (100,000*g* supernatant) containing 12 to 15 $\mu$g protein. The reaction was initiated by the addition of 2 mM ADP and proceeded for 15 min at 23°. The enzyme activity was determined spectrophotometrically by measuring the rate of disappearance of NADH at 340 nm.

Protein concentration was determined by the method of Lowry *et al.* (9) after the enzyme preparation was dissolved in 1 N NaOH solution.

Plasma glucose concentration was determined colorimetrically using *O*-toluidine reagent (Sigma Tech. Bull. No. 635).

The statistical significance of the data was evaluated by a two-way analysis of variance by the method of fitting constants (10). When the interaction term was significant, the main effect was tested by the method of weighted squares of means (10).

*E. coli* endotoxin (lipopolysaccharide B) was purchased from Difco Laboratories. Alloxan monohydrate was supplied by Nutritional Biochemical Co. ATP, ADP, NADP, NADH, fructose 6-phosphate,

fructose 1,6-diphosphate, phosphoenolpyruvate, β-mercaptoethanol, acetylcysteine, glycylglycine, EDTA, and maleate were products of Sigma Chemical Company. Purified enzymes such as aldolase, glycerophosphate dehydrogenase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and triose-phosphate isomerase were also obtained from Sigma Chemical Company. Other chemicals and reagents were of analytical grade.

**Results.** Following endotoxin administration to normal dogs, the fasting plasma glucose concentration exhibited a typical biphasic response, namely a hyperglycemia followed by a progressive hypoglycemia; the values (mean ± SEM) were 100 ± 4.0, 130 ± 10.6, 85 ± 6.6, 72 ± 7.8, and 66 ± 7.9 mg% at 0, 1, 2, 3, and 4 hr postendotoxin, respectively ($n$ = 6). The changes in blood glucose concentrations at 1, 3, and 4 hr were statistically significant. The fasting plasma glucose concentration (mean ± SEM) of diabetic dogs ($n$ = 4) was 360 ± 20 mg% and was decreased to 315 ± 17.5, 275 ± 18, 287 ± 15, and 290 ± 20 mg% at 1, 2, 3, and 4 hr, respectively, following endotoxin administration. It should be pointed out that the mean blood glucose value at each time point represents a different group of dogs.

Figure 1 shows the effects of *E. coli* endotoxin administration on heart hexokinase activity in normal and diabetic dogs. The data were analyzed by two-way analysis of variance. Hexokinase activity was not significantly different between the normal and diabetic dogs ($P$ = 0.09). Although hexokinase activity tended to increase with time after endotoxin injection ($P$ = 0.056), statistical analysis provided no reason to believe that it responded differently between the two groups, normal versus diabetic ($P$ = 0.29).

Figure 2 depicts heart phosphofructokinase activity in normal and diabetic dogs following endotoxin administration. There was no indication that phosphofructokinase activity was different between normal and diabetic dogs ($P$ = 0.49). Phosphofructokinase activity was depressed at 2 hr in both groups but it returned nearly to the control values 4 hr after endotoxin injection ($P$ = 0.48). There was no evidence that this response with time differed between normal and diabetic dogs ($P$ = 0.64).

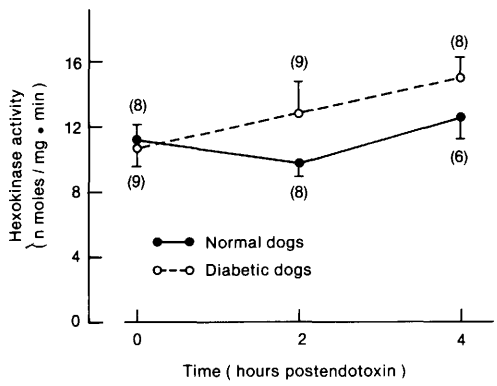


FIG. 1. Effect of endotoxin administration on hexokinase activity in normal and diabetic dog hearts. Hexokinase activity was assayed as described under Materials and Methods. The ordinate indicates enzyme activity in nmoles/mg protein per min. The abscissa represents the time course after endotoxin injection. Vertical bars indicate standard errors of the mean. Number of experiments is shown in parentheses. Solid line indicates values obtained from normal dogs while broken line represents those obtained from diabetic dogs.

Figure 3 shows the influence of endotoxin administration on heart pyruvate kinase activity in normal and diabetic dogs. In normal dogs, pyruvate kinase activity was increased by 152.8% at 2 hr but then returned to the control value 4 hr after endotoxin administration. In alloxan-diabetic

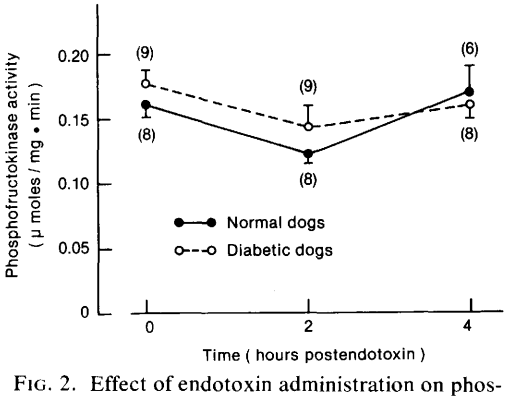


FIG. 2. Effect of endotoxin administration on phosphofructokinase activity in normal and diabetic dog hearts. Phosphofructokinase activity was assayed as described under Materials and Methods. The symbols are identical to those in Fig. 1.

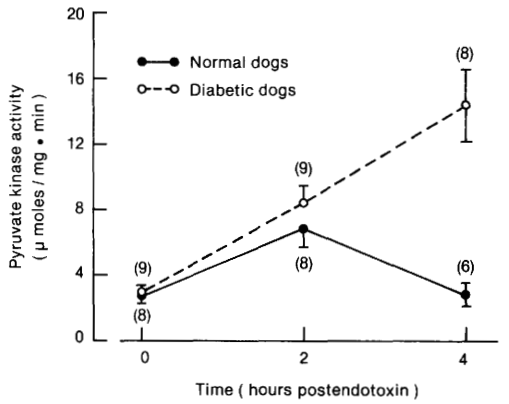


FIG. 3. Effect of endotoxin administration on pyruvate kinase activity in normal and diabetic dog hearts. Pyruvate kinase activity was assayed as described under Materials and Methods. The symbols are identical to those of Fig. 1.

dogs, pyruvate kinase activity was increased by 216.8% at 2 hr and then further increased to 425.4% at 4 hr after endotoxin injection. Analyses of variance showed that pyruvate kinase activity was significantly higher in the diabetic dogs ($P < 0.01$) and that the change in pyruvate kinase activity with time differed significantly between the two groups (normal versus diabetic) ($P < 0.01$).

**Discussion.** The mechanism responsible for the increase in pyruvate kinase activity during endotoxic shock is not clearly understood. Pyruvate kinase in liver is known to be subject to nutritional and hormonal control; its activity is increased by high carbohydrate diet and insulin (11) but decreased by high protein feeding (11) and glucagon or epinephrine (12, 13). It has been postulated that the altered pyruvate kinase activity is a result of a modification of the enzyme by phosphorylation and dephosphorylation of the enzyme moiety or by an interconversion between the phosphorylated (active) and dephosphorylated (inactive) forms through cyclic AMP modulation (14). In our experiments, heart pyruvate kinase activity was found to be unaltered in the diabetic state, suggesting that insulin deficiency and/or high blood glucose may not play a role in regulating pyruvate kinase activity in the myocardium. The increase in pyruvate kinase activity 2 hr following endotoxin administration appeared not to be associated with the lowering of plasma glucose concentration since the enzyme activity remained increased during the diabetic state in which blood sugar was high. However, the fact that the stimulatory action of endotoxin on pyruvate kinase was potentiated further and lasted longer during the diabetic state indicates that either insulin deficiency or high blood glucose or the combination of both renders the myocardium more susceptible to endotoxin action. Whether or not endotoxin stimulates myocardial pyruvate kinase activity by activating the enzyme molecule through the cyclic AMP modulated phosphorylation–dephosphorylation cycle remains to be elucidated.

Since the plasma glucagon concentration has been shown to increase during diabetes (15) and endotoxic shock (16), and since the increase in glucagon concentration is known to decrease pyruvate kinase activity (17), one would expect pyruvate kinase activity in diabetic dogs after endotoxin administration to be decreased if the change in enzyme activity is mediated through glucagon. However, this was found not to be the case as reported in this study (Fig. 3). Therefore, it is suggested that the action of endotoxin on myocardial pyruvate kinase is not mediated through a change in glucagon concentration.

Certain glycolytic intermediates are known to be potent regulators of pyruvate kinase (18, 19). The increase in pyruvate kinase activity, as reported in this study, during endotoxic shock could be the result of an alteration in metabolite concentration. Since fructose 1,6-diphosphate (activator), dihydroxyacetone phosphate (activator), and ATP (inhibitor) concentrations were found to remain unchanged in rat hearts 5 hr after *E. coli* endotoxin injection (20), it is unlikely that the endotoxin-induced changes in myocardial pyruvate kinase activity are mediated through an alteration in the substrate or metabolite concentration.

Although the findings reported here appear to eliminate certain substrates and hormones as mediators of the specific effect of endotoxin on cardiac pyruvate kinase, we do not rule out the possibility that com-

binations of changes in substrates and hormones could be associated with the effect of endotoxin.

In conclusion, *E. coli* endotoxin administration affects myocardial key glycolytic enzymes by stimulating pyruvate kinase and this stimulatory effect was further potentiated and was longer lasting during the diabetic state. Since endotoxin increases glucose oxidation independently of the transport step (3), the increase in pyruvate kinase activity appears to be responsible at least in part for the augmented glycolysis in the myocardium during endotoxic shock.

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