Glucose transport and glucose transporter GLUT4 are regulated by product(s) of intermediary metabolism in cardiomyocytes

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Alternative substrates of energy metabolism are thought to contribute to the impairment of heart and muscle glucose utilization in insulin-resistant states. We have investigated the acute effects of substrates in isolated rat cardiomyocytes. Exposure to lactate, pyruvate, propionate, acetate, palmitate, β -hydroxybutyrate or α -oxoglutarate led to the depression of glucose transport by up to 50%, with lactate, pyruvate and propionate being the most potent agents. The percentage inhibition was greater in cardiomyocytes in which glucose transport was stimulated with the α -adrenergic agonist phenylephrine or with a submaximal insulin concentration than in basal or fully insulin-stimulated cells. Cardiomyocytes from fasted or diabetic rats displayed a similar sensitivity to substrates as did cells from control animals. On the other hand, the amination product of pyruvate (alanine), as well as valine and the aminotransferase inhibitors cycloserine and amino-oxyacetate, stimulated glucose transport about 2-fold. In addition, the effect of pyruvate was counteracted by cycloserine. Since reversible transamination reactions are known to affect the pool size of the citrate cycle, the influence of substrates, amino acids and aminotransferase

inhibitors on citrate, malate and glutamate content was examined. A significant negative correlation was found between alterations in glucose transport and the levels of citrate ($P < 0.01$) or malate ($P < 0.01$), and there was a positive correlation between glucose transport and glutamate levels ($P < 0.05$). In contrast, there was no correlation with changes in [$1\text{-}^{14}\text{C}$]pyruvate oxidation or in glucose-6-phosphate levels. Finally, pyruvate decreased the abundance of GLUT4 glucose transporters at the surface of phenylephrine- or insulin-stimulated cells by 34% and 27% respectively, as determined by using the selective photoaffinity label [^3H]ATB-BMPA [^3H]2-*N*-[4-(1-azi-2,2,2-trifluoroethyl)benzoyl]-1,3-bis-(*D*-mannos-4-yloxy)propyl-2-amine]. In conclusion, cardiomyocyte glucose transport is subject to counter-regulation by alternative substrates. The glucose transport system appears to be controlled by (a) compound(s) of intermediary metabolism (other than glucose 6-phosphate), but in a different way than pyruvate dehydrogenase. Transport inhibition eventually occurs via a decrease in the amount of glucose transporters in the plasma membrane.

INTRODUCTION

In heart and skeletal muscle, the utilization of glucose is counter-regulated by alternative substrates of energy metabolism, such as fatty acids, ketone bodies and lactate [1–7]. This phenomenon is part of a complex pattern of interactions between carbohydrate and lipid metabolism known as the glucose fatty acid cycle (for reviews, see [8,9]). By inhibiting muscle glucose metabolism, alternative substrates have a glucose-sparing effect which helps to maintain a constant level of glycaemia under conditions of relative glucose shortage such as starvation [8]. On the other hand, this inhibition may contribute to the impairment of muscle glucose uptake and oxidation in diabetes and other insulin-resistant states [8,10–12].

Inhibition of glucose utilization by substrates was demonstrated to occur through the production of metabolic intermediates which eventually affect two key steps of glucose metabolism: the phosphofructokinase and pyruvate dehydrogenase (PDH) reactions [9]. Thus the substrate-induced increase in the cellular level of citrate, for instance, was shown to cause glycolytic inhibition by depressing phosphofructokinase activity directly [13], as well as indirectly (via a drop in the phosphofructokinase activator fructose 2,6-bisphosphate) [14].

In addition, alternative substrates were shown to affect the first step of heart and muscle glucose metabolism, namely the

transport of the sugar across the plasma membrane [1,15,16]. This inhibitory effect may also be crucial with respect to the overall utilization of glucose in these tissues since, under many conditions, transmembrane transport limits the uptake of glucose in muscles [17] and heart [18,19]. However, the signal(s) mediating the inhibition of glucose transport by substrates, and the mechanism involved, are still unknown. The purpose of the present study was, therefore, to explore the cellular mediator(s) and the mechanism underlying the effects of fatty acids and other substrates on muscle cell glucose transport. To this end, we have used cardiomyocytes (isolated from adult rats), which have proved to be a sensitive and reliable model for investigations on the regulation of cardiac glucose transport [20–23].

MATERIALS AND METHODS

Chemicals

All chemicals were of the highest purity grade available. Chemicals for media used for cell isolation, glucose transport assays and labelling experiments were purchased from Merck (Darmstadt, Germany); pyruvate, amino acids, BSA (fraction V; fatty acid-free) and all standards and enzymes for metabolite measurements were from Boehringer (Mannheim, Germany); all

Abbreviations used: dGlc, 2-deoxy-D-glucose; MeGlc, 3-O-methyl-D-glucose; ATB-BMPA, 2-*N*-[4-(1-azi-2,2,2-trifluoroethyl)benzoyl]-1,3-bis-(*D*-mannos-4-yloxy)propyl-2-amine; PDH, pyruvate dehydrogenase.

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substrates (except pyruvate), L-phenylephrine, D,L-cycloserine and hydroxymalonic acid were from Sigma (Deisenhofen, Germany); dichloroacetic acid was from Aldrich (Steinheim, Germany); purified bovine insulin was kindly donated by Professor Axel Wollmer (University of Aachen, Germany). 2-Deoxy-D-[^3H]glucose ([^3H]dGlc), 3-O-methyl-D-[^3H]glucose (Me[^3H]Glc) and [1- ^{14}C]pyruvic acid were from Amersham (Braunschweig, Germany). The photoaffinity label [^3H]ATB-BMPA {[^3H]2-N-[4-(1-azi-2,2,2-trifluoroethyl)benzoyl]-1,3-bis-(D-mannos-4-yloxy)propyl-2-amine} used to quantify the glucose transporters was prepared as described elsewhere [24]. Palmitate was bound to fatty acid-free BSA in a final molar ratio of 2.7:1.

Isolation of cardiomyocytes and glucose transport assays

Cardiomyocytes from adult Sprague–Dawley rats (180–220 g, fed *ad libitum*) were obtained as previously described [20]. Treatment of cardiomyocytes for all experiments (including photoaffinity labelling) was performed in medium A, containing 6 mM KCl, 1 mM Na_2HPO_4 , 0.2 mM NaH_2PO_4 , 1.4 mM MgSO_4 , 128 mM NaCl, 10 mM Hepes, 1 mM CaCl_2 and 2% (w/v) BSA, pH 7.4, at 37 °C, equilibrated with 100% oxygen. Preliminary experiments showed that the rate of cardiomyocyte glucose transport upon treatment with phenylephrine and insulin was about 30% higher when these agents were added after a 30 min preincubation at 37 °C than when the cells were stimulated immediately upon resuspension in assay medium (in addition, there was a slight decrease in basal glucose transport over the first 10 min of this preincubation). In all experiments described in this study, the cells were therefore preincubated for 30 min at 37 °C before substances such as substrates, and thereafter phenylephrine or insulin, were added. The rates of carrier-mediated dGlc and MeGlc uptake were determined as described in [20]. dGlc transport was measured over a period of 30 min. The measurement of MeGlc transport was performed over 10 s, 30 s and 90 s in insulin-stimulated, phenylephrine-stimulated and non-stimulated (basal) cells respectively, at the final concentrations indicated in Table 2. Preliminary experiments and previous studies [20] had shown that the uptake of MeGlc is linear under these conditions.

Induction of diabetes

Male Wistar rats weighing 250–300 g were fed *ad libitum* on a standard diet (Sniff; Svest) and had free access to water. Diabetes was induced by streptozotocin (Boehringer; 60 mg/kg body weight) dissolved in 0.1 ml of citrate buffer, pH 4.5, immediately before use, injected intraperitoneally as previously described [25]. Blood glucose was routinely checked, and was 34 ± 6 mM at the time the animals were killed for experimentation. Experiments were performed 8–10 weeks after streptozotocin injection. Age-matched non-diabetic littermates were used as controls.

Metabolite measurements

Upon treatment with substrates, amino acids or amino-transferase inhibitors (see the legends of Tables 1 and 6), aliquots of cardiomyocytes (containing $\sim 1.5 \times 10^5$ cells) were rapidly centrifuged (45 s, 14 g), washed once with 0.9% NaCl and centrifuged again. The pellet from this centrifugation was dissolved either (i) in 400 μl of cold ethanol (4 °C, 70%; for malate, glucose 6-phosphate and citrate measurements), or (ii) in 400 μl of cold perchloric acid (4 °C, 0.3 M; for glutamate measurements), and homogenized for 15 s with a sonifier (Branson). The ethanolic samples were then acidified by addition

of 15 μl of conc. formic acid and centrifuged for 10 min at 10000 g. The supernatants from this centrifugation were evaporated at 70 °C under a stream of nitrogen gas. The dry material obtained was dissolved in 150 μl of 0.1 M K_2HPO_4 , pH 10 (malate assay), in 150 μl of 0.1 M K_2HPO_4 , pH 7.6 (glucose 6-phosphate) or in 250 μl of 0.1 M Tris/HCl, pH 8.2 (citrate). Malate and glucose 6-phosphate were measured by luminometry (50 μl of dissolved material) [26], and citrate was determined fluorimetrically (100 μl) as described elsewhere [27]. The precipitates from sonicated perchloric acid samples were centrifuged (5 min, 10000 g), and the supernatant was neutralized with KOH/ KHCO_3 before glutamate was assayed by photometry as described in [28]. All measurements were done in duplicate on two independent samples in each experiment.

For the calculation of the corresponding cellular concentrations, the water distribution space of cardiomyocytes was calculated using a previously determined factor of 2.07 μl per mg of cell protein [20].

Measurement of $^{14}\text{CO}_2$ production from [1- ^{14}C]pyruvate

Flux through PDH was determined by monitoring the production of $^{14}\text{CO}_2$ from [1- ^{14}C]pyruvate using a protocol adapted from [29,30]. In brief, cardiomyocytes were exposed to the agents to be investigated for 30 min at 37 °C in 20 ml vials sealed with rubber stoppers (under the same conditions as for the glucose transport assays, i.e. in a total volume of 1.5 ml and with ~ 1.5 mg of cell protein per sample). The oxidation reaction was then started by injecting 0.1 μCi of [1- ^{14}C]pyruvate (0.1 mM final concentration) into the cell suspension. After another 10 min at 37 °C, the incubation was terminated by injection of 300 μl of 0.3 M perchloric acid. The released $^{14}\text{CO}_2$ was trapped (over 18 h at 4 °C) in 0.3 ml of ethanolamine/ethylene glycol (1:1, v/v) contained in a polypropylene centre well (placed in the vials before the incubations). Radioactivity was measured in a liquid scintillation counter (Wallac 1409). Non-specific values (determined by injecting perchloric acid into parallel cell samples before adding [1- ^{14}C]pyruvate) were subtracted from all sample values. The quenching caused by the ethanolamine/ethylene glycol mixture was determined in each experiment and taken into account for the calculation of pyruvate oxidation rates.

Photoaffinity labelling of glucose transporters GLUT1 and GLUT4 at the surface of cardiomyocytes

Cardiomyocytes were treated with or without pyruvate and then exposed to phenylephrine or insulin as described in the legend to Table 10. The labelling of glucose transporters present at the surface of these cells was subsequently performed as previously described in detail [22]. In brief, the cells were quickly washed with assay medium containing the same additions as in the preceding treatment (e.g. pyruvate and phenylephrine) and then resuspended in 500 μl of medium (with the same additions); 60 μl of the non-permeant photoreactive bismannose compound [^3H]ATB-BMPA (300 μCi) was then added, and the samples were irradiated for 3 min with a mercury light. Following irradiation the cells were washed, solubilized in phosphate buffer containing 2% Thesit and subjected to sequential immunoprecipitation with rabbit anti-GLUT4 serum and then with anti-GLUT1 serum, coupled to Protein A–Sepharose. The immuno-adsorbed material (with the glucose transporters) was subjected to PAGE. The lanes were cut into pieces, dissolved in 30% H_2O_2 containing 2% NH_3 , and the radioactivity in each piece was counted. To quantify the amount of labelled glucose transporters in each sample, the background of the gel slices was subtracted

from the (only) peak appearing in the lanes; note that this peak has an apparent molecular mass of around 50 kDa, corresponding to the known molecular masses of GLUTs. Moreover, labelling was abolished by the specific glucose transport inhibitor cytochalasin B.

Calculations and statistics

Cell protein used to express the glucose transport values and to calculate the cellular concentrations of metabolites (see above) was measured by the biuret method. Statistical comparison of values was carried out with a paired Student's *t* test, unless indicated otherwise. The IC_{50} values of glucose transport inhibition were calculated from the data shown in Figure 1 as fitted according to $y = 100 - x(100 - y_{min})/(x + IC_{50})$, where *y* is the relative rate of glucose transport (expressed as a percentage of the maximal, non-inhibited, rate), y_{min} is the transport rate under maximal inhibition and *x* is the substrate concentration. Correlation analysis of the data presented in Figure 2 (i.e. glucose transport data versus the corresponding concentrations of citrate, malate or glutamate) was done by a non-parametric Spearman test, using computer software supplied by SAS (Heidelberg, Germany).

RESULTS

Effects of alternative substrates on glucose transport

The effects of alternative substrates of energy metabolism on glucose transport have been investigated in freshly isolated cardiomyocytes under three conditions: in the basal, non-stimulated state and under the influence of two different types of glucose transport stimuli, namely the α -adrenergic agonist phenylephrine and insulin. Substrates of various types were tested, including carbohydrates (pyruvate, lactate), a ketone body (β -hydroxybutyrate), organic acids (acetate, propionate), middle- and long-chain fatty acids (octanoate and palmitate respectively) and an α -oxoacid (α -oxoglutarate, which is also a citric acid cycle intermediate). As documented in Table 1, all these substances, with the exception of octanoate, significantly decreased the rate of dGlc uptake by up to 50% when compared with control values measured in the absence of substrate. Note that some

substrates, e.g. pyruvate, lactate and propionate, were clearly more effective than others, in particular palmitate (Table 1). As for octanoate, it had no inhibitory action on phenylephrine- or insulin-stimulated dGlc transport, and even slightly increased the basal rate of dGlc transport (Table 1). It is also worth pointing out that phenylephrine-stimulated glucose transport was more sensitive to the action of alternative substrates, in terms of percentage inhibition, than was glucose transport measured under non-stimulated (basal) conditions or in the presence of a saturating insulin concentration (18 nM) (Table 1). In addition, inhibition of insulin-dependent glucose transport was more pronounced at a submaximal (0.3 nM) than at a supramaximal concentration of the hormone, as shown for pyruvate and β -hydroxybutyrate (Table 1).

Although transmembrane transport (and not the phosphorylation) of dGlc was previously shown to determine the rate of uptake of this sugar under the experimental conditions used in our assay [19,20], it was conceivable that the substrates exert a strong inhibition on the hexokinase reaction such that this step may become rate-limiting. We therefore also investigated the action of pyruvate on the uptake of MeGlc, a glucose analogue which is transported via glucose carriers but is not (or barely) phosphorylated by the hexokinase. As shown in Table 2, pyruvate inhibited the uptake of MeGlc to the same extent as that of dGlc (see Table 1 for comparison). Inhibition of MeGlc transport was also observed at a physiological glucose concentration (5 mM), at least in phenylephrine-stimulated cells, but not in the presence of insulin (Table 2).

The concentration-dependence of glucose transport inhibition by pyruvate, lactate and β -hydroxybutyrate is illustrated in Figure 1. The corresponding IC_{50} values calculated from these data were (mM): 0.74 ± 0.29 for pyruvate, 0.93 ± 0.35 for lactate and 0.73 ± 0.17 for β -hydroxybutyrate. Further experiments showed that maximal inhibition by pyruvate (3 mM) was achieved after less than 10 min of treatment with this substrate (results not shown).

As shown in Table 3, the effects of three different types of substrates, i.e. pyruvate, propionate and β -hydroxybutyrate, on phenylephrine-stimulated glucose transport were not additive, suggesting that these agents act through a common mechanism. Thus combined treatment with, for instance, pyruvate and

Table 1 Effects of alternative substrates on the rate of dGlc uptake in basal, phenylephrine-stimulated and insulin-stimulated cardiomyocytes

Cardiomyocytes were preincubated as described in the Materials and methods section before addition of pyruvate (3 mM), lactate (3 mM), propionate (4 mM), octanoate (3 mM), palmitate (0.8 mM), β -hydroxybutyrate (3 mM), acetate (3 mM) or α -oxoglutarate (10 mM) for 15 min. Phenylephrine (100 μ M) or insulin (0.3 or 18 nM, as indicated) was then added for another 30 min, and the rate of dGlc uptake was determined as described in the Materials and methods section. dGlc uptake values are means $\pm$ S.E.M., and are expressed as pmol/h per mg of protein. The numbers of independent experiments are indicated in parentheses. Percentage and *P* values were determined by comparison with the corresponding control measured in the absence of substrate. Significance of differences: **P* < 0.05; ***P* < 0.01; ****P* < 0.001. N.D., not determined.

Additions	Rate of dGlc uptake							
	Basal		+ Phenylephrine		+ Insulin (0.3 nM)		+ Insulin (18 nM)	
	(pmol/h per mg)	(%)	(pmol/h per mg)	(%)	(pmol/h per mg)	(%)	(pmol/h per mg)	(%)
None (control)	24.4 $\pm$ 1.4 (24)	100	87.4 $\pm$ 4.8 (21)	100	72.2 $\pm$ 13.0 (5)	100	260.5 $\pm$ 12.1 (20)	100
Pyruvate	16.5 $\pm$ 1.4 (17)***	68	41.6 $\pm$ 4.0 (21)***	48	41.7 $\pm$ 7.6 (4)*	58	213.1 $\pm$ 12.5 (15)***	82
Lactate	18.3 $\pm$ 2.1 (11)*	75	55.0 $\pm$ 4.4 (5)***	63	N.D.	—	211.5 $\pm$ 9.4 (5)**	81
Propionate	19.2 $\pm$ 0.4 (4)***	79	53.4 $\pm$ 6.1 (8)***	61	N.D.	—	208.4 $\pm$ 16.4 (11)**	80
Octanoate	29.0 $\pm$ 1.2 (6)**	119	75.3 $\pm$ 8.7 (6)	86	N.D.	—	247.7 $\pm$ 12.5 (4)	95
Palmitate	22.7 $\pm$ 3.9 (8)	93	69.1 $\pm$ 7.5 (8)*	79	N.D.	—	241.2 $\pm$ 7.0 (7)*	93
β -Hydroxybutyrate	20.5 $\pm$ 2.2 (9)*	84	64.7 $\pm$ 9.4 (8)*	74	51.9 $\pm$ 4.3 (5)**	72	222.5 $\pm$ 15.6 (12)*	85
Acetate	16.0 $\pm$ 2.0 (8)**	66	62.9 $\pm$ 6.6 (8)**	72	N.D.	—	180.0 $\pm$ 22.7 (8)**	69
α -Oxoglutarate	21.5 $\pm$ 1.6 (5)	88	64.7 $\pm$ 3.4 (5)**	74	N.D.	—	247.5 $\pm$ 18.8 (8)	95

Table 2 Effect of pyruvate on the rate of MeGlc uptake in basal, phenylephrine-stimulated and insulin-stimulated cardiomyocytes

Cardiomyocytes were preincubated as described in the Materials and methods section in the absence or in the presence of D-glucose (5 mM), and then pyruvate (3 mM) was added for 15 min, as indicated. At the end of this period, phenylephrine (180 μ M) or insulin (18 nM) was added for 30 min, and the rate of MeGlc uptake was determined as described in the Materials and methods section. Values are means $\pm$ S.E.M., expressed as pmol/s per mg of protein. The numbers of independent experiments are indicated in parentheses. Percentage and *P* values were determined as compared with the corresponding control measured in the absence of substrate. Significance of differences: **P* < 0.05; ***P* < 0.01. N.D., not determined.

Conditions	Rate of MeGlc uptake					
	Basal		+ Phenylephrine		+ Insulin	
	(pmol/s per mg)	(%)	(pmol/s per mg)	(%)	(pmol/s per mg)	(%)
No D-glucose + 3 mM MeGlc						
No substrate (control)	8.8 $\pm$ 1.1 (8)	100	50.1 $\pm$ 6.0 (4)	100	152.3 $\pm$ 8.0 (4)	100
Pyruvate	6.7 $\pm$ 1.1 (8)*	76	21.9 $\pm$ 7.1 (4)*	44	134.8 $\pm$ 9.3 (4)*	89
5 mM D-glucose + 0.1 mM MeGlc						
No substrate (control)	0.16 $\pm$ 0.02 (6)	—	0.67 $\pm$ 0.04 (6)	100	3.42 $\pm$ 0.19 (7)	100
Pyruvate	N.D.	—	0.38 $\pm$ 0.08 (6)**	57	3.41 $\pm$ 0.33 (7)	99

Figure 1 Concentration-dependence of the inhibition of phenylephrine-stimulated glucose transport by pyruvate, lactate and β -hydroxybutyrate

Cardiomyocytes were preincubated as described in the Materials and methods section, and then pyruvate, lactate or β -hydroxybutyrate was added for 20 min at the indicated concentrations. The cells were then stimulated with phenylephrine (100 μ M) for 30 min, and then the rate of dGlc transport was determined as described. Data are means $\pm$ S.E.M. from 3–5 independent experiments. The curves correspond to the fittings obtained as detailed in the Materials and methods section.

propionate did not inhibit glucose transport to a higher degree than that with either agent alone. Similarly, with pairs of substrates containing β -hydroxybutyrate, the rate of glucose transport was not lower than that with pyruvate or propionate alone (but was lower than that with β -hydroxybutyrate alone, which is less effective than the other two agents) (Table 3).

Effects of substrates in cardiomyocytes from fasted and diabetic rats

In the next series of experiments, we examined the sensitivity of glucose transport to the inhibitory effects of substrates in cardiomyocytes isolated (i) from rats fasted for 48 h, and (ii) from rats made diabetic with streptozotocin. In cells from fasted animals (Table 4), the degree of substrate-induced depression of basal and phenylephrine-stimulated glucose transport was similar

Table 3 Effects of treatment of cardiomyocytes with combinations of pyruvate, propionate and β -hydroxybutyrate on phenylephrine-stimulated glucose transport

Cardiomyocytes were preincubated as described in the Materials and methods section before addition of pyruvate (3 mM), propionate (4 mM) and/or β -hydroxybutyrate (3 mM) for 20 min, as indicated. The cells were then exposed to phenylephrine (100 μ M) for another 20 min, and the rate of dGlc uptake was determined. Data are means $\pm$ S.E.M. from the numbers of independent experiments indicated in parentheses.

Additions	Rate of dGlc uptake (pmol/h per mg of protein)
None (basal)	27.8 $\pm$ 3.7 (4)
Phenylephrine	79.7 $\pm$ 5.7 (4)
+ Pyruvate	50.8 $\pm$ 4.9 (4)
+ Pyruvate + propionate	44.5 $\pm$ 6.3 (3)
+ Pyruvate + β -hydroxybutyrate	56.5 $\pm$ 7.7 (3)
+ Propionate	45.4 $\pm$ 7.6 (4)
+ Propionate + β -hydroxybutyrate	38.7 (2)
+ β -Hydroxybutyrate	68.5 $\pm$ 1.6 (3)

to that found in cardiomyocytes from fed rats (see Table 1). In contrast, significant inhibition of insulin-stimulated transport was not detected in the former cells.

Since the animals made diabetic with streptozotocin were male Wistar rats (instead of Sprague–Dawley females as used in all the other studies), a corresponding control group (i.e. cardiomyocytes from healthy male Wistar rats) was also used. The effects of substrates in these control cells (Table 5) were similar to those observed in myocytes from Sprague–Dawley rats (Table 1). As shown in Table 5, the percentage inhibition of phenylephrine-dependent glucose transport was approximately the same in cells from control and diabetic animals. In insulin-stimulated cells from diabetic rats, the depression of glucose transport was weaker or even lost when compared with control cells (Table 5), in analogy with our findings in cells from starved rats (Table 4).

Signals involved in glucose transport inhibition

The following series of experiments was aimed at defining the potential cellular signal(s) involved in mediating the observed

Table 4 Effects of alternative substrates on dGlc uptake in cardiomyocytes from fasted rats

Rats were starved for 48 h (with free access to drinking water) before cardiomyocytes were isolated. The isolated cells were preincubated as described in the Materials and methods section before addition of pyruvate (3 mM), propionate (4 mM), octanoate (3 mM) or β -hydroxybutyrate (3 mM) for 15 min. Phenylephrine (100 μ M) or insulin (18 nM) was then added for another 30 min, and the rate of dGlc uptake was measured. Values are means $\pm$ S.E.M., expressed as pmol/h per mg of protein. The number of independent experiments is indicated in parentheses. Percentage and *P* values were determined as compared with the corresponding control measured in the absence of substrate. Significance of differences: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Additions	Rate of dGlc uptake					
	Basal		Phenylephrine		Insulin	
	(pmol/h per mg)	(%)	(pmol/h per mg)	(%)	(pmol/h per mg)	(%)
None (control)	20.0 $\pm$ 3.2 (6)	100	42.2 $\pm$ 7.1 (6)	100	219.2 $\pm$ 8.5 (6)	100
Pyruvate	12.3 $\pm$ 1.3 (5)**	62	25.7 $\pm$ 4.2 (6)**	61	205.5 $\pm$ 7.7 (5)	94
Propionate	15.5 $\pm$ 1.5 (5)*	78	23.9 $\pm$ 2.0 (6)***	57	214.4 $\pm$ 6.2 (5)	98
Octanoate	17.7 $\pm$ 1.4 (6)	88	44.0 $\pm$ 3.0 (6)	104	199.3 $\pm$ 15.7 (5)	91
β -Hydroxybutyrate	14.7 $\pm$ 1.9 (6)*	73	36.2 $\pm$ 1.9 (6)*	86	221.0 $\pm$ 6.5 (5)	101

Table 5 Comparison of the effects of substrates on dGlc uptake in cardiomyocytes from healthy and diabetic Wistar rats

Cardiomyocytes isolated from either healthy male Wistar rats or from streptozotocin-diabetic littermates were preincubated as described in the Materials and methods section before the addition of pyruvate (3 mM), propionate (4 mM), β -hydroxybutyrate (3 mM) palmitate (0.8 mM) or α -oxoglutarate (10 mM) for 15 min. Phenylephrine (100 μ M) or insulin (18 nM) was then added for another 30 min, and the rate of dGlc uptake was determined. Values are means $\pm$ S.E.M., and are expressed in pmol/h per mg of protein. The numbers of independent experiments are indicated in parentheses. Percentage and *P* values were determined as compared with the corresponding control measured in the absence of substrate. Significance of differences: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Additions	Rate of dGlc uptake			
	Cardiomyocytes from healthy rats		Cardiomyocytes from diabetic rats	
	(pmol/h per mg)	(%)	(pmol/h per mg)	(%)
Basal	18.2 $\pm$ 3.5 (4)		7.3 $\pm$ 1.8 (5)	
Phenylephrine	85.2 $\pm$ 5.7 (3)	100	38.2 $\pm$ 2.5 (4)	100
+ Pyruvate	45.5 $\pm$ 10.4 (3)*	53	20.1 $\pm$ 4.5 (4)*	53
+ Propionate	39.8 $\pm$ 1.5 (3)***	47	15.6 $\pm$ 1.9 (4)***	41
+ Palmitate	81.8 $\pm$ 6.1 (3)	96	37.2 $\pm$ 4.3 (4)	97
+ β -Hydroxybutyrate	70.9 $\pm$ 6.3 (3)	83	31.4 $\pm$ 2.1 (4)*	82
+ α -Oxoglutarate	72.2 $\pm$ 3.7 (3)*	85	29.3 $\pm$ 2.0 (4)*	77
Insulin	196.9 $\pm$ 11.6 (4)	100	106.7 $\pm$ 4.3 (5)	100
+ Pyruvate	160.2 $\pm$ 19.2 (4)	81	98.3 $\pm$ 9.9 (4)	92
+ Propionate	176.4 $\pm$ 7.2 (4)*	90	107.8 $\pm$ 5.3 (4)	101
+ Palmitate	169.3 $\pm$ 14.6 (4)	86	114.6 $\pm$ 7.3 (4)	108
+ β -Hydroxybutyrate	150.9 $\pm$ 11.4 (4)*	77	102.6 $\pm$ 3.3 (4)	96
+ α -Oxoglutarate	168.9 $\pm$ 13 (4)	86	95.4 $\pm$ 7.2 (4)	89

effects of alternative substrates. A first approach ensued from our previously reported observation that amino acids such as L-alanine increase glucose transport in cardiomyocytes [31]. Alanine formation from pyruvate occurs in heart through a reversible transamination reaction [32–34]. It was therefore conceivable that the opposite effects of alanine and pyruvate on glucose transport are mediated by the same reaction, the direction in which it takes place determining whether a stimulation or an inhibition occurs. We consequently decided to examine the role

Table 6 Effects of aminotransferase inhibitors, and of alanine and valine, on basal and phenylephrine-stimulated glucose transport

Cardiomyocytes were preincubated as described in the Materials and methods section before addition of D,L-cycloserine (0.5 mM), amino-oxyacetate (0.1 mM), L-alanine (3 mM) or L-valine (10 mM) for 15 min, as indicated. The cells were then further incubated in the absence (basal) or in the presence of phenylephrine (100 μ M) for another 30 min, and the rate of dGlc uptake was measured (pmol/h per mg of protein). Data are means $\pm$ S.E.M. of the numbers of independent experiments indicated in parentheses. Percentage and *P* values were determined as compared with the corresponding control measured in the absence of inhibitors or amino acids. Significance of differences: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Additions	Rate of dGlc uptake			
	Basal		Phenylephrine	
	(pmol/h per mg)	(%)	(pmol/h per mg)	(%)
None (control)	24.4 $\pm$ 1.4 (6)	100	90.0 $\pm$ 4.8 (6)	100
Cycloserine	52.4 $\pm$ 3.9 (6)**	215	133.2 $\pm$ 13.9 (3)*	148
Amino-oxyacetate	60.1 $\pm$ 4.6 (6)***	246	208.3 $\pm$ 41.5 (5)*	231
Alanine	41.7 $\pm$ 2.4 (6)***	171	117.1 $\pm$ 8.7 (6)**	130
Valine	48.0 $\pm$ 2.9 (3)**	197	124.5 $\pm$ 5.7 (4)**	138

of aminotransferases in the action of pyruvate on glucose transport by using selective inhibitors of these enzymes, namely D,L-cycloserine [35] and amino-oxyacetate [36]. As illustrated in Table 6, these inhibitors alone (i.e. in the absence of phenylephrine or insulin) caused a rise in glucose transport in basal cells by 115% and 146% respectively. These effects were also detected in myocytes stimulated with phenylephrine (Table 6). Similarly, alanine and valine induced increases in glucose transport in basal (as previously found [31]) and phenylephrine-stimulated cells (Table 6).

The influence of cycloserine treatment on the inhibitory action of pyruvate was also examined. As shown in Table 7, depression of glucose transport by pyruvate in phenylephrine- or insulin-stimulated cells was, at least partially, counteracted by the aminotransferase inhibitor. In contrast, a selective inhibitor of pyruvate carboxylation, i.e. hydroxymalonate [37], failed to affect the rate of pyruvate-inhibited glucose transport (Table 7).

Transamination reactions are known, on the one hand, to influence the pool size of the citrate cycle (e.g. through the

Table 7 Influence of cycloserine and hydroxymalonate on the effect of pyruvate on phenylephrine- and insulin-stimulated glucose transport

Cardiomyocytes were preincubated as described in the Materials and methods section before addition of D,L-cycloserine (0.5 mM) or hydroxymalonate (3 mM) for 15 min, as indicated. The cells were then exposed for another 15 min to pyruvate (3 mM), followed by the addition of phenylephrine (100 μ M) or insulin (18 nM) for 30 min. Subsequently, the rate of dGlc uptake was determined. Data are means $\pm$ S.E.M. of the numbers of independent experiments indicated in parentheses. Percentage and *P* values were determined as compared with the corresponding control measured in the presence of pyruvate alone. Significance of differences: **P* < 0.05; ***P* < 0.01.

Additions	Rate of dGlc uptake			
	Phenylephrine		Insulin	
	(pmol/h per mg)	(%)	(pmol/h per mg)	(%)
None (control)	87.4 $\pm$ 8.9 (6)	100	275.0 $\pm$ 12.7 (6)	100
Cycloserine	129.4 $\pm$ 13.9 (3)	148	295.1 $\pm$ 12.3 (4)	107
Pyruvate	51.7 $\pm$ 8.4 (5)	59	205.7 $\pm$ 35.5 (5)	75
Cycloserine + pyruvate	106.5 $\pm$ 18.8 (5)**	122	269.2 $\pm$ 21.7 (5)*	98
Hydroxymalonate	97.9 $\pm$ 8.8 (6)	112	305.3 $\pm$ 32.5 (4)	111
Hydroxymalonate + pyruvate	49.0 $\pm$ 11.4 (6)	56	214.2 $\pm$ 33.5 (4)	78

formation of malate) [32–34]; citrate itself was shown, on the other hand, to mediate the inhibition of glycolysis by alternative substrates (at the level of the phosphofructokinase) [13,14]. We have therefore examined changes in the cellular content of citrate, malate and glutamate as induced by substrates, amino acids and aminotransferase inhibitors. These substances produced measurable alterations in the levels of citrate, malate and/or glutamate (under the same conditions as those found to influence glucose transport) (Table 8). Note that the metabolite concentrations measured, as well as the effects of substrates on citrate cycle intermediates, are in good agreement with reported studies using intact hearts [33,34,38–40]. For instance, pyruvate, acetate and palmitate produced an increase, and amino-oxyacetate a decrease, in the citrate concentration (Table 8) comparable with the changes seen in hearts [33,34,39].

Apart from some exceptions mentioned below, substrates that are inhibitory with respect to glucose transport, such as pyruvate and propionate, increased the citrate and malate concentrations, and tended to decrease the glutamate concentration in cardiomyocytes. Conversely, transport-stimulating agents such as alanine and valine induced a decrease in citrate and malate, and tended to increase glutamate (Table 8). When the rate of glucose transport measured in the presence of substrates was plotted as a function of the metabolite concentrations found under the same conditions, a negative correlation between glucose transport and citrate content (Figure 2A) or malate content (Figure 2B), and a positive one with glutamate content (Figure 2C), became apparent. This correlation was assessed by non-linear regression analysis and was found to be significant (glucose transport versus citrate: $r = -0.79$, $P < 0.01$; glucose transport versus malate: $r = -0.80$, $P < 0.01$; glucose transport versus glutamate: $r = 0.70$, $P < 0.05$). Similar correlations were obtained for phenylephrine- and insulin-stimulated cardiomyocytes ($r = -0.81$ and -0.72 for citrate, $r = -0.83$ and -0.70 for malate, and $r = 0.81$ and 0.81 for glutamate respectively).

Although these correlations are indicative of some relationship between the observed changes in metabolite concentrations and glucose transport activity, there are deviations from the simple scheme defined above. Thus palmitate caused a large increase in the level of citrate, but hardly affected glucose transport, while substrates with a more modest effect on citrate (such as acetate or lactate) were most efficient in inhibiting glucose transport (Figure 2A). Furthermore, these latter two substrates had no influence on the cellular glutamate content, but they produced the same inhibition of glucose transport as propionate, which dramatically decreased glutamate levels (Figure 2C). Another discrepancy is seen with respect to malate: as indicated in Figure 2(B), octanoate markedly decreased the level of this metabolite without affecting glucose transport, whereas agents that bring about a similar fall in malate (e.g. valine) clearly stimulated the uptake of glucose.

In view of the well known allosteric effect of citrate on phosphofructokinase activity, it was possible that the observed changes in citrate induced by most substrates would also affect glycolysis, and possibly also the concentration of glucose 6-

Table 8 Effects of alternative substrates, amino acids and aminotransferase inhibitors on the cellular concentrations of citrate, malate, glutamate and glucose 6-phosphate in non-stimulated cardiomyocytes

Basal cardiomyocytes were treated with substrates, amino acids or aminotransferase inhibitors (cycloserine, amino-oxyacetate) as described in the legends of Tables 1 and 6, and citrate, malate, glutamate and glucose 6-phosphate levels were determined as described in the Materials and methods section. Values are means $\pm$ S.E.M. (expressed as content per litre of cell water); the numbers of independent experiments are indicated in parentheses. Significance of differences compared with corresponding control: **P* < 0.05; ***P* < 0.01; ****P* < 0.001. N.D., not determined.

Additions	Citrate (μ mol/l)	Malate (μ mol/l)	Glutamate (mmol/l)	Glucose 6-phosphate (μ mol/l)
Control (no addition)	136 $\pm$ 8 (20)	100 $\pm$ 4.3 (34)	3.60 $\pm$ 0.35 (10)	203 $\pm$ 26 (19)
Pyruvate	1299 $\pm$ 206 (8)***	641 $\pm$ 60 (20)***	1.47 $\pm$ 0.24 (5)**	312 $\pm$ 38 (10)***
Lactate	281 $\pm$ 34 (8)***	197 $\pm$ 24 (6)**	3.70 $\pm$ 0.54 (5)	215 $\pm$ 29 (5)
Propionate	242 $\pm$ 52 (8)*	313 $\pm$ 45 (5)**	0.84 $\pm$ 0.11 (4)*	208 $\pm$ 35 (5)
Octanoate	152 $\pm$ 11 (5)	37 $\pm$ 6 (5)***	2.75 $\pm$ 0.39 (4)*	194 $\pm$ 39 (3)
Palmitate	618 $\pm$ 52 (7)***	110 $\pm$ 10 (6)	N.D.	220 $\pm$ 33 (4)
β -Hydroxybutyrate	228 $\pm$ 40 (6)	90 $\pm$ 29 (6)	2.41 $\pm$ 0.42 (5)*	219 $\pm$ 20 (3)
Acetate	237 $\pm$ 35 (5)*	155 $\pm$ 35 (6)	3.58 $\pm$ 0.64 (3)	260 $\pm$ 63 (3)
α -Oxoglutarate	406 $\pm$ 71 (8)**	861 $\pm$ 129 (6)***	3.29 $\pm$ 0.32 (4)	283 $\pm$ 57 (3)
Alanine	87 $\pm$ 10 (9)***	45 $\pm$ 10 (6)**	3.95 $\pm$ 0.74 (5)	176 $\pm$ 77 (3)
Valine	115 $\pm$ 14 (8)	40 $\pm$ 14 (4)*	5.49 $\pm$ 0.66 (4)*	280 (2)
Cycloserine	98 $\pm$ 11 (9)*	56 $\pm$ 16 (5)	4.38 $\pm$ 0.56 (6)	239 (2)
Amino-oxyacetate	92 $\pm$ 20 (5)*	28 $\pm$ 11 (4)**	N.D.	187 $\pm$ 41 (3)

Figure 2 Correlation between substrate-dependent changes in glucose transport and in the cellular levels of citrate, malate and glutamate, or in the rate of [$1\text{-}^{14}\text{C}$]pyruvate oxidation

Basal cardiomyocytes were treated with substrates, amino acids or aminotransferase inhibitors as described in the legends to Tables 1 and 6, and the following parameters were determined as detailed in the Materials and methods section: the rate of dGlc transport, the cellular concentrations of citrate, malate and glutamate, and the rate of $^{14}\text{CO}_2$ production from [$1\text{-}^{14}\text{C}$]pyruvate. Glucose transport data are from the experiments presented in Tables 1 and 6, the citrate, malate and glutamate concentrations are taken from Table 8, and pyruvate oxidation data are those shown in Table 9.

phosphate. We therefore measured the glucose 6-phosphate content of cardiomyocytes under the influence of the substrates. However, apart from the increase seen upon treatment with pyruvate, no significant changes were detected (Table 8).

Besides phosphofructokinase, another key site in the control of glucose utilization by alternative substrates is the PDH complex. We have examined the influence of substrates on the rate of $^{14}\text{CO}_2$ production from [$1\text{-}^{14}\text{C}$]pyruvate (which is a measure of pyruvate decarboxylation via PDH) under conditions where the described effects on glucose transport occur. As shown in Table 9, some of the agents tested increased the rate of pyruvate oxidation up to 2.5-fold compared with the control. Among these were glucose transport stimulators (valine, cycloserine) as well as transport inhibitors (β -hydroxybutyrate, α -oxoglutarate, propionate). By comparison, the known PDH activator dichloroacetate [41] caused a 3.5-fold rise in pyruvate oxidation in these cells (Table 9). Dichloroacetate also produced a 1.8 ± 0.2 -fold increase in glucose transport (results not shown). Thus no correlation between the effects of substrates on PDH activity on the one hand, and on glucose transport on the

other, appear to exist under the experimental conditions used (Figure 2D).

Influence of pyruvate on cell-surface amounts of the GLUT4 and GLUT1 glucose transporters

We finally investigated the mechanism involved in the changes in glucose transport. In principle, the rate of glucose transport may be determined by the number of glucose transporters in the plasma membrane, and/or by the intrinsic activity of these transporters (affinity changes do not appear to be relevant in mammalian tissues). To differentiate between these two possibilities, we have used the selective non-permeant photo-affinity label ATB-BMPA in order to quantify the abundance of GLUTs at the surface of cardiomyocytes previously incubated in the presence or in the absence of pyruvate. As shown in Table 10, pyruvate treatment resulted in a decrease in the labelling of the so-called insulin-regulatable transporter GLUT4 in these cells. Thus the amount of labelled GLUT4 was significantly reduced to

Table 9 Effects of alternative substrates, amino acids, cycloserine and dichloroacetate on the rate of $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]$ pyruvate

Basal cardiomyocytes were treated for 30 min at 37 °C with agents (used at the same concentrations as detailed in the legends of Tables 1 and 6) or with dichloroacetate (2 mM), and then the rate of $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]$ pyruvate was measured. Values are means $\pm$ S.E.M. of the numbers of independent experiments indicated in parentheses. Percentage and *P* values were determined as compared with the control. Significance of differences: **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Additions	Rate of $^{14}\text{CO}_2$ production from $[1-^{14}\text{C}]$ pyruvate	
	(nmol/10 min per mg of protein)	(%)
None (control)	1.64 $\pm$ 0.17 (21)	100
Valine	2.76 $\pm$ 0.51 (10)*	168
Alanine	1.51 $\pm$ 0.13 (6)	92
Cycloserine	3.00 $\pm$ 0.57 (3)*	183
β -Hydroxybutyrate	2.12 $\pm$ 0.39 (5)*	129
Palmitate	1.22 $\pm$ 0.30 (6)	74
Acetate	1.70 $\pm$ 0.21 (8)	104
α -Oxoglutarate	2.06 $\pm$ 0.31 (9)*	126
Propionate	4.08 $\pm$ 0.53 (6)**	249
Dichloroacetate	5.71 $\pm$ 0.68 (16)***	348

Table 10 Effect of pyruvate on the content of glucose transporters GLUT4 and GLUT1 at the surface of cardiomyocytes

Cardiomyocytes were preincubated as described in the Materials and methods section, and pyruvate (3 mM) was added for 15 min before addition of phenylephrine (100 μM) or insulin (30 nM) for a further 45 min. The content of cell-surface glucose transporters GLUT4 and GLUT1 was then determined by using the selective photoaffinity label ATB-BMPA, as described in the Materials and methods section. Values are means $\pm$ S.E.M. of the numbers of independent experiments indicated in parentheses. Percentage and *P* values were determined as compared with the corresponding control measured in the absence of pyruvate. Significance of differences: **P* < 0.05.

Additions	GLUT4 content at cell surface		GLUT1 content at cell surface	
	(arbitrary units)	(%)	(arbitrary units)	(%)
Phenylephrine	1.37 $\pm$ 0.08 (4)	100	1.59 $\pm$ 0.19 (4)	100
Phenylephrine + pyruvate	0.90 $\pm$ 0.21 (4)*	66	1.10 $\pm$ 0.22 (4)*	69
Insulin	4.90 $\pm$ 0.83 (7)	100	2.77 $\pm$ 0.31 (5)	100
Insulin + pyruvate	3.58 $\pm$ 1.18 (7)*	73	2.62 $\pm$ 0.87 (5)	95

66% and 73% of the control value (no pyruvate) in phenylephrine- and insulin-stimulated cells respectively. Similarly, cell-surface labelling of another transporter expressed in cardiomyocytes (GLUT1) was reduced to 69% of control in phenylephrine-treated cells, but with no detectable change in insulin-treated cells (Table 10).

DISCUSSION

The present study substantiates the notion that cardiomyocyte glucose transport is subject to counter-regulation by alternative substrates of energy metabolism. Importantly, it was found that glucose transport is depressed not only by fatty acids and ketone bodies, as described in studies on heart [1,15] and skeletal muscle [16], but also, and to a larger extent, by other substrates and metabolites such as pyruvate, lactate and propionate. Moreover, the present work demonstrates for the first time that there is a

tight coupling between intermediary metabolism and the glucose transport system in these cells.

Mechanism of action of pyruvate on glucose transport

Our experiments using the selective non-permeant photoaffinity label ATB-BMPA clearly showed that pyruvate induces a decrease in the amount of GLUT4 (and, in phenylephrine-stimulated cells, also of GLUT1) in the plasma membranes of cardiomyocytes (Table 10). Importantly, the percentage changes in ATB-BMPA labelling were roughly the same as those in glucose transport (Tables 1 and 2), indicating that no additional process (such as a change in the intrinsic activity of the transporters) is needed to explain the full extent of transport inhibition by pyruvate. Since the abundance of GLUTs in the plasma membrane is governed by their rates of exocytosis and endocytosis [42], our results indicate that a substrate-dependent signal interferes with some step of cellular GLUT trafficking. Bearing in mind (i) that substrates inhibited non-stimulated as well as stimulated glucose transport, and (ii) that the stimuli used (phenylephrine and insulin) affect glucose transport via different signalling pathways [23,43], it appears likely that substrates act on a basic process involved in GLUT trafficking and not at the level of a stimulus-specific signal.

Coupling of intermediary metabolism and glucose transport

Two lines of evidence show that the glucose transport system is closely linked to intermediary metabolism: (i) a correlation between the levels of intermediary metabolites (citrate, malate, glutamate) and the rate of glucose transport, and (ii) indications that aminotransferase reactions are involved in at least some of the observed effects.

As detailed in the Results section, close examination of the correlations illustrated in Figure 2 reveal some exceptions from a simple rule linking, for instance, an increase in citrate levels with a decrease in glucose transport. These exceptions may indicate that none of citrate, malate and glutamate represents the direct effector modulating glucose transport. Alternatively, since these metabolites are known to exist in at least two compartments (cytosol and mitochondria), it is conceivable that overall concentrations measured in this study do not accurately reflect crucial changes occurring in the relevant compartment, although, in the case of citrate, it has been shown that the cytosolic and mitochondrial concentrations change in parallel under all conditions tested in perfused hearts [44].

Another novel finding from the present study is the influence of aminotransferase reactions on glucose transport. First, we observed that selective inhibitors of aminotransferases, i.e. cycloserine and amino-oxyacetate, themselves had stimulatory effects on glucose transport (Table 6). This, along with the similar effects of the amino acids alanine and valine, suggests (i) that, in the absence of substrates, transamination reactions normally lead to the formation of, for example, alanine or valine, and (ii) that this process is somehow causing glucose transport inhibition. Secondly, inhibition of glucose transport by pyruvate was counteracted by cycloserine (but not by hydroxymalonate, an inhibitor of pyruvate carboxylation) (Table 7). Thirdly, we had previously found that the stimulatory effect of alanine was suppressed by pyruvate [31], its deamination product. Interestingly, the concentrations of two other metabolites involved in reversible transaminations, i.e. α -oxoglutarate and aspartate, have been shown to undergo dramatic changes under the influence of amino-oxyacetate, in terms of a decrease in α -oxoglutarate and a concomitant increase in aspartate [34]. Conversely, substrates (such as lactate, pyruvate, acetate and

propionate) induce an elevation in α -oxoglutarate and a fall in aspartate in heart [32–34,40,45]. Moreover, increased cardiac work, which is known to stimulate glucose transport [15], was reported to markedly increase the aspartate level [45]. In view of all these observations, further investigations should be focused on a potential role of products of transamination reactions as effectors of the glucose transport system.

The present investigation has already ruled out a potential signal that often reflects changes in intermediary metabolism, namely glucose 6-phosphate. Previous reports had suggested that glucose 6-phosphate may act as a regulator of glucose transport in fat cells and muscle [46,47]. However, we could not detect significant changes in the concentration of glucose 6-phosphate in cardiomyocytes (with one exception) (Table 8) under conditions where glucose transport was greatly affected. In line with this observation, no correlation was found between glucose transport and the levels of glucose 6-phosphate in heart and muscles under the influence of different substrates [48].

Finally, it appears likely that the changes in the levels of citrate cycle intermediates (citrate, malate) are accompanied by changes in the signals known to control pyruvate decarboxylation through PDH, mainly the NADH/NAD⁺ ratio [9,38,40] and the acetyl-CoA/CoA ratio [9,30,38,40]. However, we found no correlation between changes in the rate of [1-¹⁴C]pyruvate oxidation on the one hand, and effects on glucose transport on the other (Figure 2). Thus propionate, for instance, had a marked stimulatory effect on pyruvate oxidation (Table 9), in keeping with observations in perfused hearts [40], whereas it was one of the most efficient inhibitors of glucose transport (Table 1). This lack of a correlation shows that the processes of glucose uptake and oxidation are differentially regulated, at least under the conditions prevailing in the present study.

Physiological relevance of the effects of substrates on glucose transport

The present investigation using isolated cells does not allow us to define exactly under which physiological conditions the effects of substrates on glucose transport may be operative or relevant *in vivo*. However, some of our observations may be of interest in this regard.

First, we found that the effects of substrates on glucose transport are more manifest in the presence of a submaximal insulin concentration than a supramaximal dose of the hormone (Table 1). This is in line with early observations reported by Randle and co-workers on the action of β -hydroxybutyrate on glucose transport in heart [1]. This result suggests that inhibition of glucose transport by substrates is of little relevance in the postprandial state, where insulinaemia is high. In addition, at saturating concentrations of the hormone, glucose transport is no longer the rate-limiting step of glucose utilization in heart [18,19], so that depression of transport may barely affect the overall rate of glucose disposal in this situation.

On the other hand, the sensitivity of glucose transport to inhibition may be largely affected by other factors, such as adrenergic activation. In accordance with this notion, we found that the rate of glucose transport stimulated by the α -adrenergic agonist phenylephrine was most responsive to the action of substrates (Table 1). Contractile activity may be another factor determining the sensitivity of the glucose transport system to inhibitory effects. In keeping with this idea, inhibition of basal and insulin-stimulated glucose transport by palmitate or β -hydroxybutyrate is greater in beating perfused hearts (35–70% decrease [1,15]) than in quiescent cardiomyocytes as used in the present study (7–16% decrease; Table 1). Furthermore, the

effect of palmitate on MeGlc transport was found to be much more pronounced at a high perfusion pressure (where contractile activity is increased) than at a low one [15].

Another result worth pointing out is that lactate, which along with glucose and palmitate is a major physiological substrate of the myocardium, was one of the most potent inhibitors of glucose transport in this study (Table 1). Moreover, half-maximal inhibition by lactate occurred at ~ 1 mM (Figure 1), which lies within the normal range of plasma concentrations (0.5–1.0 mM in the resting state [49]). Since the level of blood lactate can increase dramatically during exercise (up to 12 mM [49]), the effect of this substrate on glucose transport may become important in controlling cardiac glucose disposal.

Since phenylephrine- or workload-stimulated glucose transport is substantially depressed by substrates, and given the extent and concentration-dependence of the effect of lactate, it is tempting to speculate that physical exercise represents a physiological situation in which the inhibitory mechanism described here may be operative.

Starvation and diabetes are known to impair glucose uptake and metabolism in heart and skeletal muscle, which is probably due to the combined influence of several defects. We found that glucose transport was no more sensitive in cells from fasted or diabetic rats than in those from fed control animals (Tables 4 and 5). This indicates that no intrinsic change in cardiomyocytes due to diabetes or starvation appears to contribute to, or to promote, an inhibition of glucose transport by substrates (at least, no change that is preserved upon cell isolation). On the other hand, starvation and diabetes cause large increases in the plasma levels of palmitate and β -hydroxybutyrate (e.g. up to ~ 1.5 mM in rats [4,50,51]). The IC₅₀ of transport inhibition by the latter substrate was ~ 0.75 mM (see Figure 1). It is also noteworthy that alterations in cardiac dGlc uptake *in vivo* display the same temporal pattern (but in the opposite direction) as changes in the plasma levels of fatty acids and β -hydroxybutyrate in different phases of starvation [4,50]. Thus glucose transport inhibition by these substrates may well contribute, along with other effects or defects, to decreased glucose utilization in starvation and diabetes.

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