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Biochimica et Biophysica Acta 1283 (1996) 106–110

BBA Biochimica et Biophysica Acta

Symmetry and pH dependency of the lactate/proton carrier in skeletal muscle studied with rat sarcolemmal giant vesicles

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Received 28 February 1996; revised 26 April 1996; accepted 26 April 1996

Abstract

Muscle lactate transport was studied in rat sarcolemmal giant vesicles, in which the membrane orientation was similar to that of intact cells. The lactate concentration dependency was studied using influx and efflux experiments under equilibrium exchange, zero-*trans*, and infinite-*cis* conditions. At constant external and internal pH the carrier behaved symmetrically with regard to lactate transport from the two sides of the membrane. Lactate at the *trans* side stimulated the lactate tracer flux (*trans*-acceleration). Both in zero-*trans* influx and efflux experiments the lactate flux was stimulated in a symmetric way by H^+ at the *cis* side, consistent with protons being the substrate for the carrier. In contrast, the lactate flux was symmetrically inhibited by H^+ at the *trans* side (*trans*-inhibition), the underlying mechanism is likely that the protonated carrier with no lactate bound is unable to reorient in the membrane. These findings have implications both for the kinetic model and for understanding the physiological function of the lactate/proton transporter.

Keywords: Lactate/proton transport; pH; Symmetry; (Rat sarcolemmal giant vesicle)

1. Introduction

The lactate/proton carrier in skeletal muscle has been studied in intact and isolated muscle [8,10,15,21], L6 myotubes [6], and with two model systems: the classical small vesicles [14,18], and the sarcolemmal giant vesicles [11–13]. The transport kinetic parameter K_m for lactate transport in muscle is within the range 12–41 mM, which is higher than most other tissues (for review see Poole and Halestrap [17]). Similarly, the V_{max} for muscle lactate transport has been shown to be high, usually in the range 140–420 nmol mg^{-1} min^{-1} [11,14,18].

The functional symmetry of the lactate/proton carrier has not yet been elucidated. In order to study this issue it is necessary to compare the transport kinetics for both influx and efflux in a system with a known membrane orientation. Intact muscles are not useful for such a study because of compartmentation and internal lactate metabolism. Furthermore, measurements of K^+-stimulated phosphatase activity have indicated that the membrane orientation of the small vesicles is only 60–71% 'right-side-out' [2,14,18]. In contrast, a uniform 'right-side-out' orientation of the giant vesicular membranes has been confirmed from light and electron microscopic studies, as well as immunolabelling of dystrophin [20,23], patch-clamp studies of rectifying channels [3,4], and labelling of Na,K-ATPase with [^{3}H]ouabain [16].

Therefore, the aim of the present study was to examine the symmetry of the lactate/proton carrier with regard to both substrates (lactate and protons). By using the sarcolemmal giant vesicle technique, K_m values for lactate tracer influx (both equilibrium-exchange, zero-*trans* and infinite-*cis* experiments) can be compared to previously reported K_m values for lactate tracer efflux. In addition, experiments were performed at various external and internal pH values in order to characterize the effect of protons at both sides of the membrane.

2. Materials and methods

2.1. Definitions

The terms influx and efflux denote both the direction of the flux across the vesicular membrane and the orientation in the intact cells. The *cis* side is defined as the side from which the tracer starts, and the *trans* side, the side towards which the tracer moves. *cis*-pH and *trans*-pH denote the

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PII S0005-2736(96)00084-3

pH values at the *cis* and *trans* side, respectively. The K_m for lactate transport was determined for equilibrium-exchange conditions (with the same lactate concentration at the two sides of the membrane), zero-*trans* conditions (with lactate only at the *cis* side of the membrane), and infinite-*cis* conditions (with a high lactate concentration at the *cis* side, and various concentrations at the *trans* side).

2.2. Vesicles

Rat sarcolemmal vesicles were obtained according to the method described by Juel [11]. The vesicular membranes have previously been characterized by marker enzyme analysis and SDS-PAGE electrophoresis [16,22].

2.3. Influx

Ten μl of vesicles were placed in a test tube and incubated with 100 μl of unlabelled lactate, [^{3}H]lactate and [^{14}C]sucrose, the latter being an extravesicular marker. The influx was stopped after 5 s by means of 1.5 ml ice-cold stop solution containing 0.5 mM pCMBS (*p*-chloromercuribenzenesulfonate). The vesicles were immediately spun down (20000 × *g*, 30 s), the upper solution removed and the tip of the tube with the sedimented vesicles cut off and placed in a counting vial. The initial lactate flux (nmol min^{-1} (mg protein)$^{-1}$) was calculated from the uptake within the 5 s incubation period subtracted a zero-time sample obtained by fast mixing of vesicles, incubation medium, and stop solution. The carrier-mediated flux was determined by subtracting the simple diffusion obtained from measurements in the presence of 5 mM cinnamate + 0.5 mM pCMBS [11]. When needed, 60 min of preincubation was used to ensure steady internal pH. Protein content was determined according to Bradford using bovine serum albumin as a standard.

2.4. Measurements of pH

Vesicular pH was measured with the fluorescent H^+ indicator BCECF (Molecular Probes, Eugene, OR, USA). In order to ensure constant internal pH, the vesicles were incubated twice for 30 min with 1 μM BCECF-AM at the new pH and then washed at the same pH in order to remove the external BCECF and BCECF-AM. The measurements were carried out with a Hitachi F-2000 fluorescence spectrophotometer, which had a computer-based cation measurement program using a 500/440 nm signal. The calibration of the intravesicular pH response was carried out with the protonophore CCCP according to the method of James-Kracke [9]. The changes in pH_i were converted to H^+ flux by means of the buffer capacity (35 mmol of H^+ l^{-1} (pH)$^{-1}$) calculated from the pH displacement obtained with 10 mM NH_4Cl. The H^+ flux equals the lactate flux, since the lactate-to-proton transport ratio via the lactate/proton transporter is 1:1 [11], and any activity of the Na^+/H^+ and bicarbonate-dependent exchange systems can be ruled out, as Na^+ and bicarbonate are not present.

3. Results

3.1. Lactate dependency

Equilibrium-exchange and zero-*trans* influx experiments were carried out at pH 7.4, with lactate concentrations from 5 to 60 mM, and the lactate flux corrected for

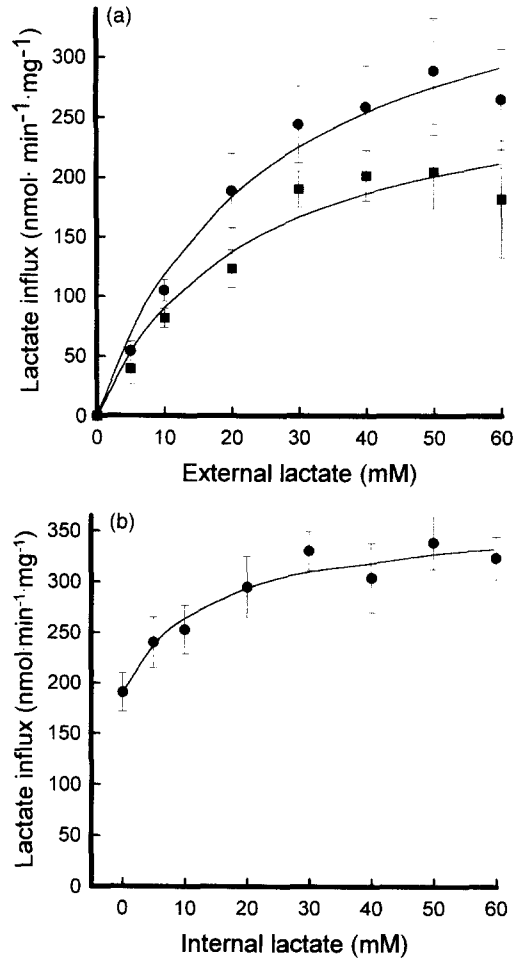

Fig. 1. (a) Lactate influx–concentration dependency. The figure shows initial lactate influx values from experiments with lactate only at the outside (zero-*trans* conditions) (■), and from experiments with the same lactate concentration at the inside and outside (equilibrium-exchange conditions) (●). The curves depicted are the best Michaelis–Menten fit to the data. Values are mean ± S.E., $n = 6$–24 (zero-*trans*) or 6–20 (exchange). (b) *trans*-Acceleration. The figure shows results from infinite-*cis* experiments with the tracer moving from the outside. The external lactate concentration was kept constant at 60 mM, and the internal lactate concentration varied from 0 to 60 mM. The *trans*-acceleration effect must be considered to be zero at 0 mM external lactate, therefore, the Michaelis–Menten fit (full line) to the data was carried out with all values subtracted the initial lactate influx (190 nmol mg^{-1} min^{-1}) obtained with no internal lactate. Values are mean ± S.E., $n = 10$–15. The values in both a and b represent carrier-mediated transport (total transport subtracted simple diffusion of undissociated lactic acid).

Table 1
A comparison of K_m values for influx and efflux; mean ± S.D. (mM)

	Tracer influx	Tracer efflux [a]
Equilibrium exchange	25.0 ± 6.1	23.7 ± 2.6
Zero-*trans*	22.2 ± 9.7	20.9 ± 5.0
Infinite-*cis*	13.6 ± 4.9	13.4 ± 5.9

[a] Calculated from Ref. [11].

lactic acid diffusion (Fig. 1a). The initial equilibrium-exchange flux exceeded the zero-*trans* flux at all lactate concentrations tested. Both curves demonstrated saturation at high lactate concentrations.

The lactate flux in infinite-*cis* experiments with both tracer and 60 mM lactate (considered to be saturating) initially at the outside, and the internal lactate concentration varying from 0 to 60 mM (Fig. 1b), demonstrates that increasing the lactate concentration at the inside (*trans* side) stimulates the isotopic influx (*trans*-acceleration). Figuratively, changing the lactate concentration at the *trans* side from 0 to 60 mM corresponds with a shift from the endpoint of the zero-*trans* to the endpoint of the equilibrium-exchange curves in Fig. 1a.

In Table 1 the K_m values (determined from a Michaelis–Menten fit to the data), for zero-*trans*, equilibrium-exchange, and infinite-*cis* influx experiments, are compared to the corresponding values obtained in previously reported efflux studies (Table 1).

The similar K_m values found for lactate tracer efflux and influx at constant pH indicates that the transporter is symmetric with regard to lactate affinity.

3.2. Dependency of pH

In the following experiments the concentration of lactate was kept constant at 10 mM, whereas pH varied in order to study the effect of protons on the lactate/proton carrier.

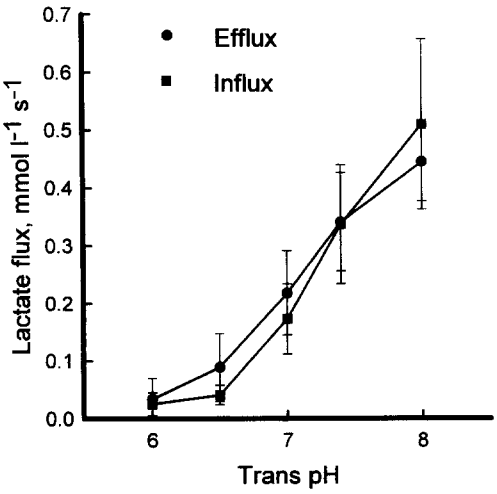

Fig. 2. Effect of *trans*-pH on lactate flux. Data from efflux (●) and influx (■) experiments with *cis*-pH constant at 7.4 and *trans*-pH varying from 6.0 to 8.0. Values are mean ± S.D., $n = 13–14$ (efflux), $n = 11$ (influx).

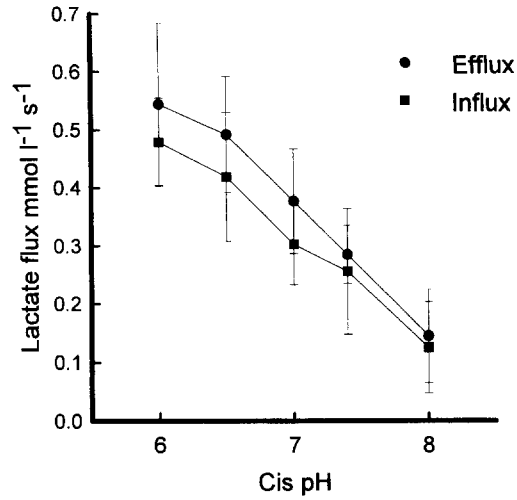

Fig. 3. Effect of *cis*-pH on lactate flux. Data from efflux (●) and influx (■) experiments with *cis*-pH varying from 6.0 to 8.0 and *trans*-pH remaining constant at 7.4. Both efflux and influx values are mean ± S.D., $n = 10–17$.

3.2.1. Effect of trans-H^+

Lactate efflux and influx measurements were carried out with a *cis*-pH constant at 7.4, while *trans*-pH varied from 6.0 to 8.0 (Fig. 2). The two similar curves demonstrated that the lactate flux in both influx and efflux experiments was inhibited at low *trans*-pH, and stimulated at high *trans*-pH. Thus the lactate/proton transporter behaved symmetrically with regard to pH changes at the *trans* side.

3.2.2. Effect of cis-pH

Lactate efflux and influx experiments were carried out at a constant *trans*-pH (7.4), while *cis*-pH varied from 6.0 to 8.0 (Fig. 3). Both the initial lactate influx and efflux were dependent on *cis*-pH; the influx was stimulated at low *cis*-pH and inhibited at high *cis*-pH. Therefore, the lactate/proton transporter behaved symmetrically with respect to *cis*-pH.

4. Discussion

The present findings demonstrate that the lactate/proton carrier is symmetric with regard to the affinity of the two substrates; lactate and protons. In addition, lactate/proton transport is stimulated by lactate at the *trans* side (*trans*-acceleration), but inhibited by protons at the *trans* side (*trans*-inhibition). These effects were both symmetric.

In contrast to the present findings of symmetry, Brown and Brooks [2] claimed that the lactate transport behaves asymmetrically. This was based on experiments using

small vesicles made from rat muscle. However, they compared the K_m for *trans*-stimulation in efflux experiments with the K_m for zero-*trans* lactate uptake. As both experiments characterise lactate binding from the outside, the difference is probably due to the different experimental conditions. In addition, in low-*cis* (1 mM inside) experiments with 100 mM lactate at the *trans* side (outside), the influx of lactate in the measuring period will increase the internal lactate concentration considerably, thereby diluting the tracer.

The symmetry, with respect to lactate affinity reported in the present study, is also in contrast to the asymmetry reported for the carrier present in erythrocytes and cardiac myocytes [5,19]. As the latter systems also differ from the muscle lactate/proton carrier with respect to inhibitor sensitivity and transport kinetic parameters [17,22], the different symmetry properties regarding lactate may therefore be taken as an argument for the existence of various isoforms of the lactate/proton transporter in different tissues.

The effects of changing proton concentrations also demonstrated symmetry with regard to stimulation of the lactate transport. The stimulatory effect of H^+ at the *cis* side (Fig. 3) is in agreement with findings from experiments using small vesicles [14,18] and intact muscle [1]. The underlying mechanism is probably that low *cis* pH increases the probability for binding of protons, one of the substrates for the transporter, although nonspecific H^+-mediated changes in membrane proteins could also be involved.

The inhibitory effect of H^+ at the *trans*-side both in zero-*trans* lactate efflux and influx experiments (Fig. 2) is in accordance with the results reported by Roth and Brooks [18]. The explanation might be that the protonated carrier cannot reorient or reorients slowly from the *trans* side when no lactate is available for binding. Based on the pronounced inhibition at low pH (6.0 and 6.5), it appears that the protonated carrier is totally immobile.

It is important to note that protons and lactate at the *trans* side have opposite effects on lactate transport. Protons at the *trans* side act inhibitorily (*trans*-inhibition) in zero-*trans* experiments, whereas the presence of lactate at the *trans* side stimulates the isotopic lactate flux (*trans*-acceleration). The explanation for the different effects of lactate and protons is likely that the binding of the two substrates is ordered [5], and that binding at one side of the membrane is independent of binding from the other side. Thus, the observations are consistent with a model in which protons bind first. Consequently, if no lactate is present (as in the zero-*trans* experiments), the carrier is blocked in the immobile protonated form. The reason for the *trans*-acceleration occurring with high lactate concentrations at the *trans* side could be an increased probability for lactate binding (when a proton is already bound) and the faster reorientation of the loaded compared with the unloaded carrier. This model is consistent with the observed higher equilibrium-exchange than zero-*trans* lactate flux (Fig. 1a). The present observations, therefore, support the kinetic model originally suggested for the erythrocyte lactate carrier [5] and later adapted for the muscle lactate transporter [11].

4.1. Physiological significance

It appears that the pH sensitivity of the lactate/proton transporter could be of major importance during high-intensity exercise. The pH reduction due to lactic acid accumulation in active muscle may reach half a pH unit, which according to Fig. 3 will accelerate the flux rate by approximately 50%. The increased lactate and proton efflux during muscle activity may reduce both the lactate accumulation and limit the decline in internal pH, thereby delaying the development of fatigue (for review see Ref. [7]) associated with low muscle pH.

Low plasma pH associated with lactate and proton accumulation in blood may stimulate lactate uptake in resting muscle, thereby counteracting the accumulation of lactate and protons in blood and stimulating the lactate metabolism of the receiving cells.

Acknowledgements

This study was supported by the Danish Natural Research Council and The Danish National Research Foundation.

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