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Evidence for facilitated lactate uptake in lizard skeletal muscle

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Summary

To understand more fully lactate metabolism in reptilian muscle, lactate uptake in lizard skeletal muscle was measured and its similarities to the monocarboxylate transport system found in mammals were examined. At 2 min, uptake rates of 15 mmol l^{-1} lactate into red iliofibularis (rIF) were 2.4- and 2.2-fold greater than white iliofibularis (wIF) and mouse soleus, respectively. α-Cyano-4-hydroxycinnamate (15 mmol l^{-1}) caused little inhibition of uptake in wIF but caused a 42–54 % reduction in the uptake rate of lactate into rIF, suggesting that much of the lactate uptake by rIF is *via* protein-mediated transport. *N*-ethymaleimide (ETH) (10 mmol l^{-1}) also caused a reduction in the rate of uptake, but measurements of adenylate and phosphocreatine concentrations show that ETH had serious effects on rIF and wIF and may not be appropriate for transport inhibition studies in reptiles. The higher net uptake rate by rIF than by wIF agrees with the fact that rIF shows much higher rates of lactate utilization and incorporation into glycogen than wIF. This study also suggests that lactate uptake by reptilian muscle is similar to that by mammalian muscle and that, evolutionarily, this transport system may be relatively conserved even in animals with very different patterns of lactate metabolism.

Key words: monocarboxylate transporter, lizard, *Dipsosaurus dorsalis*, muscle fibre type, lactic acid.

Introduction

Lactate plays numerous roles in skeletal muscle. It is a metabolic end-product of glycolysis, a major source of energy for oxidative fibers, a mitochondrial redox shuttle compound and a substrate for replenishment of glycogen stores (Brooks, 1991; Dubouchaud et al., 2000; Gladden, 2001). The changes in pH concurrent with the build-up of lactate in the muscle and blood are associated with fatigue of the muscle and inhibition of the metabolic pathways required for continued muscle function and recovery after activity (Juel, 1996; Roth, 1991). In the light of the role of lactate as both end-product and substrate, and because of the effects of lactate accumulation, study of its metabolism and transport is important to understand how it affects the activity physiology of animals.

Research on lactate production and removal during activity and recovery has shown different patterns for reptiles and mammals (Gleeson, 1996). In mammals, lactate produced during activity is primarily oxidized in muscle and is removed relatively quickly during recovery (Brooks and Gaesser, 1980). Brooks has described a system in which lactate produced by some fiber types is exported to and oxidatively removed by other fiber types (Brooks, 1986). There is also some evidence that glycogenic removal of lactate occurs in rodent muscle following intense, brief exercise (Nikolovski et al., 1996). Reptiles tend to rely upon glycolytic pathways to support most of their vigorous activity and, as a result, they can accumulate more lactate, and at a faster rate, than mammals (Gleeson, 1996). During recovery, much of this lactate is used for glycogenesis to restore muscle glycogen levels, and little is oxidized (Gleeson, 1991). These patterns of metabolism imply a need for some sort of transport system for the removal of lactate from sites of production and uptake at sites of metabolism.

Studies in various mammalian tissues have shown that lactate can be transported across the cell membrane by three mechanisms: by free diffusion, by a monocarboxylate transporter (MCT), which works as a lactate/H^+ symporter, or by anionic antiports such as lactate/Cl^--HCO_3^- exchangers (Deutick et al., 1982; Poole and Halestrap, 1993). The latter pathway does not seem to play a significant role in mammalian skeletal muscle (Juel and Wibrand, 1989; Roth and Brooks, 1990a; McDermott and Bonen, 1994). The undissociated acid can easily diffuse across the membrane (Poole and Halestrap, 1993) but, at physiological pH, more than 95 % of the lactic acid is dissociated as a lactate anion and a proton (pKa=3.86). It appears that the majority of lactate transport in mammals is due to MCT-protein-mediated transport (Juel and Wibrand, 1989; Roth and Brooks, 1990a,b; Bonen and McCullagh, 1994; McDermott and Bonen, 1994).

The MCT proteins transport short-chain monocarboxylates, such as lactate and pyruvate, and are stereospecific for L-lactate

over D-lactate. They have been shown to be inhibited both by non-specific protein modifiers and by non-transportable monocarboxylate analogs (Juel and Wibrand, 1989; Roth and Brooks, 1990a; Poole and Halestrap, 1993; McDermott and Bonen, 1994). A number of different isoforms of the protein have been identified, with specific isoforms being associated with different tissues (Halestrap and Price, 1999). MCT1 content correlates with a muscle's oxidative capacity, while MCT4 is expressed more in glycolytic fibers (McCullagh et al., 1996; Pilegaard et al., 1999; Halestrap and Price, 1999; Bonen et al., 2000).

Despite the work done on mammals, only a small number of studies have investigated the transport of lactate in other animals. A study of crustaceans suggests a lack of any facilitated lactate transport (Kinsey and Ellington, 1996). Studies of lactate efflux in rainbow trout muscle seem to show transport similar to that observed in mammals, but studies of uptake indicate some significant differences in responses to inhibitors and suggest the possibility of active transport, with lactate being taken up against its electrochemical gradient (Wang et al., 1997; Laberee and Milligan, 1999). These processes have yet to be examined in reptiles, and their study will help develop our understanding of the evolutionary history of lactate transport. Because of the high degree of dependence on glycolytic metabolism for activity of many reptiles (Bennett, 1978), knowledge of the mechanism of lactate transport in reptilian skeletal muscle will also be a key to understanding the physiological limitations on the activity capabilities of this class of animals.

The goal of this study was to examine the reptilian lactate transport system in the context of the recovery metabolism of the desert iguana *Dipsosaurus dorsalis*. This study attempted to describe lactate uptake in both oxidative and glycolytic skeletal muscle fibers of *D. dorsalis*. The responses of these muscles to various inhibitors that have been shown to inhibit the MCT proteins of mammals were measured to determine whether they have a lactate transport systems similar to those of mammals.

Materials and methods

Animals care/tissue preparation

Fifteen desert iguanas (*Dipsosaurus dorsalis*) were collected near Cathedral City, CA, USA, on permit from California Fish and Game and housed in sandy cages with 24-h access to a heat lamp and ample hiding places; they were segregated by sex. They were fed a diet of lettuce mixed with powdered rat chow three times weekly. Lizards were killed by decapitation. The iliofibularis muscle (IF) was removed and placed in a lizard Ringer's preparation solution (Guillette, 1982), with final concentrations of solutes (in mmol l^{-1}) as follows: NaCl, 157.0; KCl, 4.6; $NaHCO_3$, 12.0; $CaCl_2$, 1.45; $NaH_2PO_4.1H_2O$, 2.6; $MgSO_4$, 1.1. The solution was bubbled with 95 % O_2 and 5 % CO_2. The IF has distinct red and white sections (rIF and wIF, respectively), which are composed primarily of oxidative and glycolytic fibers, respectively (Putnam et al., 1980). These sections were separated from each other, further divided into two (rIF) or three (wIF) bundles and cleaned of damaged fibers under 7.5× magnification. Each bundle was pinned at resting length through its tendons to Tygon mounts.

Five mice (*Mus domesticus*) from an outbred stock (CF-1) were obtained from Sasco Inc., housed in a common cage on a 12 h:12 h light:dark cycle and given food and water *ad libitum*. The mice were killed by cervical dislocation. The soleus muscle (SOL) was removed from the limb and placed in Ringer's preparation solution (Dawson et al., 1998) bubbled with 95 % O_2 and 5 % CO_2. The muscle was separated into two fiber bundles, which were then cleaned of damaged fibers and mounted. All procedures were approved by the animal care and use committee of the University of Colorado.

Incubation media

Radiochemicals were obtained from ICN Pharmaceuticals (L-[U-^{14}C]lactic acid and D-[U-^{14}C]lactic acid) and NEN (sucrose[fructose-1-^{3}H(N)]). All other chemicals were purchased from Sigma Chemical. All solutions described below were made using the Ringer's (either mouse or lizard, as appropriate) solutions described for use in the tissue preparation above, plus additional chemicals as indicated below. All the solutions were equilibrated with 95 % O_2 and 5 % CO_2.

After mounting, muscle bundles were placed for 30 min in a pre-incubation solution with 5 mmol l^{-1} lactate to establish an intracellular concentration in all the fiber bundles similar to that found in resting *D. dorsalis* muscle (Crocker et al., 2000). The pre-incubation solution also contained no inhibitor (control), 15 mmol l^{-1} α-cyano-4-hydroxycinnamate (CIN) or 10 mmol l^{-1} *N*-ethymaleimide (ETH). A decrease in the rate of lactate uptake in the presence of ETH, which is a general sulfhydryl modifier, and CIN, which acts as non-transportable monocarboxylate analog, is frequently used as an indicator of protein-mediated transport (Watt et al., 1988; Roth and Brooks, 1990a; McDermott and Bonen, 1994; Laberee and Milligan, 1999). Both ETH and CIN were dissolved directly in the Ringer's incubation solution. The solutions for all incubations were at 37 °C and pH 7.4 for mouse muscle fiber bundles and 40 °C and pH 7.2 for lizard muscle fiber bundles.

After pre-incubation, muscle fiber bundles were used in one of three separate procedures. The first two tested how incubation time and external lactate concentration affected estimates of rates of lactate uptake. In the first procedure, muscle bundles were moved from the control pre-incubation solution into a 15 mmol l^{-1} lactate incubation solution containing 18.5 kBq of [^{14}C]lactate and 37 kBq of [^{3}H]sucrose and were incubated for 1–15 min. In the second procedure, bundles were incubated with radiolabeled sucrose and either L- or D-lactate as above for 2 min in L- or D-lactate concentrations of 5–100 mmol l^{-1}. To maintain the same osmolarity in all incubations and prevent any effects of osmotic change, these solutions had their NaCl concentrations decreased in proportion to the amount of sodium lactate added. The third procedure determined the effects of different lactate

concentrations and different inhibitors on lactate uptake in lizard muscle. In this procedure, bundles were moved from the pre-incubation solution into solutions containing either 5 or 15 mmol l^{-1} lactate, [^{14}C]lactate, [^{3}H]sucrose and either the same inhibitor as in their pre-incubation or a non-inhibitor control. These incubations were carried out for 2 min. After each incubation, muscle bundles were immediately blotted, frozen in liquid N_2 and stored at –70 °C until analyzed (usually within 3 days). A sample of each incubation solution was also frozen for later analysis.

Lactate uptake measurement

Pre-weighed frozen muscle samples were dropped into 0.3 ml of hot 1 mol l^{-1} NaOH, vortexed for 5 s and placed in boiling water bath for 20 min. This solution was neutralized with 0.3 ml of 1 mol l^{-1} HCl and allowed to cool for 20 min. To measure lactate uptake, 0.4 ml of the neutralized solution was added to 3 ml of Scintiverse II scintillation cocktail. The incubation medium was prepared for analysis by adding 0.3 ml to 3 ml of Scintiverse cocktail. An LKB Wallac, model 1204 liquid scintillation counter, which has integrated software capable of counting in multiple emission spectrum windows, was used to measure ^{3}H and ^{14}C radioactivity concurrently in each sample. Lactate uptake was calculated from ^{14}C uptake, using the [^{3}H]sucrose as an extracellular space marker to correct for the ^{14}C in the extracellular space (Juel and Wibrand, 1989). The following equation was used to determine [^{14}C]lactate uptake in units of disintegrations per minute (disints min^{-1}):

$$[^{14}\text{C}]\text{lactate uptake} = [^{14}\text{C}]\text{lactate}_{m} - ([^{14}\text{C}]\text{lactate}_{inc}/[^{3}\text{H}]\text{sucrose}_{inc})([^{3}\text{H}]\text{sucrose}_{m}), \quad (1)$$

where the subscripts m and inc indicate total disints min^{-1} for muscle and incubation solution, respectively. The disints min^{-1} values were then converted into nmoles of lactate using the specific activity of lactate in the incubation solution.

Metabolite assays

Muscle samples were analyzed for adenylates and phosphagens using high-performance liquid chromatography (HPLC), at rest, immediately after dissection and after each of the incubation treatments. Resting samples were obtained by freeze-clamping the entire leg immediately after decapitation and then dissecting out the frozen muscle. Post-dissection samples were obtained by freezing the muscle fiber bundles in liquid N_2 immediately after they had been mounted on the Tygon strip.

Frozen sections of each muscle were ground in a 10-fold dilution of 6 % ice-cold $HClO_4$ and then neutralized with 5 mol l^{-1} K_2CO_3. This solution was centrifuged for 5 min, and the supernatant was decanted and centrifuged for 6 min at 10 000 revs min^{-1}, frozen, thawed and centrifuged again for 6 min at 10 000 revs min^{-1}. This last supernatant was and filtered through a Rainin 0.45 μm filter. Supernatant (10 ml) was injected into a mobile phase consisting of 65 mmol l^{-1} KH_2PO_4 and acetonitrile (1:2.1), at a pH of 6.9, which flowed through a Microsorb MV-100A amino propyl column at a rate of 1.0 ml min^{-1}. The effluent was analyzed with a Rainin Dynamax spectrophotometer (model UV-C) at 245 nm for AMP, ADP and ATP in series with a Millipore Lambda Max spectrophotometer (model 481) at 205 nm for creatine and phosphocreatine. Standards were prepared and analyzed in the same manner as samples, and their metabolite concentrations did not change significantly with multiple freeze/thaw cycles. Lactate concentrations in the muscles after the 30 min pre-incubation were measured spectrophotometrically (Gleeson, 1985).

Adenylate energy charge (AEC) was calculated as:

$$\text{AEC} = ([\text{ATP}] + \tfrac{1}{2}[\text{ADP}]) / ([\text{ATP}] + [\text{ADP}] + [\text{AMP}]) . \quad (2)$$

Statistical analyses

The results were analyzed using the program StatView ver. 5.0.1 (SAS Institute Inc.). Multiple regression analysis was used to test the effects of incubation time, external lactate concentration and muscle characteristics on lactate uptake. A one-group *t*-test was used to determine whether the intracellular lactate concentration was significantly different from 5 mmol l^{-1} after the pre-incubation. Analysis of variance (ANOVA) with Fisher's PLSD analysis was used to compare the following; rIF, wIF and SOL uptake rates at 2 min, control lactate uptake rates between, and the inhibitor effects within, the 5 and 15 mmol l^{-1} lactate incubations, and the AEC and creatine, phosphocreatine concentrations throughout the procedure. All rates and concentrations reported are means ± S.E.M. All effects were considered significant at $P \leqslant 0.05$.

Results

Neither the mass of the animal nor the diameter or mass of the muscle fiber bundle (Table 1) was found to have any significant correlation to the rates of lactate uptake in the time-

Table 1. *Data for the animals and muscle bundles used in incubations*

	Mean	S.E.M.	*N*
Animal mass (mg)			
Mouse	30.72	3.63	5
Lizard	67.96	20.80	10
Muscle mass (mg)			
Soleus	4.35	1.13	20
Red IF	13.63	5.51	38
White IF	26.55	13.30	55
Muscle diameter (mm)			
Soleus	1.02	0.10	3
Red IF	1.22	0.25	36
White IF	1.48	0.39	48

IF, iliofibularas.

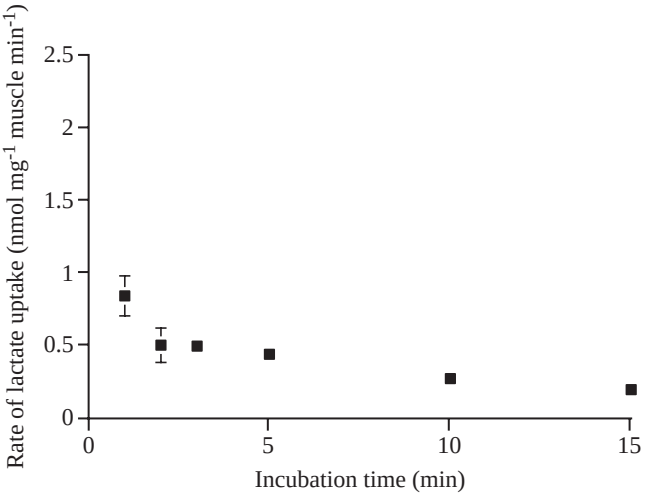

Fig. 1. Mean rate of lactate uptake by mouse soleus muscle during 1–15 min incubations. Values are means ± S.E.M., *N*=3–5, except at 10 min where *N*=2. At some points error bars are smaller than the symbols.

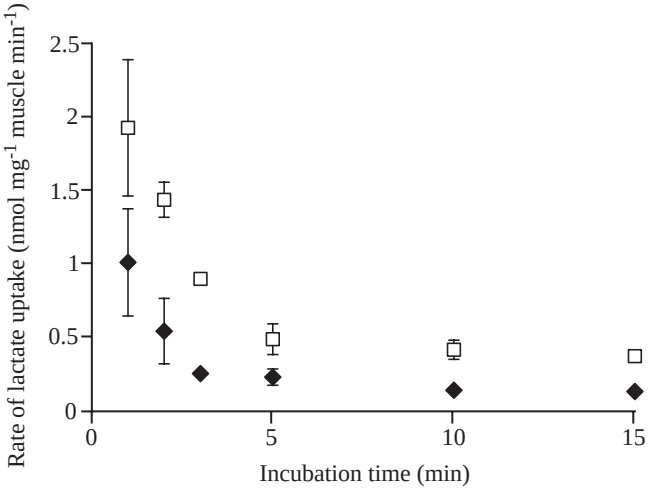

Fig. 2. Mean rate of lactate uptake by *Dipsosaurus dorsalis* red iliofibularis (rIF) (□) and white iliofibularis (wIF) (◆) during 1–15 min incubations. Values are means ± S.E.M., *N*=3–4 for rIF and wIF at each incubation time, except at 15 min where *N*=2. At some points error bars are smaller than the symbols.

course experiments for *D. dorsalis* (partial regression coefficient *P*-values: for animal mass, *P*=0.35; for bundle mass, *P*=0.93; for bundle diameter, *P*=0.77). As a result, these variables were not included in subsequent analyses of the effects of inhibitors on the rate of lactate uptake. Each muscle bundle was considered to be an independent sample because of the 30 min pre-incubation treatment. Pre-incubation of bundles in 5 mmol l^{-1} lactate established an intracellular lactate concentration of 4.7±1.2 mmol l^{-1} (*N*=10), which was not significantly different from 5 mmol l^{-1} (*P*=0.51).

To test the efficacy of our measurements of reptilian lactate uptake, uptake in mouse SOL was measured for comparison with values from other studies. The uptake rate of lactate by mouse SOL (Fig. 1) decreased significantly over time (*P*=0.013) and appeared to stabilize at 0.26±0.05 nmol mg^{-1} muscle min^{-1} by 10 min of incubation. Analysis showed that the decrease was linear (*P*=0.10 for the time×time interaction).

The uptake rate of lactate by the lizard IF (Fig. 2) decreased as a function of time (*P*<0.001). Across all incubation times, lactate uptake by rIF was higher than that by wIF (*P*<0.001), but uptake rates for rIF and wIF were not affected differently by increased incubation time (*P*=0.16 for the time×fiber interaction). At 2 min, the uptake by rIF was higher than the rates of both wIF (2.4-fold, *P*=0.01) and SOL (2.2-fold, *P*=0.009), while the rates of uptake by wIF and SOL did not differ (*P*=0.87). To estimate the uptake rate of the whole IF, the uptake rates of the rIF and wIF were multiplied by their percentage contribution to the whole IF and summed. The muscle composition was estimated as 71.1 % wIF and 28.9 % rIF (Putnam et al., 1980). At 2 min, the uptake rate of the whole IF was approximately 1.5 times faster than that of the SOL muscle.

The effects of lactate concentration on uptake measurements are shown in Fig. 3 and Table 2. A multiple regression analysis of the data for L-lactate uptake show that, for rIF, there was a significant effect of lactate concentration on the rate of lactate uptake (*P*<0.001) and that this effect decreased as lactate concentration increased (*P*=0.024 for the [lactate]×[lactate] interaction), suggesting a saturable transport process. For wIF, increasing L-lactate concentration caused a linear increase in uptake rates (*P*=0.019). WIF showed no saturation kinetics (*P*=0.88 for the [lactate]×[lactate]

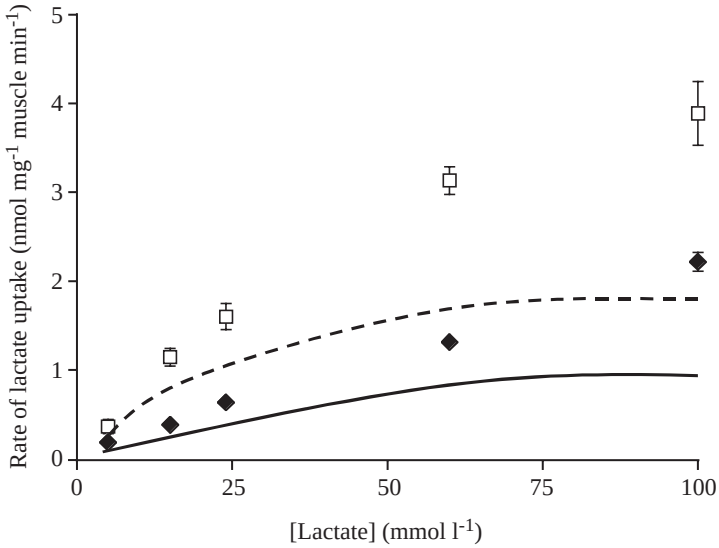

Fig. 3. Mean rate of lactate uptake for *Dipsosaurus dorsalis* red iliofibularis (rIF) (□) and white iliofibularis (wIF) (◆) during 2 min incubations with 5 mmol l^{-1} intracellular and 5–100 mmol l^{-1} extracellular lactate. The lines are the diffusion-corrected uptake rates for rIF (dashed line) and wIF (solid line). Values are means ± S.E.M., *N*=4–6 for rIF and *N*=6–10 for wIF. At some points error bars are smaller than the symbols.

Table 2. *Rates of uptake of* L*-lactate and* D*-lactate*

Concentration (mmol l^{-1})	Uptake rates (nmol mg^{-1} min^{-1})	
	L-Lactate	D-Lactate
rIF		
5	0.36±0.08	0.05±0.01
15	1.14±0.10	0.13±0.03
24	1.60±0.15	0.26±0.01
60	3.13±0.16	0.46±0.11
100	3.89±0.36	1.31±0.30
wIF		
5	0.18±0.04	0.12±0.01
15	0.38±0.05	0.35±0.01
24	0.63±0.06	0.56±0.13
60	1.31±0.08	1.39±0.15
100	2.22±0.11	2.32±0.55

Values are means ± S.E.M., $N \geqslant 4$.
rIF, red iliofibularis; wIF, white iliofibularis.

interaction). The uptake rates of D-lactate were linear for both rIF and wIF and were significantly lower than the corresponding uptake rates for L-lactate (P=0.0012 for rIF and P=0.025 for wIF) (Table 2). These data were used to correct the L-lactate uptake rates for diffusion, and the resulting curves (Fig. 3) were fitted to a Lineweaver–Burke plot. These plots gave an apparent K_m of 78.66 mmol l^{-1} and a V_{max} of 4.16 nmol mg^{-1} muscle min^{-1} for rIF and a K_m of 31.44 mmol l^{-1} and a V_{max} of 0.94 nmol mg^{-1} muscle min^{-1} for wIF.

The lactate uptake rates for rIF and wIF were 0.85 and 0.28 nmol mg^{-1} muscle min^{-1} for the 15 mmol l^{-1} incubation and 0.36 and 0.18 nmol mg^{-1} muscle min^{-1} for the 5 mmol l^{-1} incubations. The 15 mmol l^{-1} incubation uptake rates were significantly greater than the 5 mmol l^{-1} rates for rIF (P=0.004) but not for wIF (P=0.098). Analysis of control lactate uptake rates in the inhibition experiments showed that rIF had a higher uptake rate than wIF in both the 5 and 15 mmol l^{-1} lactate incubation conditions (P<0.001) (Fig. 4A,B). In the 15 mmol l^{-1} lactate incubations, CIN significantly decreased uptake relative to the uninhibited control in rIF by 0.35 nmol mg^{-1} muscle min^{-1} (P<0.001) but not in wIF (decrease of 0.07 nmol mg^{-1} muscle min^{-1}, P=0.105). ETH also significantly decreased uptake in rIF by 0.56 nmol mg^{-1} muscle min^{-1} (P<0.001) but not in wIF (decrease of 0.05 nmol mg^{-1} muscle min^{-1}, P=0.27). In the 5 mmol l^{-1} incubations, CIN and ETH significantly decreased the uptake rate by rIF (decrease of 0.19 and 0.28 nmol mg^{-1} muscle min^{-1}, respectively; P<0.005) and wIF (decrease of 0.10 and 0.08 nmol mg^{-1} muscle min^{-1}; P=0.017 and P=0.038, respectively).

The adenylate energy charge decreased significantly in both the rIF and wIF ETH incubations (P<0.001) but there were no significant decreases at any other point in the experiment (Table 3). Creatine concentrations dropped significantly in all cases except in the rIF ETH incubations, whereas phosphocreatine concentration decreased only in the wIF ETH incubation (P=0.006).

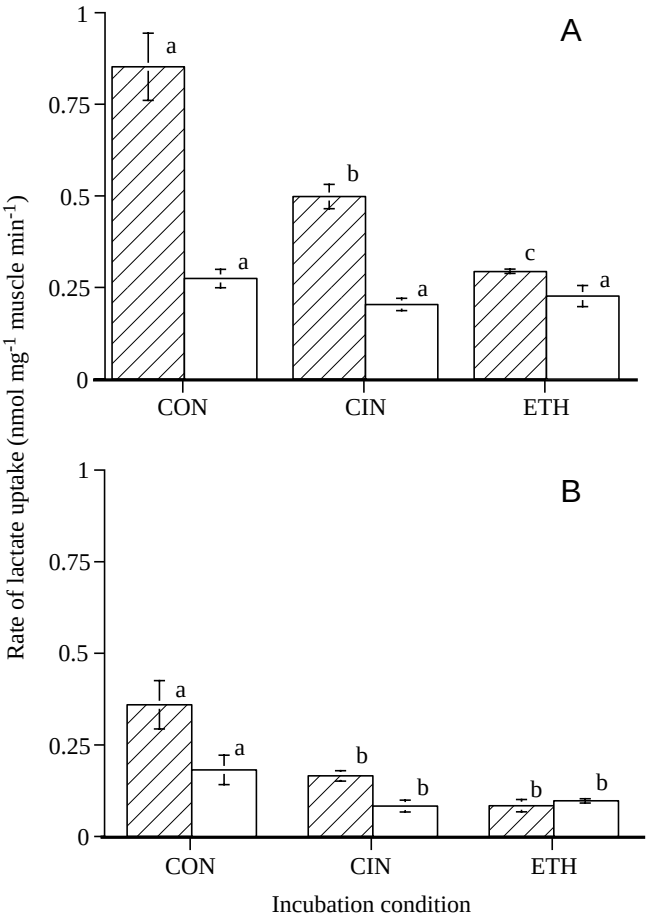

Fig. 4. Rates of lactate uptake in (A) a 5 mmol l^{-1} intracellular:15 mmol l^{-1} extracellular lactate concentration gradient and (B) a 5 mmol l^{-1} intracellular:5 mmol l^{-1} extracellular concentration gradient in the presence of the monocarboxylate transporter inhibitors *N*-ethymaleimide (ETH) and α-cyano-4-hydroxycinnamate (CIN). Values are means ± S.E.M. for red iliofibularis (rIF) (hatched columns) and white iliofibularis (wIF) (open columns); N=4–8 in A and N=3–6 in B for each fiber type in each incubation condition. Comparisons were made only within [lactate] conditions and within fiber types. Columns with different superscripts were significantly different ($P \leqslant 0.05$) for statistical comparisons made between inhibitor conditions, within a muscle type. CON, control.

Discussion

The findings of this study show that facilitated transport is present in the skeletal muscles of *D. dorsalis* and that this process plays an important role in transporting lactate at concentrations seen during typical activities. Lactate metabolism has been shown to play an important part in the physiology and energetics of lizards, and there has been much study of lactate production, its removal and its metabolic fate in different tissues. Mechanisms of lactate transport to and

Table 3. *Metabolite concentrations and adenylate energy charge in red and white iliofibularis muscle throughout the uptake inhibition experiment*

Metabolite	*In vivo* resting (mmol l^{-1})	Post-dissection (mmol l^{-1})	Control incubation (mmol l^{-1})	CIN incubation (mmol l^{-1})	ETH incubation (mmol l^{-1})
rIF					
AEC	0.92±0.02	0.91±0.02	0.87±0.04	0.92±0.01	0.12±0.06*
ATP	5.08±0.91	3.15±0.36	2.53±0.71	2.88±0.90	0.07±0.18*
ADP	0.33±0.10	0.15±0.05	0.22±0.07	0.13±0.08	0.06±0.04
AMP	0.24±0.03	0.24±0.06	0.24±0.05	0.19±0.03	0.14±0.03
PCr	22.69±7.62	21.52±5.66	20.67±6.78	27.76±4.49	17.29±7.21
Cr	18.40±3.39	5.06±2.53*	3.57±1.57*	3.63±1.27*	8.18±3.50*
wIF					
AEC	0.92±0.03	0.90±0.03	0.91±0.02	0.90±0.03	0.67±0.04*
ATP	8.89±1.45	6.03±0.65	4.89±1.06	5.38±1.47	1.03±0.01*
ADP	0.32±0.12	0.33±0.18	0.19±0.05	0.40±0.17	0.10±0.00
AMP	0.55±0.04	0.50±0.10	0.37±0.09	0.35±0.06*	0.51±0.11
PCr	37.34±4.23	29.42±2.56	30.07±3.08	29.57±8.44	11.47±3.71*
Cr	23.90±3.69	5.51±0.58*	5.14±1.81*	7.90±1.60*	4.51±1.41*

Values are means ± S.E.M., N=3 (except for rest, N=5).
AEC, adenylate energy charge; Cr, creatine; PCr, phosphocreatine; rIF, red iliofibularis; wIF, white iliofibularis.
*Indicates a significant difference from the resting value.

from these areas of production and utilization have not yet been examined. Our demonstration of active transport of lactate opens a new area of study into the activity physiology of lizards and the factors that regulate their muscular function.

Assessment of uptake procedure

To assess the overall effects of the incubation procedure and inhibitors on muscle condition, AEC and creatine and phosphocreatine concentrations were measured at various points in the 5 mmol l^{-1} inhibition procedure. These results (Table 3) show that, except for the muscles in the ETH incubations, all the muscle fiber bundles appeared to maintain an energy charge close to resting levels throughout the procedure and at the end of the incubations. However, the diminished PCr concentrations and AEC of the ETH-inhibited muscles suggest pathological effects on the physiology of the muscle cells beyond a simple inhibition of lactate transport. This raises questions about whether ETH is an appropriate inhibitor for studying protein-mediated transport in reptilian muscle fibers. Our results for the ETH incubations should not, therefore, be used to draw any conclusions about the processes of lactate transport in the muscles examined because they may not be an indication of the actual sensitivity of reptilian lactate transport proteins to ETH inhibition and instead simply be the result of deleterious effects of ETH on reptilian cellular homeostasis.

The difference between resting and experimental condition creatine concentrations merits comment. Phosphocreatine levels remained elevated; however, creatine levels were low relative to resting values (Table 3). Creatine loss was not associated with a similar decline in the concentration of any adenylate. Lower creatine concentrations in incubated muscle do not appear to be due to a loss of membrane integrity because sucrose partitioning persisted in these tissues. Experiments involving electrical stimulation in which the IF was subjected to identical dissection and similar handling showed that the muscles remained capable of normal contractions for several hours (T. T. Gleeson, unpublished data) further suggesting that membrane viability remained throughout our incubation procedure. Creatine production and breakdown have not been well studied in lizards, but in mammals they have been shown to involve multiple steps, some reversible and some not, which eventually lead to the formation of creatinine (Wyss and Kaddurah-Daouk, 2000). It is possible that some of the degradative steps proceed at a greater rate in lizards than in mammals, leading to greater loss over time.

Measurements of lactate uptake in the mouse SOL were primarily to provide an assessment of our procedure. Our results for these measurements (Fig. 1), while somewhat lower than values reported elsewhere for similar incubation conditions (Bonen and McCullagh, 1994; Juel and Wibrand, 1989; McDermott and Bonen, 1994), are of the same magnitude and show that our methods were sound.

Time- and concentration-dependency of lactate uptake

Measurements of lactate uptake by rIF over time show a pattern similar to that in mammals. Initial rates were rapid and decreased linearly for the first 3 min before leveling off (Fig. 2). Fig. 3 shows both the total and diffusion-corrected lactate uptake rates. At physiological concentrations, it appears that approximately 26–35 % of the transport is *via* diffusion in both rIF and wIF, while at higher concentrations (100 mmol l^{-1}) nearly 60 % of the transport is due to

diffusion. The K_m and V_{max} measured for the rIF are much higher than values reported for mammalian skeletal muscle, suggesting a high-capacity, low-affinity transport system relative to mammalian muscle (Watt et al., 1988; Juel and Wibrand, 1989; McDermott and Bonen, 1994). Physiological lactate concentrations in reptiles are often higher than in mammals (Gleeson, 1996), and these greater transport capacities could be beneficial in aiding recovery after activity.

Inhibitor effects

CIN (5 mmol l^{-1}) has been shown to inhibit uptake of 1 mmol l^{-1} lactate into isolated mouse SOL (McDermott and Bonen, 1994) and perfused rat hindlimb (Watt et al., 1988) by 22 %. Our data show inhibition of lactate uptake into rIF by 15 mmol l^{-1} CIN of 54 % and 42 % in the 5 and 15 mmol l^{-1} lactate treatments, respectively, and of 54 % for wIF in the 5 mmol l^{-1} lactate treatment (Fig. 4A,B). The fact that the inhibition measured here is greater than has been recorded in mammalian muscle may indicate either that the oxidative skeletal muscle of *D. dorsalis* has a higher percentage of its lactate uptake *via* a protein-mediated system or that its proteins simply have a higher affinity for these inhibitors.

The significant inhibition of uptake into rIF fibers by CIN in both 5 and 15 mmol l^{-1} lactate incubations suggests that facilitated transport plays a major role in the uptake of lactate at both resting and post-activity lactate concentrations. The significant inhibition of uptake into wIF by CIN in the 5 mmol l^{-1} lactate incubation and the apparent saturation kinetics of the diffusion-corrected lactate uptake suggest that MCT-like facilitated transport may also play a role in lactate uptake into these fibers. However, there was no significant inhibition of the uptake rates into wIF for the 15 mmol l^{-1} incubations, and L-lactate uptake showed no saturation up to 100 mmol l^{-1}. This could indicate that facilitated transport may play a major role in lactate uptake in wIF under resting conditions but that it may play a lesser role in steeper lactate concentration gradients. The low K_m and V_{max} obtained for wIF, relative to rIF, support this possibility.

To the best of our knowledge, the effects of CIN on mammalian white muscle have yet to be addressed. There are data on the effect of CIN on lactate transport in trout white muscle suggesting that CIN inhibits lactate efflux but not uptake. These data suggest that different pathways of protein-mediated uptake and efflux are present in trout. Trout, however, show a pattern of active lactate retention in white muscle that is very different from the pattern of rapid release into the bloodstream seen in reptilian and mammalian white muscle and that makes comparisons of CIN inhibition between trout and other taxa difficult (Laberee and Milligan, 1999; Wang et al., 1997). Taken together, the inhibition by CIN at 5 mmol l^{-1} and the diffusion-corrected saturation kinetic data suggest that lizard white muscle utilizes MCT-mediated transport for uptake but not to the same degree as appears to be the case for rIF.

While MCT transport may play a limited role in uptake into wIF, this does not rule out the presence of MCT-facilitated lactate efflux in lizard glycolytic muscle. In mammalian muscle, there is evidence that lactate uptake and efflux tend to occur *via* different MCT isoforms (MCT1 and MCT4, respectively) which show different distributions in oxidative and glycolytic muscles (McCullagh et al., 1996; Wilson et al., 1998; Bonen et al., 2000). If this is also the case in lizards, it would be necessary to examine the effects of transport inhibitors on lactate efflux from wIF to assess more fully the role of MCT transport in this muscle.

Correlation between uptake rates and lactate production/utilization rates

Post-exercise lactate concentrations in *D. dorsalis* rIF and wIF can be raised to 34.5 and 47.9 mmol kg^{-1}, respectively (Gleeson and Dalessio, 1990). Dealing with this lactate is vital both for acid–base balance and for recovery of depleted glycogen stores. After 2 h of recovery from exhaustive activity, incorporation of lactate into glycogen *in vivo* is approximately 48 nmol kg^{-1} in rIF and 18 nmol kg^{-1} in wIF (Gleeson and Dalessio, 1990), and skeletal muscle glycogen synthesis accounts for approximately 50 % of the lactate removal during this period (Gleeson and Dallesio, 1989). The high rates of lactate uptake measured for rIF and the lower rates for wIF relate well to their respective rates of lactate utilization for glycogen synthesis. Glycolytic muscle is the major site of lactate production, and the intracellular concentrations post-activity are likely to be high enough to support the low rates of glycogen synthesis of these fibers during recovery. Although there appears to be little facilitated lactate uptake, there may be lactate efflux from these fibers at a significant rate during recovery to speed the restoration of intracellular pH and make lactate available to the oxidative fibers for metabolism. Again, measurements of lactate efflux would be required to determine any role of MCT proteins in this process.

Comparison of rIF and wIF with oxidative and glycolytic muscles in other animals

Numerous studies have shown that mammalian glycolytic muscles have lower rates of lactate uptake than oxidative muscles (Juel et al., 1991; Bonen and McCullagh, 1994; McCullagh et al., 1997), and our results for reptilian muscle are similar. Across a range of rat and mouse skeletal muscles, there seems to be a strong correlation between the rates of lactate uptake and the oxidative capacity of the muscles (McCullagh et al., 1996). On the basis of citrate synthase activities, lizard rIF has an oxidative capacity similar to or lower than that of these mammalian oxidative muscles (Gleeson and Harrison, 1988; Moerland and Kushmerick, 1994; McCullagh et al., 1996). Therefore, lizard rIF might also be expected to have the same or a lower uptake rate. Our results indicate that rIF has a significantly higher lactate uptake rate than the mouse SOL (Figs 1, 2), suggesting that the correlation seen between oxidative capacity and lactate uptake in mammals is not present in *D. dorsalis*.

Concluding remarks

This study demonstrates that a protein-mediated lactate transport system functions in reptilian skeletal muscle. It is the first report of such transport in this vertebrate class. Lactate uptake rates are higher in oxidative than in glycolytic muscle, and lactate uptake is more sensitive to inhibitors in oxidative muscle. The data presented here also suggest that rates of uptake may be higher in reptilian oxidative muscle than they are in mammalian muscle of equivalent, or higher, oxidative capacity. The uptake may be due to a similar group of MCT proteins to that described in mammalian muscle.

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