L-(+)-Lactate translocation into the red blood cells of the skipjack tuna (*Katsuwonus pelamis*)

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Red blood cells from skipjack tuna, *Katsuwonus pelamis*, were incubated *in vitro* in various concentrations of L-(+)-lactate. There was no significant effect of pH between 6.6 and 8.1 on inward lactate translocation. Because there was a linear response between translocation rate and lactate added (up to 30 mM), we propose that a diffusive mechanism is the primary mode of lactate translocation into tuna red blood cells at least over the range of concentrations tested. This mechanism is unlike that reported for lactate influx in other animal cell types and may result from the reported differences in the control of proton movements across the red blood cell membrane of fish species.

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Des hématies de la Thonine à ventre rayé, *Katsuwonus pelamis*, ont été gardées *in vitro* à diverses concentrations de L-(+)-lactate. Le pH, dans l'intervalle 6,6 à 8,1, reste sans effet sur la translocation vers l'intérieur du lactate. Puisqu'il y a une relation linéaire entre le taux de translocation et la quantité de lactate ajoutée (jusqu'à 30 mM), il est probable qu'un mécanisme de diffusion soit le principal mode de translocation du lactate dans les hématies de la thonine, au moins dans les conditions de l'expérience. Ce mécanisme diffère de celui qui est évoqué pour expliquer l'influx de lactate dans d'autres cellules animales, peut-être à cause des différences observées dans le contrôle des movements de protons à travers les membranes des hématies de poissons.

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Introduction

Lactate translocation across membranes of mammalian cell types, including human red blood cells (Dubinsky and Racker 1978), hepatocytes (Monson et al. 1981; Fafournoux et al. 1985), and tumor cells (Spencer and Lehninger 1976; Johnson et al. 1980), is dependent upon extracellular pH (pH_e) and involves a specific monocarboxylate transporter coupled to either a H^+-symport or an OH^--antiport. Cell types of other vertebrates, including fish, have not been investigated in this regard.

This study examines lactate translocation across the red blood cell membrane of the skipjack tuna, *Katsuwonus pelamis*. This species can produce high white muscle lactate concentrations

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during burst activity (Guppy et al. 1979), with peak circulating lactate levels exceeding 25 mM (Guppy et al. 1979; Perry et al. 1985), and displays lactate disappearance rates comparable to those of mammals (Weber et al. 1986). Perry et al. (1985) reported that blood pH drops by approximately 0.5 pH units during exhausting exercise, but that both protons and lactate are rapidly removed from the blood (less than 50 min). These significant fluctuations in plasma pH and lactate concentrations make this species ideally suited for investigations of lactate translocation into red blood cells.

Materials and methods

Skipjack tuna, *Katsuwonus pelamis*, were captured on hook and line by local fishermen, transported to the Kewalo Research Facility, and held in large circular outdoor tanks supplied with rapidly flowing seawater at 25°C. The fish were not fed and were used within the first 4 days of capture. Each fish (weight, 1.5–3.0 kg) was netted and stunned by a blow to the head, the heart was exposed, and blood was collected into heparinized syringes (syringes were rinsed with 5000 U sodium heparin/mL) from the exposed heart; this procedure took less than 2 min. The blood was centrifuged (Sorvall SS-1 Superspeed; approximately 1500 rpm) at 18°C and the red blood cells were gently resuspended and washed two times with cold 0.15 M NaCl according to Kim and Isaaks (1978). The buffy layer was carefully removed during this procedure. Little or no hemolysis was noted during these procedures. Experiments were performed with the blood of individuals; pooling was not necessary.

Lactate flux measurements were carried out according to Kim and Isaacks (1978). The red blood cells were resuspended in a modified teleost Ringer's solution (150 mM NaCl, 2.4 mM KCl, 5 mM $NaHCO_3$, 2 mM $MgCl_2$, 2.6 mM NaH_2PO_4, 1.4 mM $CaCl_2$, pH adjusted to 6.6, 7.6, or 8.1) and the pH was checked with a standard electrode; any adjustments were made by adding weak acid or base to the suspension. The hematocrit was adjusted to approximately 10%, or about 25% of normal (Perry et al. 1985; this study, data not reported). The final hematocrit was estimated by weighing 1 mL of resuspension, centrifuging for 5 min (Eppendorf model 3200 microcentrifuge; Brinkmann Instruments), removing the plasma including the adhering fluids, and reweighing the pellet.

Incubations were carried out at 20°C in 50-mL Erlenmeyer flasks, open to the atmosphere, which were frequently shaken. L-(+)-Lactate was added as the sodium salt and [^{14}C(U)]lactate (New England Nuclear, Boston; specific activity 109 mCi·mmol^{-1}; 1mCi = 37 kBq) was added to obtain a final activity of 0.2 μCi·mL^{-1} of medium; the specific activity of the incubation medium was based on the measured lactate concentration and the counts in an aliquot of the medium at time zero. All experiments were completed within 3 h of removing the red blood cells from the animal.

A 0.4-mL aliquot of the cell suspension was layered onto 0.8 mL of dibutylphthalate and immediately centrifuged (Eppendorf microcentrifuge) for 1.5 min. The upper layer along with a small amount of the oil was removed with a pasteur pipet; the tube and oil surface were rinsed twice with approximately 0.75 mL of distilled water before the remaining oil was carefully removed. The tube walls were wiped dry with a Kimwipe. The red blood cells were deproteinized by adding 0.4 mL of 8% perchloric acid, mixing with a vortex, and allowing the suspension to sit for 30 min on ice before centrifuging as above. An aliquot of the clear, acidified supernatant was added to scintillation fluid (ACS II, Amersham Corporation, IL) and counted for radioactivity (Beckman scintillation counter model 3600 with internal standard quench correction). Trapped extracellular volume was not estimated, but is reported to be less than 5% using similar procedures (Kim and Isaacks 1978; Houston and Tun 1986; T. W. Moon, unpublished data). Transport rates were estimated from the specific activity of the medium at time zero and the radioactivity in the cell pellet, and related to the mass of red blood cells as calculated from the final hematocrit value.

L-(+)-Lactate concentrations of the standard solutions were estimated enzymatically (Bergmeyer 1974).

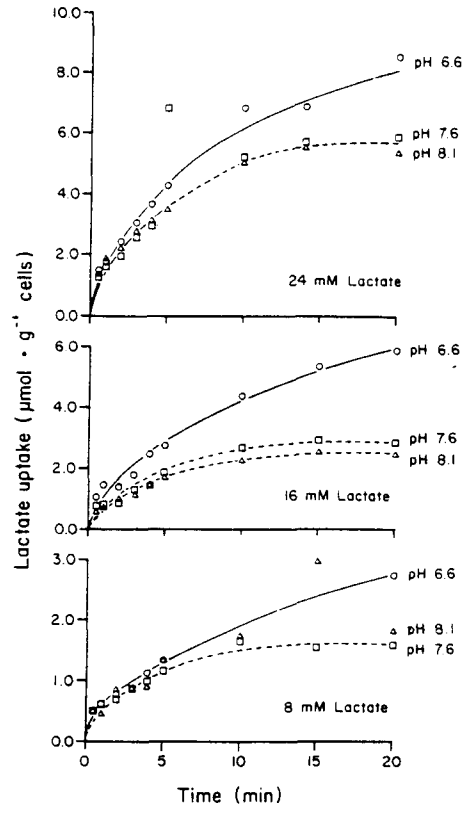

FIG. 1. Time course of lactate uptake and accumulation into skipjack tuna red blood cells as a function of pH and lactate concentration. Incubations were at 20°C and a hematocrit of approximately 10%. Each point represents a single sample.

Results

Lactate translocation into washed, resuspended skipjack red blood cells was curvilinear with time (Fig. 1), as it is in mammalian cells (Spencer and Lehninger 1976; Fafournoux et al. 1985). There were no differences in initial rates of translocation when the medium or extracellular pH (pH_e) was decreased from 8.1 to 7.6; a steady state was observed within 10 min at these pHs. A general trend for rates to increase at pH 6.6 occurred after the initial 10-min incubation time; this was a function of substrate concentration. These differential pH effects with time suggest that uptake rates do not follow simple saturation isotherms.

Initial rates of lactate translocation are a linear function of external lactate concentrations between 1 and 30 mM, and independent of extracellular pH between 6.6 and 8.1 in skipjack red blood cells (Fig. 2). The curvilinear uptake rates shown in Fig. 1 made the selection of a time for kinetic studies difficult and somewhat arbitrary as reported in other slowly transporting lactate systems (Spencer and Lehninger 1976). A 3-min period was selected (Fig. 2), as initial rates were of interest for comparison with mammalian studies. If 20 min had been selected, rates at pH 6.6 would have been higher, but not significantly so when compared with pH 7.6 or 8.1, nor would the shape of the kinetic response have been altered. These data

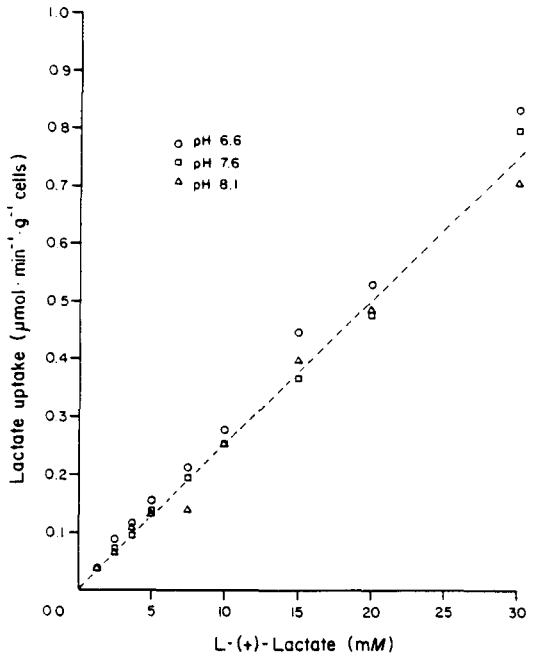

FIG. 2. Kinetics of lactate uptake into skipjack tuna red blood cells. Cells (approx. 10% hematocrit) were incubated for 3 min at 20°C at the appropriate concentration of L-(+)-lactate before being separated through oil (see Materials and methods). Each value represents the mean of two separate experiments.

suggest a prime diffusive component for lactate uptake in tuna red blood cells unlike the situation in mammalian cells.

Discussion

The interpretation of these tuna red blood cell data is made difficult by the lack of studies reported in this area. In addition, many factors affect the composition of teleost red blood cells in suspension (see Houston and Tun 1986; Nikinmaa 1986). The methods used here, however, are consistent with those of other studies in the literature, and should not affect the transport characteristics of these cells. Measurements of intracellular pH would have aided the interpretation of the data, but unfortunately we did not have access to the appropriate equipment for such an analysis.

The absence of a significant pH_e effect on the translocation rate, except possibly at very low pHs, and a linear response between the translocation rate and lactate concentration suggest significant differences in these processes between the skipjack tuna red blood cell and the mammalian cell systems studied to date. A sharp increase in translocation rates at all time intervals and substrate concentrations with pH decreases have been reported in mammalian cells at pHs between about 8.0 and 6.0 (Spencer and Lehninger 1976; Dubinsky and Racker 1978; Fafournoux et al. 1985). This result supports the role of the transmembrane pH or proton gradient in the lactate uptake mechanism (Spencer and Lehninger 1976; Dubinsky and Racker 1978). Although a diffusive translocation component may exist *in vivo* in mammalian cells, its contribution to lactate influx is thought to be small at physiological lactate concentrations of 1–5 mM (Spencer and Lehninger 1976; Fafournoux et al. 1985). Lactate translocation in these mammalian cell systems occurs primarily by a monocarboxylate transporter which is coupled to proton movements and thus is strongly pH dependent. Thus, differences between the tuna and mammalian systems may involve the distribution of protons across the cell membrane and the regulation of intracellular pH (pH_i), although other possibilities cannot be excluded.

It is generally accepted that protons are passively distributed across the non-nucleated mammalian red blood cell according to a simple Donnan equilibrium. This is due to the high efficiency of the anion transport system of the band 3 protein, and is unique to the red blood cell (Roos and Boron 1981; Borgese et al. 1986). With few exceptions (see Hemming et al. 1986), a similar situation is thought to occur in the fish red blood cell. However, differences have been reported in the response of pH_i to changes in pH_e between these red blood cell types. The slope of the pH_i–pH_e relationship for the mammalian red blood cell is −0.37 (Freedman and Hoffman 1979; Zock et al. 1980), while the value for rainbow trout red blood cells varies between −0.47 (Hemming et al. 1986) and −0.90 (Perry and Vermette 1987) with a value of about −0.70 the most frequently reported (Albers and Goetz 1985; C. L. Milligan and C. M. Wood, personal communication). The consequence of these different slope values is that the proton gradient across the mammalian red blood cell will be greater at low pH_e than that of the fish cell. A reduction in pH_e stimulates lactate uptake into mammalian red blood cells (Dubinsky and Racker 1978), probably through this increase in the magnitude of the proton gradient across the cell membrane. The minor pH_e effect on lactate translocation in tuna red blood cells reported here (Figs. 1 and 2) could be explained if the pH_i–pH_e relationship for tuna is in the range reported by Perry and Vermette (1987) for trout.

Active fish species, such as the trout and the tuna, show significant decreases in pH_e after exhaustive exercise (Wood and Perry 1985). This plasma acidification results in minor changes in pH_i (Nikinmaa et al. 1984; Primmett et al. 1986; C. L. Milligan and C. M. Wood, personal communication). Epinephrine and norepinephrine released into the circulation during exercise are thought to accomplish this regulatory response by activating the red blood cell sodium–proton antiport mechanism leading to cell alkalinization and the maintenance of hemoglobin–oxygen binding capacity (Baroin et al. 1984; Nikinmaa 1986). This *in vivo* response can be duplicated in rainbow trout red blood cells *in vitro* by adding epinephrine to the suspension (Nikinmaa 1986; Perry and Vermette 1987). Should epinephrine *in vivo* or *in vitro* lead to similar changes in tuna red blood cells, proton gradients across the membrane would increase. In this way, the tuna red blood cell may more closely resemble the mammal cell, with lactate translocation correlated with the extent of the proton gradient.

These experiments suggest that a major difference exists between the *in vitro* translocation of lactate across the red blood cell membrane of mammals and tuna. The precise explanation for this difference is not known, although epinephrine sensitivity may be important. Further studies are necessary to evaluate these transport differences, especially with respect to known inhibitors of anion and cation transport systems and catecholamines. A recent study (P. J. Walsh, personal communication) has found that lactate translocation into isolated rainbow trout hepatocytes is also a diffusion-limited process and quite unlike that reported in mammalian hepatocytes.

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