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# Possible involvement of alanine and pyruvate in the regulation of glucose transport in heart muscle cells

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In isolated rat heart muscle cells, addition of L-alanine (1.5 mmol/l) or of L-valine (3 mmol/l) resulted in either a ca 1.5- or 1.3-fold increase in glucose transport, resp. half-maximal stimulation was observed in the presence of L-alanine, but not of L-valine, within a physiological plasmatic range of concentrations. D-Alanine (1.5 mmol/l) was ineffective and the stimulating effect of L-alanine could be prevented by an excess of L-serine (15–30 mmol/l). L-Alanine produced an increase in 3-O-methyl-D-glucose transport $V_{max}$ (from 44.6 to 81.5 pmol·s$^{-1}$·mg protein$^{-1}$) without affecting the $K_m$ (12.2 in control vs 12.8 mmol/l in alanine-treated cells). Pyruvate (1.5 mmol/l) inhibited glucose transport by 20% and prevented the stimulating action of L-alanine (1.5 mmol/l). These results suggest that the effect of L-alanine in cardiac myocytes occurs through the interaction with an intracellular site and that both alanine and pyruvate may play a role in the regulation of glucose transport in these cells.

Sugar transport; Glucose; Heart; Alanine; Pyruvate

## 1. INTRODUCTION

Alanine represents a major gluconeogenic precursor for the liver. Numerous lines of evidence indicate that this amino acid is specifically released by skeletal [1–4] and heart muscle [5] in the fasted state, especially under conditions of augmented glucose utilization in these tissues [2,6]. We recently found that alanine is, in part, responsible for the stimulating effect of partially purified samples from a yeast extract on glucose transport in isolated cardiac myocytes [7,8]. This observation supports the idea that the relevance of alanine in carbohydrate metabolism may not be restricted to its release from cardiac and skeletal muscles and subsequent captation by the liver in the fasted state, but may also involve a direct effect of this amino acid on a peripheral tissue under certain conditions. In this context, it is of interest to note that alanine is taken up (and not released) by skeletal muscle in fed rats [3]. Moreover, the exercise-induced release of alanine is prevented by an increased glucose availability in humans [6]. It was further shown that alanine is a readily oxidizable substrate in peripheral tissues [9,10]. The aim of the present study was to characterize the stimulating action of alanine on glucose transport in isolated cardiomyocytes. Further, we examined the possible involvement of intracellular pyruvate in this effect. The data presented here suggest that, in cardiomyocytes, the uptake of glucose may be regulated by products of intermediary metabolism, like alanine and pyruvate.

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## 2. MATERIALS AND METHODS

### 2.1. *Chemicals*

3-O-methyl-D-Glucose, phloretin and cytochalasin B were purchased from Serva (Heidelberg, FRG); all amino acids and pyruvate were obtained from Merck (Darmstadt, FRG); 3-O-[$^3$H]methyl-D-glucose and 2-deoxy-D-[$^3$H]glucose were from Amersham (Braunschweig, FRG). Silicon oil (Abil AV 200) was from Franken Chemie (Wedelstein, FRG); liquid scintillation fluid (Quickszint 212) was obtained from Zinsser (Frankfurt, FRG). Concentrated stock solutions of phloretin and cytochalasin B (in dimethylsulfoxide) were prepared as appropriate aliquots, stored at −20°C, and diluted just prior to addition to the isolated cardiomyocytes. Final concentrations of dimethylsulfoxide were typically 0.02–0.1% in the transport assays and did not affect basal transport activity.

All other chemicals and buffers were freshly prepared immediately before use.

### 2.2. *Isolation of calcium-resistant cardiomyocytes*

Calcium-resistant, rod-shaped cardiomyocytes from adult female Sprague–Dawley rats (180–220 g fed ad libitum) were obtained by a modification [11] of a method previously described [12]. These cells were characterized by a very low basal (i.e. non-stimulated) glucose uptake rate, that was increased 8- to 20-fold increase by insulin (10 nM) [11]. Prior to the experiments, the isolated myocytes were washed 3 times with assay medium (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 $CaCl_2$, 2% bovine serum albumine, fatty acid free, pH 7.4, 37°C, equilibrated with oxygen) and resuspended in the same medium at 2–5 mg cell protein per ml.

### 2.3. *Determination of 2-deoxy-D-glucose uptake*

1 ml of washed cell suspension was incubated with 0.5 ml of assay medium (basal) or of an appropriate dilution of the amino acid to be assayed (or pyruvate), in flat-bottomed 20 ml-vials, at 37°C in a shaking water bath (180 strokes/min, 5 cm/stroke), for 30 min. The transport assay was then started by adding 70 μl 2-deoxy-D-[$^3$H]glucose (DOG; 3 μCi/ml; final sugar concentration: 1.4–2 μM). The samples were incubated in the presence of DOG for an additional 30 min. Subsequently, sugar uptake was stopped by adding 100 μl 6.8 mM phloretin (400 μM final concentration); the samples were quickly

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vortexed, 400 μl-aliquots immediately taken and placed on top of 2 ml-centrifuge vials containing 1 ml silicon oil (density 1.03 g/ml) and 12 μl of 35% perchloric acid (on the bottom). These aliquots were then centrifuged at 10 000 × g for 1 min to separate the cells from the less dense incubation medium. The supernatants were removed, the tubes cut off and the bottoms (containing the cell pellets) were counted in a liquid scintillation counter. Specific, i.e. glucose-carrier-mediated DOG uptake was estimated by subtraction of uptake rates monitored in the presence of 400 μM phloretin from values measured in the absence of this inhibitor.

2.4. *Kinetics of 3-O-methyl-D-glucose (3-OMG) uptake*

175 μl washed cardiomyocytes were incubated in the absence (control) or the presence of alanine (1.5 mM) in a total volume of 275 μl in flat-bottomed, 4 ml-tubes, for 30 min at 37°C in a shaking water bath (see above). At the end of this preincubation, 50 μl of a prewarmed (37°C) solution containing 1.1 μCi 3-O-[$^3$H]methyl-D-glucose and an appropriate amount of unlabelled 3-O-methyl-D-glucose were added. The samples were immediately swirled 3–5 times (in about 3 s) and shaken for the rest of the assay time (90 s for basal, and 45 s for alanine-stimulated cells) in the water bath (37°C). The assay was terminated by rapidly adding 100 μl 2.6 mM phloretin (600 μM final concentration) and vortexing the samples 3 times. The cells (of a 300 μl-aliquot) were then separated from the incubation mixture as described above (DOG-assay). The extent of cell-bound radioactivity at zero time was measured in samples pretreated with the specific glucose transport inhibitor cytochalasin B (20 μM), in which the assay was stopped immediately upon 3-OMG-addition. The carrier-mediated, initial transport velocities were calculated by subtracting the uptake rates measured in the presence of cytochalasin B (0–420 s) from the values obtained in the absence of inhibitor.

## 3. RESULTS

### 3.1. *Effects of L-alanine and L-valine on glucose transport*

As previously reported [8], L-alanine and L-valine increase the rate of 2-deoxy-D-glucose (DOG) uptake in calcium-resistant isolated rat cardiomyocytes 1.5- or 1.3-fold, resp. (Table I). The concentration-dependence of these effects is shown in Fig. 1. Half-maximal stimulation was observed at approximately 0.2 mmol/l with alanine and 0.6–0.7 mmol/l with valine. Interestingly, alanine is effective in a range of concentrations (0.05–1 mmol/l) that corresponds to values measured in plasma samples of rats [3,9,13], dogs [14] and humans [1,2,5,15]. In contrast, the valine concentrations necessary to elicit a significant stimulation of glucose transport (0.3–1.5 mmol/l) lie above the physiological values in rats [16] and humans [2,5,15].

Table I

Effect of L-alanine, D-alanine and L-valine on basal 2-deoxy-D-glucose transport and influence of an excess of L-serine on L-alanine-induced transport stimulation

| Addition | 2-deoxy-D-glucose transport rate (% of basal) |
|---|---|
| None (basal) | 100 ± 10 |
| L-alanine (1.5 mM) | 151 ± 9 |
| D-alanine (1.5 mM) | 92 ± 11 |
| L-serine (15 mM) | 92 ± 7 |
| L-serine (15 mM) + L-alanine | 119 ± 7 |
| L-serine (30 mM) | 109 ± 7 |
| L-serine (30 mM) + L-alanine | 102 ± 15 |
| L-valine (3 mM) | 130 ± 12 |
| L-valine (3 mM) + L-alanine | 137 ± 13 |

DOG transport was measured in cardiomyocytes previously exposed (30 min, 37°C) to one or two amino acids, as indicated. For samples with both L-serine and L-alanine, the cells were incubated for 2 min with L-serine prior to L-alanine addition. Values represent relative uptake (as compared to 100% in basal cells). Data are means from 2 to 3 independent experiments ± SD (each experiment was done in triplicate).

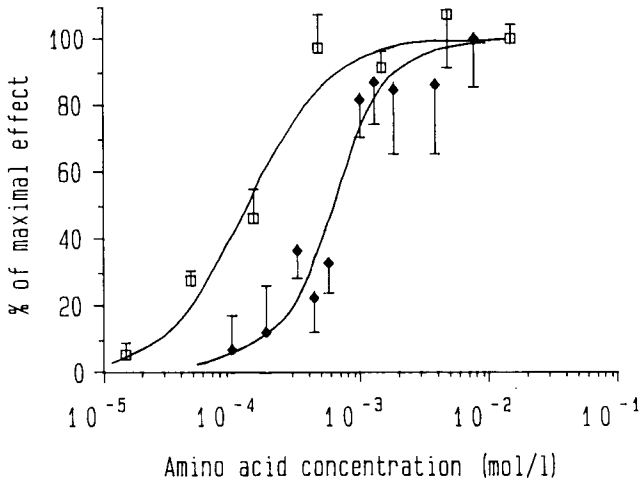


Fig. 1. Concentration dependence of the effect of L-alanine and L-valine on 2-deoxy-D-glucose transport. Data are expressed as percentage of the maximal effect induced by the corresponding amino acid in each experiment; L-alanine (□) and L-valine (◆). Data are means (± SE) from at least 2 separate experiments (each experiment in triplicate).

Further, the effects of alanine and valine are not additive (Table I). No significant changes in basal glucose transport could be detected in the presence of the other branched-chain amino acids L-leucine or L-isoleucine (0.5–10 mmol/l) (not shown).

### 3.2. *Effects of D-alanine on basal glucose transport and of L-serine on L-alanine-induced stimulation*

We next addressed the question as to whether L-alanine has to be taken up by the cardiomyocytes to induce its stimulating effect on glucose transport in these cells. The isomer D-alanine, that does not penetrate intact cells (owing to the stereospecificity of amino acid transport systems), had no detectable influence on DOG transport at a concentration (1.5 mmol/l) at which the natural L-isomer is maximally effective (Table I). As L-serine and L-alanine enter mammalian cells via a common amino acid transport system (the so-called ASC-system; [17]), an excess of one of these amino acids should inhibit the uptake of the other one. As shown in Table I, a large excess of L-serine (15–30 mmol/l) suppressed the effect of 1.5 mmol/l L-alanine.

### 3.3. *Kinetics of 3-O-methyl-D-glucose transport in alanine-stimulated cardiomyocytes*

To further characterize the action of alanine on glucose transport, we determined the kinetic parameters

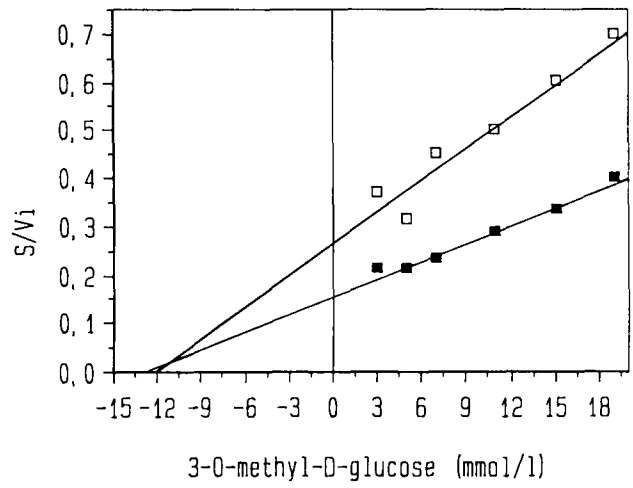


Fig. 2. Hanes plot of 3-O-methyl-D-glucose transport kinetics in basal and alanine-stimulated cells. The cytochalasin-B-sensitive, initial rate of 3-O-methyl-D-glucose uptake was determined at the concentrations indicated, in the absence (basal, □) or in the presence of 1.5 mM L-alanine (■). Each point represents the mean of at least 2 separate experiments ($n$ = 7 in each experiment).

of the uptake of the non-metabolizable glucose analog 3-O-methyl-D-glucose (3OMG) in basal (i.e. non-stimulated) and alanine-stimulated cardiomyocytes. $V_{max}$ and $K_m$ values were calculated by linear regression of data (initial 3OMG uptake) transformed in a Hanes plot (Fig. 2). Alanine (1.5 mmol/l) raised the maximal transport velocity from 44.6 (basal) to 81.5 pmol $3OMG \cdot s^{-1} \cdot mg\ protein^{-1}$, without significantly affecting the $K_m$ (12.18 in basal vs 12.84 mmol/l in alanine-treated cells).

### 3.4. *Effect of pyruvate on basal and alanine-stimulated glucose transport*

In order to investigate the possible involvement of intracellular pyruvate in the stimulation observed upon alanine addition, we tested the effect of pyruvate (1.5 mmol/l) (that is readily taken up by cardiac tissue; [18]) added alone or in combination with alanine (1.5 mmol/l), on the uptake of 2-deoxy-D-glucose. The data presented in Table II clearly show that pyruvate not only significantly inhibits basal DOG transport but also prevents the stimulating effect of alanine. Further, alanine did not produce a detectable change in the total intracellular pyruvate content (not shown).

Table II

Effect of pyruvate on basal and alanine-stimulated 2-deoxy-D-glucose transport

| Addition | 2-deoxy-D-glucose transport rate (% of basal) |
|---|---|
| None (basal) | 100 ± 11 |
| L-alanine (1.5 mM) | 140 ± 7 (**) |
| Pyruvate (1.5 mM) | 81 ± 9 (*) |
| Pyruvate + alanine | 79 ± 8 (*) |

DOG transport was measured in cardiomyocytes previously exposed (30 min, 37°C) to the agents, as indicated. Data represent relative transport, as compared to 100% basal. Values are means from 2 independent experiments. (*) Values are significantly different from basal at the 0.5% level; (**) at the 0.1% level (calculated with Student's $t$-test).

## 4. DISCUSSION

The role of alanine, released by skeletal [1–4] and heart [5] muscles in the fasted (but not in the fed state; [3]), as an essential precursor for hepatic gluconeogenesis is well-established [19]. Our observation that this amino acid stimulates glucose transport in cardiac myocytes (a step that is likely to be limiting for glucose metabolism) possibly reflects a new regulatory mechanism in carbohydrate metabolism of a peripheral tissue.

The fact that alanine is effective at concentrations that correspond to the physiological range in the plasma of mammals (Fig. 1 and [1–3,5,13–15] supports the idea that the effect we now observed may be of physiological relevance. Interestingly, it was recently reported that alanine inhibits the uptake and oxidation of glucose in isolated brown adipocytes [20]. This and our finding indicate that alanine may exert tissue-specific effects.

The action of valine was less pronounced than that of alanine (Table I). Moreover, valine only produced an increase in glucose transport at concentrations that markedly exceed the plasma values measured in rats [16] and humans [2,5,15]. The finding that alanine and valine do not act additively (Table I), along with the fact that branched-chain amino acids are the major source of nitrogen for pyruvate transamination [4,21–24] suggest that the effect of valine may be secondary to alanine formation in myocytes. However, the other branched-chain amino acids leucine and isoleucine failed to induce a significant stimulation of glucose transport in cardiomyocytes (unpublished observation). Thus, the metabolic basis of the valine effect remains to be clarified.

The results presented in Table I further demonstrate that alanine has to be taken up by the cardiomyocytes to elicit its effect. However, the alanine-induced stimulation is unlikely to involve the sodium influx concomitant to the alanine uptake, since serine, that is taken up via the same sodium-dependent amino acid carrier as alanine [17], had no effect on glucose transport (Table I).

The fact that alanine alters the $V_{max}$, but not the $K_m$, of glucose transport (Fig. 2) makes a direct interaction of this amino acid with glucose carriers unlikely, since a higher maximal transport velocity must involve either a larger number of functional carriers in the plasma membrane or a higher intrinsic activity of these carriers. Assuming that a high alanine uptake rate may also lead to an increase in the intracellular pyruvate concentration, we directly assayed the effect of pyruvate addition on glucose transport. Surprisingly, this metabolite

significantly inhibited the uptake of glucose and completely suppressed the stimulating action of alanine (Table II). Furthermore, no change in the total intracellular pyruvate concentration could be detected upon alanine addition (not shown). These findings are compatible with two types of explanations. The first possibility is that alanine and pyruvate modulate glucose transport independently, the former being stimulatory and the latter inhibitory. However, the advantage of this dual regulation is not easily conceivable since concentration changes of one of these metabolites should readily affect the concentration of the other one, owing to the high alanine aminotransferase (EC 2.6.1.2.) activity present in muscle tissue [25].

Alternatively, alanine and pyruvate may modulate some kind of metabolic signal via a common pathway, but in an antagonistic manner. They may, for instance, influence the flux through the cytosolic malate dehydrogenase (EC 1.1.1.37.) via transamination reactions involving the alanine aminotransferase and the aspartate aminotransferase (EC 2.6.1.1.) [4]. Thus, alanine would raise and pyruvate would lower the cytosolic NADH/NAD ratio. In adipocytes, alanine was indeed shown to increase the cellular glutamate and aspartate and decreases the malate and $\alpha$-ketoglutarate levels [26]. Changes in the cytosolic redox potential may, in turn, represent a signal for glucose transport regulation.

Further investigations will be required to examine these different possibilities. Nevertheless, our present results suggest that, in cardiac muscle cells, some form of metabolic coupling may exist between pyruvate and alanine, on one hand, and glucose transport, on the other hand.

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