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L(+)-Lactate transport in perfused rat skeletal muscle: kinetic characteristics and sensitivity to pH and transport inhibitors

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We have examined lactate uptake (as the rate of net muscle lactate accumulation) and unidirectional inward transport (measured by a paired-tracer dilution method) in muscle of the perfused skinned rat hindlimb. Inhibition of tracer influx (fractional uptake at 1 mM L(+)-lactate, 43.3 ± 3.1% but only 32.9 ± 1.8% at 50 mM lactate) suggested some competition between tracer and native forms of the carboxylate for transport. D(−)-lactate (50 mM) did not inhibit uptake of tracer L(+)-lactate. Pyruvate (25 mM), but none of five other monocarboxylates, inhibited uptake of tracer lactate, by 22% ($P < 0.01$). Altering perfusate pH from 7.4 to 6.8 caused a 36% increase ($P < 0.001$) in the unidirectional L(+)-lactate transport at 1 mM L(+)-lactate, whereas increasing pH to 7.7 reduced transport by 18% ($P < 0.01$). Tracer lactate influx was inhibited by 500 μM 4-acetamido-4′-isothiocyanostilbene (SITS) (19%), 5 mM α-cyano-4-hydroxycinnamic acid (CIN) (20–30%), 1 mM amiloride (27%) and by a thiol group reagent *p*-chloromercuribenzenesulphonic acid (pCMBS) (26%). Overall the results indicate that at least two processes are involved in the transfer of lactate: one, saturable, with a V_{max} of 0.84 μmol · min^{-1} · g^{-1} and an apparent K_m of 21 mM was sensitive to SITS, CIN, and a thiol group reagent; the other was non-saturable and insensitive to SITS and CIN with an apparent rate constant of 0.1 min^{-1}.

Introduction

L(+)-Lactate may be produced by glycolysis in skeletal muscle whenever the rate of ATP provision by oxidative metabolism falls below the requirement of the tissue. This may occur during contractile activity or at rest if oxygen supply is limiting. It has been demonstrated that muscle, especially resting cardiac and fast red muscle [1,2], can take up lactate for oxidation and produce glycogen from lactate. Lactate produced in excess of the capacity for oxidation via the Krebs cycle or taken up for glycogen synthesis could be exchanged across the sarcolemmal membrane via one or both of two possible routes: diffusion through the lipid membrane surface (either directly or possibly through a channel) or via a specific carrier molecule.

There are a number of advantages to rapid transfer of lactate across muscle cell membranes. For example, accumulation of protons associated with lactic acid production may have a number of deleterious effects on the contractile function of muscle, since the Ca^{2+} activation of actomyosin

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ATPase is pH-dependent [3] and a fall in intracellular pH could decrease the activity of the phosphofructokinase step in glycolysis [4] and thereby reduce the capacity for ATP provision. There are also circumstances in which rapid lactate uptake by muscle, liver and heart would be an advantage, e.g., during replacement of whole body glycogen stores after exercise, especially during fasting.

A simple diffusive pathway has been demonstrated to be present in several cell types; in the human erythrocyte diffusion accounts for only 5% of the total lactate flux [5,6], but in toadfish hepatocytes it accounts for all the transfer of lactic acid [7]. However, in rat hepatocytes, simple diffusion accounts for only 20% of lactate flux [8], with most of the transport occurring by a facilitated, carrier-mediated process for lactic acid and/or lactate.

With intense physical exercise, skeletal muscle lactate concentrations may rise to 50 mM. Removal by transport and metabolism must, therefore, be smaller than production. As the rate of metabolism of lactate (conversion to glycogen and oxidation) in skeletal muscle is low, such observations may be indicative of a saturable transport process and this has indeed been suggested [9,10]. However, the presence of such a saturable process has not been demonstrated for skeletal muscle. Facilitated transport processes have often been indirectly implicated in tissue lactate transport by the results of application of specific inhibitor compounds [5,11,12] but, again, direct demonstration of a process with the characteristics of a facilitated, saturable carrier-mediated process are not available for rat skeletal muscle.

The extent of the possible variety of mechanisms for lactate movement and their relative contributions in skeletal muscle are not, however, well understood.

Although Mainwood et al. [13] were able to provide several systems that could be responsible for the flux, i.e., lactate/proton symport, lactate ion/anion exchange, diffusion of the undissociated acid and diffusion of the ionic species, they were unable to ascertain the importance of each in the movement of lactate across the skeletal muscle membrane.

The objectives of the present work were to examine the kinetic characteristics of rapid inward unidirectional lactate transfer across the sarcolemma of a mixed mammalian skeletal muscle preparation and to attempt to discriminate between the extent of passive diffusion and facilitated transport of lactate by carrier-mediated or channel processes.

Materials and Methods

Female Wistar rats (200 g) were used throughout (Bantin & Kingman, Hull, U.K.). The preparation of the skinned rat hindlimb for vascular perfusion and the conditions used have been described elsewhere [14]. The perfusions were carried out in thermostatically controlled cabinets at 37 °C. The perfusate consisted of Krebs-Henseleit bicarbonate buffer [15] containing 6% (w/v) bovine serum albumin (BSA; Miles Pentex), and D-glucose (5 mM). The perfusate was equilibrated with 95% O_2/5% CO_2 by pumping through a thin-walled silastic tubing 'lung' exposed to the gas mixture.

In six preparations, in which the net accumulation of lactate into muscle was measured, the hindlimb was initially perfused with a medium containing no lactate. Following an equilibration period (20 min) the perfusate lactate concentration was increased to 50 mM. Gastrocnemius and soleus muscle samples were freeze-clamped at intervals up to 60 min after the onset of lactate perfusion. The frozen muscle was prepared for analysis by grinding with a pestle in a mortar cooled with liquid N_2. Protein was precipitated from weighed aliquots of muscle powder with 0.2 M perchloric acid (5, v/w) and separated by centrifugation. L(+)-Lactate was assayed enzymatically in the neutralised supernatant [16].

Unidirectional uptake of lactate was measured by the paired-tracer dilution method [17]. [^{3}H]Mannitol was used as an extracellular space marker (New England Nuclear Radiochemcials, Dreiech, D.D.R.). L(+)-[^{14}C]Lactic acid and DL-[^{14}C]lactic acid (1 : 1) were obtained from Amersham International PLC (Amersham, U.K.). Tracers were mixed in the dpm ratio of 5 : 1 (^{3}H/^{14}C); N_2 dried tracers were reconstituted to 100 µl with perfusate. Samples of venous effluent from the single hindlimb perfusions were collected for 2 min, after the pulse injection of tracers in a

drop-wise fashion (4 drops/vial, approx. 120 μl) directly into scintillation vials. To these samples 3 ml of scintillation cocktail (Beckman EP) were added before counting in a Beckman 1800 liquid scintillation counter using a dual-channel (^{3}H and ^{14}C) program with automatic quench correction, external standard channel ratio and the Beckman H-number procedure. Data output from the scintillation counter was collected and processed on-line by an Apple IIe microcomputer.

L(+)-Lactic acid (Sigma) was added to the perfusate at steady concentrations between 0.5 and 50 mM to investigate the possibility of competition for transport between unlabelled and labelled tracer lactate. In a separate experiment the effect of the presence of 50 mM extra NaCl in the perfusate on lactate (1 mM) uptake was also investigated.

In order to characterise the process of lactate transfer further, in particular experiments the following were added to the perfusate: various substituted monocarboxylic acids (25 mM) (see Table I for details), 5 mM α-cyano-4-hydroxycinnamic acid (CIN), 500 μM 4-acetamido-4′-isothiocyanostilbene (SITS) and the thiol group reagent *p*-chloromercuribenzenesulphonic acid (pCMBS) (200 μM). The effect on lactate influx of putative inhibition of Na^+/H^+ exchange by amiloride (1 mM) was also investigated. The hindlimb was perfused with buffer containing each added chemical for at least 6 min before injection of the tracers.

Since it has been reported that CIN is able to bind to serum proteins [18], we attempted to ascertain the fraction of CIN or lactate bound to the 6% BSA present in the perfusate by CIN-determination after equilibrium dialysis of 6% BSA in Krebs-Henseleit buffer against Krebs-Henseleit buffer containing different concentrations of CIN or lactate. Dialysis continued at 4° C for 24 h with gentle stirring. The dialysis tubing (Scientific Instruments Ltd.) had a molecular weight cut-off of 12000–14000. The amounts of bound and free CIN were determined from the absorbance of equilibrium samples at 340 nm (given a molar absorption coefficient of 4480).

The olive oil/water partition coefficient for L(+)-lactate was determined from the distribution of radiolabelled L(+)-[^{14}C]lactate between olive oil and water after 12 h equilibration at pH 7.4. Samples of the aqueous and oil phases were removed and processed for liquid scintillation counting with external standard and automatic quench correction.

Chemicals, except for radiochemicals, were purchased from either Sigma Chemicals or BDH, both of Poole, Dorset, U.K.

Statistical analysis of the data by linear regression, exponential curve analysis (single and double), Student's *t*-test and paired-sample *t*-test for significance of difference was applied to data using software commercially available for the Apple IIe computer [19]. Results were taken to be significantly different at $P < 0.05$.

TABLE I

THE EFFECT OF MONOCARBOXYLIC ACIDS ON THE UNIDIRECTIONAL INFLUX OF L(+)- and DL-[^{14}C]LACTATE (pH 7.4)

The compounds were added to perfusate containing 1 mM L(+)-lactate. Influx was calculated as: U_{max} = perfusate [lactate] × flow rate/perfused tissue weight. U_{max} was the maximal uptake (U) of lactate relative to the extracellular space marker mannitol ($U = 1 - C_{lactate}/C_{mannitol}$, where $C_{lactate}$ and $C_{mannitol}$ are the fractional recoveries of the radiolabelled tracers). Values are mean ± S.D. of at least three observations. Comparisons were made with 1 mM L(+)-lactate influx. ** $P < 0.001$, * $P < 0.05$, n.s. not significantly different.

Compound		Lactate influx ($\mu mol \cdot min^{-1} \cdot g^{-1}$)
L(+)-Lactate	(1 mM)	0.144 ± 0.009
DL-Lactate	(1 mM)	0.107 ± 0.012 *
L(+)-Lactate	(5 mM)	0.653 ± 0.039 **
L(+)-Lactate	(25 mM)	3.042 ± 0.236 **
L(+)-Lactate	(50 mM)	5.569 ± 0.231 **
D(−)-Lactate	(50 mM)	0.149 ± 0.005 n.s.
Pyruvate	(25 mM)	0.113 ± 0.018 *
Formate	(25 mM)	0.136 ± 0.016 n.s.
Acetate	(25 mM)	0.143 ± 0.015 n.s.
Propionate	(25 mM)	0.135 ± 0.015 n.s.
2-Hydroxybutyrate	(25 mM)	0.151 ± 0.011 n.s.
3-Hydroxybutyrate	(25 mM)	0.140 ± 0.017 n.s.

Results

Accumulation of lactate (Fig. 1)

Data for the net accumulation of lactate into muscle, soleus and gastrocnemius, were corrected

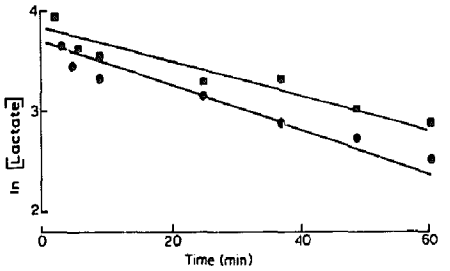

Fig. 1. Accumulation of L(+)-lactate into perfused hindlimb muscle. Rat hindquarters were perfused with 50 mM L(+)-lactate (pH 7.4) and samples of gastrocnemius and soleus muscle were taken at different time points for analysis of total lactate concentration. Plot of ln maximal muscle [lactate] minus muscle [lactate] against time. Soleus (●) $t_{1/2} = 27.9 \pm 5.6$ min, $k = 0.025 \pm 0.005$ min^{-1}. Gastrocnemius (■) $t_{1/2} = 41.3 \pm 9.7$ min, $k = 0.017 \pm 0.004$ min^{-1}.

for the presence of extracellular lactate by subtraction of the extracellular compartment from total lactate (assuming that the extracellular space accounted for 27% of the total fluid space [20]) and fitted to single- and double-exponential curves. There were no significant improvements in the correlation coefficients for the results from the soleus and gastrocnemius when fitted with a double rather than a single exponent. The rate of accumulation of lactate for soleus ($k = 0.025 \pm 0.005$ min^{-1}, $t_{1/2} = 27.9 \pm 5.6$ min) was not significantly faster than that for gastrocnemius ($k = 0.017 \pm 0.004$ min^{-1}, $t_{1/2} = 41.3 \pm 9.7$ min) ($P > 0.05$).

Surface area and permeability coefficients

The possibility that the movement of lactate could be due either to simple diffusion or some faster, possibly carrier-mediated, process was examined by calculating a theoretical value for the permeability surface area product (PS) of hind limb muscle and comparing this to experimentally obtained values, determined from the following formula [21]:

$$PS = -F \ln(1 - U)$$

where P = permeability coefficient; S = surface area available for exchange, F = flow rate through the vascular bed, and U = fractional uptake of lactate (see Table I).

The total surface area for sarcolemma and the T-tubule system in rat muscle has been estimated as 0.21 m$^2 \cdot$ g^{-1} [22–24]. The surface of the sarcolemma would be expected to be the limiting area for exchange in these transport studies because the extracellular marker, [^{3}H]mannitol, is able to traverse capillary pores freely [25]. The permeability of lipid to lactic acid may be estimated from the partition coefficient of its distribution between water and olive oil, since the two parameters are closely correlated for many organic substances [26]. We found the partition coefficient of lactate between water and olive oil to be $(0.88 \pm 0.08) \cdot 10^{-3}$ ($n = 10$) at 22°C, 5 mM lactate. From the correlation between log P and log partition coefficient [26], P was calculated as $2.93 \cdot 10^{-7}$ cm $\cdot$ s^{-1}. With this value and the flow used in this experiment (33 ml $\cdot$ 100 g$^{-1} \cdot$ min^{-1}) the expected uptake of tracer lactate would be 10.6 ± 0.9%, assuming that the sarcolemmal and T-tubular surface was the exchanging membrane and that the entire surface behaved as lipid. The observed uptake of 5 mM lactate in the perfused hindlimb was 38.6 ± 4% ($n = 7$) at pH 7.4. From the above calculations it appears that passive diffusive processes could explain some of the observed uptake into muscle, but the exact proportion is difficult to define a priori.

Characteristics of unidirectional lactate transport

Organic acid competition for [^{14}C]lactate uptake into muscle

The isotope dilution curves of lactate and mannitol tracers and the unidirectional lactate uptake calculated from them are shown in Fig. 2. There are no differences in the shapes of the dilution profiles, particularly in their time to peak, for the two tracers, but there were differences in their fractional recoveries, indicating that lactate was taken up into the perfused muscle. The uptake of tracer lactate at 1 mM perfusate lactate (pH 7.4) was 43.3 ± 3.1%, and was unaffected by the addition of 50 mM NaCl to the perfusate, but at increased concentrations of L(+)-lactate the fractional uptake of lactate decreased to 34 ± 5% at 25 mM and 33 ± 2% at 50 mM, suggesting competition by the unlabelled lactate for some transport process. Furthermore, 50 mM D(−)-lactate had no

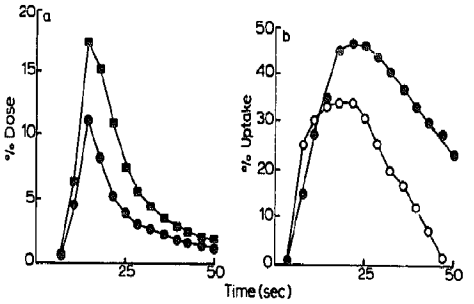

Fig. 2. (a) Venous effluent isotope dilution curve for [^{14}C]lactate (●) and [^{3}H]mannitol (■) at 1 mM perfusate lactate concentration. A single hindlimb was perfused with buffer containing 1 mM L(+)-lactate. A single rapid injection of the isotopes in 100 μl of buffer was followed by the collection of consecutive venous effluent samples. (b) Uptake curves derived from isotope dilution curves. Uptake (U) is calculated from the isotope dilution curves as: $U = 1 - (\%$ recovered lactate/% recovered mannitol$) \times 100$. (●) 1 mM lactate, (○) 50 mM lactate.

effect on the unidirectional uptake of L(+)-lactate, suggesting stereospecificity of the competition effect. The uptake of DL-[^{14}C]lactate was significantly lower (26%) than the uptake of L(+)-[^{14}C]lactate (Table I). Over the range of lactate concentrations (0.5–50 mM) used in this experiment the observed unidirectional influx was approximately linear both at pH 7.4 and pH 7.7 (Fig. 3), with substantial deviation (an apparent fall) from linearity only at pH 6.8 with 50 mM lactate.

As the greatest inhibition of [^{14}C]lactate uptake by unlabelled lactate, in these experiments, was limited to 24% (50 mM lactate), the relationship between unidirectional transport of lactate and perfusate concentration was effectively linear for the concentration range 0.5–50 mM lactate, pH 7.4. The approximate inward transport rate, calculated by linear regression analysis, was 0.111 $\pm$ 0.002 min^{-1} (pH 7.4).

Assuming that the hindlimb is composed of approx. 80% fast type fibres and 20% slow-type fibres [27] and that the soleus and gastrocnemius are good representative muscles of the two fibre types, we can compare the unidirectional influx results with the average rate of net lactate accumulation. The average rate constant calculated for the whole hindlimb at pH 7.4 should be 0.019 min^{-1}. This value is much lower than the approximate influx rate constant of 0.111 min^{-1}, suggesting either rapid re-export of most of the lactate initially transported, or its metabolism to another compound. The rate of export (efflux) of the lactate can be calculated from knowledge of the influx and net accumulation rates of lactate; i.e., accumulation = influx – efflux. Using the values for influx and accumulation obtained in this study an efflux rate of 0.092 min^{-1} was calculated.

The addition of substituted carboxylates,as possible competing agents, had little effect on the measured uptake of lactate (Table I). The presence of 25 mM formate, acetate and propionate had no significant effect on the measured influx, but the dehydro keto-acid of lactate, i.e. pyruvate, at 25 mM inhibited the influx by 22%. Hydroxy analogues of lactate (2- or 3-hydroxybutyrate) at 25 mM had no significant effect.

Effect of perfusate pH

Alteration of perfusate pH had significant effects on the unidirectional uptake rate. A reduction from pH 7.4 to 6.8 stimulated the tracer

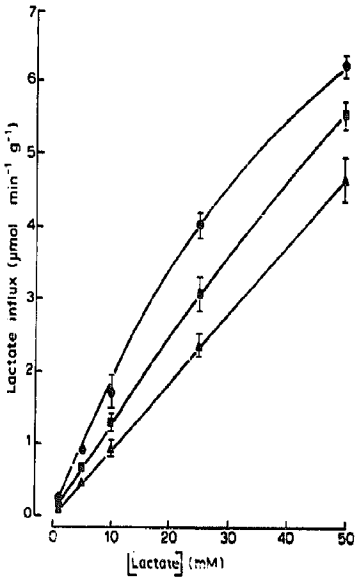

Fig. 3. The effect of perfusate pH on L(+)-lactate influx. Lactate influx was determined from the maximal uptake of radiolabelled lactate at each concentration (see Table I for calculation). (●) pH 6.8; (■) pH 7.4; (▲) pH 7.7. Values are mean $\pm$ S.D. of at least three measurements. Vertical bars represent 1 S.D. of the mean.

TABLE II

INHIBITION OF TRACER LACTATE INFLUX BY CHEMICAL AGENTS

Values are mean ± S.D., n at least 3 unless indicated otherwise.

	Lactate influx (μmol·min^{-1}·g^{-1})	Percent change
0.5 mM L(+)-lactate	0.074 ± 0.007	
0.5 mM L(+)-lactate + 5 mM CIN	0.052 ± 0.007	−30
1 mM L(+)-lactate	0.144 ± 0.009	
1 mM L(+)-lactate + 5 mM CIN	0.112 ± 0.016	−22
1 mM L(+)-lactate + CIN + pCMBS	0.075 (n = 2)	−48
1 mM L(+)-lactate + 1 mM amiloride	0.097 ± 0.008	−33
5 mM L(+)-lactate	0.653 ± 0.039	
5 mM L(+)-lactate + 0.5 mM SITS	0.530 ± 0.050	−19
1 mM L(+)-lactate + DL tracer	0.107 ± 0.012	−26
1 mM L(+)-lactate + DL tracer + pCMBS	0.085 ± 0.014	−21
1 mM L(+)-lactate + DL tracer + amiloride	0.074 (n = 2)	−31

influx by 30% at 1 mM lactate but only by 12% at 50 mM (Fig. 3). At pH 6.8 the apparent rate constant, for the concentration range 1–25 mM, was 0.16 ± 0.01 min^{-1}. Increasing perfusate pH from 7.4 to 7.7 reduced the influx by approx. 20% at both 1 mM and 50 mM lactate. The apparent rate constant (1–50 mM) fell significantly to 0.086 ± 0.004 min^{-1} ($P < 0.001$).

CIN

The competitive inhibitor of mitochondrial lactate/pyruvate transport, CIN [18] (5 mM), inhibited the uptake by 30% at 0.5 mM lactate and by 22% at 1 mM lactate (Table II). Exclusion dialysis of CIN against the perfusate normally used in the tracer uptake experiments showed half-maximal binding at 166 μM CIN and thus, although CIN does bind to albumin, the free perfusate CIN concentration in a 5 mM solution (approx. 4 mM) was still likely to be in excess of the observed K_i for other lactate transport systems, e.g., mitochondrial transport (50 μM) [18] and Ehrlich ascites tumour cells (500 μM) [28].

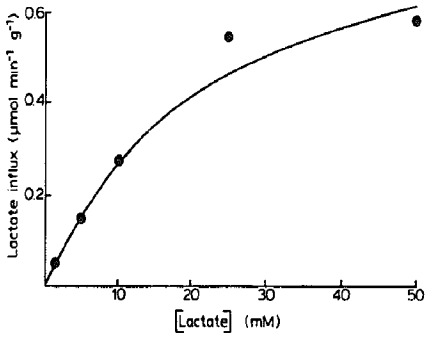

Fig. 4. Rate of lactate influx plotted against concentration to show CIN-sensitive lactate flux. Line gives best fit to single Michaelis-Menten type kinetics. $K_m = 21 \pm 4$ mM, $V_{max} = 0.84 \pm 0.01$ μmol·min^{-1}·g^{-1}.

The maximal inhibition of tracer lactate influx by CIN was 30% (0.5 mM lactate) (Table II). If the remaining influx is assumed to be non-saturable, i.e., the CIN has inhibited all of the carrier-mediated influx, then an approximation of the contribution by a saturable process can be obtained. From such calculations the non-saturable component would have a rate constant of 0.1 min^{-1} and, assuming Michaelis-Menten kinetics, the carrier-mediated influx appears to have a K_m of 21 ± 4 mM and a V_{max} of 0.84 ± 0.01 μmol·min^{-1}·g^{-1} (Fig. 4).

When the external pH was decreased in the presence of CIN, inhibition of tracer lactate influx was still present. Plots of unidirectional lactate influx (at 1 mM lactate) against perfusate pH

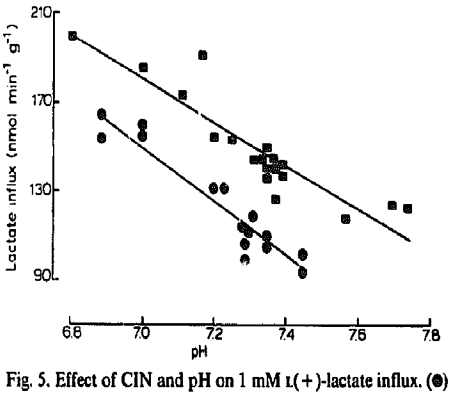

Fig. 5. Effect of CIN and pH on 1 mM L(+)-lactate influx. (●) +5 mM CIN; (■) no CIN. Each point represents the measurement in an individual experiment.

show a significant correlation with or without CIN (Fig. 5). There is no significant difference in the slopes of the two lines. Intercepts on the abscissa suggest complete inhibition at pH 8.8 (no CIN) and pH 8.4 (with CIN).

pCMBS

The presence of pCMBS significantly reduced the tracer uptake of the DL-lactate tracer by 21%, at 1 mM L(+)-lactate, but only had a significant effect on the uptake of L(+)-lactate tracer (1 mM L(+)-lactate) when 5 mM CIN was also present. In the presence of CIN and pCMBS there was a 48% inhibition of tracer uptake (Table II), 26% more than in the presence of CIN alone.

Amiloride and SITS

The presence of 1 mM amiloride in the perfusion mixture inhibited the uptake of both L(+)- and DL-lactate tracer uptake, at 1 mM L(+)-lactate, by 33% and 31%, respectively (Table II).

The presence of 500 μM SITS in the perfusate inhibited lactate influx (5 mM) by 19% (Table II).

Discussion

The major findings from the present investigation are that the transport of lactate across the sarcolemma occurs via two major pathways and is pH-dependent. The largest proportion of lactate flux is via a non-saturable (up to 50 mM) process, and could account for at least 70% of lactate movement at rest. The second major route (accounting for the remainder) appears to be saturable, is competed for by pyruvate and inhibited by CIN, SITS and pCMBS. This saturable component becomes less important at higher lactate concentrations, accounting for only 10% of the flux at 50 mM.

The rate of lactate accumulation into the gastrocnemius (rate constant 0.017 min^{-1}, $t_{1/2}$ 41.3 min) appears to be much lower than previously cited rates of efflux of lactate from lactate-loaded rat diaphragm ($k = 0.077$ min^{-1}, $t_{1/2} = 9$ min) [29]. If the net uptake is subtracted from the likely influx, obtained from the tracer studies, efflux can be calculated. This gives an efflux for the hindlimb preparation of 0.094 min^{-1}, a value closer to that from rat diaphragm [29]. This value is also quite close to the influx rate constant, possibly indicating that the processes responsible for influx and efflux are similar.

In the perfused preparation used to examine the influx of tracer lactate the site of transport may be expected to be beyond the capillary and at the blood-facing sarcolemma. The basis for this expectation is that we used, as a reference for lactate uptake, the extracellular space marker mannitol, and molecules of this size (lactate, M_r 89; mannitol, M_r 182) are not restricted by the capillary wall [25]. This view is supported by the venous effluent isotope dilution profiles (Fig. 2), which show no significant difference in the time to peak recovery or time course of dilution for the two tracers (Fig. 2).

Lactate influx by simple diffusion theoretically, according to calculations based on its lipid permeability and the sarcolemmal surface area, could account for some of the observed uptake of tracer lactate. However, there remains some uncertainty because it may be that olive oil/water partition of lactate is different from skeletal muscle membrane lipid/plasma partitioning due to the presence of complex hydrophobic substances in the lipid membrane, e.g., cholesterol. A value for the permeability coefficient (P) of lactic acid through a black lipid membrane has also been measured by Wolosin and Ginsburg [30] as $5 \cdot 10^{-5}$ $cm \cdot s^{-1}$. If that value is used then the expected fractional extraction of lactate should be 99.9%. In the same paper they observed that estimates of P made from such black lipid membranes were higher than those observed for biological membranes, by at least 10-fold. Even this error, i.e., an overestimate of P by a factor of 10, would lead to expected uptakes of 88%, still much higher than we observed. Clearly the permeability co-efficient for lactate to skeletal muscle membrane is greater than $5 \cdot 10^{-5}$ $cm \cdot s^{-1}$.

Bean et al. [31] have shown not only that the permeability for acids through an artificial lipid membrane was a function of the lipid membrane composition but that it could also be influenced by pH, being increased by increases in the proportion of the undissociated species. However, the permeability was always lower than predicted from the expected increase of undissociated acid.

If simple diffusion were a major route for lactic

acid transport, lowered pH, and thus an increase in the proportion of undissociated lactic acid, would be expected to affect the rate of influx and efflux by a similar proportion. The importance of such pH effects may be assessed on the basis that if influx was in the form of lactic acid then the unidirectional uptake should be approximately 10-fold greater at pH 6.8 than at pH 7.7. In fact the observed increase in influx was only 1.6-fold (Fig. 3), indicating either that the muscle membrane is more impermeable than predicted or that the surface area is an overestimate. In fact it is difficult to see how the possible identifiable errors might combine sufficiently to account for the 10-fold deficit. It seems likely that such errors may well make only a small contribution towards the difference, especially as we have assumed in our a priori calculation that the whole surface of the muscle acts as lipid. It would appear, therefore, that simple diffusion as lactic acid is unlikely to be a major route for tracer influx.

Another possibility resulting in increased influx at low pH is that lactate ions are the major species permeating the membrane. Lactate ions could be gaining entry via channels on which the observed pH effects occur directly, altering the charge of amino acid groups or conformation of the channel or pore molecule to allow increased lactate ion permeation. Efflux of lactate has been shown to be dependent on pH [32,33]. Assuming that transport in and out is affected similarly by pH, these findings can be reconciled by recognition that the transfer rate, in either direction, is likely to be modulated by the pH prevailing at the transporting surface. Thus, since net movement is the resultant of influx and efflux, low external pH will increase the uptake and re-uptake of lactate and reduce net efflux; high external pH will act in an opposite fashion to increase net efflux.

Although it appears that a large proportion of the lactate flux may be non-saturable, we were still able to show up to 24% inhibition of tracer lactate influx by competing perfusate concentrations of unlabelled L(+)-lactate (50 mM). This provides sufficient evidence to suggest that there may also be a carrier or specific channel in the sarcolemma for lactate.

Further evidence is provided from inhibition of tracer uptake by agents other than L(+)-lactate. Pyruvate (25 mM) caused 21% inhibition of tracer lactate influx, suggesting competition for a limited number of carriers or channels recognising lactate and pyruvate. Rat skeletal muscle appears to differ quantitatively from rat cardiac muscle in this respect, as the influx of lactate into perfused heart could be inhibited by 70% in the presence of only 10 mM L(+)-lactate or pyruvate [34].

The transport mechanisms for lactate in heart and Ehrlich ascites cells are known to be carriers of pyruvate. In the present study inhibition of lactate influx by pyruvate may be due to competition for the sarcolemmal carrier, which either cannot recognise organic acids other than pyruvate or lactate or has a lower affinity for them.

The apparent K_m of a saturable (CIN-sensitive) skeletal muscle lactate transport (21 mM) appears to be similar to that in cardiac muscle (19 mM) [34] and Ehrlich ascites tumour cells (20 mM) [28]. However, the maximal capacity for the transport process in skeletal muscle (V_{max} 0.8 $\mu mol \cdot min^{-1} \cdot g^{-1}$) is only 10% of that in cardiac muscle (V_{max} 8.4 $\mu mol \cdot min^{-1} \cdot g^{-1}$).

More evidence of the existence of a saturable membrane transport process comes from the experiments involving the D(−)-lactate and the racemic DL tracer mix. At least part of the lactate influx appears to be stereospecific, indicated by the inhibitory effect of the L form but not the D form of lactate on tracer L(+)-lactate uptake and a significantly lower tracer uptake of DL-lactate.

A further criterion for the involvement of carrier-mediated processes [35] is also fulfilled, that of inhibition of transport by protein reagents and substances not directly chemically related to lactate. Lactate influx into skeletal muscle was found to be inhibited by several agents, e.g., CIN, CIN + pCMBS, amiloride and SITS. Their use to investigate lactate transport has been described for many preparations, e.g., human erythrocytes [5,6], Ehrlich ascites cells [28], rat liver [36] and rat heart [34]. In toadfish hepatocytes [7] lactate uptake was, apparently, entirely diffusive and inhibitors, such as CIN, SITS and pCMBS, had no effect on lactate uptake. This was not the case for the present study where all of these agents had some inhibitory effect on tracer lactate influx. The extent of CIN inhibition on lactate influx, approx. 30% at 0.5 mM lactate, indicates that at least this

proportion is probably carrier-mediated and the remaining 70% is via some other mechanism. The parallel relationship between lactate influx and pH, in the presence and absence of CIN (Fig. 5), indicates that a constant proportion of the lactate influx at any pH is inhibited by CIN.

The maximal CIN inhibition seen in the present results, at pH 7.4, from perfused rat muscle, 30% at 0.5 mM lactate and 5 mM CIN, is lower than the effect of CIN in inhibiting intramuscular pH change due to lactate influx into frog skeletal muscle [37]. This may be due to differences in the relative contribution of the muscle lactic acid transporter in the different species, i.e., more of the transmembrane flux being carrier-mediated in the frog.

Further evidence of a lactate transporter in rat skeletal muscle came from the application of pCMBS in combination with CIN. This combination was found to inhibit the influx of tracer lactate by 50%. The effect of pCMBS was also to slightly reduce the tracer uptake of DL-lactate but pCMBS was found only to have a significant effect on L(+)-lactate influx in the presence of CIN. The finding that pCMBS with CIN was more effective at inhibiting lactate influx may have been due to a synergistic effect, perhaps increasing the concentration of free pCMBS.

Other agents, not related to lactate, e.g., SITS and amiloride, were also found to inhibit the influx of tracer lactate. If SITS is inhibiting an inorganic anion exchanger then the observed effect, of reduced influx, could arise from locally increased pH at the outside surface of the muscle membrane. The inhibition of influx may also arise from direct inhibition of a lactate transporter, as dihydro-DIDS, chemically related to SITS, has been shown to inhibit lactate transport in rabbit erythrocytes in a specific manner [39].

The inhibition of lactate influx by amiloride may also be due to an alteration of the local pH conditions at the muscle exchanging surface, because amiloride at this concentration appears to inhibit Na^+/H^+ exchange [40] and has been shown to affect the efflux of lactate from mouse skeletal muscle [41]. Thus SITS and amiloride may be affecting the anionic (HCO_3^-/Cl^-) and proton fluxes and either directly affecting the transport mechanism for lactate or altering the local proportions of the protonated/ionic forms, thus effecting transfer.

The transport of lactate in skeletal muscle appears to resemble, qualitatively, the transport process for lactate in Ehrlich ascites cells [28], erythrocytes [5,6], aorta [42] and liver [36], being increased by lowered pH and inhibited by the same chemical agents. Effects of pH on the uptake of lactate into rat aorta [4] and ascites tumour cells [28] has been reported to be of the same type found in the present study, i.e., an increase with pH drop. The major difference between skeletal muscle and these other tissues appears to be in the proportions travelling via various routes. In this respect the results presented here are different to those of Koch et al. [43], who showed that 75% of the rapid lactate uptake into mouse diaphragm was via a mediated transport mechanism. This cannot be the case for the perfused rat hindlimb in which the presence of greater saturation of the tracer transport at high lactate concentrations would be apparent.

From our influx results it appears that mixed mammalian skeletal muscle is capable of exchanging about $10\% \cdot min^{-1}$ of prevailing lactate over the physiological concentration range (0.5–50 mM) in skeletal muscle with little interference by saturation of any carrier-mediated mechanism. The effect of a physiological pH drop on the flux of lactate ought to be functionally significant because at the lower concentrations (1–10 mM) lactate flux showed increases of 30–35% with a fall of 0.6 pH units, the pH change observed as a result of strenuous exercise [44]. The dependence of the transport process on the prevailing pH ought to assist in the redistribution of metabolically accumulated lactate. Thus a decreased intracellular muscle pH, due to strenuous exercise, would increase the rate of lactate efflux. Conversely, the lowering of blood pH would also assist in removing lactate from the blood into liver [11,36] and into muscle not involved in the exercise [2,29]. In conclusion, it appears from our results that lactate transport in skeletal muscle may be explained by a combination of a linear process and a carrier-mediated process, both of which may be modulated by physiological factors in a way which is of advantage for the metabolic control of muscle fuel economy.

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