Myocardial Insulin Resistance During Acute Endotoxin Shock in Dogs

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Myocardial insulin responsiveness was determined in open-chest pentobarbital sodium–anesthetized dogs before and after endotoxin administration. Animals were instrumented to measure mean arterial blood pressure (MABP), heart rate (HR), and coronary blood flow. Myocardial glucose uptake and myocardial oxygen uptake (MVO_2) were determined during a basal control period and after a hyperinsulinemic-euglycemic clamp procedure over a wide range of insulin concentrations. The clamp was accomplished by intravenously infusing insulin (0–4000 mU/min) and 20% glucose in sufficient amounts to maintain arterial glucose concentrations within 5 mg/dl of the control value. In a separate series of experiments, myocardial insulin responsiveness was determined by use of a single dose of insulin (4000 mU/min). This was done to determine whether antecedent insulin infusions during the sequential clamp procedure would affect the responsiveness of the heart. In control experiments, myocardial glucose uptake increased without any changes in HR, MVO_2, or MABP. Maximum myocardial glucose uptake occurred at an insulin infusion rate between 400 and 4000 mU/min. A single concentration of insulin resulted in similar increases in myocardial glucose uptake as with the sequential clamp protocol. Acute endotoxin shock was induced by bolus injection of 1 mg/kg *Salmonella typhimurium* endotoxin (Difco Labs, Detroit, MI). One hour after administration of endotoxin, basal myocardial glucose uptake was decreased compared with the control animals. After 1 h of endotoxin shock, the heart was refractory to all concentrations of insulin, suggesting the site for altered insulin response was being mediated by a postreceptor mechanism. *Diabetes* 37:1684–88, 1988

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Decreased responsiveness to insulin stimulation of glucose and amino acid transport and metabolism has been reported in skeletal muscle (1–4), adipose tissue (5,6), liver (7), and the whole body (8) during several pathophysiologic states such as burn injury, trauma, and sepsis. Although the preponderance of data regarding insulin resistance during stress injury has focused primarily on nonvital tissues, the heart's response to insulin during acute and stress injury (i.e., burn, trauma, and sepsis) is unclear.

Although data are sparse on the effectiveness of insulin therapy on myocardial metabolism during shock, there have been several reports that detail myocardial insulin resistance during obesity. Recently, Eckel et al. (9) described insulin resistance in cardiocytes of genetically obese Zucker rats and reported the site for the decreased insulin response to reside from both receptor and postreceptor mechanisms.

Unlike skeletal muscle, myocardial glucose utilization accounts for a large portion of the energy requirement at rest. Acute endotoxin shock has been reported to result in decreased fatty acid utilization by the heart (10), which is its primary energy substrate. Under these conditions, glucose could play a major role in maintaining myocardial metabolism and function. This study was undertaken, therefore, to determine responsiveness of the heart to insulin-stimulated glucose uptake during acute endotoxin shock in the dog.

MATERIALS AND METHODS

Cardiac instrumentation. In conducting the research described, the authors adhered to the National Institutes of Health (NIH) guidelines for use of experimental animals. Twenty-two adult mongrel dogs of either sex weighing 20–25 kg were used in this study. Animals were anesthetized with pentobarbital sodium (30 mg/kg i.v.), intubated, and ventilated on room air against 3–5 cmH_2O of positive end-expiratory pressure with a Harvard Apparatus respirator. All dogs were given 1 mg/kg i.v. of the short-acting paralytic

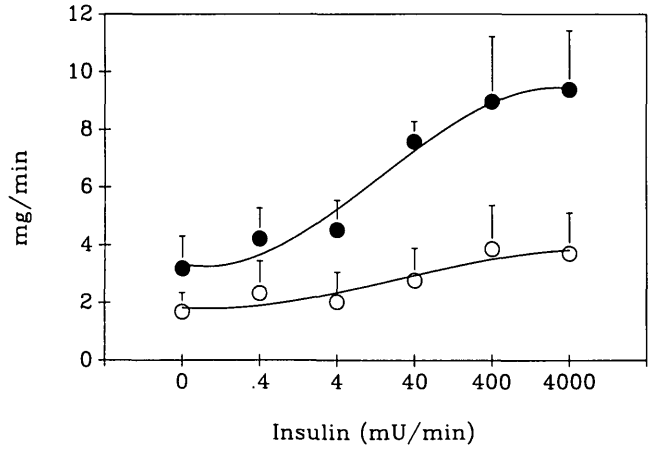

FIG. 1. Dose-response curves of insulin-stimulated myocardial glucose uptake in control (●, *n* = 5) and endotoxin (○, *n* = 4) dogs.

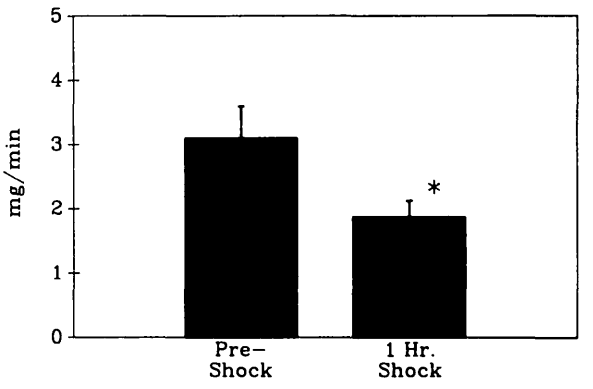

FIG. 2. Effect of endotoxin shock on myocardial glucose uptake before insulin infusion. One-hour shock value also represented in Fig. 1 during basal insulin-infusion period. *P < .05.

drug succinylcholine chloride to achieve muscle relaxation during surgical instrumentation.

A left thoracotomy was made in the fifth intercostal space. Blood flow through the circumflex artery was monitored with an electromagnetic flow probe (Gould, Oxnard, CA). A silicone rubber (Silastic) catheter was placed in the descending thoracic aorta and coronary sinus for collecting blood samples for chemical analyses and to monitor heart rate and arterial blood pressure (Gould P23 pressure transducer). Two small Tygon catheters (Cardiovascular Instrument, Wakefield, MA) were positioned in the right atrial appendage for insulin and glucose infusions.

Experimental protocol. After a stabilization period of ~30 min, cardiac hemodynamic and metabolic profiles were determined during a basal control and after sequential hyperinsulinemic-euglycemic clamp periods in control animals (*n* = 5). The clamps were achieved by infusing various concentrations of regular insulin from 0–4000 mU/min i.v. at 1-ml/min infusion rate with variable amounts of 20% glucose to maintain arterial glucose concentration within 5 mg/dl of the basal value. Animals were considered clamped when three successive arterial glucose samples, taken within 15 min, did not vary by >5 mg/dl. All insulin infusions were for a minimum of 60 min. In a separate group of animals, after basal measurements, shock (*n* = 4) was induced by a bolus injection of 1 mg/kg i.v. *Salmonella typhimurium* endotoxin (Difco, Detroit, MI). Animals were monitored for 1 h, during which glucose was infused as required to maintain plasma glucose at the preendotoxin level. At 1 h of endotoxin shock, hemodynamic and metabolic profiles were determined during a basal-shock period and then during clamp procedures by means of various insulin concentrations, as described above. In a separate group of animals, a single dose of insulin (4000 mU/min) was infused during control (*n* = 8) and after 1 h of shock (*n* = 5) to determine the response of the heart to a single insulin concentration.

Calculation and chemical analyses. Blood samples were collected from the arterial pressure and coronary sinus catheters. These samples were analyzed as follows: *1*) oxygen content with a Radiometer ABL-3 acid-base laboratory, *2*) glucose with a Yellow Springs glucose analyzer (model 23A, Yellow Springs, OH), *3*) plasma insulin was determined by standard radioimmunoassay techniques; and *4*) myocardial uptakes of glucose and oxygen were calculated as the product of the appropriate arteriocoronary sinus difference and coronary blood flow. Blood pressure was recorded continuously on a Gould 2800s physiologic recorder. Total blood volume required for all analyses ≤5 ml.

Statistical analyses. Data were analyzed with one- and two-way analysis of variance measures. Student-Newman-Keuls' or Dunnett's test was used for determining differences among means and were considered significant when yielding a *P* value of ≤.05.

RESULTS

Figure 1 graphically illustrates the changes in insulin-stimulated myocardial glucose uptake in control (*n* = 5) and shocked (*n* = 4) animals. In the control group, insulin resulted in a dose-dependent increase in glucose uptake, reaching a plateau response between 400 and 4000 mU/min insulin infusion. In contrast to the myocardial insulin response in control animals, acute endotoxin shock induced a myocardial insulin refractoriness to glucose uptake throughout the entire range of insulin concentrations. One hour of endotoxin shock also decreased the basal myocar-

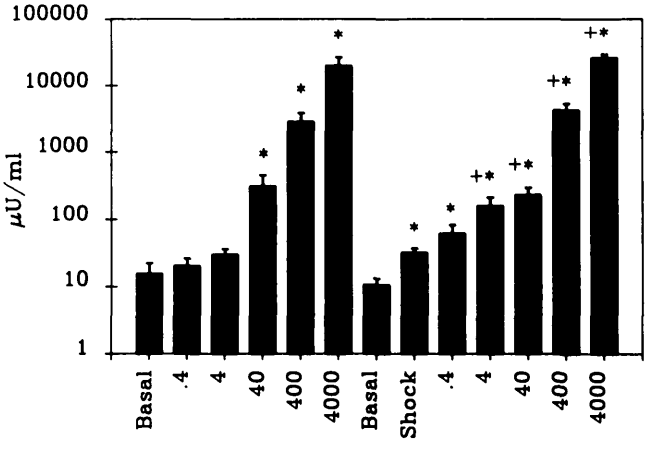

FIG. 3. Plasma insulin concentration in control (*n* = 5) and shocked (*n* = 4) animals during sequential insulin infusions (hyperinsulinemic-euglycemic clamp). *P < .05 compared with corresponding basal value; +P < .05 compared with shock value.

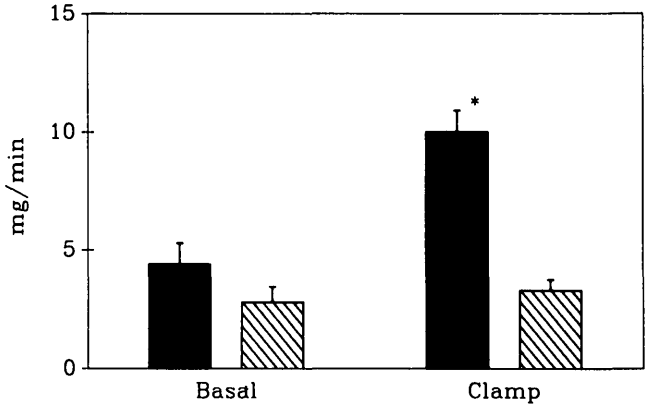

FIG. 4. Myocardial glucose uptake during basal period and after 1 h of 4000-mU/min insulin infusion (clamp). *Solid bars*, control animals (n = 8); *hatched bars*, shocked animals (n = 5). Basal period in shocked groups was 1 h after endotoxin administration. *P < .05 compared with basal value.

dial glucose uptake compared with the preendotoxin value (Fig. 2).

Figure 3 illustrates changes in plasma insulin concentration in control and shocked animals during each insulin infusion. Similar changes in plasma insulin concentration occurred in both groups of animals; however, endotoxin shock resulted in an increase in plasma insulin from 11 ± 3 to 32 ± 5 μU/ml.

In a separate group of control (n = 8) and shocked (n = 5) animals, only a single concentration of insulin (4000 mU/min) was infused during the clamp protocol (Fig. 4). In the control group, the increase in glucose uptake during this single dose of insulin infusion was similar to the glucose uptake achieved in control animals during the sequential clamp protocol at the corresponding insulin dose. A similar response also occurred in the group of shocked animals receiving a single dose of insulin; the heart was as refractory to this single dose of insulin as it was during the sequential clamp protocol.

In a few animals (n = 8), the weight of the left ventricle, perfused by the circumflex artery, was determined by infusing Monastral blue dye into the circumflex artery at the completion of the experiment. Average weight from these hearts was 55.4 ± 3.8 g.

Table 1 lists the changes in MABP, coronary flow, arterial glucose concentration, rate of exogenous glucose infusion, HR, and MVO_2 during each of the experimental periods. In the control nonshocked animals, insulin infusion resulted in an increase in coronary blood flow but did not affect HR, MABP, or MVO_2. After 1 h of endotoxin shock, MABP was reduced, with a concomitant reduction in coronary blood flow; however, HR and MVO_2 were not different from control after 1 h of endotoxin shock. Insulin infusion during shock increased MABP and coronary blood flow but did not affect HR or MVO_2. Arterial glucose concentration was maintained within 5 mg/dl of the preendotoxin values throughout the experimental protocol. To maintain arterial glucose concentration within 5 mg/dl of the basal value, exogenous glucose had to be continuously infused after endotoxin administration, resulting in a glucose infusion rate during the basal shock period of 4 ± 2 mg · kg^{-1} · min^{-1}. The rate of exogenous glucose infusion in the shocked animals was significantly above that of the control group during the 0.4- and 4-mU/min insulin infusions. Insulin infusions of 40–4000 mU/min had similar rates of exogenous glucose infusion in both control and shocked groups.

DISCUSSION

After 1 h of acute endotoxin shock in the dog, myocardial insulin responsiveness was diminished in comparison with nonshocked animals. Lack of response or refractoriness of the heart to insulin-stimulated glucose uptake occurred over a wide range of plasma insulin concentrations and was sim-

TABLE 1
Hemodynamic and metabolic changes during sequential insulin infusions

	Insulin (mU/min)					
	0	0.4	4	40	400	4000
Mean arterial blood pressure (mmHg)						
Control	109 ± 4	112 ± 5	120 ± 10	123 ± 9	121 ± 9	119 ± 11
Endotoxin shock	73 ± 12*	92 ± 10*†	104 ± 8†	101 ± 9†	102 ± 8†	105 ± 8†
Heart rate (beats/min)						
Control	165 ± 10	168 ± 8	165 ± 9	168 ± 11	163 ± 9	160 ± 11
Endotoxin shock	156 ± 12	166 ± 13	160 ± 11	160 ± 9	160 ± 9	156 ± 9
Myocardial oxygen uptake (ml/min)						
Control	5.3 ± 1	5.1 ± 1	6.1 ± 1	6.3 ± 1	5.8 ± 1	6.0 ± 1
Endotoxin shock	4.7 ± 1	5.5 ± 0.2	5.5 ± 0.5	6.0 ± 1	6.0 ± 1	7.0 ± 1
Arterial glucose (mg/dl)						
Control	64 ± 3	65 ± 3	67 ± 4	64 ± 4	62 ± 2	62 ± 2
Endotoxin shock	69 ± 2	65 ± 2	66 ± 3	64 ± 2	62 ± 3	61 ± 3
Circumflex blood flow (ml/min)						
Control	52 ± 5	44 ± 6	47 ± 4	55 ± 8	57 ± 10	66 ± 10†
Endotoxin shock	37 ± 2*	44 ± 3	51 ± 5†	50 ± 5†	53 ± 4†	63 ± 5†
Glucose infusion rate (mg · kg^{-1} · min^{-1})						
Control	0	0.28 ± 0.1†	1 ± 0.3†	9 ± 0.4†	11 ± 1†	12 ± 1†
Endotoxin shock	4 ± 2*	5 ± 1*	7 ± 2*	9 ± 0.5†	12 ± 0.8†	13 ± 2†

Values for endotoxin shock group were obtained at 1 h of shock.
*P < .05 vs. corresponding control group.
†P < .05 vs. 0 (basal).

ilar to insulin refractoriness of skeletal muscle during hyperdynamic sepsis in the dog (3) and burn injury in the rat (11).

Several reports have documented insulin resistance during stress injury such as trauma, burn, and sepsis, but these reports have generally focused on nonvital tissues such as skeletal muscle (11,12) and adipose tissue (5,6). However, a recent report by Clemens et al. (7) documented insulin resistance in the liver of septic rats.

In previous reports, insulin responsiveness was assessed by infusing insulin intra-arterially into the test organ (2,3,13). This procedure ensured that no locally infused insulin reached the systemic circulation and also prevented changes in skeletal muscle blood flow and oxygenation, because blood flow was kept constant with a blood pump. In this study, we used the hyperinsulinemic-euglycemic clamp technique to vary plasma insulin over a wide range of physiologic and supraphysiologic concentrations. This clamp technique (14) has been used by many investigators to measure in vivo insulin response during various endocrinopathies such as obesity (15), diabetes (14), renal (16) and hepatic (17) dysfunction, trauma, and burn injury (8). The euglycemic clamp technique was helpful in determining myocardial insulin resistance because it maintained a steady-state concentration of arterial glucose and thereby prevented the rapid changes in plasma glucose often seen in dogs during endotoxin shock (18).

Use of the sequential insulin dose-response technique has raised questions about the appropriateness of antecedent insulin infusions on subsequent insulin responses (19). In this study, we have shown that in separate groups of animals (control and shocked), a single dose of insulin resulted in similar glucose uptakes, and we have indicated that sequential insulin infusions from 0.4 to 4000 mU/min do not affect the responsiveness of the heart to insulin-stimulated glucose uptake, in agreement with reports with similar clamp protocols (20,21). We recently reported that myocardial glucose uptake is increased during clamping and is sustained without change for 3 h (22). During the 3-h clamp protocol, no subsequent increases in exogenous glucose infusion were required to maintain the increase in glucose uptake.

Although evidence in the literature for myocardial insulin resistance during noncardiogenic shock is lacking, myocardial insulin resistance itself is not unprecedented. Zaninetti et al. (15) recently documented insulin resistance from hearts of obese rats. Their data indicated that decreased insulin binding or downregulation of insulin receptors was not responsible for the insulin resistance state. However, their results did implicate an intracellular defect distal to the insulin receptor as the site for myocardial insulin resistance. Eckel et al. (9) also reported similar findings from isolated cardiocytes of genetically obese Zucker rats. Their data demonstrated a decrease in the number of low-affinity insulin binding sites with no change in high-affinity sites; however, despite the involvement of low-affinity sites, this mechanism could not readily explain the decreased responsiveness of cardiocytes during obesity. Results from obese rats strongly indicate a postreceptor mechanism responsible for insulin resistance during obesity (9,23,24). Zaninetti et al. (23) reported decreased basal glucose transport, no alteration in insulin sensitivity, and decreased insulin responsiveness in perfused hearts of obese rats. Eckel et al. (9) reported a decreased myocardial insulin responsiveness, suggesting a postreceptor mechanism that may include alterations in the glucose transport system. In our study, the shape of the insulin-stimulated dose-response curve in shocked animals agrees with a postreceptor mechanism (25) mediating myocardial insulin unresponsiveness during acute endotoxin shock.

Rosen et al. (26) recently described lipid metabolism–dependent and –independent mechanisms that alter glucose uptake in hearts of obese rats. In diabetic rats, data suggest that free–fatty acid (FFA) oxidation, derived from endogenous lipid stores, results in decreased myocardial glucose uptake. During endotoxin shock, exogenous free-fatty usage by the myocardium has been reported to be decreased (10), but whether endogenous lipolysis or intracellular FFAs are increased during acute endotoxin shock is unclear. Recent evidence from Dhainaut et al. (27) has shown that patients suffering from septic shock had reductions in myocardial glucose, FFA, and ketone uptakes, whereas myocardial oxygen uptake was unaltered. These data suggest that our model of acute endotoxin shock in the dog closely mimics metabolic changes seen in humans during sepsis.

The hemodynamic changes after endotoxin administration agree with previous reports (27,28). During the 1-h shock protocol, the increased glucose infusion rate required to maintain arterial glucose concentration clamped after endotoxin administration has been shown to result from an increased rate of disappearance (R_d) of glucose (29). For a discussion of the potential mechanisms responsible for increased R_d of glucose during endotoxin and bacteremic shock see Raymond et al. (13), Romanowsky et al. (30), and Raymond et al. (31).

It remains unclear whether the mechanism(s) responsible for inducing myocardial insulin resistance during obesity and diabetes can be extrapolated to hearts during endotoxin shock. Further studies are required to clarify these suggested mechanisms during endotoxin and other modalities of acute shock.

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