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ACUTE ENDOTOXIN TOLERANCE IS ACCOMPANIED BY STIMULATED GLUCOSE USE IN MACROPHAGE RICH TISSUES

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SUMMARY: A single injection of *E. coli* LPS at a dose of 10 mg/kg b.w. ("high dose") is lethal in Sprague Dawley rats. However, animals given a sublethal dose of LPS (0.5 mg/kg bw; "low dose") at time zero, followed by a second high dose injection at 48 h, display endotoxin tolerance with 100 % survival. The aim of the present study was to assess the relationship between this observed endotoxin tolerance and the endotoxin-induced glucose metabolic response in selected tissues and nonparenchymal hepatic cells. In each experimental group two injections, the first at time zero, the second at 48 h were given *in vivo*. Four experimental groups constituted these studies: A) saline followed by saline, B) low dose LPS followed by saline, C) saline followed by high dose LPS, and D) low dose LPS followed by high dose LPS. *In vivo* glucose use in tissues and cells was measured 3h after the last treatments employing the 2-deoxy-glucose tracer technique. Glucose use by liver, lung, spleen and intestine was not different between saline/saline (group A) and low dose LPS/saline injected (group B) animals. Saline/high dose LPS injection (group C) doubled glucose uptake, while the sequential LPS injections (group D) caused an additional, 2-3 fold increase in the glucose use by these tissues. Hepatic endothelial cells showed a similarly elevated glucose use *in vivo* in both group C and D. Kupffer cells from group D animals, however, displayed markedly elevated glucose use *in vivo* as compared to cells from group C. Our data indicate that high dose LPS in endotoxin tolerant animals is accompanied by a more markedly stimulated tissue glucose use than found following lethal LPS treatment alone. This increased peripheral glucose use may support cellular functions responsible for the protection of the host.

Repeated administration of nonlethal doses of lipopolysaccharide (LPS) renders the host endotoxin tolerant which is manifested in increased survival to subsequent LPS treatments. Endotoxin tolerance has been studied extensively in a variety of species,

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however, the mechanism responsible for the event is not well understood. Several studies have indicated the role of various cytokines, TNF, IL-1 and IL-6, in the development of endotoxin tolerance (1-5). It was also shown that, the altered cytokine response is accompanied by modulated nitric oxide and superoxide anion production by macrophages and endothelial cells (3-9), altered hepatic acute phase response and hemostasis (1,6,10). Stimulated glucose utilization by immune competent tissues and by the cellular elements of the native immune system is an early and important metabolic response to bacterial infections. It has been postulated that during endotoxemia, macrophages, leukocytes and endothelial cells are major contributors to the elevated glucose utilization observed in tissues rich in resident or newly recruited cells of native immunity (11,12). In LPS-stimulated hepatic macrophages and endothelial cells, the elevated exogenous uptake of glucose and its subsequent catabolism in the hexose monophosphate shunt were shown to be important in supporting both the production and elimination of oxygen derived radicals (13,14). Furthermore, glucose can be a source of energy for upregulated cellular functions. Alterations in the whole body glucose metabolic response during endotoxin tolerance has been demonstrated (15-17). However, it is not known how the *in vivo* glucose utilization is altered in individual tissues and cells of native immunity in endotoxin tolerance. Thus, in the present study, our aim was to elucidate the alterations in the *in vivo* glucose metabolic response by peripheral tissues to LPS during the endotoxin tolerant state. Our additional aim was to test the *in vivo* glucose metabolic response in hepatic endothelial and Kupffer cells which are important elements of the reticuloendothelial system.

MATERIALS AND METHODS

Male, Sprague-Dawley rats (320-350 g bw) were provided with standard rodent chow, and water ad libitum. One day before the experiment rats were anesthetized and catheters were placed in the right and left jugular veins and left carotid artery as described earlier (18,19). The experiments were performed on conscious unrestrained rats with adherence to the NIH guidelines for the use of experimental animals.

Experimental Protocol:

A/	Saline	Saline	All groups:	All groups:
B/	Low LPS	Saline		Tissue removal
C/	Saline	High LPS		and
D/	Low LPS	High LPS	2d[^{14}C]glc	Cell isolation
	↓	↓	↓	↓
time	0h	48h	51h	51.7h

Four *in vivo* treatments formed our experimental groups. In each group two injections were administered at time zero and at 48 h as follows: A/ saline followed by saline; B/ low dose LPS (0.5 mg/kg bw) followed by saline; C/ saline followed by high dose LPS (10 mg/kg bw); and D/ low dose LPS followed by high dose LPS. One hundred and eighty minutes after the last treatment, a tracer dose (10 μCi/100g bw) of 2-deoxy-[U-^{14}C]glucose (spec. act. 319 Ci/mol; Amersham, Arlington Heights, IL) was injected intravenously. During the 40 minute *in vivo* labeling period, 0.2 ml serial arterial blood samples were withdrawn at 2, 4, 8, 14, 22, 32 and 40 min after the injection of the tracer, and analyzed for radioactivity and glucose concentrations. *In vivo* glucose utilization (Rg) in individual tissues and isolated cells was assessed by the determination of the accumulated phosphorylated metabolites of 2-deoxy-D-[U-^{14}C]glucose after its intravenous injection as described previously (18,19).

Hepatic nonparenchymal cells were isolated using a modified version of collagenase perfusion (20) and pronase digestion, followed by centrifugal elutriation and gradient centrifugation as we described previously (12,13). Purity of endothelial and Kupffer cell preparations were more than 92, and 80 %, respectively. Viability of cells was over 95 % as assessed by the trypan blue exclusion. For statistical analysis of the data the ANOVA was employed, statistical difference was set at $p<0.05$.

RESULTS AND DISCUSSION

Figure 1 shows the *in vivo* glucose utilization by selected tissues following the experimental treatments. Following a single low dose LPS injection glucose use was not significantly different from saline-injected controls in any of the tissues tested. Single high dose LPS caused a significantly elevated glucose use by liver, lung, spleen and intestine while no significant changes were found in muscle and testis. However, when high dose LPS was injected to animals pretreated with low dose LPS, glucose use by macrophage rich tissues was significantly higher than after a single high dose LPS-injection. The sequential treatments by LPS did not result in significant changes in the glucose use by muscle and testis.

The glucose metabolic response to sepsis or endotoxemia consists of a markedly elevated peripheral glucose use in macrophage rich tissues. Upregulated glucose metabolism by lung, liver, spleen and intestine is maintained even during severe endotoxin shock despite decreasing blood glucose levels, suggesting that the major disturbance of glucose homeostasis is the discrepancy between elevated peripheral glucose use and impaired production of glucose by the hepatic parenchyma (21). Long term low dose endotoxin

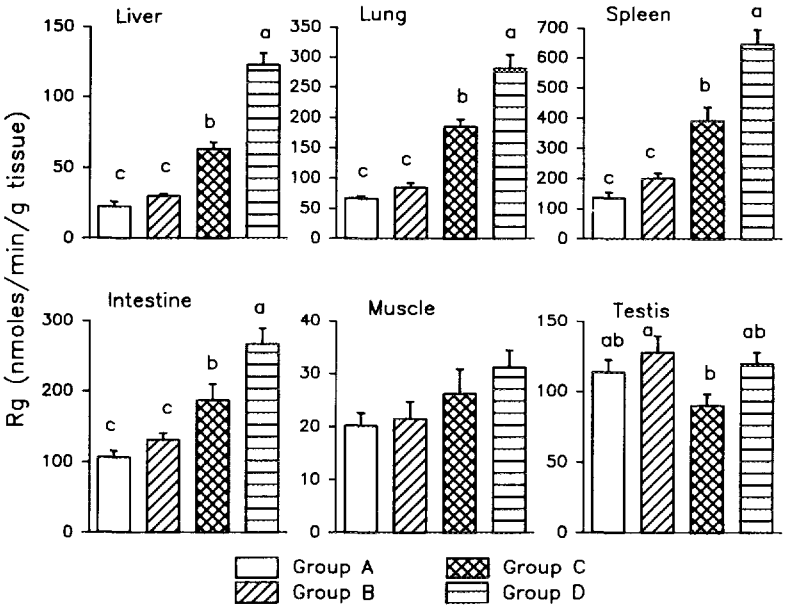

Fig. 1. Glucose utilization rates (Rg) by selected organs following sequential LPS treatments. Three hours following the last treatments, glucose utilization was determined in different tissues (for experimental details see the Materials and Methods section). Values are means, ± S.E.M, n=6. Different letter above columns indicates statistically significant difference ($p<0.05$).

infusion, or repeated endotoxin injections, however, attenuate the whole body glucose metabolic response to subsequent endotoxin treatment (16). Our present findings indicate that early endotoxin tolerance is accompanied by a primed peripheral glucose metabolism which is manifested in augmented glucose use by macrophage rich tissues following an additional endotoxin challenge.

Independent studies have suggested the role of cytokines in the development of endotoxin tolerance. IL-1 seems to play a particularly important role in the process (3,5,22). These studies showed that pretreatment with this cytokine prevent the animals from responding to endotoxin in a similar manner as observed during endotoxin tolerance caused by endotoxin pretreatments (10). TNF, IL-1 and colony stimulating factors were shown to upregulate peripheral glucose use similarly to endotoxin (23-25). One interpretation of our study is that the action of cytokines or other inflammatory mediators (complement factors, arachidonic acid metabolites, hormones, growth factors, etc.) released after the low dose LPS treatment prime the glucose metabolic pathway in competent tissues, presumably by enzyme inductions, thus glucose use will be more responsive to subsequent challenges. A direct role of TNF in the mediation of glucose metabolic response in the LPS tolerant group is not probable because the plasma TNF level was markedly decreased following the second LPS treatment as compared to the single high dose LPS treatment (8).

In order to assess the contribution of hepatic nonparenchymal cells to the observed LPS effects in the liver, we also determined the *in vivo* glucose use by Kupffer and endothelial cells (Fig 2). While the changes in glucose use by Kupffer cells were not statistically significant after low dose or high dose LPS treatment alone, *in vivo* glucose utilization by Kupffer cells was significantly elevated following the sequential LPS treatments as compared to treatment by a single dose LPS. In contrast, glucose use following high or sequential LPS treatments was not different in endothelial cells (Fig 2). These data indicate that the glucose metabolic response of Kupffer cells parallel with that measured in whole liver tissue indicating that they are important contributors to the hepatic glucose metabolic response during endotoxin tolerance. The fact that hepatic endothelial cells responded with similar increase in their glucose use under both single LPS injection or LPS challenge during endotoxin tolerance suggests that moderate endotoxemia has no major priming effects on the glucose metabolism of these cells. This fact indicates that glucose metabolism responds differently to LPS-induced mediator release in hepatic endothelial and Kupffer cells.

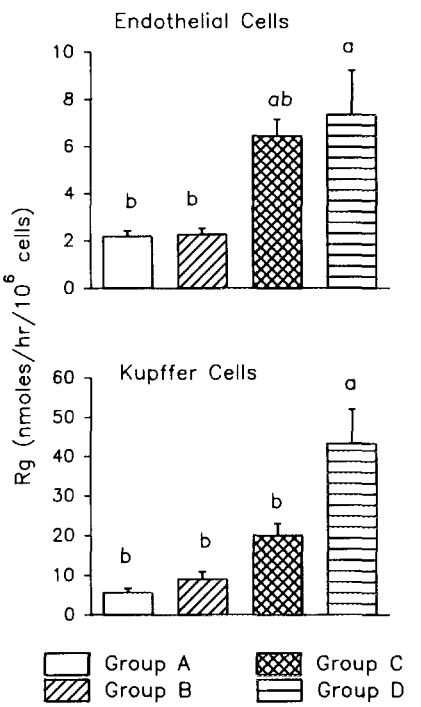

Fig. 2. Glucose utilization rates by hepatic nonparenchymal cells following sequential LPS treatments. Values are means, ± S.E.M, n=6. Different letter above columns indicates statistically significant difference ($p<0.05$).

Recent studies suggested that the upregulated glucose uptake is important for the support of a primed pentose cycle activity in resident immune competent cells of the liver (13,14). Endotoxin treatment caused the induction of glucose-6-phosphate dehydrogenase the rate controlling enzyme of the hexose monophosphate shunt (26). Glucose oxidation through this pathway produces precursors for macromolecule synthesis, and generates NADPH which can be utilized for production of superoxide anion, nitric oxide and reduced glutathione. Thus, glucose oxidation by this pathway is important for the support of elevated production, as well as, elimination of oxygen-derived radicals supporting both bacterial killing and protection against oxidative cellular injury. Because during endotoxin tolerance the production of NADPH dependent proinflammatory mediators is downregulated (8,9), we suggest that under this condition, the elevated glucose uptake and its subsequent catabolism support protective cellular functions. These may include the production of precursors for synthesis of macromolecules, or providing reduced coenzymes for peroxidases, catalase, and glutathione metabolism.

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