Bacterial endotoxin effects on carbohydrate utilization and transport

J. J. Spitzer

Department of Physiology, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, LA 70112-1393, U.S.A.

Introduction

After the early pioneering investigations of Berry [1], the results of extensive studies over the last two decades clearly identified some of the marked metabolic alterations that accompany endotoxaemia and sepsis. Although the intermediary metabolism of all three major substrates, carbohydrates, lipids and proteins, is affected, changes in carbohydrate metabolism are especially important in conditions of infection and inflammation, as glucose contributes to the maintenance of the intracellular balance between oxidants and antioxidants, in addition to serving as an oxidizable energy-yielding substrate. Although both aspects of carbohydrate metabolism, i.e. supply as well as utilization of metabolites, are altered in endotoxaemia or sepsis, this brief review will only concentrate on alterations in carbohydrate utilization. Furthermore, it will primarily attempt to summarize some of the investigations carried out in our laboratories over the last 20 years.

Total body glucose uptake

Shortly after the administration of Escherichia coli endotoxin [lipopolysaccharide (LPS)], total body glucose uptake (Rd), as determined by the primed constant infusion of [6-3H]glucose, increases [2]. Elevated Rd is also observed in rats after non-lethal doses of LPS [3]. Similar changes are noted during acute, hypermetabolic sepsis in rats [4]. The hypermetabolic septic state was induced in these animals by intraperitoneal administration of a pooled faecal inoculum [4]. In experiments seeking to ascertain the involvement of cytokines in this effect, glucose kinetics were found to be elevated in rats by the administration in vivo of conditioned media from LPS-stimulated RAW 264.7 cells, as well as by the administration of human recombinant tumour necrosis factor-α (TNF-α) [5], indicating that cytokines may play a role either directly or indirectly in the LPS- or sepsis-
induced alterations in glucose kinetics. However, endogenous TNF-α alone is not directly responsible for LPS-induced changes in glucose kinetics, as a neutralizing goat anti-TNF IgG antibody did not prevent the LPS-induced alterations in glucose kinetics [6].

Eicosanoids have been shown to play an important role in eliciting many of the haemodynamic alterations after LPS administration. Therefore, the effects of the cyclo-oxygenase and lipoxygenase pathway inhibitor, BW755C, were studied on both LPS- and sepsis-induced changes in glucose uptake. BW755C failed to abolish the LPS-induced increase in glucose kinetics, although it prevented the early hypotension after LPS administration [7]. In septic rats, arachidonic acid metabolites also do not seem to be directly involved in the increased Rd, as BW755C did not reverse the changes in glucose kinetics, although it prevented the hypothermic response [8].

Since elevation of catecholamines accompanies the effects of LPS administration, glucose kinetics were studied after adrenergic blockade by phentolamine and propranolol in order to probe the potential significance of adrenergic mechanisms in this response. The α- and β-blockade by these agents prevented the marked LPS-induced increase in Rd, while α- or β-adrenergic antagonists given separately only blunted but did not completely prevent changes in glucose kinetics [9].

Elevation of plasma glucagon is another hallmark of the effects of LPS administration [3] or hypermetabolic sepsis [4]. When hyperglucagonaemia was selectively reduced (by the administration of somatostatin) while normal insulin levels and euglycaemia were maintained, the elevated glucose kinetics were attenuated in hypermetabolic septic rats, indicating the potential importance of hyperglucagonaemia in this effect [10].

Thus, it appears that a combination of interacting cytokines, pathways utilizing α- and β-adrenergic mechanisms, as well as excessive plasma glucagon concentration, are important factors in eliciting the elevated total body glucose uptake during endotoxaemia or hypermetabolic sepsis.

**Glucose utilization by the liver**

The consideration of glucose utilization by the liver is made especially difficult because of the inhomogeneous cellular composition of this organ. In addition to the parenchymal and the several types of non-parenchymal cells, there is a marked infiltration of polymorphonuclear leukocytes (PMNs) into the liver after LPS administration that may have a major contribution to metabolic and functional alterations of the liver under post-LPS conditions. Since the individual cell-types of the liver perform a vast array of different functions, it is not surprising that the metabolic behaviour of these various cell-types also differs. Thus, in the presence of a physiological substrate mixture, less than 20% of the ATP generated from endogenous substrates is derived from glycolysis in Kupffer and sinusoidal endothelial cells, while oxidation of glutamine and palmitate serves as the main source of energy. In parenchymal cells on the other hand, lactate and palmitate oxidation is responsible for approximately 90% of the ATP production derived from the oxidation of endogenous substrates [11]. As stated earlier, glucose serves not only as an oxidizable energy-yielding substrate but also supports various functions of cells, e.g. supply of NADPH for free radical production and for the maintenance of the glutathione cycle, etc. These functions become very important in infection and in inflammatory conditions. Among the various hepatic cell-types, Kupffer cells have been studied the most, and therefore glucose uptake by these cells will be discussed most extensively.

Studies of glucose uptake in vivo by different organs (and various cells) were accomplished by using the 2-deoxyglucose tracer technique [12]. Most of these investigations were performed in fasting, conscious rats one day after the surgical implantation of indwelling vascular catheters. For a more detailed account of this technique, see [13].

The uptake of glucose by the liver (Rg) was markedly elevated after both the administration of LPS [13] and in sepsis [14]. The non-lethal hypermetabolic sepsis was produced in rats by repeated subcutaneous injections of live *E. coli* bacteria [8]. Furthermore, it was shown that within the liver, Kupffer cells are most sensitive to the effect of LPS in terms of increased glucose uptake, followed by infiltrated PMNs and endothelial cells. The increase in glucose uptake by parenchymal cells after LPS administration was much less marked [15]. Although Kupffer cells represent only a small fraction of the total hepatic cell mass, they were responsible for almost half of the LPS-induced elevation of
hepatic glucose uptake [15]. LPS administration increased glucose uptake and oxidation in Kupffer cells, but especially and very markedly elevated hexose monophosphate shunt activity and superoxide anion production [16]. Elevated hexose monophosphate shunt activity was also found in hepatic sinusoidal endothelial cells which produce very little superoxide anions under these conditions [17]. In these cells, NADPH may serve to maintain the antioxidant potential of the cell, thus affording protection against possible cellular damage by oxygen-derived free radicals. After LPS administration, a marked release of \( O_2^- \) from the perfused liver was also observed [18]. Primarily the Kupffer cells and PMNs were responsible for the \( O_2^- \) release [19].

TNF administration [20] or phagocytic challenge in vivo [21] also caused marked elevation in Rg, especially by the hepatic sinusoidal cells. TNF treatment in vivo or the addition of PMA in vitro upregulated glucose uptake by the Kupffer cells; this was accompanied by an increased glucose flux through the hexose monophosphate shunt [22]. The metabolic changes are thought to be related to the increased demand for NADPH in the course of elevated superoxide anion production by the Kupffer cells [18,23,24]. A similar relationship between glucose metabolism and superoxide radical production has also been described in rat peritoneal macrophages [25–27].

Rg was markedly increased even when a very high dose of LPS was administered to rats (LD<sub>100</sub>), and severe hypoglycaemia developed [28].

Since the first step of glucose utilization involves glucose translocation via glucose transporter activity, glucose transporter isoform content of plasma membranes from hepatic cells was determined by Western blot analysis using polyclonal antibodies. The predominant glucose transporter isoform expressed in parenchymal cells was GLUT2. GLUT1 and GLUT4 were also observed to be present; however, GLUT4 protein was expressed to a relatively minor extent and GLUT3 was not detectable. LPS injection resulted in a decrease in GLUT2, while levels of GLUT1 protein doubled. GLUT4 was not altered. In the plasma membranes of Kupffer and endothelial cells, only the GLUT1 isoform was detected. LPS treatment resulted in a several-fold increase in GLUT1 protein content in these cells. Thus, LPS treatment augments the synthesis and/or membrane translocation of GLUT1 in hepatic sinusoidal cells [29].

Endotoxaemia or sepsis via their intricate and interrelated mediated effects cause marked cell-specific adaptations within the liver that affect carbohydrate metabolism. These changes include alterations in the expression and/or membrane translocation of hepatic glucose transporters with a resultant increase in glucose uptake, primarily by the sinusoidal cells and especially by Kupffer cells. This is followed by increased hexose monophosphate shunt activity in these cells, which results in increased availability of NADPH. Coincidental with these changes, an increased superoxide anion production is also found primarily in Kupffer cells and in PMNs that migrated to the liver in response to LPS. Superoxide anion production subserves the bactericidal function of Kupffer cells and PMNs, but when produced in excessive amounts, superoxide anions can also be cytotoxic for the host. Some tissue protection may also be afforded by the increased generation of NADPH, by enabling glutathione reductase to maintain the proper activity of this cycle. This sequence of events is schematically depicted in Figure 1.

**Glucose uptake by other macrophage-rich tissues**

After the administration of a non-lethal dose of LPS, an increased glucose uptake was demonstrated in most of the tissues studied, with the notable exceptions of the brain and testes [13]. The most marked effects were displayed by tissues rich in macrophages, including the gut, skin, lungs and spleen, in addition to the liver. Among these organs, the liver showed the most...
pronounced changes that lasted for over 48 h, while the effect diminished in the other tissues over time [13]. Glucose uptake by the spleen and lungs was elevated after an LD_{100} dose of LPS, even in the face of marked hypoglycaemia [28]. In a non-lethal model of hypermetabolic sepsis, glucose uptake was also elevated in the macrophage-rich tissues [14]. It was postulated on the basis of these studies that in sepsis the mononuclear phagocyte system may be responsible for most of the increment of glucose utilization, and the latter provides metabolic support for the increased antibacterial activities of these cells [14]. TNF administration also caused marked increases in glucose uptake in the macrophage-rich tissues [30]. The acute administration of another cytokine, granulocyte–monocyte colony-stimulating factor, resulted in a transient elevation of glucose uptake by the spleen, skin and lungs, in addition to the liver [31]. The glucose-uptake-enhancing effect of TNF in the spleen, liver, skin and gut involves adrenergic-mediated modulation as it was reduced or prevented by simultaneous \( \alpha \) - and \( \beta \) -blockade [32].

Although a rather heterogeneous group of organs with widely differing functions are included in this section, the common denominator among them is that they are all rich in macrophages and play an important role in immune defence. While LPS and sepsis may have many additional organ-specific effects, they markedly increase glucose uptake in these organs.

**Glucose uptake by muscle**

Glucose uptake and lactate release by skeletal muscle were increased in anaesthetized dogs after LPS administration [33–35]. In the conscious rat, glucose uptake by the gastrocnemius, red quadriceps and white quadriceps muscles was elevated after the administration of a relatively low dose of LPS [36]. The greatest increase in glucose uptake among muscles after LPS administration was observed in the abdominal muscle and diaphragm [36]. Enhanced respiratory activity may have been at least partially responsible for the increased glucose uptake in these two muscles. The elevated glycolytic rate of glucose after LPS administration was maintained in vitro when epitracheal muscle was removed from the LPS-treated rats [37]. Recently, Vary et al. [38] have shown in a chronic intra-abdominal septic abscess model in rats that glucose uptake was markedly elevated by hindlimb muscles. Sepsis also increased the expression of GLUT1, which correlated with a similar increase in the relative abundance of GLUT1 mRNA. The amounts of GLUT4 protein and mRNA were not changed.

Thus, alterations in glucose utilization in skeletal muscle after endotoxaemia or sepsis consist of increased glucose transporter activity followed by preferential metabolism of glucose to lactate.

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20 Spolarics, A., Schuler, A., Bagby, G. J., Lang, C.
Mechanism of inhibition of hepatic gluconeogenesis by bacterial endotoxin: a role for nitric oxide?

M. A. Titheradge, R. G. Knowles*, F. S. Smith, R. A. Horton and E. D. Ceppi

School of Biological Sciences, University of Sussex, Brighton BN 1 9QG, U.K. and *Wellcome Research Laboratories, Beckenham BR3 3BS, U.K.

Introduction

Septic shock is characterized by profound alterations in glucose homeostasis, typically an initial transient hyperglycaemia followed by a progressive hypoglycaemia. A major feature of the hypoglycaemic phase of septic or endotoxic shock is a 40–50% decrease in hepatic gluconeogenesis, which can be mimicked by the administration to the intact animal of either live bacteria or the lipopolysaccharide component of the cell wall (LPS) known as endotoxin, but not by direct treatment of hepatocytes or perfused livers with LPS [1–6]. The work of Knowles et al. [6] demonstrated that the effect of endotoxin is resistant to hepatocyte isolation and is apparent with a variety of substrates including lactate, pyruvate, alanine, asparagine, glutamine and proline, but not glucose production from dihydroxyacetone, glycerol or glyceraldehyde. The effect is independent of the presence of a maximally effective concentration of glucagon and is not the result of alterations in cell viability, as judged by the cellular ATP content.

Site of action of endotoxin

From the analysis of cross-over plots of metabolites in perfused livers prepared from both normal and endotoxin-treated animals, the initial studies of Williamson et al. [2] indicated that the impaired capacity of the liver to make glucose after endotoxin treatment is the result of an increased activity of the two futile cycles catalysing the conversion of pyruvate into phosphoenolpyruvate (PEP) and fructose 6-bisphosphate into fructose 1,6-bisphosphate (Fru-1,6-P 2). It was suggested that the major defect is the result of an activation of 6-phosphofructo-1-kinase, the resultant increase in Fru-1,6-P 2 feeding back and activating pyruvate kinase, thus diminishing the conversion of PEP into glucose. Further studies have confirmed that a major effect of endotoxin

Abbreviations used: Fru-1,6-P 2, fructose 1,6-bisphosphate; Fru-2,6-P 2, fructose 2,6-bisphosphate; Fru-2,6-P 2ate, fructose-2,6-bisphosphatase; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; NO, nitric oxide; PEP, phosphoenolpyruvate; PEPCK, phosphoenolpyruvate carboxykinase; SNAP, S-nitroso-N-acetylpenicillamine; TNF, tumour necrosis factor.

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