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Cellular Stress Causes Accumulation of the Glucose Transporter at the Surface of Cells Independently of their Insulin Sensitivity

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Abstract. The stimulation of glucose transport in response to various types of stress has been studied. There is no relationship between effects of stress-inducing agents on glucose transport and their effects on cellular protein synthesis. Although the effect of stress on glucose transport appears analogous to its stimulation by insulin, cells that are slightly insulin-sensitive in terms of glucose transport (BHK cells) show a similar degree of stimulation as highly insulin-sensitive cells (differentiated 3T3-L1 cells). External labeling of the transporter protein with a photoactivatable derivative of mannose, 2-N-4-(1-azi-2,2,2-trifluoroethyl) benzoyl-1, 3-bis-(D-mannos-4-yloxy)-propylamine, shows that most of the increased glucose transport activity correlates with an increase in the amount of the transporter on the cell surface. Cells subjected to K^+-depletion, which inhibits endocytosis and results in an accumulation of receptors at the cell surface, show the same increase in glucose transport as cells exposed to stress; stressed cells show no further increase in glucose transport when subjected to K^+ depletion. These results support the view (Widnell, C.C., Baldwin, S.A., Davies, A., Martin, S., Pasternak, C.A. 1990. *FASEB J* **4**:1634–1637) that cellular stress increases glucose transport by promoting the accumulation of glucose transporter molecules at the cell surface.

Key Words: Cell surface—Glucose transporter—GLUT1—GLUT4—Insulin—Stress

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Introduction

Exposure of cells to various stresses induces an increased synthesis of the group of proteins termed heat shock proteins (Welch, 1992), some of which function as 'molecular chaperones' (Ellis & van der Vies, 1991). Another response to such stresses (e.g., hyperthermia (Reeves, 1977; Warren et al., 1986); infection by RNA (Gray et al., 1983; Garry, Bostick & Ulug, 1986; Pasternak, Whitaker-Dowling & Widnell, 1988) or DNA (Landini, 1984; Warren et al., 1986; Gray et al., 1986) viruses; exposure to arsenite (Warren et al., 1986) or to bacterial endotoxin (Lang et al., 1992) is an increased capacity of cells to take up glucose; it is a specific response in that amino acid uptake, for example, is unaffected (Gray et al., 1983; Warren et al., 1986; Lang et al., 1992). The response is not correlated with increased (Warren et al., 1986) or decreased (Pasternak et al., 1988; this paper) protein synthesis; instead, it is more similar to the insulin-stimulated uptake of glucose (Warren et al., 1986; Warren & Pasternak, 1989) that is independent of protein synthesis and involves a translocation of the glucose transporter protein from an intracellular (inactive) site to the plasma membrane where it is functional (Simpson & Cushman, 1986). Glucose transporter proteins comprise a family of isoforms (termed GLUT1, GLUT2, etc.), each with a characteristic tissue distribution (Gould & Bell 1990; Mueckler, 1990; Bell et al., 1993). Baby hamster kidney (BHK) cells, which contain predominantly GLUT1, show a similar morphological change when exposed to insulin or stress (Widnell et al., 1990); this change in the distribution of GLUT1 is reversed by removal of the stimulus in the same way as is the uptake of glucose (Pasternak et al., 1991).

To investigate the generality of the stress response in cultured cells (Warren & Pasternak, 1989) we have measured the effect of stress on cells that exhibit major differences in their response to insulin: differentiated 3T3-L1 cells which are adipocyte-like, contain GLUT1 and GLUT4, and are very sensitive to insulin (Calderhead et al., 1990; 10–20 fold stimulation of glucose transport) and BHK cells that contain mainly GLUT1, and respond weakly to insulin (Warren et al., 1986; Widnell et al., 1990; Pasternak et al., 1991; <3-fold stimulation of glucose transport). We find that sensitivity to a stress stimulus such as arsenite is independent of whether cells are insulin-sensitive or not.

We have also assessed quantitatively the distribution of the glucose transporter between cytoplasmic vesicles and the cell surface by covalent labeling of the transporter protein with a photoactivatable derivative of mannose, 2-N-4-(1-azi-2,2,2-trifluoroethyl) benzoyl-1,3-bis-(D-mannose-4-yloxy)-2-propylamine (ATB-[2-^{3}H]BMPA; Holman, Karim & Karim, 1988), and find that stressed cells show an increase in label at the cell surface that correlates with the increase in glucose uptake. Furthermore we show that K^+ depletion, which blocks endocytosis (Larkin et al., 1983) and might therefore be expected to accumulate ''cycling'' molecules such as the glucose transporter that associates with clathrin lattices (Robinson et al., 1992), does indeed increase glucose transport to the same extent as exposure to arsenite.

Taken together, the present results—which have been reported in brief (Pasternak, 1993)—provide direct evidence to support our earlier findings based on immunocytochemistry (Widnell et al., 1990; Pasternak et al., 1991) that stress induces a specific increase in the number of glucose transporter molecules at the cell surface that is independent of the insulin-sensitivity of the cell.

Materials and Methods

Materials

ATB-[2-^{3}H]BMPA (specific activity 10 ci/mmol) was prepared as described (Clark, Holman & Kozka, 1991). ^{125}I-transferrin was prepared as described (Simon et al., 1990) and all other reagents were obtained from commercial sources as described (Widnell et al., 1990; Pasternak et al., 1991).

Cells

The procedures for cell culture have been described for BHK cells (Pasternak et al., 1991) and for 3T3-L1 cells, which were stimulated to differentiate prior to use (Calderhead et al., 1990; Frost & Lane, 1985). Cells were kept in serum-free medium containing albumin (MEM/BSA) overnight prior to use (BHK cells) or for 2 hr prior to use (3T3-L1 cells) as described (Kozka, Clark & Holman, 1991); the overnight deprivation of serum in the case of BHK cells was carried out as a matter of convenience: the levels of glucose transport of stimulated cells return to basal values already within 3 hr (Pasternak et al., 1991).

Glucose and Aminoacid Transport Activity

Uptake of [^{3}H] 2-deoxy-D-glucose, and [^{14}C]-L-amino-iso-butyrate (AIB) as internal control, by BHK cells was measured as described previously (Pasternak et al., 1988). Uptake of [^{3}H] 2-deoxy-D-glucose by differentiated 3T3-L1 cells was measured as described (Kozka et al., 1991); the uptake of [^{14}C] AIB by 3T3-L1 cells was unaffected by insulin or stress.

Protein Synthesis

Protein synthesis was assessed by exposing duplicate dishes of BHK cells to 1 ml of carrier-free [^{35}S] methionine in HBS for 15 and 30 min at 37°C. Cells were washed three times with HBS and extracted with 2 × 0.5-ml samples of ice-cold 5% TCA. The extract was sonicated, sampled for ^{35}S, and spun. The pellet was extracted with 0.5 ml of 1 N NaOH and sampled for ^{35}S and protein.

Labeling with ATB-[2-^{3}H]BMPA

Labeling of cells, immunoprecipitation of the glucose transporter, and analysis of the labeled protein was carried out in Dr. G.D. Holman's laboratory (Yang & Holman, 1993). The method—which works for GLUT1 of BHK cells as well as it does for GLUT1 and 4 of rat adipocytes (Holman et al., 1990) or differentiated 3T3-L1 cells (Yang et al., 1992)—has been tested by comparing the total number of glucose transporter molecules in basal and stimulated states assessed by photolabeling, with that assessed by extraction of transporters from BHK membranes followed by immunoprecipitation and quantitation by Western blotting (Widnell et al., 1990): in each case, approx 4 pmol of GLUT1/mg protein is found.

K^+ depletion of BHK Cells

Cells were subjected to hypotonic shock by exposure to MEM/H_2O 1:1 for 20 min at 37°C, and then cultured in 0.05 M HEPES pH 7.4 0.1 M NaCl, 1% BSA, 0.1% D-glucose, 1 mM $MgCl_2$, 1 mM $CaCl_2$ (HSAG) for various times at 37°, essentially as described (Larkin et al., 1983). Cells cultured in HSAG supplemented with 5 mM KCl (HSAGK) served as controls, and in some experiments, cells in MEM/BSA were used as additional controls. The rates of glucose transport and transferrin uptake were similar in the two groups of controls.

Uptake and Release of ^{125}I-transferrin

The procedures used to study the uptake of ^{125}I-transferrin, and the distribution of transferrin between the cell surface and intracellular membranes, have been described (Simon et al., 1990). To study the release of transferrin, cells were treated with ^{125}I-transferrin for 15 min at 37°, and washed 5 times with HSAGK at 37°. At this time ~70% of the ^{125}I-transferrin was internal, and release from the cell was determined, during the subsequent 20–30 min, by analyzing radioactivity in the cells and medium. In some experiments, the cells were also trypsinized at 2° to determine the fraction of the ^{125}I-transferrin that was located within the cells. At all times ~80% of the cell-associated ^{125}I-transferrin was internal.

Table 1. Increased glucose transport by BHK cells is independent of protein synthesis

Treatment		Protein synthesis	Glucose transport
Control		100	100
Arsenite	(0.1 mM)	28	155
	(0.3 mM)	27	262
Mercaptoethanol	(10 mM)	72	148
	(30 mM)	30	351
Dithiothreitol	(10 mM)	49	139
	(30 mM)	35	257
Insulin	(6 μM)	111	157
Serum	(10%)	148	211

BHK cells were exposed to the treatments shown for 2 hr and protein synthesis and glucose transport then measured as described in Materials and Methods. The values for protein synthesis are means of the % of [^{35}S] methionine that is TCA-precipitable relative to a control value of 6.25%. The values for glucose transport are means of rate of glucose uptake relative to a control value of 5.9 pmol/min/mg protein.

Results

Increased Glucose Transport is Independent of Protein Synthesis

The increase in glucose uptake following exposure of BHK cells to stress is unlikely to be due to synthesis of new glucose transporter molecules, since stress stimuli such as arsenite or thiols inhibit—rather than increase—protein synthesis (Table 1); although other inducers of glucose uptake such as insulin or serum do increase protein synthesis somewhat, there is no correlation between the two effects (Table 1). Moreover Western blotting of BHK cells exposed to these treatments shows no increase in the total amount of glucose transporter molecules present (Widnell et al., 1990). Taken together with the previous observations that inhibition of protein synthesis by cycloheximide does not increase glucose uptake (Pasternak et al., 1988), and that cycloheximide does not affect arsenite—or heat—stimulated glucose uptake (Warren et al., 1986), it is clear that changes in the rate of cellular protein synthesis do not underly changes in the rate of glucose uptake. This is in contrast to situations like that in L6 muscle cells, where an increased glucose transport in response in hypoxic stress is prevented by cycloheximide (Bashan et al., 1992).

Stress-induced Glucose Uptake involves Accumulation of Transporter Molecules at the Plasma Membrane

Use of the bis-mannose photo-activatable probe ATB[^{3}H]BMPA (Table 2),shows that stress stimuli such as arsenite, heat shock or infection with Semliki Forest virus (SFV)—as well as exposure to serum or insulin—increase the proportion of glucose transporter at the plasma membrane to about the same extent (viz 2- to 3-fold increase) as the uptake of glucose. Because of dish-to-dish variation within a particular experiment, the totals (column 1 of Table 2) vary somewhat; nevertheless it is clear that the increases in surface label (column 2 of Table 2) cannot be accounted for by changes in total labeling [except for experiment 3, for reasons that are not clear; even in this experiment, however, there is good correlation between % label at the surface and the uptake of glucose].

The stress stimuli that lead to an increased glucose uptake in BHK cells also do so in differentiated 3T3-L1 cells [Warren (1988) and results obtained in conjunction with J. McLenithan and M.D. Lane which showed 6-fold stimulation by infection (40 p.f.u./cell; 6 hr) with SFV; 5-fold stimulation by arsenite (0.2 mM for 30 min); 1.5-fold stimulation by heat shock (45° for 30 min)]. Table 2 shows that in these cells, also, there is good correlation between % label at the surface and the uptake of glucose (for cells exposed to arsenite). It is to be noted that GLUT1 and GLUT4 accumulate at the cell surface to the same extent.

Interpretation of the results from experiments in which cell surface—exposed glucose transporters are labeled with ATB[^{3}H]BMPA has been criticized (Harrison et al., 1992) on the grounds that if glucose transporters at the cell surface exist in active and inactive forms distinguished by alterations in the glucose-binding site, only the active form would become labeled since ATB[^{3}H]BMPA binds at the same site as glucose. In that case an increase in labeling might not reflect an increased number of transporter molecules at the cell surface. Such an explanation is rendered unlikely by the fact that the total amount of label associated with transporter molecules (i.e., as measured in digitonin-treated cells) is not systematically increased by stress in BHK cells (GLUT1) or 3T3-L1 cells (GLUT1 and GLUT4).

Regulation of Membrane Trafficking in BHK Cells

Studies on GLUT4 in adipocytes and 3T3-L1 cells indicate that the glucose transporter is continuously recycled between the cell surface and an internal compartment (Satoh et al., 1991; Jhun et al., 1992; Czech & Buxton, 1993). The same appears to be the case for GLUT1 in BHK cells (Widnell et al., 1990; Pasternak et al., 1991). Confirmation that this is so and that stress leads to an accumulation of glucose transporters at the cell surface of BHK cells was obtained by comparing the effect of arsenite with that of blocking endocytosis. Because endocytosis by BHK cells occurs entirely via clathrin coated pits (Marsh & Helenius, 1989; Davoust, Gruenberg & Howell, 1987), prevention of clathrin-mediated

Table 2. Photolabeling of glucose transporter in BHK and 3T3-L1 cells

		Transporter localization (dpm per 100 μC added)			dGlc uptake (pmol/min/mg protein)
		Total	Surface	Surface (%)	
Exp 1:	Control	1704	360	21	7.6
BHK	Arsenite	2008	1268	63	26.7
Exp 2:	Control	1525	461	30	13.6
BHK	Arsenite	1765	1027	58	33.4
	Heat	1645	807	49	26.9
Exp 3:	Control	1551	327	21	3.1
BHK	Serum	2136	648	30	10.5
	SFV	2718	1050	39	23.9
Exp 4:	Control	2238	338	15	6.8
BHK	Insulin	2386	738	31	15.8
	SFV	2022	956	47	29.0
Exp 5:	Control	2809	649	23	
3T3-L1	Arsenite	2183	1448	66	
GLUT 1					18.6
GLUT 4	Control	2881	185	6	67.5
	Arsenite	1823	646	35	

BHK cells and differentiated 3T3-L1 cells were exposed to arsenite (0.1 mM for 2 hr), heat shock (45°C for 30 min), serum (10% new born calf serum for 2 hr), Semliki Forest virus (SFV) infection (14 hr after infection at m.o.i. of 5) or insulin (6 μM for 2 hr). One dish corresponding to each condition was then used for assay of [^{3}H] deoxyglucose uptake. Two other dishes were used for photolabeling with ATB[^{3}H]BMPA (Calderhead et al., 1990): one dish was irradiated directly for 1 min at 18°C to assess *surface* GLUT1 (BHK and 3T3-L1 cells) or GLUT4 (3T3-L1 cells); the other dish was exposed to ATB[^{3}H]BMPA in the presence of 0.025% digitonin for 8 min at 18°C in the dark prior to irradiation for 1 min to assess *total* GLUT1 or GLUT4. Each dish was then treated exactly as described (Yang et al., 1992) in order to isolate labeled GLUT1 or GLUT4.

endocytosis by hypotonic shock followed by K^+ depletion (Larkin et al., 1983), should lead to an increase in glucose uptake. When BHK cells are subjected to hypotonic shock and K^+-depletion, the uptake of transferrin is inhibited by ~90% (Table 3). This treatment results in a parallel increase in the number of transferrin receptors on the cell surface and an increased glucose transport activity (Table 3). In contrast, cells first treated with arsenite and then subjected to K^+-depletion show no further increase in the rate of glucose transport (Table 4). The effect of K^+-depletion is reversible: when cells are returned to MEM/BSA after 2 hr in K^+-free medium, the rate of glucose transport returns to control values within 2 hr (Table 4). Amino acid transport—assessed by uptake of the alanine analogue amino-iso-butyrate—does not increase, and, in fact shows a variable decrease at 2 hr of K^+-depletion (Tables 3 and 4), and a marked decrease at 4 hr (*not shown*); this confirms previous observations (Warren et al., 1986; Pasternak et al., 1988) that amino acid uptake by cultured cells is not controlled in the same manner as is that of glucose. The results in Tables 3 and 4 are from one experiment; two others, which studied different times after K^+-depletion, yielded essentially the same results.

We next studied the rate of release of transferrin from BHK cells (Mayor et al., 1993) to determine whether arsenite or phenylarsine oxide (PAO; Frost & Lane, 1985) affects constitutive exocytosis. Table 5 (one experiment, confirmed with different time points in two further experiments) shows that transferrin is released from arsenite-treated cells at the same rate as from control cells. This shows that recycling of the transferrin receptor is unaffected by arsenite stress and suggests that the increased recycling of the glucose transporter is a specific—not a generic—response. As anticipated from previous experiments (Pasternak et al., 1991), PAO causes an inhibition of transferrin release, for reasons that are not yet clear.

Discussion

Our present results extend earlier findings (Warren & Pasternak, 1989; Pasternak et al., 1991) that examined the relationship between the stimulation of glucose transport in response to insulin or serum, and the stimulation in response to stresses such as hyperthermia, hypothermia (*unpublished*), azide (i.e., anoxia), virus infection or

Table 3. Effect of K^+-depletion on glucose transport by BHK cells

Treatment of cells	Glucose transport (pmol/min /mg protein)	AIB transport (pmol/min /mg protein)	Surface-bound transferrin (ng/mg protein)	Internalized transferrin (ng/mg protein)
Control (MEM/BSA)	9.9	14.8	27	65
K^+-depleted, 0.5 hr[a]	15.0	16.6	34	21
K^+-depleted, 1 hr	19.0	18.1	38	15
K^+-depleted, 2 hr	24.4	12.0	43	6
K^+-supplemented, 1 hr[b]	10.1	16.8	28	63
K^+-supplemented, 2 hr	11.7	16.5	25	66

The uptake of glucose and AIB were determined as described in Materials and Methods. The surface binding and internalization of transferrin was determined as described in Materials and Methods, after incubating the cells with ^{125}I-transferrin at 37°C in the same medium as that used for their treatment.
[a] Cells were subjected to hypotonic shock and then cultured in K^+-deficient medium (HSAG), as described in Materials and Methods.
[b] Cells were subjected to hypotonic shock and then cultured in K^+-supplemented medium (HSAGK), as described in Materials and Methods.

Table 4. Effect on K^+ depletion on glucose transport by BHK cells pretreated with arsenite

Treatment of cells	Glucose transport (pmol/min/ mg protein)	AIB transport (pmol/min/ mg protein)
Control (MEM/BSA)	10.6	13.5
Arsenite, 2 hr[a]	24.0	10.8
Arsenite, 2 hr; then K^+-depleted, 1 hr[b]	24.5	15.6
Arsenite, 2 hr; then K^+-depleted, 2 hr	22.8	9.5
Arsenite, 2 hr; then MEM/BSA, 2 hr	12.1	12.3
K^+-depleted, 2 hr[c]	21.8	14.2
K^+-depleted, 2 hr then MEM/BSA 0.5 hr	17.8	18.1
K^+-depleted, 2 hr; then MEM/BSA 2 hr	12.7	14.6

The uptake of glucose and AIB were determined as described in Materials and Methods.
[a] Cells were treated with 100 μM arsenite in MEM/BSA.
[b] Cells were treated with 100 μM arsenite in MEM/BSA, subjected to hypotonic shock and then cultured in K^+-deficient medium (HSAG), as described in Materials and Methods.
[c] Cells subjected to hypotonic shock and cultured in K^+-depleted medium (HSAG) for 2 hr; these cells internalized 8% of the ^{125}I-transferrin internalized by controls.

arsenite. First, we have shown that the stimulation of glucose transport by stress occurs under conditions where protein synthesis is inhibited, i.e., in a situation opposite to that elicited by insulin or serum (Table 1). Since slightly insulin-sensitive cells [i.e., BHK cells, Swiss 3T3 cells, undifferentiated 3T3-L1 cells, chick or rat embryo fibroblasts (Warren, 1988); <3-fold stimulation of glucose uptake by insulin] show a similar response to arsenite, hyperthemia or SFV infection as highly insulin-sensitive cells [i.e., differentiated 3T3-L1 cells or rat adipocytes (Pasternak, 1991); 10-20-fold stimulation of glucose uptake by insulin); the effect of stress appears to bypass the insulin receptor, as previously postulated (Warren & Pasternak, 1989), but to converge at some subsequent state in the regulation of vesicle trafficking. Because insulin-induced and stress-induced glucose uptake are equally sensitive to thiol inhibitors like NEM and PAO (Warren & Pasternak, 1989; Frost & Lane, 1985), identification of the molecules with which they react should prove fruitful for elucidating postreceptor events in the case of insulin action. We have ruled out changes in intracellular Ca^{2+} or protein kinase C (Warren & Pasternak, 1989) as well as pH (no effect of acidifying or alkalinizing cytoplasm by addition of 10 mM sodium acetate or 10 mM ammonium chloride respectively) or an effect of NO (no effect of 0.1 mM or 1 mM N_{ω} nitro-L-arginine methyl ester) as possible ''second messengers'' of stress-induced glucose uptake in BHK cells.

Second, we have obtained results that validate the conclusions drawn from our earlier cytochemical evidence (Widnell et al., 1990; Pasternak et al., 1991), that stress induces an alteration in the disposition of the glucose transporter between an intracellular location and the cell surface (Table 2). The fact that the redistribution of GLUT1 in BHK cells is observed both after inhibition of endocytosis and after exposure to arsenite (Tables 3 and 4) supports such a conclusion and demonstrates clearly that recycling of glucose transporters is not a special feature of GLUT4.

Third, our results indicate that selective trafficking of GLUT1 is responsible for the regulation of glucose transport in response to stress. Our earlier results (Pas-

Table 5. Release of ^{131}I-transferrin from BHK cells

Experiment	Treatment of cells	Reculture (min)	Cell-associated transferrin	
			ng/mg protein	% released
A	None (control)	0	73	0
		6	40	45
		12	24	67
		20	17	77
	Arsenite[a]	0	71	0
		6	42	41
		12	34	53
		20	28	61
B	None (control)	0	55	0
		5	35	36
		15	15	73
		30	12	78
	PAO[b]	5	53	4
		15	45	18
		30	44	20

The uptake and release of transferrin were determined as described in Materials and Methods.
[a] Cells were treated with 0.2 mM arsenite for 2 hr before the addition of transferrin.
[b] Cells were treated in the same way as controls until after the addition of transferrin, when 0.1 mM PAO was included throughout the period of release.

ternak et al., 1988; Simon et al., 1990; Pasternak et al., 1991) have clearly dissociated changes in glucose transport from the general bulk flow of membrane, as judged by the recycling of the transferrin receptor and by fluid-phase pinocytosis. First, the stimulation of glucose transport by arsenite occurs without affecting fluid-phase endocytosis, as measured by uptake of [^{14}C] sucrose (Warren & Pasternak, 1989), and with a scarcely detectable effect on the uptake of transferrin and the amount of the transferrin receptor present on the cell surface (Pasternak et al., 1991). Second, in cells at the early stages of infection with VSV, there is a marked inhibition of fluid-phase pinocytosis and transferrin uptake, together with an accumulation of transferrin receptors at the cell surface (Simon et al., 1990) well before any change in glucose transport could be detected (Pasternak et al., 1988).

Further evidence that the regulation of glucose transport in stressed cells requires the specific trafficking of GLUT1 was obtained in the present study. Cells in which endocytosis was inhibited by K^+-depletion showed a parallel increase in glucose transport and the amount of the transferrin receptor on the cell surface. The inhibition of bulk membrane flow either by K^+-depletion or by VSV infection causes the transferrin receptor to accumulate at the cell surface at essentially the same rate, since the results in Table 3 are very similar to those described by Simon et al. (1990). In contrast, the increase in the rate of glucose transport occurs at an earlier time in arsenite-treated cells, and later in VSV-infected cells. As expected, when GLUT1 has accumulated at the surface of arsenite-treated cells, K^+-depletion has no additional effect.

There are now several lines of evidence that the regulation of GLUT4 in response to insulin is mainly the result of a stimulation of exocytosis, i.e., the fusion of membrane vesicles containing GLUT4 with the plasma membrane (Satoh et al., 1991; Yang et al., 1992; Robinson et al., 1992; Cain et al., 1992; Holman et al., 1994). It will be important in future studies to define the relative contribution of exocytosis and endocytosis to the regulation of GLUT1, and to determine how the trafficking of GLUT1 is segregated from that of other recycling plasma membrane proteins.

The increased movement of the glucose transporter in stressed cells is physiologically significant, since the increase in glucose transport is matched by an increase in lactate production (Pasternak et al., 1991). An increased glycolytic rate, first noted in heat-shocked cells by Burdon, Kerr, Cutmore, Munro and Gill (1984) and by Schlesinger (1985), is likely to be a consequence of any stress stimulus that inhibits respiration and hence meets the demand for ATP synthesis by accelerated glycolysis. However an increase in glucose transport is not shown by all anaerobically stressed cells: cells that have an intrinsically high rate of glucose uptake, such as hepatocytes, erythrocytes or malignant cells, do not show an increase when exposed to arsenite, azide or hyperthermia (experiments with A Burchall). In avian erythrocytes, the rate of glucose uptake appears to be controlled by activation of transporter resident at the cell surface,

rather than by accumulation of transporter molecules at the cell surface (Diamond, Hebert & Carruthers, 1993). The fact that lactate production in hepatocytes *is* stimulated by azide or other stress stimuli without an effect on glucose transport (experiments with A Burchall) is compatible with the well established concept that in such cells lactate production is controlled by an enzyme of glycolysis (Krebs, 1972), rather than by the rate of glucose uptake; another example of this situation is afforded by the increased synthesis of pyrurate kinase in heat-shocked *Xenopus* embryos (Marsden et al., 1993). Our results with stressed cells in culture suggest that in intact organisms, also, increases in glucose uptake in response to stresses such as sepsis and endotoxin (Lang et al., 1992) may be due to translocation of glucose transporter proteins to the cell surface.

In conclusion, the present results show that a variety of treatments can cause glucose uptake by two different cell types to be increased by a mechanism similar to that induced by insulin. This has implications both for the elucidation of the molecules that may be involved in that mechanism (e.g., Laurie et al., 1993, Piper et al., 1992, Ismail-Beigi, 1993; Hudson et al., 1993; Okada et al., 1994; Hara et al., 1994) and for the synthesis of novel drugs that may prove clinically useful in situations of insulin resistance or insensitivity where the insulin receptor is impaired.

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