Pyruvate shuttle in muscle cells: high-affinity pyruvate transport sites insensitive to \( trans \)-lactate efflux

Raymond Mengual,2 Kaoukib el Abida,1 Nassima Mouaffak,1 Michel Rieu,1 and Michele Beaudry1

1Laboratoire de Physiologie des Adaptations, Unité de Formation et de Recherche Cochin Port Royal, Université René Descartes, 75014 Paris; and 2Institut de Pharmacologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique, 06560 Sophia Antipolis Valbonne, France

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Pyruvate shuttle in muscle cells: high-affinity pyruvate transport sites insensitive to \( trans \)-lactate efflux. Am J Physiol Endocrinol Metab 285: E1196–E1204, 2003. First published August 12, 2003; 10.1152/ajpendo.00034.2003.—The specificity of the transport mechanisms for pyruvate and lactate and their sensitivity to inhibitors were studied in L6 skeletal muscle cells. \( Trans- \) and \( cis- \)lactate effects on pyruvate transport kinetic parameters were examined. Pyruvate and lactate were transported by a multisite carrier system, i.e., by two families of sites, one with low affinity and high capacity (type I sites) and the other with high affinity and low capacity (type II). The multisite character of transport kinetics was not modified by either hydroxycinnamic acid (CIN) or \( p \)-chloromercuribenzylsulfonic acid (PCMB), which exert different types of inhibition. The transport efficiency (TE) ratios of maximal velocity to the \( trans \)-activation dissociation constant \( (K_t) \) showed that lactate and pyruvate were preferentially transported by types I and II sites, respectively. The \( cis \)-lactate effect was observed with high \( K_t \) values for both sites. The \( trans \)-lactate effect on pyruvate transport occurred only on type I sites and exhibited an asymmetric interaction pattern \( (K_t \text{ of inward lactate} > K_t \text{ of outward lactate}) \). The inability of lactate to \( trans \)-stimulate type II sites suggests that intracellular lactate cannot recruit these sites. The high-affinity type II sites act as a specific pyruvate shuttle and constitute an essential relay for the intracellular lactate shuttle.

THE PRODUCTION OF LACTATE by glycolysis occurs in several tissues (e.g., skeletal muscle cells) and results from anoxia (6–8, 20, 24, 26–28, 30, 35, 36). As a consequence, lactate-proton removal is dependent on monocarboxylate transport systems. A large family of isoforms of monocarboxylate transporter (MCT), proton-linked transporters, has been identified (21, 24, 40, 41). Skeletal and cardiac muscles express (1, 2, 9–11, 20, 24, 28) the major isoform MCT1, but MCT2 and MCT4 isoforms are also present in these tissues. There is a fiber specificity in skeletal muscle with a predominant expression of MCT1 in oxidative fibers (10, 19, 24). Homeostasis of pH in muscle cells is regulated by the lactate-to-pyruvate ratio, which controls a metabolic and redox equilibrium (3, 28) among extracellular, cytosolic, and mitochondrial compartments (13–17). The function of these transporters is to maintain metabolic equilibrium among the various cellular compartments. During exercise, for example, a high lactate concentration is produced. Under these conditions, the internal lactate influences the inward transport of pyruvate (18), and conversely, the internal pyruvate acts on lactate influx (29, 34). Cell-to-cell shuttles and intracellular shuttles provide the means through which glycolytic and oxidative pathways are linked and metabolic equilibrium is maintained. The stereospecificity of the pyruvate/lactate carrier has been demonstrated to behave symmetrically with regard to lactate and proton binding (28).

Our aim was to determine the mechanism by which skeletal muscle cells are able to accumulate pyruvate when pyruvate transporters are blocked or, alternatively, recruited by the excess of intracellular lactate. Because carriers are shuttles that restore this altered equilibrium, our first approach was to compare the pyruvate and lactate transport systems by use of transport kinetic analysis. This kinetic approach has been investigated previously by us (4, 5, 39) in L6 skeletal muscle cells. In the present studies, we examined and compared the transport capacities by analyzing the transport efficiency (TE), estimated as the ratio of maximal velocity \( (V_{\text{max}}) \) to the \( trans \)-activation dissociation constant \( (V_{\text{max}}/K_t) \) for these two metabolites. Because previous studies have shown that cloned isoforms exhibit different sensitivities to inhibitors (24), we analyzed the effects of \( \alpha \)-cyano-4-hydroxycinnamic acid (CIN) and \( p \)-chloromercuribenzenzylsulfonate (PCMB) on pyruvate transport.

In addition, to determine the specificity of the pyruvate transport system, we conducted \( trans \)-stimulation experiments to examine the influence of a high internal \( L \)-lactate concentration. We conclude with the physiological advantage of such a pyruvate multicarrier system in skeletal muscle cells.
**MATERIALS AND METHODS**

**Cell Cultures**

Undifferentiated muscle cells of the L6 line were used as previously described. L6 is an established cell line obtained from rat muscle. Cultures were grown in Dulbecco’s modified Eagle’s medium (Seromed) containing 5% fetal calf serum and 1% penicillin-streptomycin (10,000 U/ml) under humidified air containing 5% CO2. The standard medium was changed every 2 days. The cells were cultured until myotubes were obtained. When the myoblasts reached confluence, they stopped dividing and fused to form multinucleated myotubes.

**Transport Studies**

The transport experiments were performed as previously described (5). Myoblasts and myotubes were obtained after 2 and 14 days of culture, respectively. The culture medium was removed, and cells were washed with PBS, pH 7.4; then the cells were incubated with buffer or inhibitors for 1 min. The transport experiments were performed at 20°C. The buffer was aspirated and replaced with 200 μl of PBS containing 15 mM HEPES (pH 7.4) and 11 kBq/ml [14C]pyruvate or 1-[U-14C]lactate (Amersham) per assay and unlabeled pyruvate or 1-lactate (0.01–25 mM) with or without inhibitors. Pyruvate uptake was terminated after 3 s (initial velocity) by addition of ice-cold buffer (1 ml, 4 times). The ice-cold stopping solution was aspirated before addition of 500 μl of 0.1 N NaOH. To assay the radiolabeled compounds, 400 μl were mixed with 4 ml of scintillation liquid (Readysafe; Beckman), and radioactivity was determined using a liquid scintillation counter (1600-Ca-Packard; Tricarb, Canberra, Australia). The radioactivity obtained was normalized with respect to pyruvate or lactate specific activity and protein concentration for each cell sample (100 μl in 0.1 N NaOH). The Bio-Rad Protein Assay adapted to 0.1 N NaOH (pH 12.5) medium was used in the range of 20–40 μg of protein. Transport inhibitors were CIN and PCMBs, used at the concentrations (previously determined) producing a maximum percentage of inhibition: 2.5 mM CIN and 5 mM PCMBs (corresponding to 25 × Ki).

For trans-stimulation of pyruvate transport studies, cells were preloaded for 15 min (time value at equilibrium) with 30 mM lactate (saturating concentration). Then, transport experiments, applied to a range of pyruvate concentrations from 5 μM to 30 mM, were carried out for 3 s (initial velocity conditions), and the cells were washed as before. In a cis-inhibition procedure, pyruvate transport was determined with radiolabeled pyruvate in the presence of 30 mM lactate. For these experiments, the osmotic pressure (300 mOsM) was maintained by PBS buffer. All data are expressed as means ± SE of three or four samples. Four separate experiments were carried out, and all showed the same velocity curve characteristics.

The velocity curves were analyzed using software applied to hyperbolic and sigmoidal plots. A linear or curvilinear regression (using software) of Hill plots, log[V/Vmax − V] vs. log[PYR] (where [PYR] is pyruvate concentration), was carried out to obtain information about the type and level of cooperativity [from the slope nH (Hill number)]. The S0.9/S0.1 ratio corresponds to substrate concentrations observed for two fractions of Vmax, 0.9 vs. 0.1 Vmax. This ratio depends on the value of n (Hill number or degree of cooperativity), and a value <1 (Segel-Enzyme Kinetics 1985) corresponds to an allosteric or multisite transport system.

For the determination of kinetic parameters, nonlinear regression algorithms specific for two-site Scatchard plot were applied (Software Cigale-IPMC-CNRS-UNSA, Valbonne, France). This software gives the equations of the asymptotes (linear plot) for each branch in a biphasic Scatchard plot

\[
\frac{V}{[PYR]} \text{ vs. } -\frac{1}{[PYR]} = \frac{1}{K_p} + \frac{[PYR]}{K_p} + \frac{[PYR]}{K_p}.
\]

where Kp is the dissociation constant when the pyruvate concentration is expressed as [PYR]n. The nth root was applied to express the Kp value.

The inhibition constant Ki was derived for a mixed-type inhibition from the expression (app, apparent)

\[
V_{\text{max}} \text{ app vs. } V_{\text{max}}/[1 + (1/Ki)].
\]

where α equals (Ki app/Ki), and (1) equals lactate concentration, and for a competitive inhibition from the relation

\[
K_{\text{app}} = K_i[1 + (1/Ki)]
\]

The pyruvate transport trans-stimulation by lactate is described by the velocity equation of a random bireactant system (Segel-Enzyme Kinetics 1985)

\[
V_{\text{max}} = \frac{[LAC]}{[PYR]} \frac{[LAC]}{[PYR]} + \frac{[PYR]}{[PYR]} + \alpha K_{\text{app}}[LAC][PYR] + [LAC][PYR].
\]

The Kp LACin (dissociation constant of lactate on the inner cellular face) is calculated from the equation

\[
V_{\text{max}} \text{ app} = V_{\text{max}}/[1 + (\alpha K_{\text{LACin}}/[LAC])]
\]

The ratio Vmax/Kp expressed as nanomoles per minute per milligrams of protein per millimolar transported substrate, was used to compare lactate and pyruvate kinetics and was considered the TE parameter.

**RESULTS**

**Pyruvate Transport and Sensitivity to Inhibitors**

In the absence of inhibitor. The pyruvate uptake rate, like that of lactate, appeared to be concentration dependent (Fig. 1A). Simple diffusion was subtracted from corresponding values (0.8 ± 0.013 nmol·min⁻¹·mg protein⁻¹·mM PYR⁻¹) for all data.

The Vmax app (125 ± 8 nmol·mg⁻¹·min⁻¹) in the absence of any inhibitor was reached at 15 mM pyruvate, although it continued to increase slightly up to 30 mM pyruvate; this is the first index of a multisite system. The ratio S0.9/S0.1 = 9 also suggests that pyruvate is carried by a multisite carrier in the presence or absence of inhibitor. These properties were confirmed by the Hill plot (not shown) with a mean value for nH of 1.3 and the best regression giving a curve with two branches in both the absence and presence of the two inhibitors tested.

Because the mean value of nH was 1.3, the Scatchard plot V/[PYR]1.3 vs. V is presented (Fig. 1B). The regression analysis of these data resulted in a biphasic curve. Asymptotes of each component of the pyruvate transport were calculated using this biphasic curve, and kinetic parameters are reported in Table 1. These results indicate that pyruvate transport was mediated by two systems: a family of type I sites with low affinity (Ki = 7.83 mM) and a high transport capacity (Vmax = 155 nmol·mg⁻¹·min⁻¹) and a family of type II sites.
with a high affinity \((K_t = 59 \mu M)\) and a low capacity of transport \((V_{\text{max}} = 2.5 \text{ nmol} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}; \text{Table 1})\).

In the presence of inhibitor. Three cis-inhibitors of pyruvate transport were analyzed: CIN, PCMBS, and L-lactate.

**VELOCITY CURVE ANALYSIS.** Figure 1A shows that inhibition by PCMBS was effective both at low and high extracellular pyruvate concentrations. However, the CIN inhibition was effective only at high pyruvate concentration, i.e., >5 mM. The velocity curve showed that CIN inhibited the \(V_{\text{max app}}\) of pyruvate transport by 44%, whereas PCMBS reached 60% inhibition.

The effect of lactate (30 mM, saturating concentration) on the initial velocities of pyruvate transport is reported in Fig. 2A. In this case, lactate (30 mM) was applied in the uptake medium for cis inhibition. The \(V_{\text{max app}}\) in Fig. 2A was inhibited by 68%.

**SCATCHARD PLOT ANALYSIS.** With regard to inhibition of type I sites (Fig. 1B), CIN produced 44 and 46% inhibitions of \(V_{\text{max}}\) and \(K_t\), respectively, corresponding to a noncompetitive inhibition (with an increase of the affinity; Table 1). With PCMBS (Fig. 1C), we observed that, on the type I sites, \(V_{\text{max}}\) was inhibited by 62%, whereas \(K_t\) increased by 43%. These last effects characterized a mixed inhibition. The cis-lactate inhibition exhibited a mixed inhibition with derived constant \(K_{1 \text{LACout-I}} = 11.1 \text{ mM} \) (Fig. 2B).

With regard to inhibition of type II sites, in the presence of CIN, \(V_{\text{max}}\) and \(K_t\) were unchanged. The major effect of PCMBS was a 63% inhibition of \(V_{\text{max}}\) by a noncompetitive mode (Table 1), with no effect on \(K_t\). When cis-lactate was applied (Fig. 2B), it exerted a competitive inhibition with a \(K_{1 \text{LACout-II}} = 5.4 \text{ mM} \).

**Pyruvate Transport Trans-Stimulated by Lactate**

Type I sites. Velocity curves (Fig. 2A) and two Scatchard plots of pyruvate transport \((V/\text{[PYR]}^{1.3} \text{ vs. } V, \text{ expanded in Fig. 2C, inset})\) obtained in the absence and presence of trans-lactate are presented, and their kinetic parameters are reported in Table 1.

In the trans-stimulation mechanism, the trans-lactate gradient (30 mM) stimulated pyruvate influx by a pre-steady-state “burst” in the first step. This rapid first equilibrium of the lactate binding-debinding step drives a large number of carriers toward the outside for the uptake step of external pyruvate. From our results of the Scatchard curves, where the intercept of the asymptotes is to the left of the ordinate axis, the more consistent and clear-cut interpretation comes from the
PYRUVATE SHUTTLE IN MUSCLE CELLS

Table 1. Kinetic parameters of pyruvate and lactate transports in absence or presence of MCT effector in L6 muscle cells

<table>
<thead>
<tr>
<th>Transported Monocarboxylate/Effecter</th>
<th>Types of Site</th>
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<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$V_{\text{max}}$</td>
<td>$K_i$, mM</td>
<td>TE</td>
</tr>
<tr>
<td>Pyruvate</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Without</td>
<td>155 ± 6</td>
<td>7.83 ± 0.9</td>
<td>19.70</td>
</tr>
<tr>
<td>cis-CIN</td>
<td>86 ± 7</td>
<td>4.24 ± 0.5</td>
<td>20.30</td>
</tr>
<tr>
<td>cis-PCMBs</td>
<td>58.6 ± 5</td>
<td>11.19 ± 1.2</td>
<td>5.20</td>
</tr>
<tr>
<td>cis-Lactate</td>
<td>66 ± 4</td>
<td>15.92 ± 1.4</td>
<td>4.20</td>
</tr>
<tr>
<td>trans-Lactate</td>
<td>576 ± 10</td>
<td>13.33 ± 1.2</td>
<td>43.00</td>
</tr>
<tr>
<td>Lactate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without</td>
<td>67 ± 7</td>
<td>8.2 ± 0.7</td>
<td>18.17</td>
</tr>
<tr>
<td>cis-CIN</td>
<td>23 ± 3</td>
<td>7.9 ± 0.6</td>
<td>2.91</td>
</tr>
<tr>
<td>cis-PCMBs</td>
<td>31 ± 4</td>
<td>8.3 ± 0.6</td>
<td>3.73</td>
</tr>
</tbody>
</table>

|                                      | INH Type     |  |        |
|                                      |              |        |        |
|                                      | $V_{\text{max}}$ | $K_i$, mM | TE     |
| Pyruvate                             |              |        |        |
| Without                              | 155 ± 6      | 7.83 ± 0.9 | 19.70  |
| cis-CIN                              | 86 ± 7       | 4.24 ± 0.5 | 20.30  |
| cis-PCMBs                            | 58.6 ± 5     | 11.19 ± 1.2 | 5.20  |
| cis-Lactate                          | 66 ± 4       | 15.92 ± 1.4 | 4.20  |
| trans-Lactate                        | 576 ± 10     | 13.33 ± 1.2 | 43.00 |
| Lactate                              |              |        |        |
| Without                              | 67 ± 7       | 8.2 ± 0.7 | 18.17  |
| cis-CIN                              | 23 ± 3       | 7.9 ± 0.6 | 2.91   |
| cis-PCMBs                            | 31 ± 4       | 8.3 ± 0.6 | 3.73   |

Values are means ± SE (SE of fitted parameter value). Maximal velocity ($V_{\text{max}}$) is expressed in nmol·min$^{-1}$·mg protein$^{-1}$ and the trans-activation dissociation constant ($K_i$) of pyruvate (PYR) in mM and mM and and $K_i$ of lactate (LAC) in mM. TE, transport efficiency ratio ($V_{\text{max}}/K_i$PYR or $K_i$LAC); INH, inhibition type; CIN, α-cyano-4-hydroxycinnamic acid; PCMBS, p-chloromercuribenzoic acid sulfonate; NO, no inhibition; C, competitive; NC, noncompetitive; MX, mixed type.

simultaneous model (random bireactant system). The rate-limiting step of pyruvate transport (i.e., actual $V_{\text{max}}$) was concentration dependent on the ternary-like complex C-LAC-PYR (where C is carrier). The pyruvate uptake follows a cyclic mechanism as described in Fig. 3.

In the presence of trans-lactate, the external $K_{i\text{PYR app}}$ (13.33 mM) for type I transport site (low affinity) was increased by a factor $\alpha = 1.7$ (13.33/7.83). These significant results indicated that the type I transport site was involved in the pyruvate and lactate transport mechanism.

The actual $V_{\text{max}}$ was $576 ± 10$ nmol·min$^{-1}$·mg protein$^{-1}$, the $V_{\text{max app}}$ was $155 ± 6$ nmol·min$^{-1}$·mg protein$^{-1}$, and the internal $K_i$LAC ($K_i$LACin) was 48.0 mM. The asymmetry ratio of lactate affinity (inner vs. outer) in these experimental conditions can be calculated from $K_i$LACin (48 mM) vs. external lactate $K_i$LACout ($K_i$LACout) obtained either by lactate transport (8.2 mM; see next paragraph) or by lactate $K_i$ (11.1 mM) from pyruvate transport kinetics, and these values are 5.8 and 4.3, respectively.

Type II sites. In contrast to the type I sites, as seen the Scatchard plot (Fig. 2C), in the presence of trans-lactate, the slopes (1/$K_i$PYR) were similar, and the $V_{\text{max}}$ was 2.3 with and 2.5 without trans-lactate, respectively. (Table 1 and Fig. 2C, inset). These results indicate that trans-lactate has no effect on the type II site of the pyruvate carrier.

Lactate Transport in L6 Myotubes

Under the same experimental conditions as applied previously on L6 muscle cells (5), we determined L-[U-14C]lactate transport kinetic parameters both with and without the presence of cis inhibitors CIN or PCMBS (Table 1).

Pyruvate and Lactate Transport Efficiency vs. Kinetic Specificity

Pyruvate transport efficiency. We observed (see Table 1) that the two families of sites have different TE ratios ($V_{\text{max}}/K_i$), the low-affinity transport type I sites being less than one-half the TE value of the high-affinity type II sites (19.7 vs. 42.4).

Although CIN inhibited pyruvate transport (non-competitive inhibition), the efficiencies of types I and II transport sites were not affected. In contrast, PCMBS decreased the efficiency ratio of both sites by a factor of 3–4.

Lactate was applied with [14C]pyruvate on the cis side; it inhibited the TE of the types I and II sites by factors of 4.2- and 5.4-fold, respectively. We observed that lactate did not significantly increase the pyruvate uptake efficiency ratio on type II sites, conversely to type I sites, which appear more sensitive to lactate trans-stimulation of pyruvate influx, with the TE ratio increasing from 19.7 to 42.1. This meant that the turnover of type I transport sites was at least doubled by lactate trans-stimulation.

Lactate TE. Table 1 shows the kinetic parameters obtained for [14C]lactate transport. Both types I and II sites presented lower TE values of 8.2 and 4.6, respectively (i.e., 3 to 8 times lower than for pyruvate). Moreover, CIN and PCMBS did not show the same effects as observed for pyruvate. Indeed, the TE ratio was inhibited up to 70% (on type I sites) and 66% (on type II sites) in the presence of CIN and 56% (on type I sites) and 69% (on type II sites) with PCMBS.

Comparative analysis of TE. An overall analysis of TE ratios showed that pyruvate was more efficiently carried than lactate in these muscle cells. Irrespective of the monocarboxylate transported, the type II site exhibited the higher transport ability. On the other hand, the type I site possessed twice the efficiency for
lactate transport, which is twice that of the type II sites; conversely, type II sites showed a TE for pyruvate that is twice that of type I sites.

**DISCUSSION**

Analysis of monocarboxylate transport kinetics is necessary for revealing whether more than one MCT isoform was present in the same cell and whether there is a general pattern in various cell and tissues types such as muscle, neuronal, hepatic, renal, and intestinal cells (24). Because lactate and pyruvate have distinct functions in the metabolic pathway, their respective concentrations vary with the physiological status of the muscle cells and could influence their transport.

In these studies, we took advantage of the opportunity to examine functionally, on the same cellular type, the role of lactate in the multicarrier system of pyruvate transport. This study revealed the physiological importance of the high-affinity type site specific for pyruvate uptake, as suggested by its insensitivity to lactate trans-efflux.

**Multicarrier System and Pyruvate Transport**

In this study, we have specifically studied the kinetics of pyruvate and lactate transport in skeletal muscle cells. Our results show that, in contrast to lactate, pyruvate undergoes significant simple diffusion. The major conclusion on the existence of a multicarrier system is supported by the allosteric character of velocity curves, curvilinear Hill plot, and nonlinear Scatchard plots with a squared pyruvate concentration or with an exponent of 1.3. Our findings on this pyruvate multicarrier system are in accord with three major findings. First, a family of MCT isoforms was identified (24). Second, on the basis of results with the FRET technique [fluorescence resonance energy transfer (29, 41)], it was proposed that a functional unit of transport is constituted by a di-isomeric complex consisting of two entities of CD147 glycoproteins (24, 29) associated with two molecular entities of MCT1 or MCT4, which are coexpressed in plasma membranes. Third, various studies have shown that several MCT isoforms can be coexpressed (MCT1 and MCT4) on muscle cells of the same fiber type (23, 31). The asymptotic lines on Scatchard plots presenting different kinetic parameters are consistent with a mechanism of a multimeric carrier complex in the presence or absence of an inhibitor such as described for the diisomeric complex [CD147]2[MCT1 or MCT4]2 (29, 41).

The transport specificity between the different families of sites was revealed. Type I transport sites (low affinity, high capacity) are more specific for lactate, and type II sites (high affinity, low capacity) are more specific...
for pyruvate. This is confirmed by the TE/site I/TE/site II ratios. Moreover, the mean values obtained from the TE ratio of both sites together are 30 for pyruvate and 5 for lactate; i.e., L6 muscle cells have a sixfold more efficient transport system for pyruvate than for lactate. These findings extend our previous results obtained on lactate and pyruvate transport in primary culture of rat myotubes (4–6). The distribution analysis by Western blots of MCT isoforms revealed their differential expression in oxidative (MCT1) and glycolytic (MCT4) white muscle fibers (11, 24, 28, 31). A single saturable transport system was demonstrated to be dependent on MCT1 (in cultured rat brain astrocytes) or MCT2 (in neuron-rich primary culture), but when these experiments were also carried out in Xenopus oocytes injected with MCT1 cRNA, kinetic results showed two carrier systems (12). In adult brain cortical synaptosomes, several carrier systems have been characterized: lactate is carried by only one low-affinity system and pyruvate by two systems, one with low affinity, high capacity, and a second one with high affinity, low capacity (33). In the kidney (in proximal renal LLC-PK1 cells and brush border membrane vesicles), only one family of independent carrier has been found (37).

Consistent with previous observations in this and other biological tissues, the transport sites for lactate had lower \( V_{\text{max}} \) (Table 1) and higher \( K_i \) values than for pyruvate. Interestingly, in contrast to pyruvate, the higher lactate TE was associated with low-affinity, type I sites, regardless of the presence of inhibitors. The variation of the kinetic parameters observed for both transport sites and their different sensitivity to CIN and PCMBs are also in agreement with results obtained for other transport isoforms MCT1, MCT2, and MCT4 (24). The PCMBs sensitivity confirms that thiol groups play a more determinant role in lactate and pyruvate interaction with both sites.

This comparative analysis of pyruvate and lactate transport shows that the family with high pyruvate affinity really corresponds to a specific site for pyruvate molecules and not based only on the difference of affinity between lactate and pyruvate to the site. It is known that pyruvate is carried with a higher affinity than lactate in the majority of tissues and plasma membranes. Our data show clearly that skeletal L6 muscle cells have a major capacity for pyruvate uptake via the high-affinity, type II site, whereas they preferentially transport lactate more specifically (TE) via the type I site. Although our aim was not to attribute to each functional site a defined isoform (MCT1 or MCT4), we have correlated cell functions and double-carrier equipment of the skeletal L6 muscle cells: one more specialized in pyruvate uptake and useful for the oxidative function and the second specialized for lactate exchange produced by glycolytic activity.

**Asymmetry of Cis- and Trans-Lactate Effects on Both Families of Pyruvate Transport Sites**

Kinetic studies on pyruvate transport have described and revealed that lactate did not induce the same effect on both pyruvate transport families and was dependent on whether lactate is applied to the cis or trans side relative to the pyruvate influx.

**Cis-lactate effect.** The mixed inhibition type by lactate on the type I site (TE inhibition of 78%) is the result of a broad specificity in contrast to the competitive inhibition on type II sites (TE inhibition of 87%), which expressed a higher specificity of pyruvate to these sites (Table 1).

**Trans-lactate effect.** Only the type I sites were sensitive to trans-stimulation (type II are not; Table 1). The trans-lactate produced a higher increase in \( V_{\text{max}} \) than in \( K_i \) for pyruvate transport. This decrease of pyruvate affinity due to trans-recruitment of pyruvate transport sites is dependent on the low affinity for lactate on the internal side of the plasma membranes. Indeed, the kinetic model described in Fig. 3 and its equations enable the derivation of a lactate trans-stimulation constant, \( K_i \text{LAC}_{\text{in}} = 48 \) mM. The asymmetry for lactate (\( K_i \text{in}/K_i \text{out} \) ratio) shows that the half-saturating concentration value is 4.3–5.8 times higher during the trans-stimulated influx than for influx. This property indicates that type I sites are more specialized for lactate influx.

In the case of type II sites, kinetic analysis by Scatchard plots indicates that trans-lactate did not modify

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**Fig. 3.** King-Altman diagram describing trans-stimulating sequences consistent with the type of curves (Scatchard plots) from our results; i.e., the apparent mechanism is a random bireactant system in which the lactate gradient initializes the first step of this cyclic equilibrium. In this model, maximal velocity \( (V_{\text{max}}) \) is dependent on a ternary-like complex \([\text{C-LAC-PYR}]\) in the presence of lactate (C, carrier) and reached in the presence of the 2 substrates (in maximal concentration). Pyruvate affinity \( (K_i, \text{dissociation constant}) \) in the presence of lactate is \( \alpha K_i \text{PYR}_{\text{out}} \) and \( (\alpha = K_i \text{ PYR}_{\text{app}} \text{ (with lactate)})/(K_i \text{ PYR}_{\text{out}}) \) app, Apparent. We assume that \( V_{\text{max}} \) app of pyruvate transport is observed when the lactate concentration is low or near zero and the pyruvate affinity constant is \( K_i \text{ PYR}_{\text{out}}-\).
the transport kinetic parameters of pyruvate. Lactate does not influence type II site transport function (TE = 42.1) and shows only a weak influence on pyruvate uptake. On this pyruvate affinity site, the cis-lactate $K_i$ of 5.4 mM is 100 times higher than the $K_t$ PYR and demonstrates again the low affinity of lactate for this site. In other studies, the ability of lactate to trans-activate radiolabeled lactate efflux (pH dependent) was also observed in rat sarcolemmal giant vesicles (28). By use of the same membrane model as that used previously, the stimulated efflux was only 110% of baseline (18) when lactate efflux (1 mM) was tested in the presence of trans-pyruvate (100 mM). In another cell membrane system, renal brush borders, a monocarboxylate is transported by single family site, borne by a transporter that also displays kinetic asymmetry: a $K_{in}/K_{out}$ ratio of 32 [measured by pyruvate transport trans-stimulation ($K_{in} = 36$ mM and $K_{out} = 1.1$ mM)] (34). Our results on the asymmetry of lactate $K_t$ strengthen the evidence that pyruvate and lactate transport pathways are specific, each one being dependent on its main MCT isoform.

**Physiological Advantage of High-Affinity Pyruvate Transport Sites (Not Trans-Stimulated by Lactate) in Muscle Cells**

Kinetic studies of lactate cis-inhibition and trans-stimulation reported here extend our previous results and together provide a better understanding of the transporter's role (summarized in Fig. 4). The specificity of each carrier gives L6 muscle cells the capacity to maintain the oxidative pathway from pyruvate, necessary for energy function, independently of the intracellular lactate concentration. In other words, the high-affinity pyruvate carrier bypasses the lactate dehydrogenase activity that acts to lower the NADH/NAD$^+$ ratio to synthesize high levels of lactate. As a consequence of the trans-lactate not modifying the TE of type II sites, these sites act as specific pyruvate shuttles and constitute the first essential relay to compensate for other shuttles that import lactate (14–17).

This selectivity is essential for oxidative turnover and metabolic steps described for the lactate shuttle (15). This high-affinity pyruvate shuttle constitutes a physiological advantage. The high-affinity type II site exerts control over the homeostatic mechanism for the basal energetic metabolism, as evidenced by elevated lactate concentrations during muscular exercise and hypoxic or ischemic shocks. It should be noted that the affinity of this pyruvate shuttle (50 μM) corresponds to the pyruvate concentration found in venous blood. This property ensures that the muscle cells are still able to transport pyruvate to maintain mitochondrial metabolism under conditions where the low-affinity transport site is removing intracellular lactate.

Finally, we draw attention to the importance of having types I and II sites on the same skeletal muscle cell, one for lactate extrusion and the second for pyruvate uptake necessary for energy metabolism. The MCT expression studied by immunoblotting reveals that MCT1 is more specific to type I muscle fibers and MCT4 to fiber types IIa and IIb (23–25, 28). MCT1 and MCT4 have shown differences in regulation of pretranslational or posttranscriptional processes (9–11, 25). It was shown that these two types of sites are subject to various regulatory mechanisms involved in cell maturation (4) and exercise training (11) and as-

![Fig. 4. General schema showing the 2 families of sites, their sensitivity, and the insensitivity of pyruvate carrier (high-affinity site) to extreme concentrations of intracellular lactate.](http://ajpendo.physiology.org/)
associated with acidosis. Kinetic analysis such as that used here allows one to demonstrate the relevance of different isoforms in muscular physiopathology (24, 28, 32, 35, 38). Our kinetic results provide evidence that the function of these transporters in muscle homeostasis (lactate extrusion and pyruvate uptake) is dependent on distinct lactate and pyruvate shuttles.

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REFERENCES


