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Lactate transport by rainbow trout white muscle: kinetic characteristics and sensitivity to inhibitors

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Wang, Yuxiang, Pamela M. Wright, George J. F. Heigenhauser, and Chris M. Wood. Lactate transport by rainbow trout white muscle: kinetic characteristics and sensitivity to inhibitors. *Am. J. Physiol.* 272 (*Regulatory Integrative Comp. Physiol.* 41): R1577–R1587, 1997.—This study used an isolated-perfused tail-trunk preparation of rainbow trout to examine the uptake and release of lactate (Lac) and metabolic protons (ΔH_m^+) in resting and exercised fish white muscle. In exercised muscle, L(+)-Lac efflux was inhibited (~40%) by 5 mM α-cyano-4-hydroxycinnamate (CIN), but not by 0.5 mM 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) or 0.1 mM amiloride. These results suggest that Lac release occurs through a Lac^--H^+ symport and the free diffusion of lactic acid (HLac) or Lac^-, but not via the Lac^-/HCO_3^--Cl^- antiporter. Lac efflux was accompanied by ΔH_m^+ influx in all treatments, and increased ΔH_m^+ influx occurred after SITS treatment. In resting muscle, Lac uptake rates were greater than Lac efflux rates in the postexercise preparation. L-Lac influx exhibited partial saturation kinetics, whereas D(−)-Lac influx was linearly related to its extracellular concentration (0–32 mM). At 16 mM extracellular L-Lac, with a negligible transmembrane L-HLac gradient and an outwardly directed net driving force on L-Lac^-, CIN, and SITS reduced net L-Lac uptake by 75 and 45%, respectively. At 16 mM extracellular concentration, D-Lac influx was 64% of the net L-Lac influx. These results suggest that in trout muscle at 16 mM extracellular L-Lac, the Lac^--H^+ symport accounts for 30–36%, the Lac^-/HCO_3^--Cl antiport for 39–45%, and diffusion for 19–25% of uptake, although the latter is probably overestimated and the former underestimated for methodological reasons. Net L-Lac efflux was not affected by extracellular D-Lac concentration and/or D-Lac influx, implying the existence of a concurrent L-Lac efflux during L-Lac influx. The D-Lac influx kinetics data indicated that the Lac^-/HCO_3^- antiport was not saturable in the extracellular D-Lac concentration range of 0–32 mM. This study clearly demonstrates the involvement of carrier-mediated transport in transmembrane Lac movement in fish muscle and supports the "active lactate retention" mechanism proposed by Turner and Wood (*J. Exp. Biol.* 105: 395–401, 1983).

exercise; metabolic hydrogen ion; kinetics; perfusion; 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid; α-cyano-4-hydroxycinnamate; amiloride

IN HIGHER VERTEBRATES the exchange of lactate across skeletal muscle cell membranes is regarded as an important process in the regulation of its production, redistribution, and utilization (2, 5). Thus, instead of simply being treated as a terminal waste product of "anaerobic glycolysis," causing fatigue and acid-base disturbance and eventually being eliminated from the synthesis sites (e.g., skeletal muscles), the released lactate may be transferred, as a precursor, to red muscle, cardiac muscle, or even other less activated white muscles for oxidation. Hepatic gluconeogenesis (i.e., the Cori cycle) and in situ glyconeogenesis in white and red muscle may also occur, using lactate as a substrate (for reviews see Refs. 28 and 29), although the latter remains controversial (24). The glycolytic fibers (white muscle), once thought to be simply the production site of lactate, are also involved in the removal of extracellular lactate from other tissues or exogenous sources (for reviews see Refs. 2 and 22). Transmembrane lactate (Lac, refers to total lactate) movement has been found to occur via passive diffusion of undissociated lactic acid (HLac) or ionic Lac^-, as well as through carrier-facilitated transport, namely, Lac^--H^+ symports and Lac^-/HCO_3^--Cl^- antiports. The contribution of each route, however, varies depending on species, pH gradient, Lac concentration ([Lac]) gradient, age, training, metabolic state, and hormonal conditions (2, 6, 30).

In fish, in contrast to higher vertebrates, our understanding of Lac transport mechanisms remains fragmentary. Recent studies have suggested that the major portion of the postexercise lactate load is retained in the white muscle by active or passive mechanisms and is metabolically removed in situ by oxidation and/or glycogen resynthesis (for reviews see Refs. 6, 18, 21, 30, 36). This clearance of the postexercise lactate load from fish white muscle can take >12 h in contrast to <1 h in higher vertebrates. The nonrelease or slow release of Lac from white muscle is thought to be advantageous for fish for several reasons (36): *1*) The fish body is composed of as much as 60% white muscle, and extracellular fluid (ECF) is poorly buffered and small in volume compared with muscle intracellular fluid (ICF) (7, 20). *2*) During exhaustive exercise in fish, the white muscle fibers are uniformly activated and loaded with lactate; consequently, the removal of lactate to less exercised white muscle for metabolic processing, as occurs in mammals, may not be an option. *3*) Lac oxidation in cardiac muscle and hepatic gluconeogenesis play a very minor part in total lactate clearance (18, 21). However, the pertinent question remains: Does efflux of Lac and H^+ occur at rates well below the membrane-specific transferring capacity due to the "equilibrium limitation," i.e., ECF concentrations are elevated to a level that gains an electrochemical equilibrium with ICF concentrations (7), or is there an active lactate retention mechanism involved in the process, as originally proposed by Turner and Wood (31)?

Recently, we developed an isolated-perfused tail-trunk preparation of the rainbow trout for the study of

metabolite fluxes in white muscle (33, 34). The objectives of the present study were to utilize this preparation to characterize the transmembrane movement of Lac and metabolic H^+ (ΔH_m^+) in postexercised and resting white muscle. Specific inhibitors of carrier-mediated transport and manipulations of perfusate concentrations of L(+)-Lac and its isomer D(−)-Lac were employed to identify the roles of carrier-mediated transporters and passive diffusion in Lac release and uptake in white muscle.

MATERIALS AND METHODS

Experimental Animals

Rainbow trout (*Oncorhynchus mykiss*) of either sex (400–600 g) were obtained from a local trout hatchery and held as described previously (34). During the holding period of 2–4 mo, trout were fed ~2% (wet body wt/day) of trout grower floating pellets (Aquaculture Zeigler Bros., 50% protein, 15% lipid) until they reached the desired size (800–1,000 g). Fish were acclimated to the experimental temperature of 15 ± 1°C for 5–7 days without feeding before experimentation. Fish were placed for 48 h in darkened acrylic flux chambers supplied with flowing dechlorinated water to establish "resting conditions." "Exercised" fish were then manually chased in a 150-liter tank for 6 min to exhaustion. The resting or exercised fish was then anesthetized with a high dose of MS-222 (0.5 g/l) neutralized with NaOH and killed within 1 min without struggling. The portion of the fish posterior to the anus was cut off for the tail-trunk perfusion study. At this time, an initial white muscle sample (3–5 g) from the area between the dorsal fin and the lateral line immediately anterior to the point of section was preserved by freeze-clamping (see below) to establish the metabolic condition of the preparation before the start of perfusion.

Perfused Tail-Trunk Preparation and Experimental Design

The isolated-perfused tail-trunk preparation used in this study has been described previously (see Refs. 33 and 34 for detailed setup and evaluation). The study consisted of two series: *1*) a postexercise series, which was designed to examine Lac transport mechanisms during postexercise Lac release, and *2*) a resting series, which was designed to characterize lactate uptake mechanisms and their kinetics.

Cortland salmonid saline, supplemented with 3% bovine serum albumin (Sigma Chemical, fraction V), was used as the perfusate. The saline was equilibrated with 0.25% CO_2-99.75% O_2, and the desired pH (resting plasma pH ~8.0; exercised pH ~7.5) was achieved by varying the saline $NaHCO_3$ concentration from 7 to 2 mmol/l under a constant P_{CO_2} (P_{CO_2} ~2 Torr). Inflowing P_{O_2} was kept in the range of 480–530 Torr. Arterial and venous sampling ports were implanted into the caudal artery and caudal vein, respectively, to allow the collection of inflowing and outflowing perfusate samples into gastight Hamilton syringes at 30 and 60 min after the perfusion was started (see Fig. 2 of Ref. 34 for schematic diagram of setup). The perfused trunk preparation was placed in a 15 ± 0.5°C water bath, and the perfusion rate of 2 ml·min^{-1}·100 g tail wt^{-1} was delivered by a peristaltic pump. In all treatments the perfusate, for the first 30 min, consisted of heparinized Lac-free saline to facilitate blood purging and to secure a steady state and a red blood cell-free preparation. Experimental treatments were introduced in the second 30 min of perfusion. A pressure transducer, attached to the constant-flow perfusion line, monitored variations in the vascular resistance of the preparation. In our previous studies the acid-base and metabolic conditions of the perfusion preparation were proven to be satisfactory over the experimental period (33, 34), and the exercise protocol was shown to be sufficient to elevate intracellular Lac concentration ($[Lac]_i$) to a level comparable to that seen in vivo after exhaustive exercise (18, 36).

At 30 and 60 min into the perfusion, arterial and venous samples (2 ml each) of perfusate were collected, and pH, total CO_2 (T_{CO_2}), P_{O_2}, and protein content were analyzed immediately. A portion (300 μl) of the saline was deproteinized with two portions of 8% perchloric acid (PCA). The supernatant of this PCA extract was then stored at −70°C for later analysis of [Lac]. The remainder of the perfusate sample (500 μl) was used to measure Na^+ ($[Na^+]$), K^+ ($[K^+]$), and Cl^- concentrations ($[Cl^-]$).

Immediately after the termination of the 60-min perfusion, a white muscle tissue sample (3–5 g) from the area above the lateral line of the tail trunk was preserved by freeze-clamping in liquid N_2 with a pair of precooled aluminum tongs. The muscle samples were stored in liquid N_2 before further analysis for $[Lac]_i$, intracellular pH (pH_i), $[Cl^-]$, $[Na^+]$, $[K^+]$, and water content.

Postexercise series (efflux experiments). This series continued the approach of our previous work, in which the effects of transmembrane potential and pH gradients on Lac efflux were studied (33). In the present study, various pharmacological inhibitors were applied to postexercise trunks to identify the transport processes involved in Lac efflux. In each experiment, Lac-free saline, containing one of the following inhibitors, was used: 5 mM α-cyano-4-hydroxycinnamic acid (CIN; Sigma Chemical), 0.5 mM 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS; Sigma Chemical), or 0.1 mM amiloride (Sigma Chemical). An extra 5 mM $NaHCO_3$ was added to the saline containing CIN to neutralize the acidification introduced by this acid, such that the fluid pH and HCO_3^- concentration ($[HCO_3^-]$) were ~7.5 and ~2 mmol/l, respectively. CIN is considered a specific competitive blocker of the Lac^--H^+ symport and a noncompetitive blocker of the Lac^-/HCO_3^--Cl^- antiport. SITS, at 0.5 mM, blocks only the anion antiport (22). Amiloride blocks the Na^+/H^+ antiport, and this antiport may influence the acid-base status across the muscle cell membrane and thereby indirectly affect the pattern of Lac flux (22).

Inasmuch as the amiloride tests were performed on a different batch of trout with quantitatively different postexercise responses, a separate control series was performed for comparison to the amiloride treatment. Inasmuch as dimethyl sulfoxide (DMSO, 0.5% vol/vol) was required to dissolve the pharmacological agents in the saline, control experiments, with or without DMSO (0.5% vol/vol) in the perfusate, were also conducted on the postexercise series to identify any potential effects introduced by this solvent.

Resting series (influx experiments). The resting series, consisting of five experiments, was designed to study mechanisms of transmembrane Lac uptake in white muscle.

Two parallel sets of experiments, with various extracellular concentrations of L(+)- or D(−)-Lac ($[L\text{-}Lac]_e$ and $[D\text{-}Lac]_e$, respectively; 0, 1, 2, 4, 8, 16, and 32 mM), were performed to characterize the kinetics of Lac uptake via carrier-mediated transporters and free diffusion in the resting muscle. L(+)- and D(−)-Lac (Sigma Chemical) were added as sodium salts. The Lac^--H^+ symport is considered to be stereospecific for the L-Lac isomer, whereas the anionic antiport (Lac^-/HCO_3^--Cl^-) does not discriminate between L- and D-Lac (22).

The roles of carrier-mediated monocarboxylate transporters and free diffusion in Lac uptake in white muscle were also

assessed by applying pharmacological blockers (5 mM CIN or 0.5 mM SITS) at [L(+)-Lac]$_e$ of 16 mM.

In addition, a low extracellular pH treatment (pH_e ~7.6) at 16 mM L(+)-Lac was conducted to study the effect of pH_e depression on Lac uptake. The rationale behind the low pH treatment was to evaluate whether HLac diffusion is involved in Lac uptake by increasing the extracellular HLac concentration ([HLac]).

Analytic Protocols

The pH_e and P_{O_2} were measured in arterial and venous saline at 15°C using a Radiometer microelectrode (model E5021) and acid-base analyzer (model PHM-84) and a Radiometer P_{O_2} electrode (model E5046) connected to an O_2 meter (model OM-200, Cameron Instrument), respectively. Perfusate T_{CO_2} was measured using a T_{CO_2} analyzer (model 965, Corning Canada). P_{CO_2} and [HCO_3^-] were calculated using the Henderson-Hasselbalch equation with appropriate constants (α_{CO_2} and pK') for trout true plasma at 15°C (1). The perfusate electrolytes (Na^+, K^+, and Cl^-) were measured by means of an AVL specific electrode (model 983-S electrolyte analyzer). The specific L- and D-Lac hydrogenase (Sigma Chemical) NAD/NADH assays were used to analyze the perfusate [L-Lac] and [D-Lac], respectively. Perfusate total protein and water content were measured with a refractometer (American Optical). The non-HCO_3^- buffer capacities (β) of salines containing DMSO, CIN, SITS, or amiloride were determined according to the methods described previously (34); L- and D-Lac had no measurable effect on β.

Freeze-clamped muscle samples were pulverized in liquid N_2 with a mortar and pestle. A portion of the frozen muscle tissue (200–300 mg), without pulverization, was used to determine the tissue water content (C_w) by drying in an oven at 70°C for 48 h to constant weight. The muscle tissue powder was then used for pH_i measurements or lyophilized for 64 h to a dry powder for analysis of [Lac] and ion concentrations. Muscle pH_i was measured by the homogenization technique described elsewhere (23, 34). The freeze-dried muscle powder (~20 mg) was weighed, then extracted with 1 ml of 8% PCA. The supernatant of the PCA-extracted tissue was used to determine tissue L- or D-Lac levels enzymatically. L-Lac measurements on saline and muscle tissue were also carried out in the D-Lac series to monitor the potential effects of D-Lac treatment on L-Lac movement. For tissue ion measurements, the freeze-dried tissue powder (~20 mg) was weighed, then extracted in 1 N HNO_3 (1 ml) at 50°C for 48 h. Flame atomic absorption spectrometry (model AA-1275, Varian) was used to determine [Na^+] and [K^+] in the diluted supernatant, whereas [Cl^-] was analyzed by coulometric titration. Common standards were used to calibrate all the instruments involved in saline and tissue ion analyses.

Calculations

Flux rates for Lac, ΔH_m^+, T_{CO_2}, HCO_3^-, O_2, and ions were calculated according to the Fick principle using the perfusion rate (2 ml·100 g^{-1}·min^{-1}) and the measured concentration differences between the arterial and venous perfusate sample.

The muscle ECF volume (ECFV, ml/g) was estimated by the "Cl^--K^+ space" equation of Conway (see Refs. 3 and 34 for details) based on tissue C_w, muscle [K^+], and muscle [Cl^-], whereas the ICF volume (ICFV, ml/g) was given by the difference between C_w and ECFV. All concentrations of substances in the muscle tissue were corrected for the level in trapped ECF and expressed per liter of ICFV (i.e., mmol/l ICFV).

The arteriovenous difference in "metabolic acid" (ΔH_m^+) was calculated according to the following equation (20)

$$\Delta H_m^+ = [HCO_3^-]_a - [HCO_3^-]_v - \beta \times (pH_a - pH_v) \quad (1)$$

where subscripts a and v denote measured values in arterial and venous saline, respectively.

The total lactate (Lac) is the sum of the ionic (Lac^-) and nonionic (HLac) forms and, with a pK' of ~3.75, exists largely as Lac^- under physiological pH (6.0–8.0) according to the following equation

$$pH = pK' + \log \frac{[Lac^-]}{[HLac]} \quad (2)$$

On the basis of the above relationship, [HLac] can be estimated as

$$[HLac] = \frac{[Lac^-]}{1 + 10^{(pH - pK)}} \quad (3)$$

Therefore, at constant [Lac], a decrease in pH results in a higher [HLac]. Under "normal" resting and postexercise conditions, there will always be an outwardly directed HLac gradient across the muscle cell membrane, owing to the ~0.6 unit lower pH_i than pH_e and a higher total [Lac]$_i$.

If the muscle cell membranes are freely diffusible only to Lac^-, then the diffusive Lac flux should be a linear function of the net driving force (NDF) on Lac^-

$$NDF = E_m + \frac{RT}{ZF} \ln \frac{[Lac^-]_e}{[Lac^-]_i} \quad (4)$$

where R is the gas constant, T is absolute temperature, Z is the valence of the ion species, and F is the Faraday constant (96,500 C/mol). As in our previous studies (33, 34), the transmembrane potential (E_m) of the white muscle was estimated from measured intra- and extracellular [K^+], [Na^+], and [Cl^-] according to the Goldman-Hodgkin-Katz equation (8).

Statistics

Values are means ± SE, and significance was taken at $P \leq 0.05$ in all tests. Within each treatment group, Student's paired t-test was used to examine the differences between 30- and 60-min values. One-way analysis of variance was used to test for significant differences among the means in the various treatments in the exercised and resting experiments, then post hoc comparison by means of Duncan's critical and multiple range test was performed between the control and each treatment at common times. The two-tailed Student's t-test for nonpaired data was also used to test corresponding points in the L- and D-Lac series (Statistica, Statsoft). Linear regression was carried out on the data of the D-Lac experiment using the least squares regression method. Curve fits to other data sets were accomplished using nonlinear least squares regression methods (SigmaPlot, Jandel Scientific).

RESULTS

Perfusion pressures (10–16 cmH_2O), and therefore vascular resistances, at constant flow of 2 ml·100 g^{-1}·min^{-1} were similar in the resting and postexercise series and remained generally stable throughout the 60 min of perfusion. The rate of O_2 uptake of the preparation (0.8–1.0 mmol·kg^{-1}·h^{-1}) did not vary significantly over the course of the experiment, and there was no significant difference between the resting and exercised series (results not shown).

Postexercise Series

The exhaustive exercise protocol used in this study introduced a pronounced intracellular acidosis, intracellular Lac accumulation, and partial depolarization in white muscle at 0-min postexercise in all treatment groups (Table 1). Over the 60 min of perfusion, there was no correction of pH_i or reduction of $[Lac]_i$, but E_m repolarized slightly in all treatment groups (Table 1).

In control experiments the presence or absence of 0.5% DMSO had no significant effect on postexercise responses. However, control experiments for the two different batches of trout ("control-1" accompanied the CIN and SITS series; "control-2" accompanied the amiloride series) yielded quantitatively different results and, therefore, have been presented separately. The trout of the second batch did not appear to exercise as intensively as those of the first batch, as evidenced by lower $[Lac]_i$ and higher pH_i in the muscle samples obtained before and after perfusion (Table 1).

Lac efflux. Postexercise muscle $[Lac]_i$ was ~75 mM ICF in the control-1, CIN, and SITS treatments (Table 1). However, in the control-2 and amiloride treatments, postexercise $[Lac]_i$ was only ~70% of this level (Table 1). In all five treatments, Lac efflux rates were similar (~2 mmol·kg^{-1}·h^{-1}) after 30 min of perfusion (Fig. 1). After another 30 min of perfusion, Lac flux rate did not change in either of the control groups or the SITS group, whereas CIN treatment resulted in a significant 40% decrease relative to its respective control group (control-1; Fig. 1). Although net Lac efflux tended to fall in the amiloride group in the second 30 min (Fig. 1), the difference was not statistically significant with respect to the relevant control group (control-2).

Table 1. *White muscle pH_i, E_m, and $[Lac]_i$ before and after 60 min of perfusion in postexercise treatments*

	n	pH_i	E_m, mV	$[Lac]_i$, mM
Control-1	8			
0 min		6.453 ± 0.057	−69.9 ± 1.3	74.05 ± 4.31
60 min		6.444 ± 0.077	−74.6 ± 1.2*	74.05 ± 5.66
CIN	9			
0 min		6.413 ± 0.029	−62.5 ± 3.8	71.08 ± 7.08
60 min		6.415 ± 0.045	−75.6 ± 1.8*	74.45 ± 8.62
SITS	10			
0 min		6.450 ± 0.028	−71.4 ± 1.2	75.41 ± 5.28
60 min		6.431 ± 0.031	−76.4 ± 2.5*	78.89 ± 6.36
Control-2	9			
0 min		6.488 ± 0.057	−68.8 ± 1.8	46.04 ± 4.61
60 min		6.452 ± 0.061	−78.4 ± 1.8*	50.76 ± 5.88
Amiloride	11			
0 min		6.530 ± 0.036	−68.3 ± 0.9	51.52 ± 7.71
60 min		6.508 ± 0.039	−80.0 ± 1.3*	52.44 ± 3.22

Values are means ± SE; n, no. of preparations. Control-1 accompanied α-cyano-4-hydroxycinnamate (CIN) and SITS series; control-2 accompanied amiloride series. Intracellular lactate concentration ($[Lac]_i$) was expressed in mM intracellular fluid water; 0-min values were corrected for [Lac] in extracellular fluid and extracellular fluid volume obtained from a previous in vivo study (32). Transmembrane potential (E_m) was calculated according to Goldman-Hodgkin-Katz equation (see Refs. 8 and 34 for equations). pH_i, intracellular pH. *Significant difference ($P \leq 0.05$) from corresponding 0-min (preperfusion) value.

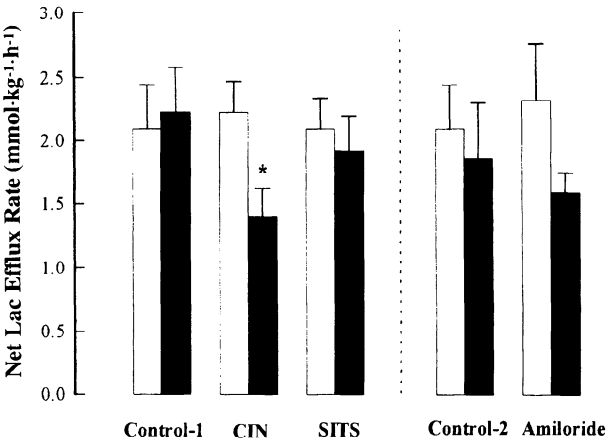

Fig. 1. Net efflux rates of lactate (Lac) from postexercise perfused tail-trunk preparation in various treatment groups after 30 min (open bars) and 60 min (solid bars) of perfusion. Control-1 accompanied α-cyano-4-hydroxycinnamate (CIN) and 4-acetamido-4′-isothiocyanostilbene-2,2′-disulfonic acid (SITS) series; control-2 accompanied amiloride series. Tail-trunk preparations were taken from exhaustively exercised trout. Values (mmol·kg wet wt tail-trunk^{-1}·h^{-1}) are means ± SE; n = 8, 9, 10, 9, and 11 for control-1, CIN, SITS, control-2, and amiloride groups, respectively. *Significant difference ($P \leq 0.05$) from corresponding control.

Metabolic acid flux. As in our previous study (34), the uncoupling of ΔH_m^+ and Lac movements was again very clearly demonstrated, because Lac efflux was always accompanied by ΔH_m^+ influx (Figs. 1 and 2*A*). Postexercise ΔH_m^+ flux was inwardly directed (net uptake into muscle) after 30 and 60 min of perfusion in all treatments (Fig. 2*A*). However, the ΔH_m^+ influx was reduced by 50% after 60 min of perfusion in the control-1, control-2, and CIN groups, whereas there was no such decrease after SITS treatment. In fact, ΔH_m^+ influx was significantly elevated by SITS relative to control-1 at 60 min. The amiloride treatment depressed ΔH_m^+ influx to a level not significantly different from zero or control-2 after 60 min of perfusion (Fig. 2*A*). Despite the constancy of O_2 uptake, T_{CO_2} efflux rate dropped over the postexercise perfusion period in all treatments, but the values did not vary significantly among these treatments at 30 or 60 min (Fig. 2*B*). At 60 min the acid-base status of the venous effluent perfusate and arteriovenous differences in T_{CO_2}, P_{CO_2}, and $[HCO_3^-]$ were very similar in the two control and all three experimental groups (Table 2).

Resting Series

In resting preparations at 60 min the white muscle pH_i (~7.25) and $[Lac]_i$ (~3 mM) were generally similar throughout the various [Lac] in the L-Lac (without inhibitors) and D-Lac series (see Table 3). These values were not significantly different from their corresponding preperfusion levels at 0 min (data not shown) or from the in vivo resting values (32). In comparison with the 16 mM L-Lac group, $[Lac]_i$ increased ~75 and 80%, respectively, in the CIN- and SITS-treated groups (see Table 3), despite the fact that their preperfusion $[Lac]_i$ were not different from the typical resting values reported above. In contrast, at arterial [L-Lac] ($[L\text{-Lac}]_a$)

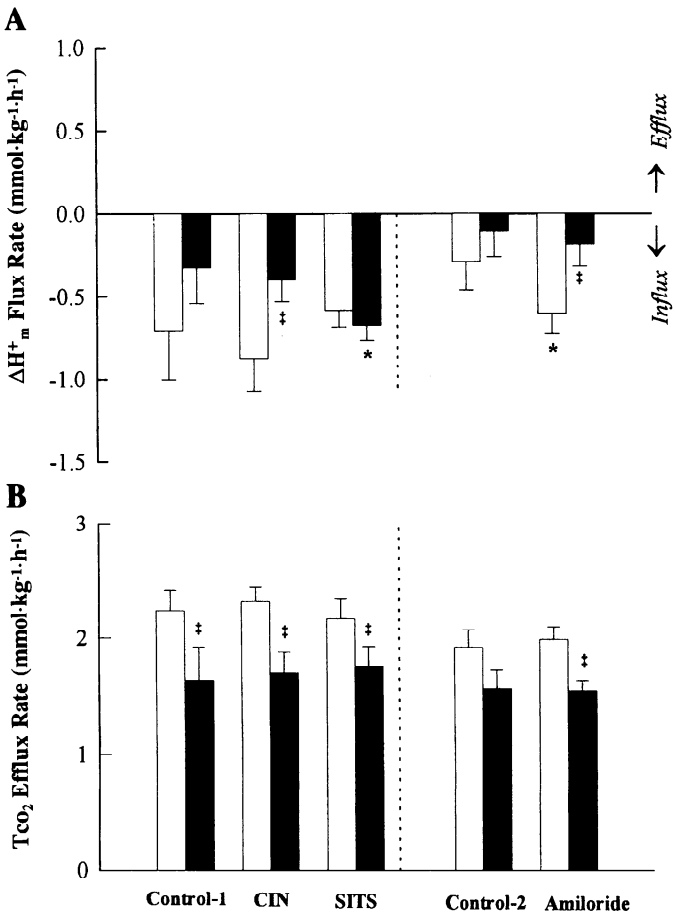

Fig. 2. *A*: transmembrane metabolic H^+ (ΔH_m^+) flux rates (*Eq. 1*) across postexercise perfused tail-trunk preparation after 30 min (open bars) and 60 min (solid bars) of perfusion in control-1, CIN, SITS, control-2, and amiloride groups. *B*: total CO_2 (T_{CO_2}) efflux rates across preparation after 30 min (open bars) and 60 min (solid bars) of perfusion in various postexercise treatment groups. See Fig. 1 legend for further information. *Significant difference ($P \leq 0.05$) from corresponding control. ‡Significant difference ($P \leq 0.05$) from 30 min.

of 16 mM, lower arterial pH (pH_a; pH 7.68 + 16 mM) did not result in significant changes in $[Lac]_i$ (see Table 3). Notably, these treatments, which raised $[Lac]_i$, did not lower pH_i and, in the case of lower pH_a, actually caused a slight rise in pH_i.

Lac influx. As shown in Fig. 3*A*, net L-Lac influx into resting white muscle was actually slightly negative (i.e., efflux) at low extracellular $[L\text{-}Lac]_a$ (0, 1, and 2 mM), in accord with the outwardly directed concentration gradients under these conditions (cf. Table 3). Net L-Lac influx became positive at higher $[L\text{-}Lac]_a$. Influx started to level off at a rate of ~5 $mmol \cdot kg^{-1} \cdot h^{-1}$ at $[Lac]_a$ of 16–32 mM, suggesting saturation kinetics. Notably, this absolute net flux rate in the inward direction was more than twice that (~2 $mmol \cdot kg^{-1} \cdot h^{-1}$; Fig. 1) in the outward direction in the postexercise series. This difference occurred despite the fact that the inwardly directed [L-Lac] and electrochemical gradients in these resting preparations were far lower (or even negative) than the large outwardly directed gradients in the postexercise series. For example, the simple chemical concentration gradient for L-Lac was ~50–70 mM outward in the postexercise series (Tables 1 and 2), a difference that would be further increased by the highly negative value of E_m. These observations suggest that L-Lac transport is preferentially rectified in the inward direction in trout white muscle.

D-Lac net influx exhibited a pattern different from L-Lac influx. D-Lac influx was never negative and increased in a simple linear fashion with extracellular concentration (Fig. 3*A*). Unlike L-Lac, D-Lac is not naturally present in vertebrate tissue. Thus, theoreti-

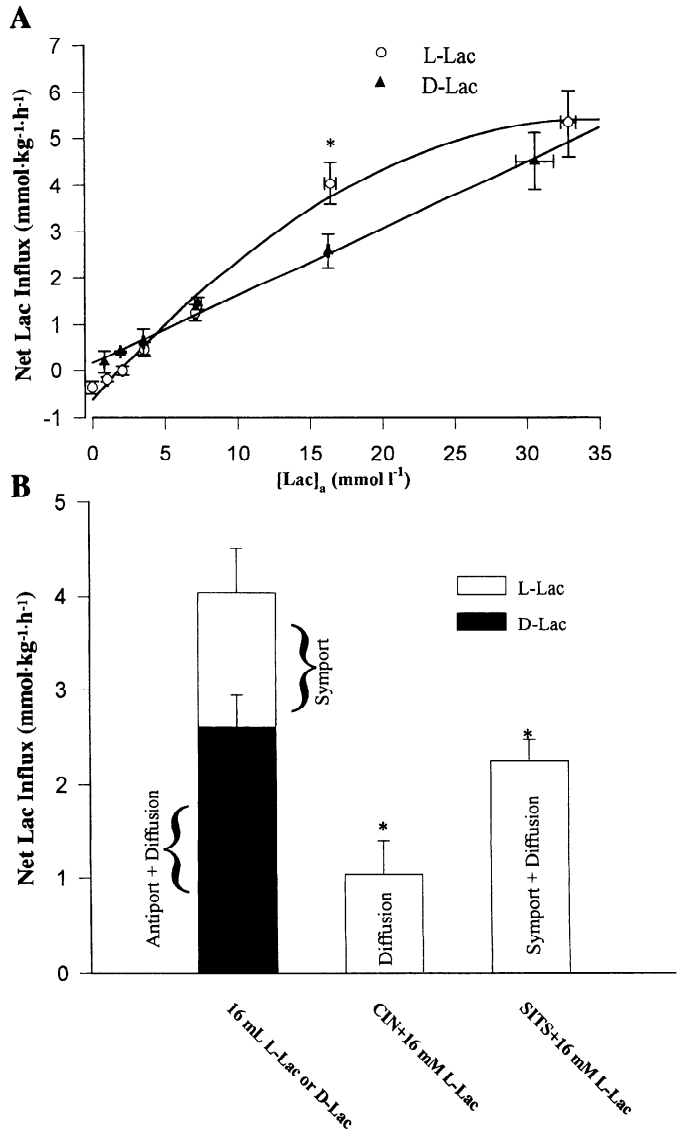

Fig. 3. *A*: net uptake rates of L- and D-Lac after 60 min of perfusion as a function of measured inflowing arterial L- or D-Lac concentration ($[Lac]_a$). *Significant difference between corresponding L- and D-Lac flux rates. See Table 3 for number of trunk preparations in each treatment. L-Lac flux presented nonlinear kinetics: L-Lac flux = $-5.04e^{-3} \times [L\text{-}Lac]_a^2 + 0.35 \times [L\text{-}Lac]_a - 0.61$ ($r^2 = 0.99$). D-Lac flux presented linear kinetics: D-Lac flux = $0.144 \times [D\text{-}Lac]_a + 0.179$ ($r^2 = 0.99$). *B*: comparison of net L- and D-Lac influx rates with 16 mM Lac (L- or D-Lac) in inflowing arterial perfusate. Effects of CIN and SITS with 16 mM L-Lac in perfusate are also illustrated, together with a tentative breakdown of relative contributions of transport mechanisms involved. *Significant difference ($P \leq 0.05$) from L-Lac flux rate at 16 mM arterial Lac without inhibitor treatment. See Table 3 for number of trunk preparations.

Table 2. *pH, T_{CO_2}, P_{CO_2}, $[HCO_3^-]$, and [Lac] in arterial and venous perfusate after 60 min of perfusion in postexercise series*

	n	pH	T_{CO_2}, mM	P_{CO_2}, Torr	$[HCO_3^-]$, mM	[Lac], mM
Control-1	8					
Arterial		7.543 ± 0.007	2.83 ± 0.17	2.26 ± 0.16	2.71 ± 0.17	0
Venous		7.317 ± 0.039	4.20 ± 0.30	5.87 ± 0.75	3.88 ± 0.27	1.85 ± 0.30
CIN	9					
Arterial		7.495 ± 0.019	2.73 ± 0.11	2.43 ± 0.12	2.38 ± 0.21	0
Venous		7.341 ± 0.020	4.15 ± 0.16	5.33 ± 0.35	3.07 ± 0.17	1.17 ± 0.18
SITS	10					
Arterial		7.513 ± 0.032	2.49 ± 0.21	2.09 ± 0.09	2.38 ± 0.21	0
Venous		7.331 ± 0.040	3.96 ± 0.17	5.26 ± 0.44	3.67 ± 0.17	1.60 ± 0.23
Control-2	9					
Arterial		7.472 ± 0.025	3.10 ± 0.17	2.99 ± 0.25	2.97 ± 0.15	0
Venous		7.264 ± 0.039	4.35 ± 0.24	7.05 ± 0.91	3.78 ± 0.08	1.55 ± 0.36
Amiloride	11					
Arterial		7.447 ± 0.027	2.87 ± 0.11	2.97 ± 0.32	2.71 ± 0.10	0
Venous		7.236 ± 0.026	4.16 ± 0.09	6.88 ± 0.49	3.78 ± 0.08	1.32 ± 0.14

Values are means ± SE; n, no. of preparations. Control-1 accompanied CIN and SITS series; control-2 accompanied amiloride series. Total CO_2 (T_{CO_2}), HCO_3^- concentration ($[HCO_3^-]$), and [Lac] were expressed in mM extracellular fluid water.

cally, $[D\text{-Lac}]_i$ was zero. Even after 30 min of D-Lac perfusion at various $[D\text{-Lac}]_a$ levels, muscle $[D\text{-Lac}]_i$ was not detectable with the enzymatic assay employed in this study. However, $[L\text{-Lac}]_i$ values of the D-Lac series have been presented in Table 3 to illustrate the stable metabolic state of the preparation. The different levels of D-Lac in the ECF did not affect the negative net L-Lac "influx," which remained stable at -0.24 ± 0.05 $mmol \cdot kg^{-1} \cdot h^{-1}$. Thus there must normally be a small L-Lac efflux occurring concurrently during D-Lac uptake.

When compared at a common extracellular concentration of 16 mM, D-Lac influx was 64% of net L-Lac influx, a significant difference (Fig. 3*A*). Inasmuch as the Lac^--H^+ symport is considered to be stereospecific for the L-Lac isomer whereas other mechanisms are not (22), a simple interpretation is that 36% of L-Lac influx occurs by symport and 64% by anionic antiport (i.e., $Lac^-/Cl^-/HCO_3^-$) and/or diffusion. CIN treatment, reported to block the symport and anionic antiport, resulted in a 75% inhibition of net L-Lac influx, suggesting a 25% contribution from diffusion (Fig. 3*B*). In accord with this breakdown, SITS treatment, reported to block only anionic exchange, reduced net L-Lac influx by 45% (Fig. 3*B*). Overall, these experiments suggest that the symport contributes 30–36%, the anion exchanger 39–45%, and diffusion 19–25%.

Table 3. *Muscle pH_i, $[Lac]_i$ and $[L\text{-Lac}]_a$ or $[D\text{-Lac}]_a$ after 60 min of perfusion in resting treatments*

Treatment	n	pH_i	$[L\text{-Lac}]_i$, mM	$[L\text{-Lac}]_a$ or $[D\text{-Lac}]_a$, mM
L-Lac, mM				
0	5	7.291 ± 0.025	3.04 ± 0.59	0
1	8	7.231 ± 0.021	3.25 ± 0.20	1.02 ± 0.04
2	8	7.207 ± 0.014	3.73 ± 0.37	2.07 ± 0.05
4	8	7.259 ± 0.015	2.67 ± 0.38	3.58 ± 0.07
8	7	7.226 ± 0.011	3.19 ± 0.36	7.08 ± 0.12
16	7	7.209 ± 0.020	3.74 ± 0.73	16.42 ± 0.41
32	5	7.301 ± 0.014	3.06 ± 0.94	32.90 ± 0.50
CIN + 16 mM	6	7.235 ± 0.016	6.50 ± 0.95*	16.12 ± 0.49
SITS + 16 mM	7	7.237 ± 0.031	6.78 ± 0.45*	14.80 ± 0.44*
pH 7.68 + 16 mM	7	7.333 ± 0.032*	4.90 ± 0.65	12.80 ± 0.38*
D-Lac, mM				
1	6	7.306 ± 0.011	2.61 ± 0.46	0.83 ± 0.03
2	4	7.325 ± 0.025	2.52 ± 0.62	1.93 ± 0.03
4	5	7.388 ± 0.020	2.89 ± 0.50	3.53 ± 0.08
8	10	7.318 ± 0.012	4.01 ± 0.50	7.27 ± 0.22
16	5	7.330 ± 0.012	3.43 ± 0.62	16.25 ± 0.18
32	5	7.246 ± 0.025	3.06 ± 0.96	30.56 ± 1.29

Values are means ± SE; n, no. of preparations. $[L\text{-Lac}]_i$ was expressed in mM intracellular fluid water. Extracellular [L-Lac] or [D-Lac] ($[L\text{-Lac}]_a$ or $[D\text{-Lac}]_a$) was expressed in mM extracellular fluid water. *Significant difference from corresponding values in 16 mM $[L\text{-Lac}]_a$ treatment.

Net L-Lac influx occurred at extracellular $[L\text{-Lac}]_a$ of 4 and 8 mM (Fig. 3*A*), even though the HLac gradient (Fig. 4*B*) and the NDF on $L\text{-Lac}^-$ (Fig. 4*A*) were outwardly directed. Not until $[Lac]_a$ reached 32 mM did the L-HLac gradient become inwardly directed, whereas the D-HLac gradient was always inwardly directed owing to the lack of intracellular D-Lac (Fig. 4*B*). For L- and D-Lac fluxes, there were approximately linear relationships between net flux rate and the respective L- and D-HLac gradients, with the former displaced laterally to the left (Fig. 4*B*). The pH 7.68 + 16 mM treatment, designed to make the L-HLac diffusion gradient more positive, did not result in an increase in net L-Lac influx but, rather, a significant decrease and, therefore, plotted well off the regression line (Fig. 4*B*).

Metabolic acid flux. In L- and D-Lac groups, pH_a was maintained between 7.9 and 8.0 in each treatment (with a grand mean of 7.920 ± 0.012, $n = 83$; data not shown), whereas venous pH (pH_v) averaged ~7.8 (with a grand mean of 7.806 ± 0.012, $n = 83$; data not shown).

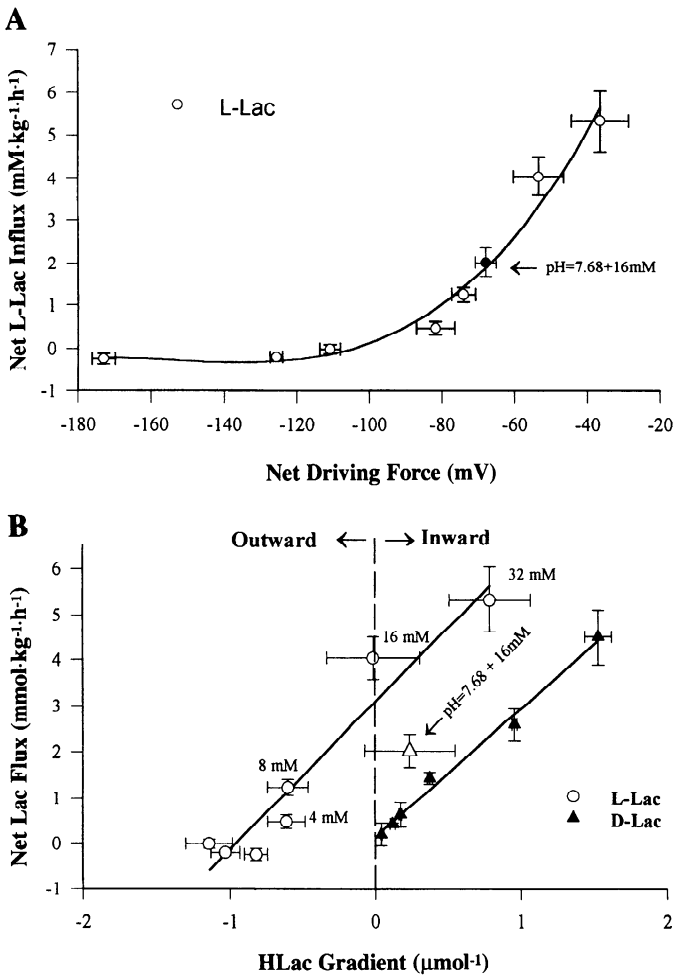

Fig. 4. *A*: net L-Lac influx rates as a function of calculated net driving force (NDF) on Lac^- (*Eq. 4*). NDF on Lac^- was based on measured transmembrane Lac^- gradient between arterial inflow perfusate and muscle intracellular fluid (ICF) after 60 min of perfusion. Curve is a third-order polynomial regression line: Lac flux = $3.98e^{-6} \times NDF^3 + 1.80e^{-3} \times NDF^2 + 0.27 \times NDF + 13.42$ ($r^2 = 0.97$). Negative NDF on Lac^- opposes Lac^- movement into ICF via free diffusion. *B*: net L- or D-Lac flux rates as functions of corresponding L- or D-HLac concentration gradient between arterial perfusate and muscle ICF after 60 min of perfusion. Linear regression lines have been fitted: L-Lac flux = $3.22 \times$ HLac + 3.09 ($r^2 = 0.92$). D-Lac flux = $2.80 \times$ HLac − 0.13 ($r^2 = 0.99$). Negative values indicate an outwardly directed HLac gradient; positive values suggest an inwardly directed gradient. Net L-Lac uptake can occur even in presence of an outwardly directed gradient.

ΔH_m^+ flux rates remained near zero in the L-Lac series as $[L\text{-Lac}]_a$ was raised from 0 to 8 mM, whereas significant influx occurred in the 16 and 32 mM $[L\text{-Lac}]_a$ treatment groups (Fig. 5*A*).

At $[L\text{-Lac}]_a$ of 16 mM , CIN completely blocked ΔH_m^+ influx, whereas SITS only partially blocked the influx (Fig. 5*A*). The ΔH_m^+ influx rate in the pH 7.68 + 16 mM group was comparable to that at 16 mM at normal pH. Unlike their L-Lac counterparts, in the D-Lac experiments the high $[D\text{-Lac}]_a$-induced ΔH_m^+ influx did not occur. In fact, there was very little, if any, net ΔH_m^+ flux (Fig. 5*A*).

T_{CO_2} efflux rates in the resting groups with low or zero $[L\text{-Lac}]_a$ (Fig. 5*B*) were ~50% of the rates measured in postexercise preparations (Fig. 2*B*), despite the fact that O_2 consumption rates (data not shown) were the same as in postexercise preparations. Unlike the postexercise preparations, T_{CO_2} efflux rates remained unchanged over the 60 min of the perfusion period. The T_{CO_2} efflux rate was not affected by elevated $[L\text{-Lac}]_a$ up to 8 mM but increased significantly in the 16 and 32 mM groups (Fig. 5*B*). However, at $[L\text{-Lac}]_a$ of 16 mM with CIN or SITS, the T_{CO_2} efflux rates did not increase (Fig. 5*B*). Meanwhile, in the D-Lac series the T_{CO_2} efflux rate remained constant (Fig. 5*B*).

DISCUSSION

Isolated-Perfused Tail-Trunk Preparation

In previous studies (33, 34) we assessed the physiological condition of the isolated-perfused tail-trunk preparation and its utility for the type of experiments performed here. We have concluded that the preparation is stable with respect to acid-base status (stable

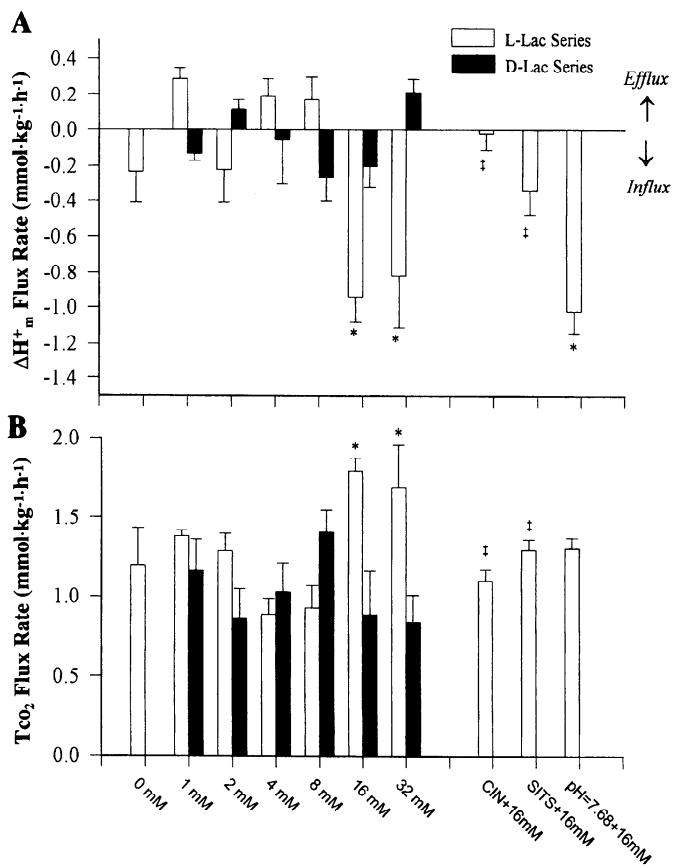

Fig. 5. *A*: net ΔH_m^+ flux rates across white muscle cell membranes in L- and D-Lac series of resting perfused tail-trunk preparation after 60 min of perfusion. *B*: T_{CO_2} efflux rates across white muscle cell membranes in L- and D-Lac series of resting perfused tail-trunk preparation after 60 min of perfusion. Positive values indicate efflux; negative values indicate influx. Flux rates are expressed as mmol·kg wet tail $wt^{-1} \cdot h^{-1}$. L-Lac series consists of treatments with different [L-Lac] in inflowing perfusate: 0, 1, 2, 4, 8, 16, and 32 mM, CIN + 16 mM, SITS + 16 mM, and pH = 7.68 + 16 mM. D-Lac series consists only of treatments with different [D-Lac] in arterial inflowing saline: 1, 2, 4, 8, 16, and 32 mM. *Significant difference ($P \leq 0.05$) from corresponding value in 0 mM treatment. ‡Significant difference from corresponding value in 16 mM treatment. See Table 3 for number of trunk preparations in each treatment.

pH_i), metabolic status (stable O_2 consumption rate and $[Lac]_i$), and ionic status (stable intracellular electrolytes and fluid volumes) over the 1-h perfusion and mimics in vivo values extremely well for these parameters in resting and postexercise situations. Of particular importance for the present experiments is the conclusion that postexercise Lac efflux rates from the perfused preparation (34) are broadly similar to those measured in vivo by [^{14}C]lactate turnover (19).

However, quantitative differences were seen between the two batches of trout tested in the present study. The fish used in the control-2 and amiloride series displayed responses very similar to those of our previous studies (33, 34). The trout used in the control-1, CIN, and SITS tests exhibited higher $[Lac]_i$ (~75 vs. ~50 mM), lower pH_i (~6.45 vs. ~6.5), and partial depolarization of E_m, the latter associated with a reduction of intracellular $[K^+]$. Inasmuch as all these differences were present at *time 0*, before the start of perfusion but after the end of exercise, we attribute them to preexisting differences. Presumably, this batch of fish had greater anaerobic capacity for HLac production, probably because of greater on-board glycogen stores.

An isolated-perfused preparation such as that used in this study offers advantages and limitations. It differs from vesicles, dispersed muscle cells, and isolated cells in better duplicating the true in vivo situation where perfusion and diffusion limitations may be important in determining Lac and H^+_m transfers (6). Thus Lac and acid-base equivalents moving between muscle and blood/perfusate must transit sarcolemmal and endothelial cell membranes as well as the interstitial fluid space. The [Lac] and acid-base composition of the blood/perfusate changes as it flows through the preparation. If movements of any of these substances are rapid relative to the flow rate, "equilibrium limitation" may occur, especially at the level of the venous capillary (6, 7). Inasmuch as the perfusion rate chosen was comparable to or higher than in vivo blood flow estimates in intact postexercise trout (discussed in Ref. 33), vascular resistance was lower than in vivo (37), and the inflowing perfusate was lactate free, perfusion limitation for Lac was likely less than in vivo. However, this may not be the case for H^+_m, because the perfusate lacked carbonic anhydrase activity and provided a non-HCO_3^- capacity (β) only ~40% of that of trout blood (20). Therefore, this study was not designed to define the in vivo rates of various Lac and H^+_m transport mechanisms but, rather, to verify their presence or absence and relative importance.

The present study showed that O_2 uptake during postexercise recovery does not significantly differ from the resting values, despite an increase in CO_2 efflux (Figs. 2*B* and 5*B*). The absence of a postexercise increase in O_2 uptake together with unchanged $[Lac]_i$ over time (Table 2) suggests that Lac oxidation was negligible during this 1st h of postexercise recovery. The increase in T_{CO_2} efflux without the corresponding changes in O_2 uptake seen in the present study could result from an O_2-independent shunt to form acetyl-CoA from the exercise-elevated acetylcarnitine pool, as seen in vivo after exercise (32).

Lac Release From Postexercise Muscle

The most important finding of the postexercise study is that carrier-mediated transport and passive diffusion are involved in Lac release from exercised trout white muscle. The significant 40% depression in Lac efflux caused by CIN, in combination with the insignificant effect of SITS treatment, clearly suggests the involvement of a Lac^--H^+ symport in postexercise Lac release from fish white muscle (Fig. 1), whereas the role of Lac efflux through the Lac^-/Cl^--HCO_3^- antiporter, if any, is minimal. If it is assumed that the CIN concentration was high enough to block all the transporters on the cell membrane (36), the remaining part of Lac release (~60%) occurs through free diffusion of HLac or Lac^-. In the case of passive diffusion, Lac should move according to the NDF for Lac^- (heavily influenced by E_m, *Eq. 4*) or the [HLac] gradient (heavily influenced by the transmembrane pH gradient, *Eq. 3*). Our previous study on Lac efflux from postexercise trout muscle indicated the importance of electroneutral mechanisms, such as Lac^--H^+ symport and/or HLac diffusion, and strongly discounted the importance of the free diffusion of Lac^- according to NDF (34). In particular, partial depolarization of E_m by experimental elevation of extracellular $[K^+]$ did not result in the expected decrease in Lac efflux (34). Similar observations have been made on rat muscle sarcolemmal vesicles (27). This leaves the passive movement of HLac as the only diffusive mechanism, and this conclusion has been supported by many studies in higher vertebrates where changes in transmembrane pH gradient did impose a marked influence on Lac efflux (11, 13, 15, 35). In our earlier study on postexercise trout muscle (34), we found that net Lac efflux responded to the HLac gradient, but not in a proportionate manner. This finding is now understandable in view of our current finding that a carrier-mediated Lac^--H^+ symport is also involved and that the relatively small net Lac efflux is occurring against a background of simultaneous Lac uptake activity (see below).

Although the passive diffusion of HLac or Lac^- was regarded as the predominant route for Lac efflux from muscle ICF to ECF (6), our results, along with other studies on mammals and amphibians, indicate that a considerable portion of the Lac release from exercised muscle occurs through the saturable Lac^--H^+ symport, but not the Lac^-/anion antiport (11, 13, 14). In an early study on the perfused trout trunk, Turner and Wood (31) observed that SITS actually increased postexercise Lac efflux, suggesting that Lac^-/Cl^--HCO_3^- antiporters may play a more important role in concurrent active Lac retention than in the release process during postexercise recovery. At first glance, this seems inconsistent with the lack of observed effect of SITS on net Lac efflux in the present study. However, the lower perfusion rate (<40% of the rate used in the present study) and the larger portion of the trunk used by Turner and Wood resulted in much higher Lac levels in the venous

effluent. This higher $[Lac^-]_e$ may have facilitated Lac^- uptake via the band 3-mediated anion exchange. As a result, SITS blockade under these circumstances would increase net Lac efflux.

The SITS-triggered enhancement of ΔH_m^+ influx (equivalent to a depression of HCO_3^- efflux) observed in the present study (Fig. 2*A*) provides circumstantial evidence to support the idea that Lac uptake mediated by the Lac^-/HCO_3^- antiport is occurring in the postexercise preparation. If we assume the above scenario to be true, with CIN treatment completely eliminating Lac efflux via the symport and Lac uptake via the anion antiport, then the reduced Lac efflux that persists after CIN treatment (Fig. 1) should represent the "free diffusion portion" of Lac efflux. Under all other circumstances, simultaneous Lac uptake will usually result in an underestimated efflux rate.

Lac transport, either by free diffusion of HLac or by carrier-facilitated mechanisms, is often pH gradient sensitive (11, 13, 26). Therefore, the Na^+/H^+ antiport could serve as an important coregulator for muscle pH_i and indirectly alter the apparent stoichiometry of Lac^-/H^+ transport. However, in the present preparation, the decreased Lac efflux in the amiloride group was not significant relative to the corresponding control-2 value at 60 min (Fig. 1). More importantly, amiloride did not result in a surge in Lac efflux as shown elsewhere (27). Although other studies have shown that local pH near the membrane could be disturbed (11, 15, 26, 35), the amiloride-induced change in ΔH_m^+ flux (Fig. 2) was not enough to affect muscle pH_i (Table 1), probably because of the high muscle ICF buffer capacity (20). The lack of hormonal support in the present preparation may also have reduced the activity of the Na^+/H^+ antiport (10).

Metabolic Acid Flux in Postexercise Muscle

The net ΔH_m^+ influx and its uncoupling from Lac efflux shown in Fig. 3*A* were consistent with our earlier findings (34). However, the observation of net ΔH_m^+ influx was somewhat puzzling, inasmuch as a release of metabolic protons is normally expected from postexercise muscle. In vivo, this ΔH_m^+ efflux normally exceeds Lac efflux (20, 32, 36). It is important, however, to appreciate that ΔH_m^+ is a compound measurement, with an influx indicating an H^+ influx and/or HCO_3^- efflux. As pointed out in earlier studies, ΔH_m^+ flux is influenced by many factors, including the transmembrane pH gradient, the E_m (through its effect on NDF for HCO_3^- and H^+), the activity of symports and antiports for H^+ and HCO_3^-, and the ECF buffer concentration (7, 13, 34). The latter may have had an important influence in the present study. As discussed earlier, the low value of β in the perfusate relative to whole trout blood, together with the absence of carbonic anhydrase, meant that the effective extracellular buffer concentration in our preparation was much lower than in vivo. Nevertheless, because our major interest was Lac release, we elected to duplicate normal in vivo postexercise pH_a at the arterial inflow. As a result, pH values (non-steady state) in the venous capillary may have been exceedingly low, thereby resulting in "equilibrium limitation" and the reversal of electrochemical gradients for ΔH_m^+ movement. In support of this interpretation is our earlier finding that ΔH_m^+ became positive (efflux) and similar to Lac efflux when an inflowing perfusate of abnormally high pH_a was used (34).

Lac Uptake in the Resting Muscle

In the resting series, pH_i and $[L\text{-Lac}]_i$ in pre- and postperfusion muscle samples (Table 3) were similar to those in in vivo resting muscle (12, 32).

The most important finding is that Lac flux appears to be preferentially rectified in the uptake direction into white muscle. When $[L\text{-Lac}]_e$ was experimentally elevated to the blood levels (e.g., 16 mM) normally seen after strenuous exercise in vivo (for reviews see Refs. 18 and 36), net L-Lac influx (Fig. 3) greatly exceeded net efflux measured in the postexercise perfusion (Fig. 1). This occurred despite the fact that the passive gradients (NDF on $[Lac^-]$ and [HLac] gradients) strongly favor Lac efflux in the postexercise situation (see Ref. 34 for detailed calculation), whereas the passive gradients for Lac uptake in resting preparations are either strongly opposed (highly negative NDF on Lac^-, Fig. 4*A*) or negligible ([HLac] gradient, Fig. 4*B*). The system appears to be designed to preferentially take up and retain Lac in white muscle, and SITS-sensitive anion exchange appears to play a role in this process, as originally suggested by Turner and Wood (31).

The hyperbolic form of the L-Lac uptake curve (Fig. 3*A*) suggests the involvement of a saturable or partially saturable component in Lac uptake. However, traditional methods (Lineweaver-Burk and Eadie-Hofstee plots) could not be applied to the L-Lac influx data to characterize the kinetic properties because of the relatively large linear component involved. Nonetheless, there appeared to be basic agreement with most previous studies on L-Lac transport kinetics in other systems, where Michaelis-Menten constants (K_m) of 4–40 mM have been reported (9, 15, 17, 25, 26, 35). Our L-Lac uptake curve therefore seems to plateau within the reasonable range (~30 mM). It is worth noting that L-Lac transport kinetics are reported to vary according to many factors (5, 10, 13, 17, 35). In contrast, the linear D-Lac uptake curve did not display any indication of saturation kinetics. Because D-Lac does not move across cell membranes through the stereospecific Lac^--H^+ symport (4, 11), its flux likely represents movement through the anion exchanger and/or by free diffusion. In higher vertebrate systems, the Lac^-/HCO_3^--Cl^- antiport is reported to be saturable only at extremely high concentration; it has very high capacity and low affinity (K_m ~ 300 mM) (22, 27). Thus Lac uptake through this pathway should not show saturation kinetics at the [Lac] (D- and L-forms) used in the present study (22). The linear relationship of D-Lac influx and concentration is therefore the expected relationship (Fig. 4*B*).

The difference in the Lac net flux between the two isomers may represent the portion transported by the symport. At $[Lac]_e$ of 16 mM, this accounted for ~36% of

the observed uptake rate. CIN treatment, which should block both symport and the anion exchanger, caused a 75% inhibition, whereas SITS treatment, which should block only the anion exchanger, caused a 45% inhibition. These experiments are internally consistent, indicating that the symport contributes 30–36%, the anion antiport 39–45%, and diffusion 19–25%. It is likely, however, that the diffusion component has been overestimated and the symport component underestimated for the following reasons. At an [L-Lac] of 16 mM, the NDF on L-Lac was strongly outward (Fig. 4*A*), whereas the inward HLac gradient was negligible due to the presence of appreciable $[L\text{-Lac}]_i$. However, such an inward [HLac] gradient did exist at [D-Lac] of 16 mM, so the difference in the net influx rates between the L- and D-Lac experiments would underestimate the symport contribution.

The rationale for decreasing pH_e in the pH 7.68 + 16 mM group was to examine whether improving the HLac-for-L-Lac gradient from essentially zero to the inward direction would lead to an increase in Lac influx. Moreover, lower pH_e can sometimes facilitate Lac uptake via Lac^--H^+ cotransport according to other studies (11, 15, 22, 27). As shown in Fig. 4*B*, lower pH_e (pH_a 7.68 + 16 mM), shifted the gradient to the inward direction only slightly, because the actual $[Lac]_a$ achieved was only 80% of the nominal 16 mM (cf. Table 3). However, the important point is that, relative to the regression relationship established by the other L-Lac data at higher pH_e (pH_a 7.92), there was no increase in Lac influx, and indeed there was a decrease. The reason for this is unknown, but it reinforces the conclusion that the contribution of HLac diffusion to influx is small or nonexistent.

The significant increases in ΔH_m^+ influx in the groups treated with $[L\text{-Lac}]_a$ at 16 and 32 mM, although still substantially lower than L-Lac influx, were in accord with the Lac flux data (Figs. 3*A* and 5*A*). Together with the CIN- and SITS-induced decrease in ΔH_m^+ influx, these results support the proposed Lac uptake mechanisms (Lac^-/H^+ symport and Lac^-/HCO_3^- antiport) and perhaps a very small contribution by the free diffusion of HLac. In the case of a $[Lac]_a$ of 16 mM, the possibility of HLac diffusion was minimized as mentioned previously. Furthermore, the significant increases in T_{CO_2} efflux in the groups treated with $[L\text{-Lac}]_a$ of 16 and 32 mM indicated that Lac oxidation was accelerated due to greater Lac influx, which caused higher CO_2 excretion, or greater HCO_3^- efflux occurred as the result of increased Lac^-/HCO_3^- antiport (Fig. 5*B*). The CIN- and SITS-triggered decreases in T_{CO_2} efflux support either of these possibilities (Fig. 5*B*), but the latter is favored by the constancy of O_2 uptake in the various treatments, as well as by the acid-base status of the venous effluent perfusate. The pH_v values were significantly higher in the 16 and 32 mM L-Lac treatments than at lower concentrations (data not shown), implying a decrease in Lac and CO_2 efflux and/or an increase in HCO_3^- efflux. The inhibitory effects of CIN and SITS on T_{CO_2} efflux could also be due to inhibition of mitochondrial pyruvate transport; Lac oxidation through the tricarboxylic acid cycle would be impeded, which would explain the observed accumulation of $[Lac]_i$ in muscle (Table 3).

In the D-Lac series, CO_2 and ΔH_m^+ efflux rates remained constant, despite the rising D-Lac influx (Figs. 3*A* and 5). The pH_v also remained unchanged (data not shown). Because D-Lac is not metabolized in muscle, the enhanced D-Lac uptake would not provide more fuel for Lac oxidation to elevate CO_2 production. As the result of rising D-Lac influx and unchanged HCO_3^- efflux, Lac uptake mediated by band 3 may be limited when there is an inwardly directed HLac gradient. In mammalian muscle it has been suggested that Lac uptake via passive diffusion increases with [Lac] or Lac gradient, and it eventually exceeds transport by the carrier system at high concentration (30 mM) (cf. Ref. 5).

At low or zero $[L\text{-Lac}]_e$, as in the D-Lac experiments, the simultaneous L-Lac efflux (Fig. 3*A*) was likely due to diffusion along the outward HLac gradient. Normally, this would lead to an underestimate of the true Lac influx rate effected by the carrier-mediated transporters (Fig. 3*A*). Although maximal velocity of L-Lac efflux via Lac^--H^+ cotransporter could be transstimulated by the elevated $[L\text{-Lac}]_e$ as suggested by several research groups, the net efflux should not change due to the equal molar lactate exchange according to the "transstimulation facilitated transport" model (cf. Refs. 5, 9, 22).

Transmembrane Lac transport in fish white muscle operates at a very low level in comparison with its higher vertebrate counterparts. The Lac flux rate in trout muscle was only about one-tenth and one-fifth of those observed in rats and frogs, respectively (13, 35). This study clearly demonstrates the existence of Lac uptake in resting fish muscle and provides evidence to support the idea that fish white muscle has the potential to actively retain lactate via the anionic antiport during postexercise recovery (31). Unlike the situation in mammals and amphibians, which exhibit minor or no effects of SITS on Lac uptake (5, 14, 16, 35), the involvement of the Lac^-/HCO_3^--Cl^- antiport (by no means the only carrier-mediated transporter) was clearly demonstrated. In conjunction with specific blockers, further studies with elevated and isotopically labeled Lac in the ECF would provide direct evidence for simultaneous Lac uptake in postexercise fish muscle.

Perspectives

The unique Lac-retaining abilities of fish muscle, together with its structural homogencity and hardiness when perfused (likely a function of poikilothermy), make it an attractive model system. In higher vertebrates the study of Lac flux across muscle cell membranes has exhibited major advances in the past few years as a result of several new techniques: culture of isolated muscle cells, studies on several types of sarcolemmal vesicles, and, most recently, cloning of putative lactate transporters (see Ref. 5 for a recent review). At least the first two of these techniques should be feasible for studies on fish white muscle in the very near future, and molecular studies will become feasible once mam-

malian probes are available. The combination of such in vitro approaches with further detailed study on the pharmacology and kinetics of the perfused muscle in situ will cast further light on this most interesting system.

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