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ENDOTOXIN-INDUCED ALTERATIONS IN GLUCOSE TRANSPORT IN ISOLATED ADIPOCYTES

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2-Deoxyglucose and 3-*O*-methylglucose were used to assess endotoxin-induced changes in glucose transport in rat adipocytes. 6 h after *Escherichia coli* endotoxin injection insulin-stimulated 2-deoxyglucose uptake was significantly depressed (V decreased, K_m unaltered), phosphorylation of 2-deoxyglucose was seemingly unimpaired; basal 3-methylglucose entry was significantly increased, insulin-stimulated uptake was unaltered. Insulin significantly reduced K_m in control and endotoxin-treated cells. Cytochalasin B-insensitive uptake of both 2-deoxyglucose and 3-methylglucose, a small fraction of total transport, increased significantly in endotoxic cells. Endotoxin reduced spermine- and insulin-stimulated 2-deoxyglucose uptake to a similar extent. Results are consistent with the hypotheses that (1) a site of endotoxin-induced insulin resistance is at the cell membrane level and may reflect a decrease in number or activity of effective carrier units, rather than alterations in affinity, (2) endotoxin does not compromise the hexokinase system, (3) the cell membrane-localized effect of endotoxin on hexose transport is not necessarily mediated by the insulin receptor and (4) the entry of 2-deoxyglucose and 3-methylglucose may involve two separate transport systems.

Introduction

Endotoxin shock has been demonstrated to be responsible for a number of alterations in carbohydrate metabolism. In most instances, there is an increase in glucose utilization and turnover and a reduced ability to synthesize glucose [1–4].

Associated with these metabolic changes are alterations in the glucoregulatory hormones including glucocorticoids, catecholamines, glucagon and insulin [5,6,7]. In addition to changes in hormone levels, several tissues have been demonstrated to exhibit altered hormone sensitivity [6,8]. Although the metabolic distortions associated with endotoxemia are well known, there is little information in the literature relating to their cellular basis.

Recently, Holley and Spitzer [9] demonstrated that adipocytes isolated from endotoxic rats exhibited an accelerated rate of basal glucose oxidation and reduced insulin sensitivity (insulin-induced stimulation as a percentage of basal value) and responsiveness (absolute magnitude of the insulin-stimulated function). In addition, endotoxin treatment resulted in enhanced insulin binding to the adipocytes. These studies suggested a possible intracellular as well as membrane-associated mechanism of action.

The current studies were initiated in order to look more closely at the cellular alterations produced by endotoxin in rat adipocytes. We focused on the uptake of glucose by endotoxon-treated rat adipocytes, since this is one of the earliest responses to insulin stimulation and is a prerequisite of glucose oxidation.

Materials and Methods

(1) Animals and treatment. Male Sprague-Dawley rats of approx. 200 g were obtained from ARS

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(Madison, WI) or Charles Rivers Breeding Laboratories (Wilmington, MA). Food and water were provided ad libitum. The photoperiod was maintained at 14 : 10 L : D. *Escherichia coli* endotoxin (Lipopolysaccharide B, 0127 : B8, Difco Laboratories, Detroit, MI) was injected via a tail vein under ether anesthesia. The dosage ranged from 2–4 mg/100 g body weight and was calibrated to provide an approx. LD_{50} at 6 h. Control rats received a similar injection of 0.9% saline. 6 h post-injection the animals were decapitated and trunk blood collected in heparinized tubes.

(2) Preparation of adipocytes. Following killing, epididymal fat pads were removed and digested for 30 min with collagenase (1 mg/ml, Sigma Chemical Co., St. Louis, MO) in a Krebs-Ringer bicarbonate buffer at pH 7.4. The buffer contained one-half the recommended calcium and 2% bovine serum albumin (Reheis Chemical Co., Phoenix, AZ). The method for calculating cell numbers has been previously reported [10].

(3) Glucose uptake. Glucose uptake by adipocytes was assayed using a modification of the technique described by Olefsky [11]. This assay measures the total uptake of 2-deoxy-[^{14}C]glucose and is based on the principle that, although 2-deoxyglucose is transported and phosphorylated by the same processes as D-glucose, it cannot be further metabolized. 2-Deoxy-D-[U-^{14}C]glucose and 3-*O*-methyl-D-[1-^{3}H]glucose were the two glucose analogous employed in these studies. Insulin-stimulated uptake was measured by preincubating 0.4 ml cell suspension (containing $(2–3) \cdot 10^5$ cells) in a Dubnoff metabolic shaker in the presence of insulin. Basal uptake was assayed in cells preincubated in the absence of insulin. In the 2-deoxyglucose series, cells were incubated 20 min at 37°C with 1 mU/ml porcine insulin (kindly supplied by Dr. M. Root of Eli Lilly Co., Inc.). For the 3-methylglucose experiments, the insulin concentration was 2 mU/ml for 40 min and the preincubation and assay performed at 24°C.

Following preincubation, uptake was initiated by the addition of 100 µl buffer containing the labeled hexose plus cold hexose (variable concentration). Uptake was terminated by centrifuging a 0.3-ml aliquot of the cell suspension through a layer of dinonylphthalate oil in a Beckman microcentrifuge, as described by Gliemann et al. [12]. Either [^{14}C]inulin or [^{14}C]-sorbitol were used to correct for fluid trapped in the extracellular fluid space. Radioactivity in the pellets was measured in a 2 : 1 solution of Omnifluor and Triton X-100.

(4) Separation of 2-deoxyglucose and 2-deoxyglucose 6-phosphate. Separation was carried out according to the procedure described by Tsuboi and Petricciani [13], by chromatography through a Dowex-1 (Sigma Chemical Co.) ion-exchange column (1.5 mm × 4.0 cm). Free 2-deoxyglucose was eluted with 3 ml deionized water, 2-deoxyglucose 6-phosphate with 3 ml 0.5 M formic acid, 0.01 M ammonia acetate solution. Radioactivity in the eluates was counted directly in Aquasol. To verify the separation, standard solutions of 2-deoxy[^{14}C]glucose and 2-deoxy[^{14}C]glucose 6-phosphate were chromatographed in parallel with the samples. Recoveries ranged from 95–100% for 2-deoxyglucose and 85–90% for 2-deoxyglucose 6-phosphate. Corrections were made for the small losses noted.

(5) Insulin and glucose determinations. Immunoreactive insulin was determined using an insulin radioimmunoassay kit (Amersham-Searle, Arlington Heights, IL). Serum glucose levels were determined on a Beckman automatic glucose analyzer.

(6) Statistical analysis. The data are presented as means ± S.E. Statistical analysis was performed either by two-way analysis of variance (ANOVA) or Student's *t*-test for paired or unpaired data as indicated.

Results

(1) Plasma glucose and insulin levels following endotoxin treatment. In Table I we present the plasma glucose and insulin concentrations of rats injected with an LD_{50} dose of *E. coli* endotoxin or with 0.9% NaCl 6 h prior to death. In both series of experiments using 2-deoxyglucose and 3-methylglucose to measure hexose uptake, plasma glucose and insulin were reduced by essentially the same extent. The absolute levels of either glucose or insulin did not vary significantly within the same series. The levels of the same substances, however, differed between the two series, a fact most likely due to an interim change in animal suppliers.

(2) Effects of endotoxin treatment on 2-deoxyglucose uptake. Fig. 1 illustrates the effect of endo-

TABLE I

THE EFFECT OF ENDOTOXIN INJECTION ON PLASMA GLUCOSE AND IMMUNOREACTIVE INSULIN CONCENTRATION

Experimental rats (ET) were injected with an LD_{50} dose of *E. coli* endotoxin (2–4 mg/100 g body weight) 6 h prior to sampling. Control rats (C) received injections of a 0.9% NaCl solution. Number of animals in parentheses.

	2-Deoxyglucose experiments			3-Methylglucose experiments	
	Glucose (mg/dl)	IRI [a] (μU/ml)		Glucose (mg/dl)	IRI (μU/ml)
C	153.1 ± 1.8 (43)	44.1 ± 2.7 (42)	C	144.0 ± 2.5 (27)	32.4 ± 2.7 (27)
ET	81.6 ± 3.5 (41) [b]	20.6 ± 0.9 (36) [b]	ET	64.6 ± 5.2 (28) [b]	10.1 ± 1.4 (28) [b]

[a] Immunoreactive insulin.
[b] $P < 0.01$ (Student's *t*-test) compared to control rats.

toxin treatment on both basal and insulin-stimulated 2-deoxyglucose uptake. The concentration of 2-deoxyglucose in the medium was 0.01 mM and transport was terminated at 1 min. Under these conditions intracellular free 2-deoxyglucose could not be detected at all. Therefore it was legitimate to assume

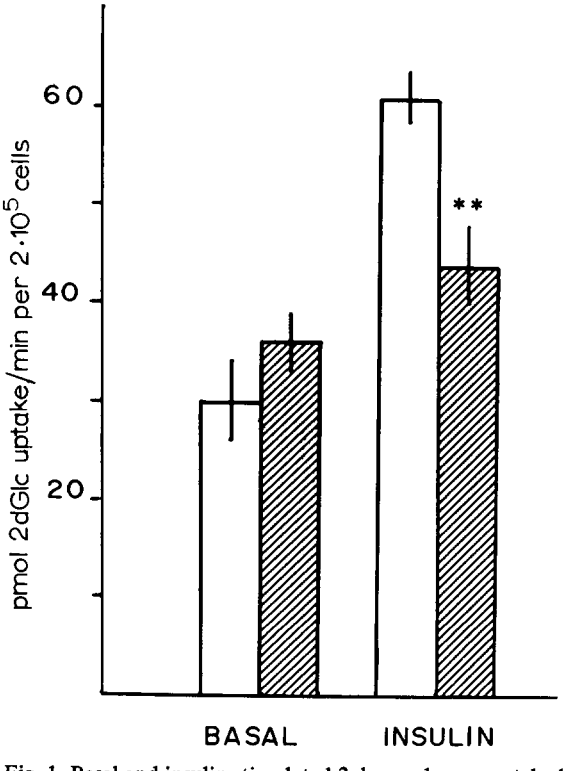

Fig. 1. Basal and insulin-stimulated 2-deoxyglucose uptake by adipocytes isolated from saline and endotoxin-injected rats. The concentration of 2-deoxyglucose in the incubation medium was 0.01 mM and the cells were incubated for 60 s. ** Significantly different from control cells, $P < 0.01$, Student's *t*-test. Open bar, control animals; hatched bar, experimental animals. ($n = 5$)

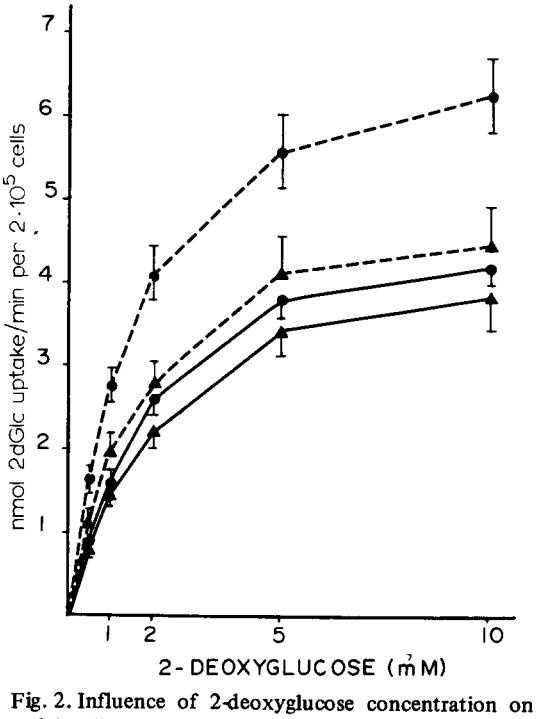

Fig. 2. Influence of 2-deoxyglucose concentration on basal and insulin-stimulated 2-deoxyglucose uptake by adipocytes isolated from saline- and endotoxin-injected rats. ●------●, control cells preincubated with 1 mU/ml insulin; ▲------▲, endotoxin-treated cells preincubated with 1 mU/ml insulin; ●——●, control cells and ▲——▲, endotoxin-treated cells, no insulin preincubation, $n = 7$. Cells were incubated for 60 s. Endotoxin-treated cells exhibited a significant decrease in insulin-stimulated hexose uptake ($P < 0.01$, two-way ANOVA).

that efflux was trivial and that transport, rather than phosphorylation, was the rate-limiting step [14]. Basal uptake was not altered significantly by endotoxin treatment; however, insulin-stimulated uptake was markedly suppressed ($P < 0.01$, Student's t-test).

(3) Kinetic experiments. In order to investigate some kinetic parameters of the endotoxin effect on 2-deoxyglucose uptake, we performed a series of experiments with variable extracellular 2-deoxyglucose concentrations (0.5 to 10.0 mM). Preliminary experiments indicated a linear rate of uptake over this concentration range for at least 2 min and our assays were routinely run for 1 min, under conditions measuring true unidirectional fluxes. The effects of endotoxin treatment on basal and insulin-stimulated uptake are shown in Fig. 2. Basal 2-deoxyglucose uptake was again not significantly altered by endotoxin treatment, although these cells exhibited a trend toward decreased uptake with higher 2-deoxyglucose substrate concentrations. Insulin-stimulated uptake was reduced by endotoxin treatment at all 2-deoxyglucose substrate concentrations tested. The magnitude of insulin-stimulated glucose transport compared with basal transport in these studies is well within the range of values reported in the literature. As observed in several laboratories [15,16], the extent of activation of sugar transport by insulin is lower (2–3-fold increase of the basal rate) than the one noted for glucose oxidation, which can be a 3–10-fold increase of the basal rate. The difference may reflect direct stimulation by insulin of intracellular glucose metabolism beyond the transport step.

Table IIA presents the kinetics analysis of the substrate curve data. Endotoxin pretreatment caused a significant decrease in V of the insulin-stimulated cells without altering K_m. No statistically significant changes occurred in either K_m or V of the non-insulin-stimulated cells from endotoxin-treated rats. It is interesting to note that we observed a significant decrease in K_m with insulin treatment in both control and endotoxin-treated cells. Since such a finding is contrary to several previous reports in the literature, it was investigated in more detail. In a second series of experiments, the relationship between 2-deoxyglucose concentration and uptake was examined in rats of an average body weight of 139 g to conform with most of the published reports. Again, we found a significant decrease in K_m and an increased V in insulin-stimulated cells (Table IIB).

TABLE II

A. KINETIC PARAMETERS CALCULATED FROM DATA IN FIGS. 2 AND 3

Units for K_m are mM, for V nmol/min per $2 \cdot 10^5$ cells.

	Control		Endotoxin-treated	
	Basal	Insulin	Basal	Insulin
K_m	2.26 ± 0.27	1.73 ± 0.05 [a]	2.25 ± 0.10	1.83 ± 0.10 [a]
V	5.37 ± 0.25	7.41 ± 0.54 [b]	4.79 ± 0.49	5.36 ± 0.52 [c]

B. K_m AND V VALUES FOR 2-DEOXYGLUCOSE UPTAKE IN YOUNG RATS

$n = 4$ in this group.

	Basal	Insulin
K_m	2.99 ± 0.20	2.01 ± 0.08 [a]
V	4.42 ± 0.07	7.31 ± 0.95 [b]

[a] Significantly different from respective basal group (paired t-test) $P < 0.05$.
[b] $P < 0.01$.
[c] Significantly different from control insulin-stimulated cells, $P < 0.02$.

As a further check on our observations, we also performed a substrate inhibition experiment assaying 2-deoxyglucose uptake (1 and 3 mM) at 1 min in the presence of variable concentration of D-glucose (0.1 to 5 mM). The resulting data were then analyzed by a Dixon plot (Fig. 3). These data indicate a basal K_i of 1.45 mM and an insulin-stimulated K_i of 0.50 mM.

(4) 2-Deoxyglucose phosphorylation and previous endotoxin treatment. In order to determine whether the endotoxin effect on insulin-stimulated uptake reflected an altered hexokinase system, intracellular 2-deoxyglucose and 2-deoxyglucose 6-phosphate were separated and measured in cells from control and endotoxin-treated rats. The time course of intracellular 2-deoxyglucose and 2-deoxyglycose 6-phosphate accumulation is depicted in Fig. 4. Here, we found a statistically significant decrease in both basal ($P < 0.05$) and insulin-stimulated ($P < 0.001$) uptake in cells from endotoxin-treated rats. In all cases, the intracellular free 2-deoxyglucose levels accounted for a small fraction of the total 2-deoxyglucose uptake. At 5 min, the 0.5 nmol 2-deoxyglucose present in

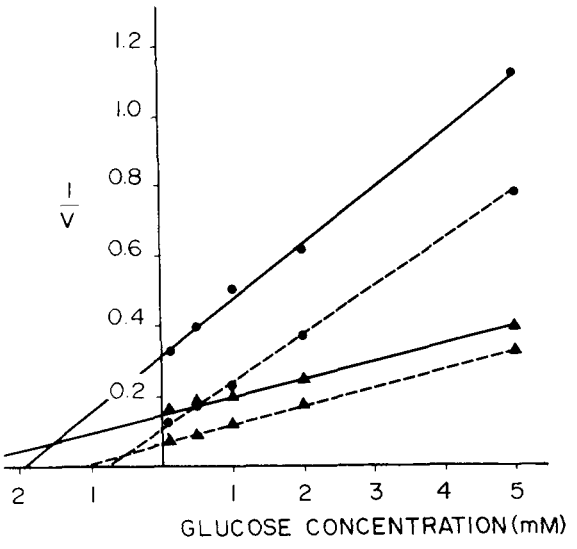

Fig. 3. Dixon plot of the inhibitory effect of D-glucose on 2-deoxyglucose uptake. The concentrations of 2-deoxyglucose were 1 mM (•) and 3 mM (△). Solid line, basal 2-deoxyglucose uptake; broken line, insulin-stimulated 2-deoxyglucose uptake. The K_i for basal cells was 1.45 mM, while that for insulin-stimulated cells was 0.50 mM. Data shown are from one experiment; each point was performed in triplicate. Data were fitted by linear regression, using the Eadie-Hofstee method. v = nmol 2-deoxyglucose uptake/min per $2 \cdot 10^5$ cells.

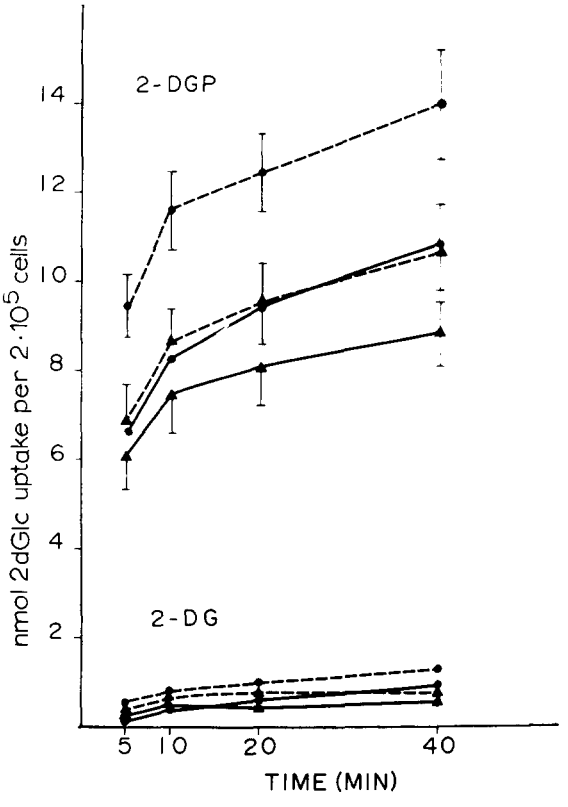

Fig. 4. Intracellular accumulation of 2-deoxyglucose (2-DG) and 2-deoxyglucose phosphate (2-DGP) in cells of control and endotoxin-treated rats. Experiments were performed with 2 mM 2-deoxyglucose in the buffer. Data were analyzed with a two-way ANOVA. Endotoxin significantly decreased 2-deoxyglucose phosphate levels in both basal and insulin-stimulated cells ($P < 0.05$ and $P < 0.01$, respectively), and 2-deoxyglucose levels in insulin-stimulated cells ($P < 0.05$). Line designations are as in Fig. 2. $n = 5$.

$2 \cdot 10^5$ cells represented about 5% of the total 2-deoxyglucose uptake of 9.5 nmol. Both basal and insulin-stimulated cells exhibited a significant decrease in 2-deoxyglucose 6-phosphate levels ($P < 0.05$ and $P < 0.01$, respectively).

Intracellular 2-deoxyglucose levels were also significantly reduced ($P < 0.05$) in insulin-stimulated cells from endotoxin-treated rats from 83 to 75% of control values. This decrease was similar to the depressed 2-deoxyglucose 6-phosphate levels which ranged from 77 to 72% of control measurements. In the basal condition, this pattern was not as consistent. At the later sample times, there was a trend toward decreased levels of both 2-deoxyglucose and 2-deoxyglucose 6-phosphate in endotoxin-treated rats; however, at 10 min, 2-deoxyglucose levels were higher than in control cells.

(5) 2-Deoxyglycose uptake in the presence of cytochalasin B. Considering the possibility that endotoxin treatment may alter membrane permeability characteristics, 2-deoxyglucose uptake was also assayed in the presence of cytochalasin B. Cells from either control or endotoxin-treated rats were preincubated with cytochalasin B (40 μM) for 5 min, followed by a 15 min incubation with 0.5 mM 2-deoxyglucose. Endotoxin treatment clearly increased the diffusional component of uptake from 11.84 ± 1.60 pmol 2-deoxyglucose uptake per min per $2 \cdot 10^5$ cells ($n = 9$) in control cells to 25.46 ± 3.49 ($n = 10$) in endotoxic cells. The difference was significant at the $P < 0.001$ level by Student's t-test.

(6) Endotoxin effects of spermine-stimulated 2-deoxyglucose uptake. The effects of endotoxin treatment on both spermine- and insulin-stimulated

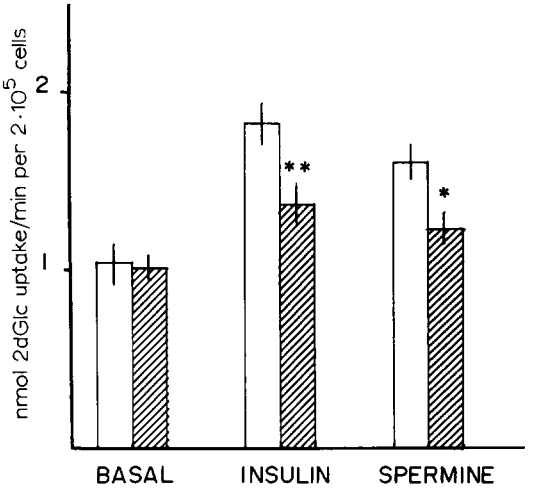

Fig. 5. Endotoxin effect on basal, insulin- and spermine-stimulated 2-deoxyglucose uptake. Cells were preincubated for 1 h in the absence or presence of insulin (1 mU/ml) or spermine (200 mM). ** Significantly different from control group, $P < 0.01$. * Significantly different from controls, $P < 0.05$ (Student's t-test). Open bar, control; hatched bar, endotoxin-treated. $n = 8$.

2-deoxyglucose uptake are shown in Fig. 5. Preincubation with spermine (200 μM) for 1 h was found to produce an increase in 2-deoxyglucose uptake comparable to that obtained by a 1-h preincubation with 1 mU/ml insulin. Endotoxin treatment significantly reduced both insulin- and spermine-stimulated 2-deoxyglucose uptake.

(7) Effect of endotoxin treatment on 3-O-methylglucose uptake in the absence and presence of cytochalasin B. A second glucose analog commonly used in studies of membrane transport is 3-methylglucose. This hexose differs from 2-deoxyglucose in that it is transported, but not phosphorylated. Fig. 6 illustrates the effects of endotoxin treatment on the time course of both basal and insulin-stimulated 3-methylglucose uptake. Basal uptake was significantly increased over the entire time course ($P < 0.05$, two-way ANOVA); however, insulin-stimulated uptake was not altered by endotoxin treatment.

The cytochalasin B-insensitive component of 3-methylglucose uptake was measured in two experiments (preincubation of cells with 40 μM cytochalasin B for 5 min). In endotoxic cells the cytochalasin B-insensitive accumulation of 3-methylglucose in 15 s increased to 0.020 nmol from values in control

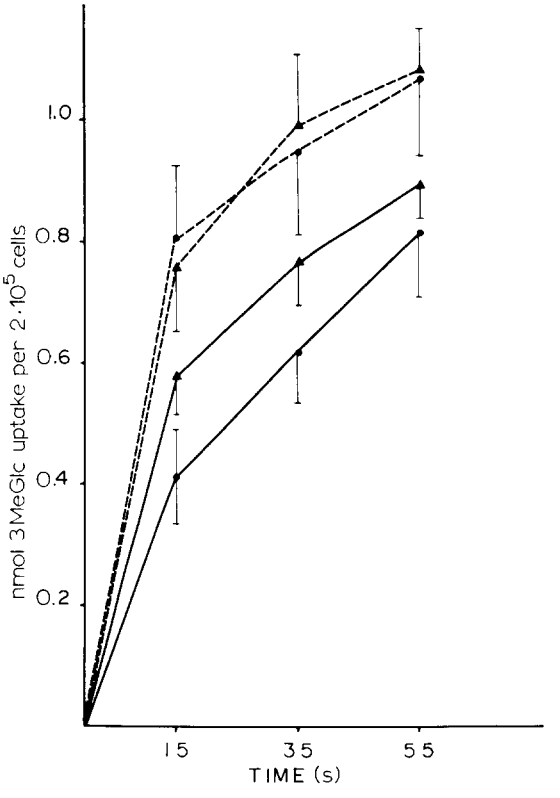

Fig. 6. Endotoxin influence on the time course of 3-*O*-methylglucose uptake. Substrate concentration was 2 mM. Endotoxin treatment significantly increased basal uptake ($P < 0.05$, two-way ANOVA) but insulin-stimulated uptake was not altered. Line designations are as in Fig. 2.

cells of 0.010 and 0.017 nmol, respectively. The corresponding 3-methylglucose uptake by endotoxic cells in the absence of cytochalasin B was 0.550 nmol. Thus, even though there was an increase in diffusion in absolute terms, this still represented only an insignificant fraction, less than 4% of the total quantity transported.

Discussion

Several bacterial products, including endotoxin, exert an insulin-like action and induce an insulin-resistant state in various mammalian cell types [2,17, 18,19]. Most of the studies demonstrating such action relied on the conversion of [^{14}C]glucose to $^{14}CO_2$ as an indicator of glucose transport. Although uptake is a necessary prerequisite of oxidation, the

alterations in glucose oxidation might represent a shift in intracellular metabolic pathways and/or changes in membrane transport.

Recently, Holley and Spitzer [9] demonstrated that *E. coli* endotoxin acted both in vivo and in vitro to increase basal glucose oxidation and reduce insulin sensitivity and responsiveness in isolated adipocytes. The apparent insulin resistance was accompanied by enhanced insulin binding to the fat cell membrane. These data led the authors to suggest a possible intracellular as well as membrane-localized endotoxin effect.

All our 2-deoxyglucose experiments demonstrate a marked reduction in insulin-stimulated uptake in adipocytes isolated from endotoxin-treated rats. In searching for an explanation of this insulin resistance, it is necessary to consider intracellular phosphorylating mechanisms. Since 2-deoxyglucose is phosphorylated after entering the cell, inhibition of hexokinase could lead to an increase in intracellular 2-deoxyglucose concentrations which might in turn act to inhibit further uptake. Our data indicate this was not the case. No intracellular 2-deoxyglucose was detectable with the usual 1-min incubation at a low extracellular 2-deoxyglucose concentration. A minimum incubation period of 5 min at a significantly higher concentration of extracellular substrate was needed to achieve measurable intracellular 2-deoxyglucose concentrations. Even beyond the 5-min time period over 90% of the 2-deoxyglucose that has entered the cell was phosphorylated (Fig. 4). Although intracellular 2-deoxyglucose 6-phosphate levels were reduced by endotoxin treatment, there was a corresponding drop in non-phosphorylated 2-deoxyglucose levels as well.

In non-insulin-stimulated cells this relationship was not as unequivocal, due perhaps in part to the difficulty of measuring free 2-deoxyglucose in the basal state. After 5 to 10 min of incubation, endotoxin-treated cells contained somewhat more 2-deoxyglucose than control cells, while the phosphorylated component tended to be lower in endotoxin-treated cells. At later times, however, comparable drops in 2-deoxyglucose and 2-deoxyglucose 6-phosphate were observed (data not shown). Even under these conditions the intracellular concentration of free 2-deoxyglucose was only 0.83 μM, whereas the 2-deoxyglucose concentration in the medium was 2.0 mM. Thus, the concentration gradient did not favor leakage out of the cells. Furthermore, this intracellular 2-deoxyglucose concentration is far removed from the K_m value for phosphorylation, which is in the range of $(3–4) \cdot 10^{-3}$ M. Our overall interpretation of these data is consistent with the hypothesis that hexokinase activity in the endotoxic cells is not compromised and a site of the endotoxin-induced insulin resistance is at the transport level.

Insulin has been found to increase V without changing K_m in studies with both 3-methylglucose [20,21] and 2-deoxyglucose uptake [11]. We found an increase in both affinity (lower K_m) and V in insulin-stimulated cells, in both immature (139 g body weight) and adult (204 g body weight) rats. Furthermore, our Dixon plot illustrating the inhibition of 2-deoxyglucose uptake by D-glucose provides additional support for an insulin-inducible change in K_m. The K_i for D-glucose has been shown to approximate the K_m [11] and indeed we observed a shift in the K_i of insulin-stimulated cells indicative of a greater carrier affinity. Our results agree well with previously reported lower K_m values for 2-deoxyglucose uptake in the presence of insulin in normal rat adipocytes [22] as well as the documented significant effect of insulin on glucose transport manifested by marked reduction in the apparent K_m or K_t in adipocytes [23] and in epididymal adipose tissue [24].

Endotoxin treatment was found to decrease V without causing any change in K_m. Thus the endotoxin-treated cells exhibited an alteration in K_m similar to that exhibited by control cells, when stimulated with insulin. These data suggest that the reduced insulin response may be mediated by a decrease in the effective number or activity of available carrier units rather than through an alteration in substrate carrier affinity. In contrast, Kuo [18] demonstrated with *Bacillus subtilis* peptidase-treated adipocytes a decreased affinity compared to control cells.

The fact that no change in K_m was observed in the endotoxin-treated cells suggests that, although endotoxin may act at the cell membrane site, this action is not necessarily mediated through the membrane-bound insulin receptor. Our observations with spermine-stimulated cells lend additional support to this hypothesis. Spermine is thought to increase glucose

uptake through a mechanism not involving the insulin receptor [25]. Endotoxin treatment reduced spermine-stimulated uptake to approximately the same extent as insulin-stimulated uptake, indicating that the defect most likely resides in the glucose transport system itself.

Although our data clearly indicate a primary site of endotoxin action on or in the cell membrane, the precise mechanism of action is not known. Recently it has been demonstrated that the action of insulin on various cellular functions, including stimulation of glucose transport, involved phosphorylation of specific membrane fractions [26,27]. In addition, in both adipose tissue and skeletal muscle, hypoxia and agents which uncouple oxidative phosphorylation were shown to deplete cellular ATP levels as well as reduce insulin sensitivity [28,29,30]. Since in vivo endotoxin treatment is known to cause massive vasodilation with vascular pooling and localized hypoxia [2], the observed insulin resistance may reflect reduced ATP levels. Spitzer and Holley [8], however, demonstrated reduced insulin sensitivity in isolated fat cells exposed to endotoxin in vitro, arguing for a direct effect of endotoxin on cellular metabolism and not simply a result of localized hypoxia. Furthermore, reduced cellular ATP levels are not likely in view of the fact that 2-deoxyglucose is phosphorylated normally.

A contributing factor in bringing about the endotoxin-induced decrease in insulin-stimulated 2-deoxyglucose uptake may be the elevated plasma corticosteroid level in endotoxin-treated rats (Kelleher, D., Bagby, G.J.and Spitzer, J.J., Personal communication) since Olefsky [31] demonstrated that glucocorticoids decrease glucose transport, as measured by 2-deoxyglucose uptake in isolated fat cell preparations. With 3-methylglucose, endotoxin significantly increased basal uptake without affecting insulin-stimulated uptake. Data obtained regarding cytochalasin B inhibition of 3-methylglucose uptake document that the increased basal uptake due to endotoxin is indeed due to increased transporter activity rather than increased diffusion.

We have demonstrated an increased cytochalasin B-insensitive component of both 2-deoxyglucose and 3-methylglucose uptake in endotoxin-treated cells. Although this represents a minor component of the total uptake, it indicates increased membrane permeability. At the same time, however, the cells did not demonstrate any swelling. We measured the intracellular fluid volume in four cell preparations from control and endotoxin-treated rats and found no difference (3.43 ± 0.59 pl/control cell vs. 2.85 ± 0.22 pl/endotoxic cell, respectively).

The divergence of the 3-methylglucose and 2-deoxyglucose uptake data suggests an intriguing aspect of the mechanism of endotoxin action at the cellular level. Several groups [11,20,21] have reported a K_m for 3-methylglucose uptake 2–3-fold higher than the K_m for 2-deoxyglucose. Moreover, Czech [20] reported a 3-methylglucose uptake component which was non-saturable, but cytochalasin B-sensitive. Evidence for the existence of two separate transport systems for the uptake of 3-methylglucose and 2-deoxyglucose, respectively, is well demonstrated in several tissues, including rat adipocytes [32–35]. Recently, more direct evidence was provided for the existence of two independent barriers in series separated by an aqueous pore, for the entry of 2-deoxyglucose and 3-methylglucose into adipocytes, with transport across the second barrier more rate-limiting for 2-deoxyglucose than for 3-methylglucose [36]. The idea that endotoxin may conceivably modulate the two transport systems in a non-parallel fashion is consistent with such findings. Alternatively, although the hexokinase reaction does not appear to be inhibited by endotoxin treatment, there may be other subtle differences in the intracellular processing of 2-deoxyglucose and 3-methylglucose which are altered by endotoxin, resulting in perturbed transport.

In conclusion, we have demonstrated that endotoxin treatment in vivo markedly influenced membrane hexose uptake processes in isolated adipocytes. Using the glucose analog 2-deoxyglucose, basal uptake was not significantly altered; however, an inappropriately low response to a submaximal dose of insulin was observed. This apparent insulin resistance did not result from an altered substrate-carrier affinity, but rather a decreased maximal velocity. Spermine-stimulated uptake was similarly depressed. Endotoxin did not appear to affect the hexokinase-mediated phosphorylating system. Experiments with 3-methylglucose revealed a significant increase in basal uptake with no alteration in insulin responsiveness. The lack of uniformity between the 3-methyl-

glucose and 2-deoxyglucose data suggests the possible reflection of the existence of two separate transport systems, differentially modulated by endotoxin.

Acknowledgements

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