Characterization of the monocarboxylate transporter 1 expressed in *Xenopus laevis* oocytes by changes in cytosolic pH

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Several laboratories have investigated monocarboxylate transport in a variety of cell types. The characterization of the cloned transporter isoforms in a suitable expression system is nevertheless still lacking. H^+/monocarboxylate co-transport was therefore investigated in monocarboxylate transporter 1 (MCT1)-expressing *Xenopus laevis* oocytes by using pH-sensitive microelectrodes and [^{14}C]lactate. Superfusion with lactate resulted in intracellular acidification of MCT1-expressing oocytes, but not in non-injected control oocytes. The basic kinetic properties of lactate transport in MCT1-expressing oocytes were determined by analysing the rates of intracellular pH changes under different conditions. The results were in agreement with the known properties of the transporter, with respect to both the dependence on the lactate concentration and the external pH value. Besides lactate, MCT1 mediated the reversible transport of a wide variety of monocarboxylic acids including pyruvate, D,L-3-hydroxybutyrate, acetoacetate, α-oxoisohexanoate and α-oxoisovalerate, but not of dicarboxylic and tricarboxylic acids. The inhibitor α-cyano-4-hydroxycinnamate bound strongly to the transporter without being translocated, but could be displaced by the addition of lactate. In addition to changes in the intracellular pH, lactate transport also induced deviations from the resting membrane potential.

INTRODUCTION

Lactic acid is a major end product of glucose metabolism in mammalian cells. The glycolytic breakdown of 1 mol of glucose yields 2 mol each of lactate and H^+. This production of lactic acid is the major source of intracellular acidification in mammalian cells. Lactate is transported in mammalian cells by monocarboxylate/H^+ co-transporters [1]. The efflux of 1 H^+ with 1 lactate releases cells from the acid load generated by glucose metabolism. Conversly, proton-dependent uptake of lactate at physiological concentrations decreases the cytosolic pH in mammalian cells [2,3].

Seven different cDNA species have been identified that encode monocarboxylate transporters in higher eukaryotes [4–8]; these were designated MCT1 to MCT7. MCT1 is found in heart, skeletal muscle, intestine and many transformed cell lines, whereas MCT2 is expressed predominantly in heart, liver, kidney, brain and testis [6]. MCT3 shows strong expression in placenta and muscle [8] but was first identified in chick retinal epithelium [7]. MCT4 and MCT5 are predominant in placenta and kidney; whereas MCT6 and MCT7 seem to be expressed almost ubiquitously [8]. The situation is further complicated by the differential cellular expression of MCTs in various tissues. In mouse and rat brain, for example, two different MCTs have been detected. Cultured astroglial cells were found to express MCT1, whereas cultured neurons expressed MCT2 [9]. In slices of adult rat brain, however, MCT2 was allocated to certain subpopulations of astrocytes [10]. The distribution of MCTs between tissues and cell types is suggestive of a vectorial transfer of monocarboxylates between, for example, muscle and liver or astrocytes and neurons [3,9]. The kinetic properties of MCT isoforms should reflect the different tasks of these transporters. Of all isoforms only MCT1 has been thoroughly characterized, owing to its abundant presence in erythrocytes and transformed cell lines [2,11]. Other cell preparations often contain more than one MCT isoform, e.g. cultured liver cells [3,12]. Characterization of MCT isoforms therefore relies strongly on a suitable expression system. Recently it was shown that MCT1 can be expressed in *Xenopus laevis* oocytes [9], a system that is well suited for the characterization of MCTs.

Here we demonstrate that the use of pH-sensitive microelectrodes and of labelled lactate are an effective method for the characterization of expressed MCTs in *X. laevis* oocytes. The use of these methods revealed that the substrate specificity of MCT1 is less restricted than expected.

EXPERIMENTAL

Materials

L-[U-^{14}C]Lactate (5.62 GBq/mmol) was purchased from Amersham Buchler (Braunschweig, Germany). Sodium L-lactate was obtained from Fluka (Deisenhofen, Germany). α-Oxoisohexanoate, D,L-β-hydroxybutyrate, α-cyano-3-hydroxycinnamate, α-oxoisovalerate and acetoacetate were purchased from Sigma (Deisenhofen, Germany). The cap analogue m^{7}G(5′)ppp(5′)G was obtained from New England Biolabs (Schwalbach, Germany) and Ultima Gold scintillation cocktail from Canberra Packard (Dreieich, Germany). Collagenase (EC 3.4.24.3; 0.6–0.8 PZ units/mg; PZ is 4-phenylazobenzyloxycarbonyl) was from Boehringer (Ingelheim, Germany). All other chemicals were of analytical grade and were bought from E. Merck (Darmstadt, Germany), Roth (Karlsruhe, Germany) and Boehringer-Mannheim (Germany).

Oocytes and injections

X. laevis females were generously supplied by Dr. P. Hausen (Max-Planck-Institut für Entwicklungsbiologie, Tübingen, Germany). Oocytes (stages V and VI) were isolated by collagenase treatment as described [13] and left to recover overnight.

Abbreviations used: cRNA, complementary RNA; MCT, monocarboxylate transporter; pH_i, intracellular pH.

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For oocyte expression a 1.9 kb *Eco*RI fragment of the rat MCT1 cDNA cloned into the vector pGEM-He [14] (kindly provided by Dr. J. Ludwig, Hamburg, Germany) was used [9]. This vector contains the 5′ and 3′ untranslated regions of the *Xenopus* β-globin interrupted by a multiple cloning site. Plasmid DNA was linearized with *Not*I and transcribed *in vitro* with T7 RNA polymerase in the presence of the cap analogue $m^7G(5')ppp(5')G$ at a concentration of 1 mM. Template plasmid was removed by digestion with RNase-free DNase. The complementary RNA (cRNA) was purified by phenol/chloroform extraction followed by precipitation with 0.5 vol. of 7.5 M ammonium acetate and 2.5 vol. of ethanol to remove unincorporated nucleotides. Integrity of the transcript was checked by denaturing agarose-gel electrophoresis. Oocytes were microinjected with either 12.5 nl of water or 12.5 nl of MCT1 cRNA in water at a concentration of 1 μg/μl, by using a microinjection device (Bachofer, Reutlingen, Germany).

Recording of intracellular pH (pH_i) values

Double-barrelled pH-sensitive microelectrodes to measure pH_i and membrane potential were prepared as described previously [15]. In brief, the electrodes were pulled in two stages and silanized by filling a drop of 5% tri-*N*-butylchlorosilane in 99.9% pure CCl_4 into the prospective ion-selective barrel and then baking the pipette on a hot plate at 475 °C for 4.5–5 min.

For pH-selective microelectrodes a small amount of H^+ cocktail (Fluka 95291) was backfilled into the tip of the silanized barrel and the remainder was filled with 0.1 M sodium citrate, pH 6.0. The reference barrel was filled with 2 M potassium acetate. Electrodes were accepted for experiments if their response exceeded 50 mV per unit change in pH; on average they responded with a change of 54 mV to a unit change in pH.

The recording arrangement was the same as described previously [15,16]. The central barrel and the reference barrel were connected by chlorided silver wires to the head stages of an electrometer amplifier. The voltage signal of the reference barrel was also used for voltage-clamping and subtracted from the signal of the ion-selective barrel to obtain direct readings of pH_i.

In some experiments pH_i was recorded with two pH-sensitive microelectrodes inserted into different compartments of an oocyte to monitor possible pH gradients and/or differences in the rate and the amplitude of the pH_i changes within the oocytes. For this mode of recording, a double-barrelled and a single-barrelled pH-sensitive microelectrode were inserted into the same oocyte. The single-barrelled pH-sensitive electrode was produced by the same principle as the pH-sensitive channel of the double-barrelled microelectrode, with 1.5 mm glass tubing. One electrode was inserted preferably close to the inner surface of the plasma membrane and the other into the bulk cytoplasm of the oocyte. The location of the electrode close to the membrane was sometimes achieved by carefully rotating the oocyte with one impaled electrode against the other electrode. All experiments were performed at room temperature (22–25 °C).

Flux measurements

For each determination, groups of seven cRNA-injected or water-injected oocytes were washed twice with 4 ml of oocyte-Ringer solution OR2+ (82.5 mM NaCl/2.5 mM KCl/1 mM $CaCl_2$/1 mM $MgCl_2$/1 mM Na_2HPO_4/5 mM Hepes, titrated with NaOH to pH 7.0) before incubation at room temperature in a 5 ml polypropylene tube containing 70 μl of the same buffer supplemented with 5 kBq of [U-^{14}C]lactate and different amounts of unlabelled substrate. Transport was stopped after different intervals by washing oocytes three times with 4 ml of ice-cold OR2+ buffer. Repeated washing steps did not result in leakage of labelled lactate; for example, when each oocyte contained 29 ± 1 pmol lactate after three washing steps, the same batch of oocytes contained 27.5 ± 1.5 pmol after one additional washing step and 28 ± 4 pmol after a total of six washing steps. Single oocytes were placed into scintillation vials and lysed by the addition of 200 μl of 10% (w/v) SDS. After lysis, 3 ml of scintillation fluid was added and the radioactivity was determined by liquid-scintillation counting.

Calculations

For the determination of kinetic parameters, non-linear regression algorithms of SIGMA-PLOT software (Jandel Scientific, Erkrath, Germany) were used. The initial slopes of pH transients from single oocytes were first fitted to the appropriate equation, then normalized by calculation of v/V_{max}. The final curve was then calculated from all v/V_{max} values. K_m values were determined by non-linear regression of values to the equation $v/V_{max} = [S]/(K_m + [S])$, the pH dependence of lactate transport by non-linear regression to the equation $J/J_{max} = 10^{-n \times pH}/(10^{-n \times pK} + 10^{-n \times pH})$, where J is the flux rate. The numbers of oocytes investigated (n) are given in the text. When pH-sensitive microelectrodes were used, single oocytes could be superfused with complete sets of concentrations (e.g. pH dependence and concentration dependence). For radioactive flux measurements, each individual data point represents the difference between the mean $\pm$ S.D. uptake activity of n MCT1-expressing and n non-injected oocytes. The S.D. of the difference was calculated by Gauss' law of error propagation.

RESULTS

Methodological considerations

For MCT expression *X. laevis* oocytes were routinely stored in OR2+ buffer (pH 7.8) for up to 7 days. Transport experiments

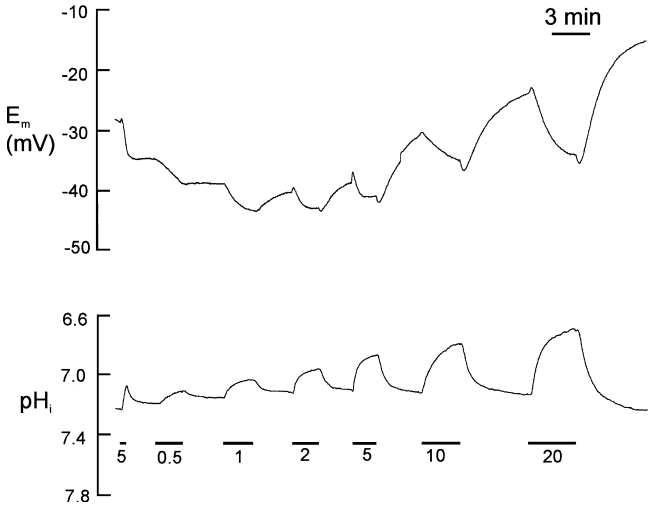

Figure 1 pH_i changes elicited in MCT1-expressing oocytes by superfusion with lactate

Each oocyte was injected with 12.5 ng of MCT1 cRNA followed by an expression period of 4–7 days. pH_i and membrane potential were recorded with a double-barrelled pH-sensitive microelectrode. In the experiment depicted a representative oocyte was superfused with lactate-containing solutions of different concentrations (marked by bars, concentrations given in mM below the bars) and lactate-free solutions (intervals between bars).

Table 1 Comparison of lactate and butyrate transport in MCT1-expressing and non-injected oocytes

Injection of oocytes with 12.5 ng of MCT1 cRNA was followed by an expression period of 4–7 days. pH_i was recorded in the oocytes during superfusion with solutions containing lactate or butyrate until equilibration was reached. To calculate ΔpH, the resting pH, which was always close to 7.3, was subtracted from pH_i at the equilibration level during superfusion. Abbreviation: n.d., not detectable.

Substrate	Concentration (mM)	Extracellular pH	Injection with ...	ΔpH in oocytes: MCT1 cRNA	ΔpH in oocytes: Water
Lactate	1	7.3		0.086	n.d.
Butyrate	1	7.3		0.066	*
Lactate	3	7.3		0.17	n.d.
Butyrate	3	7.3		0.14	0.15
Lactate	10	7.3		0.37	n.d.
Butyrate	10	7.3		0.31	0.29
Lactate	3	6.0		0.56	n.d.
Butyrate	3	6.0		0.66	0.54
Lactate	3	5.0		0.71*	0.02
Butyrate	3	5.0		1.21	1.17

* Equilibrium not reached.

were performed between the fourth and seventh days of expression. To facilitate a comparison with data from the literature and experiments with [^{14}C]lactate, measurements were performed in OR2+ buffer (pH 7.0).

The pH_i of uninjected oocytes was 7.41 ± 0.11 ($n = 8$) and the pH_i of cRNA-injected oocytes was 7.32 ± 0.08 ($n = 22$) (means $\pm$ S.D.). There was no significant difference in the steady-state pH_i at the beginning of an experiment between the different groups of oocytes ($P > 0.1$). Superfusion with OR2+ buffer (pH 7.0) of oocytes that had been stored at pH 7.8 did not result in any change of pH_i. Superfusion of MCT1-expressing oocytes with lactate resulted in a fast acidification of the oocyte (Figure 1). The amplitude and rate of acidification depended on the lactate concentration; both parameters increased with increasing lactate concentrations, in agreement with co-transport of lactate and H^+. At each lactate concentration the acidification was completely reversible. A switch back to lactate-free OR2+ buffer (pH 7.0) was immediately followed by a re-alkalinization of the oocyte, reaching equilibrium near the initial, resting pH_i of the oocyte (Figure 1). The pH changes elicited by lactate in MCT1-expressing oocytes were compared with pH changes induced by butyrate under the same conditions in MCT1-expressing and non-injected oocytes (Table 1 and Figure 2). It is generally assumed that butyric acid can cross the plasma membrane by diffusion. At low driving forces, e.g. small pH gradient and low substrate concentrations, equilibration levels of lactate and butyrate were very similar. At higher driving forces butyrate consistently generated stronger acidification of the oocytes than lactate (Table 1), suggesting that the lactate distribution did not reach thermodynamic equilibrium under these conditions.

The membrane potential was also used as a criterion of the viability of the oocytes. Only oocytes with a membrane potential of -30 mV and more negative were used for the experiments. Although monocarboxylate transport is thought to be electroneutral [17], the membrane potential changed during lactate superfusion (Figure 1). Experiments under voltage-clamp conditions confirmed that superfusion with lactate induced small currents in MCT1-expressing oocytes (Figure 3). An initial very small inward current was followed by a larger outward current. Although the small inward current was barely detectable in this experiment, it corresponded to the initial depolarization visible in non voltage-clamped oocytes during superfusion with lactate

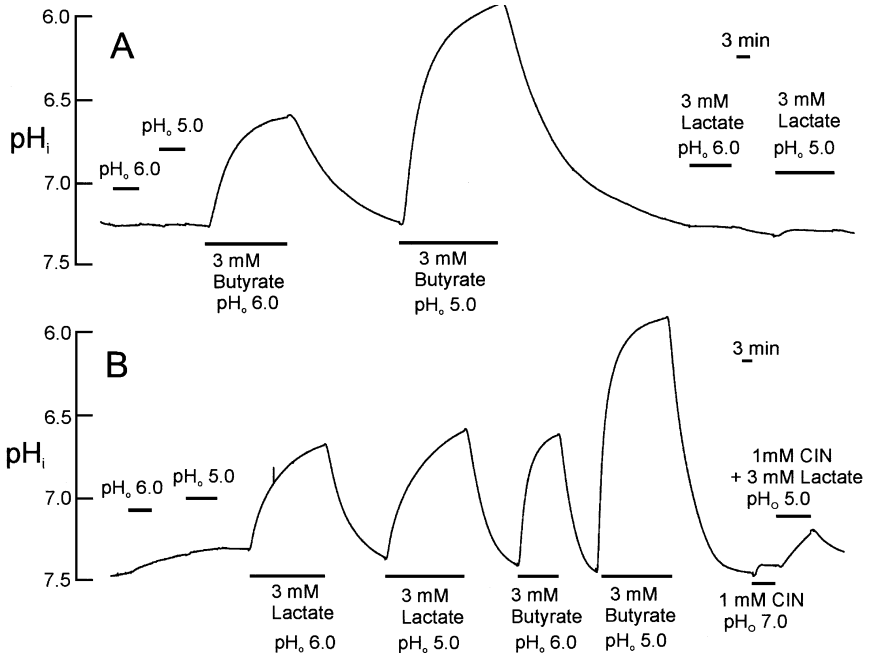

Figure 2 Changes in pH_i in non-injected and MCT1-expressing oocytes induced by lactate and butyrate

Oocytes received no injection (**A**) or were injected with 12.5 ng of MCT1 cRNA (1 ng/nl) (**B**) followed by an incubation period of 4–7 days. pH_i was recorded during superfusion with different solutions as indicated below the bars. Abbreviations: CIN, α-cyano-4-hydroxycinnamate; pH_o, external pH.

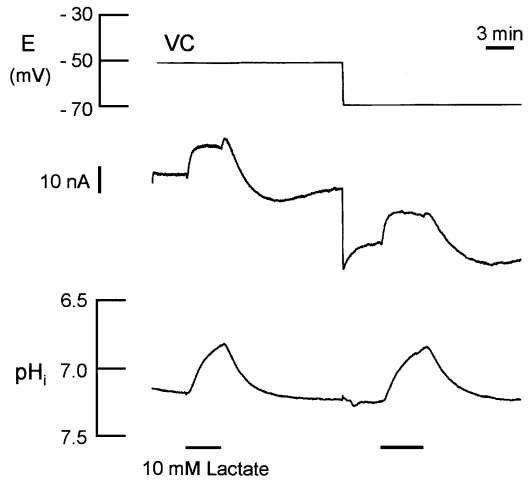

Figure 3 Lactate-induced currents in MCT1-expressing oocytes

Membrane current (middle trace) and pH_i of a voltage-clamped oocyte injected with 12.5 ng of MCT1 cRNA followed by an expression period of 6 days. The membrane potential was held at −50 and −70 mV during lactate superfusion, as marked by bars. Inward currents are visible as downward deflections. Abbreviation: VC, recording of the clamped voltage.

(Figure 1). There seemed to be little difference in the amplitude and rate of acidification when the oocyte membrane was held at −50 or −70 mV. Removal of lactate resulted in a small additional outward current followed by a recovery to the original holding current (Figure 3).

The rates of uptake of [^{14}C]lactate in oocytes of the same batch were very uniform. The slopes of the pH transients, in contrast, varied significantly between different oocytes. This might have been due partly to some inhomogeneity in the pH within the large oocyte. Therefore the pH was recorded with two pH-sensitive microelectrodes inserted at different positions of the oocyte to point-measure pH_i in different cytoplasmic locations. The distance between the electrodes was at least 300 μm. Initially only small deviations between the pH_i recordings were detected, suggesting that there was no pH_i gradient within the oocytes. However, when the tip of one pH-sensitive microelectrode was positioned close to the inner face of the cell membrane, much faster and larger pH_i changes were recorded in response to lactate (Figure 4). The differential pH signal indicated a transient overshoot and undershoot on lactate addition and lactate removal respectively, and a net difference in the total pH_i amplitude of more than 0.1 pH unit between the two recording sites.

Control experiments showed that the observed intracellular acidifications were mediated solely by monocarboxylate transport via MCT1. Exposure of water-injected or non-injected oocytes to lactate solutions (10 mM) of various pH values did not result in any acidification of the oocytes (Figure 5B). Only superfusion with solutions containing 10 mM lactate at pH 6.5 and below resulted in small changes of the pH_i in water-injected oocytes; these changes were, however, small in comparison with those recorded in MCT1-expressing oocytes under the same conditions. The amplitude of the pH_i change at an external pH of 6.0, for example, was 0.1 pH unit or less in a water-injected oocyte, as compared to more than 0.8 pH unit change recorded in the cRNA-injected oocytes over the same time (Figures 5A and 5B). When pH changes were applied in the absence of substrate, pH_i was unaffected in both MCT1-injected and non-injected oocytes (Figure 2).

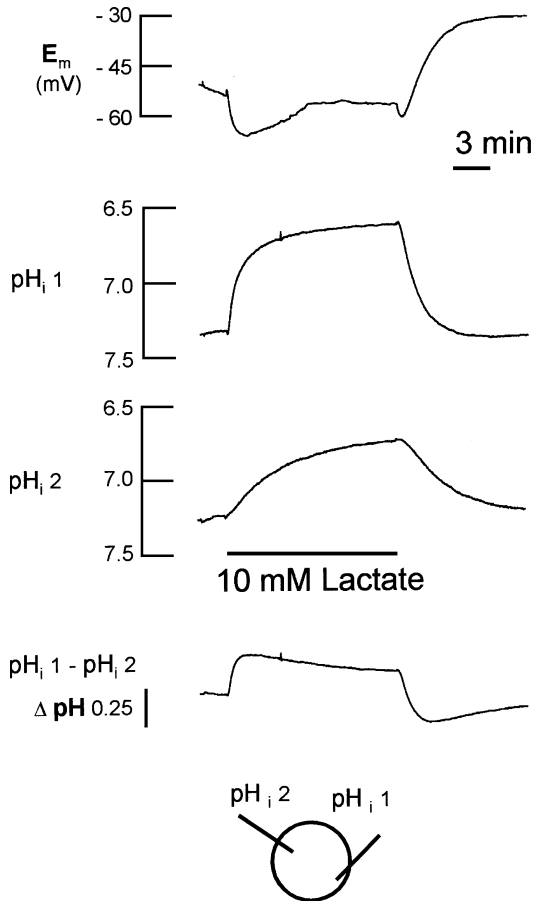

Figure 4 Recording pH_i at different sites in the oocyte cytoplasm

Each oocyte was injected with 12.5 ng of MCT1 cRNA (1 ng/nl). In the experiment depicted, cytosolic pH was recorded with two pH-sensitive microelectrodes inserted into different parts of an oocyte, one close to the inner face of the cell membrane and the other into the bulk cytoplasm (pH_i1 and pH_i2 respectively; see diagram below tracings). The difference between the two electrode recordings is given in the lowest trace, and the membrane potential (E_m) in the uppermost trace.

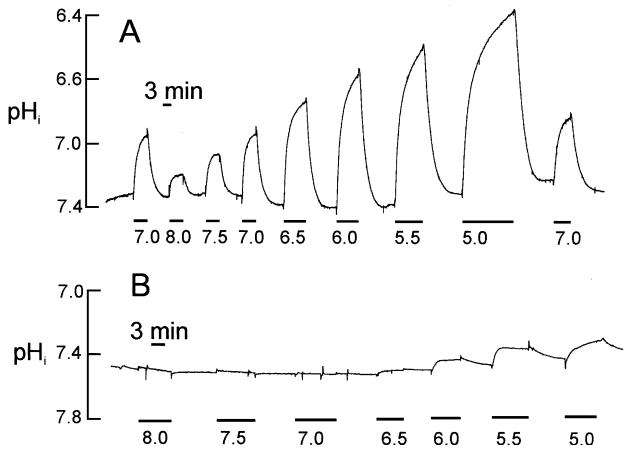

Figure 5 H^+/lactate co-transport at different external pH values in MCT1-expressing and control oocytes

Oocytes were injected with 12.5 ng of MCT1 cRNA (1 ng/nl) (**A**) or with 12.5 nl of water (**B**) followed by an expression period of 4–7 days. The cytosolic pH was recorded during superfusion with lactate (10 mM) solutions at different external pH values, as marked by bars.

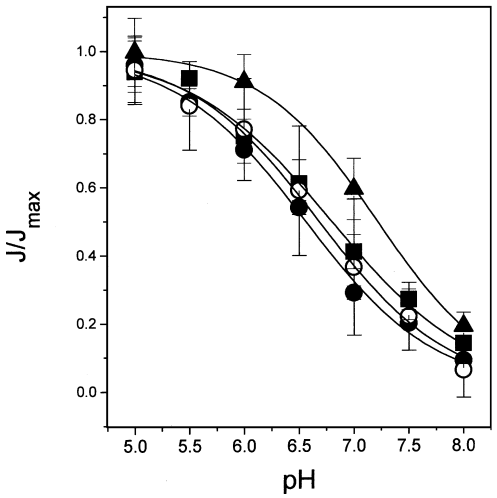

Figure 6 Dependence of lactate transport on the proton concentration in MCT1-expressing oocytes

MCT1-expressing oocytes were superfused with lactate solutions of different pH values. The dependence of lactate transport rate on pH was determined with pH-sensitive microelectrodes (○, $n = 8$) or by using [^{14}C]lactate (●,■,▲) at concentrations of 1 mM (●, $n = 7$), 5 mM (■, $n = 7$) and 10 mM (▲, $n = 7$). For the evaluation of transport rates under the different conditions each curve was first fitted by a logistic equation. All values were then divided by the maximum transport rate (J_{max}) under the given conditions.

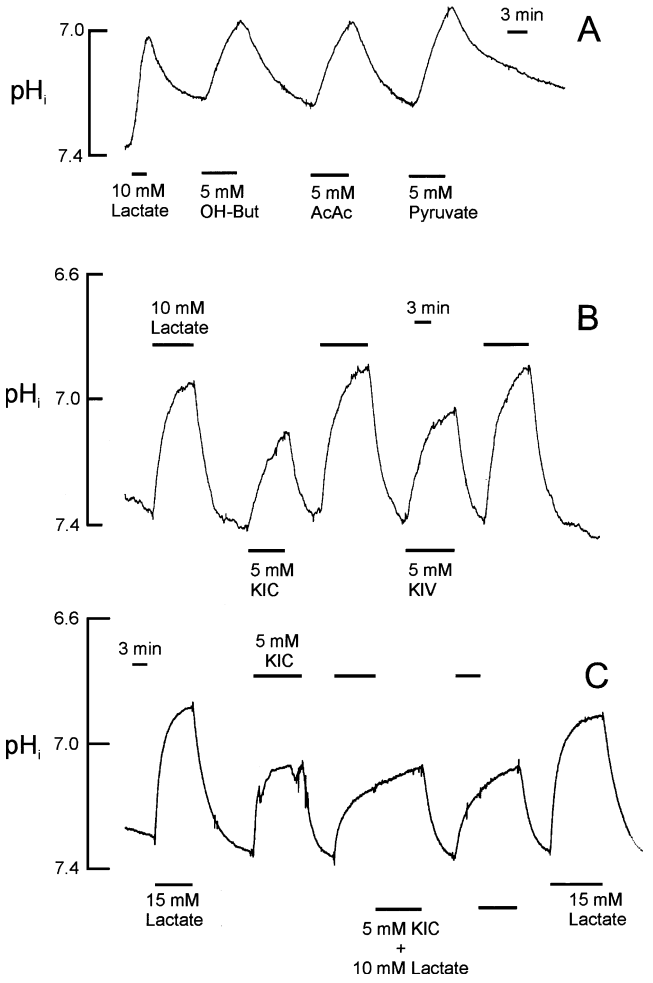

Figure 7 Substrate specificity of MCT1

Cytosolic pH values are shown of oocytes that were injected with 12.5 ng of MCT1 cRNA followed by an expression period of 4–7 days during superfusion with solutions containing different monocarboxylic acids (OH-But, D,L-3-hydroxybutyrate; AcAc, acetoacetate; KIC, α-oxoisohexanoate; KIV, α-oxoisovalerate). The traces in (**A**), (**B**) and (**C**) are recordings from different oocytes.

Kinetic parameters of monocarboxylate transport via MCT1

As stated above, deviations were found between the absolute rates of pH_i changes in response to lactate in different oocytes. To permit a statistical evaluation, rates of H^+ uptake were normalized and presented as v/V_{max}. The amplitude and rate of intracellular acidification depended on the lactate concentration (Figure 1). For lactate uptake a K_m of 3.5 ± 0.4 mM ($n = 6$) was determined from the inital rates of pH_i changes. The shapes of the pH recordings indicated that the transport of lactate via MCT1 was reversible and almost symmetrical. Although it was tempting to use the efflux branches of the pH recordings for calculating kinetic constants of lactate efflux, certain assumptions had to be made. (1) The pH gradient of 0.3 units between cytosol and superfusate should result in a 2-fold accumulation of lactate under equilibrium conditions. (2) The intracellular H^+ concentration increased with increasing intracellular lactate accumulation. At a lactate concentration of 20 mM in the superfusate (corresponding to approx. 40 mM intracellular lactate), the free H^+ concentration was three times higher than in oocytes superfused with 0.5 mM lactate. Figure 6 shows that the lactate transport rate was almost linearly dependent on the extracellular H^+ concentration between pH 6.0 and 7.2. Because this covers the observed changes of pH_i, the assumption was made that the increase in H^+ concentration resulted in a linear increase in the lactate efflux rate. Under these two premises, twofold intracellular lactate concentration and exclusion of the pH effect, a K_m of approx. 6.4 mM was determined for lactate efflux.

The H^+ concentration also had a strong influence on the rate and amplitude of the acidification (Figure 5A). Variation of the pH between 8.0 and 5.0 at a lactate concentration of 1 mM ($n = 8$) indicated a more than 5-fold increase in lactate transport, as determined from the inital rates of pH_i changes (Figure 6, ○). These results were compared with the pH dependence determined by using [^{14}C]lactate ($n = 7$) at three different lactate concentrations (Figure 6, ●,■,▼). The curves of the pH dependences resembled titration curves. The resulting apparent pK values (termed pH_{50} below) varied with the lactate concentration. With [^{14}C]lactate at concentrations of 1, 5 and 10 mM, pH_{50} values of 6.5, 6.8 and 7.2 respectively were determined. However, using pH-sensitive microelectrodes a pH_{50} of 6.7 was determined at a lactate concentration of 1 mM.

Substrate specificity of MCT1

MCT1 is a non-specific MCT. Acidification of the oocytes was elicited not only by superfusion with lactate but also by superfusion with pyruvate, acetoacetate, D,L-3-hydroxybutyrate, α-oxoisohexanoate or α-oxoisovalerate (Figures 7A, 7B and 8, and Table 2). Transport of D,L-3-hydroxybutyrate and acetoacetate reached V_{max} values similar to those for lactate, whereas the V_{max} values for pyruvate, α-oxoisohexanoate and α-oxoisovalerate were about half that for lactate. In contrast with the characteristics of monocarboxylate transport in erythrocytes

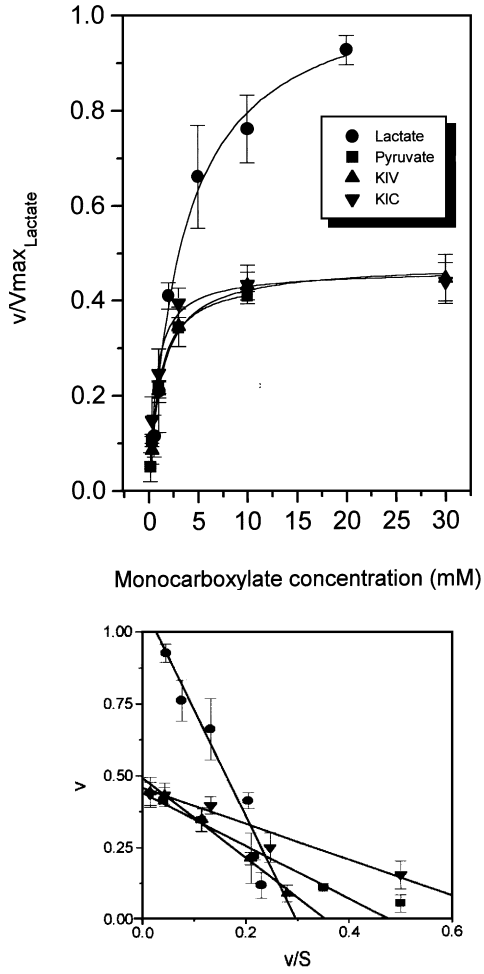

Figure 8 Determination of the K_m values of monocarboxylic acids in MCT1-expressing oocytes

Top panel: MCT1-expressing oocytes were superfused with solutions containing lactate (●), α-oxoisovalerate (▲), pyruvate (■) and α-oxoisohexanoate (▼) at various concentrations. The initial slopes of the recorded pH changes were normalized to the V_{max} of lactate and used for the calculation of transport rates. The K_m values extracted from these graphs are listed in Table 2. Bottom panel: transformation of the data by an Eadie–Hofstee plot.

Table 2 Substrate specificity of MCT1

Injection of oocytes with 12.5 ng of MCT1 cRNA was followed by an expression period of 4–7 days. pH_i was recorded in the oocytes during superfusion with solutions containing different monocarboxylates. The rates of acidification were compared with the initial pH_i changes elicited by superfusion with lactate (10 mM). In these control experiments pH_i changes ranged from 0.1 to 0.5 pH unit/min. K_m values were calculated from the results presented in Figure 8.

Substrate	Relative V_{max} (%)	K_m (mM)
Lactate	100	3.5 ± 0.4 (n = 6)
α-Oxoisohexanoate	46 ± 5 ($n = 6$)	0.67 ± 0.1 (n = 6)
α-Oxoisovalerate	48 ± 10 ($n = 6$)	1.25 ± 0.2 (n = 7)
Pyruvate	45 ± 12 ($n = 3$)	1.01 ± 0.06 (n = 3)
Acetoacetate	65 ± 19 ($n = 4$)*	*
D,L-β-Hydroxybutyrate	109 ± 29 ($n = 4$)*	*

* V_{max} values were calculated by using K_m values from [2], i.e. 5.5 mM for acetoacetate and 12.5 mM for D,L-β-hydroxybutyrate.

Table 3 Influence of α-oxoisohexanoate on lactate transport

Oocytes were injected with 12.5 ng of MCT1 cRNA followed by an incubation period of 4 days. Before the flux determination, oocytes were preincubated for 15 min with the substrates indicated. Subsequently oocytes were washed three times with OR2+ transport buffer, followed by a 10 min incubation in transport buffer of the indicated composition. All results are the uptake activity of seven oocytes, with the uptake activity of non-injected oocytes already subtracted. Abbreviation: KIC, α-oxoisohexanoate.

Preincubation for 15 min with	Substrates in transport buffer	Lactate uptake (pmol/10 min per oocyte)
No addition	[^{14}C]Lactate (5 mM)	664 ± 53
Lactate (5 mM)	[^{14}C]Lactate (5 mM)	828 ± 79
Lactate (5 mM)	[^{14}C]Lactate (5 mM) + KIC (5 mM)	386 ± 64
KIC (5 mM)	[^{14}C]Lactate (5 mM) + KIC (5 mM)	383 ± 61

[11], branched-chain oxo acids such as α-oxoisohexanoate and α-oxoisovalerate (Figure 7B) were found to be substrates of MCT1. The K_m values for branched-chain oxo acids were even lower than that for lactate (Figure 8 and Table 2). Preloading of oocytes with α-oxoisohexanoate did not affect subsequent uptake of lactate. When oocytes were incubated with 5 mM α-oxoisohexanoate for 15 min and then washed three times to remove extracellular α-oxoisohexanoate, subsequent uptake of 1 mM [^{14}C]lactate was unaffected. In preloaded oocytes an uptake activity of 220 ± 27 pmol per 5 min was found, which was similar to the uptake activity of 250 ± 44 pmol per 5 min for untreated control oocytes. It has been found [2] that branched-chain oxo acids strongly decreased the net uptake of lactate when cells were first preloaded with these compounds, followed by an incubation of the cells with a mixture of branched-chain oxo acids and lactate. A similar effect was observed in oocytes that had been superfused first with 5 mM α-oxoisohexanoate and subsequently with a mixture of 5 mM α-oxoisohexanoate and 10 mM lactate (Figure 7C). This increase in total monocarboxylate concentration resulted in a restrained further acidification of the oocyte. For flux measurements, conditions had to be slightly modified to maintain a reasonable specific activity (Table 3). Preloading with 5 mM lactate resulted in a small increase in subsequent lactate uptake. The simultaneous presence of 5 mM α-oxoisohexanoate in the transport buffer decreased lactate transport by 53%. Preloading with 5 mM α-oxoisohexanoate gave the same result (Table 3).

Despite the low substrate specificity, lactate transport via MCT1 was stereoselective. At a concentration of 10 mM, the transport of L-lactate was 2.7-fold faster than that of D-lactate. Dicarboxylic acids and tricarboxylic acids did not change the pH_i of MCT1-expressing oocytes, indicating that these acids were not transported by MCT1 (results not shown). No pH_i change was observed in non-injected oocytes during superfusion with all mentioned monocarboxylates except butyrate, showing that they did not display significant uptake activity for hydrophilic monocarboxylic acids (results not shown).

In contrast with the monocarboxylates listed in Table 1, the known inhibitor α-cyano-4-hydroxycinnamate [1] did not cause acidification of the oocytes and suppressed lactate-induced acidification (Figure 9). Binding of the inhibitor to the transporter became apparent when both α-cyano-4-hydroxycinnamate (1 mM) and lactate (10 mM) were present in the superfusate. After superfusion with α-cyano-4-hydroxycinnamate (1 mM), acidification of the oocytes by superfusion with lactate was

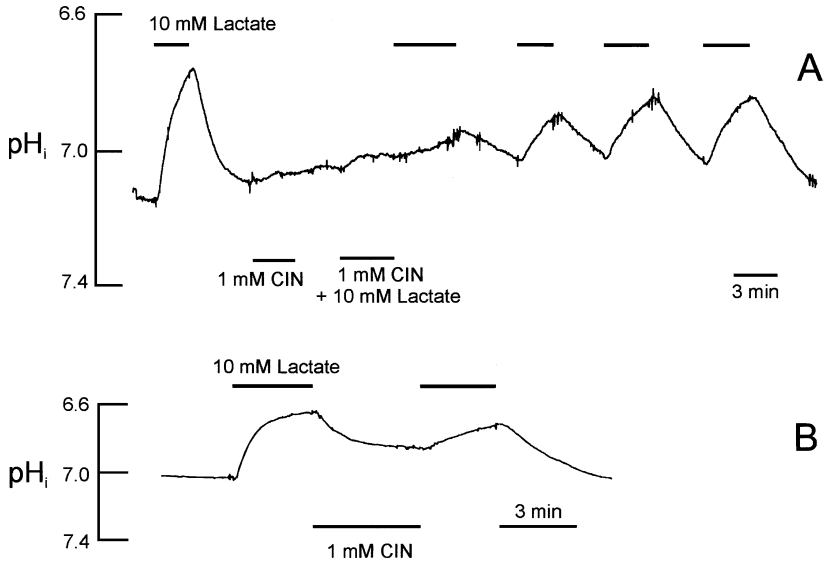

Figure 9 Inhibition of lactate transport by α-cyano-4-hydroxycinnamate

Oocytes were injected with 12.5 ng of MCT1 cRNA followed by an expression period of 4–7 days. Cytosolic pH was recorded with a pH-sensitive microelectrode. Lactate and the inhibitor α-cyano-4-hydroxycinnamate (CIN) were superfused alone, in combination (**A**) or successively (**B**). The traces in (**A**) and (**B**) are recordings from different oocytes.

strongly decreased. Repeated superfusion with lactate-containing and lactate-free solutions led to a gradual recovery from inhibition by α-cyano-4-hydroxycinnamate of the pH_i response elicited by lactate transport via MCT1 (Figure 9A). When the same protocol was performed at pH 5.0, lactate transport could not completely be inhibited by α-cyano-4-hydroxycinnamate, in comparison with pH changes induced in non-injected oocytes by the same lactate concentration (Figure 2). When oocytes were preloaded with 10 mM lactate and subsequently superfused with 1 mM α-cyano-4-hydroxycinnamate, the pH_i decrease indicating lactate efflux was initially fast, but decreased quickly (Figure 9B), suggesting that α-cyano-4-hydroxycinnamate had to enter the oocyte to inhibit transport.

DISCUSSION

Monocarboxylate transport has been investigated intensively in a variety of cell types (reviewed in [1,11,18]). The investigation of monocarboxylate transport in mammalian cells is hampered by several constraints: (1) transport of monocarboxylates is very fast, which prevents the determination of exact initial rates at 37 °C; (2) cells cannot be preloaded with labelled lactate or pyruvate because both compounds are metabolized; (3) conditions under which no substrate is present on the *trans* side (zero-*trans* conditions) can be achieved only in efflux experiments, for which, however, preloading is necessary; (iv) mammalian cells might express several routes for lactate transport, as has been shown for erythrocytes [11] and for liver cells [12], for example.

To overcome some of these limitations the pH-sensitive fluorescent dye 2′,7′-bis-(carboxyethyl)-5(6)-carboxyfluorescein was used for the determination of kinetic parameters [2,3]. Nevertheless it is difficult to exclude the possibility that several types of MCT are expressed in a given cell population. We have used *X. laevis* oocytes as an expression system with a low background activity for the characterization of MCT1, to overcome the limitations of cell culture experiments. The recording of H^+ fluxes with pH-sensitive microelectrodes instead of determining fluxes of labelled monocarboxylates extended the number of substrates that could be analysed. The kinetic characteristics of monocarboxylate transport via MCT1 were determined for the substrates lactate, pyruvate, α-oxoisohexanoate and α-oxoisovalerate under a variety of conditions. Measurements with [^{14}C]lactate were in agreement with the pH measurements with microelectrodes. Most of the monocarboxylate transport characteristics were in accordance with results gained from flux measurements in erythrocytes [11] and pH recordings from Ehrlich–Lettre tumour cells with 2′,7′-bis-(carboxyethyl)-5(6)-carboxyfluorescein [2]. A Hill plot analysis of the pH dependence of lactate transport suggested a proton-to-lactate co-transport stoichiometry of 1:1 [9]. Although the rate of lactate transport seems to be limited by the proton concentration in most circumstances [19], the pH dependence could be a complex function of several pH-dependent steps, which might become rate-limiting at different pH values. The stoichiometry was corroborated by a comparison of pH changes induced by butyrate and lactate. The comparison showed that in most cases the equilibration levels reached by both compounds were similar. However, when the internal lactate concentration was raised to levels well above the K_m, an exchange of intracellular and extracellular lactate took place preferentially. This prevented an equilibration of the lactate gradient with the proton gradient in a reasonable timescale, e.g. less than 1 h. It has been pointed out [20] that the inability of ion–solute co-transporters to reach thermodynamic equilibrium in the time scale of laboratory experiments can often be explained by strongly decreased net transport due to exchange. Closer inspection of the traces in Figure 2 confirms that the pH_i is still decreasing at a lactate concentration of 3 mM and at an extracellular pH of 5.0, whereas in the same time scale full equilibration is reached with butyrate. Although non-injected oocytes are permeable to butyrate, MCT1-injected oocytes always showed faster pH changes than non-injected oocytes (results not shown). At pH 7.0 a significant

portion of butyrate transport was mediated by MCT1 (Table 1, and results not shown).

Monocarboxylic acids with a chain length above four have previously not been considered as substrates of MCT1 [11], or were thought to inhibit the transporter [2] by binding to a high-affinity site. The oocyte experiments confirmed that net uptake of lactate, determined by pH-sensitive microelectrodes, was indeed strongly decreased when oocytes were preloaded with branched-chain oxo acids and subsequently superfused with a mixture of branched-chain oxo acids and lactate. The uptake of [^{14}C]lactate was, however, only decreased by competition with α-oxoisohexanoate. The analysis of the flux data in combination with the low K_m values displayed by branched-chain oxo acids suggest an alternative explanation for their inhibitory potential. Under the conditions used in these experiments the transporter was saturated on both sides by approx. 90% with α-oxoisohexanoate (assuming similar K_m values on both sides of the membrane). Owing to the higher affinity of α-oxoisohexanoate for MCT1, the transporter was likely to be trapped in exchange although thermodynamic equilibrium was not reached. Similarly to the experiments of the butyrate/lactate comparison, closer inspection of the traces shows that the oocytes were continously acidified during superfusion with α-oxoisohexanoate plus lactate.

During the transport of lactate, strong deviations from the resting potential of the oocyte were observed. In comparison with the lactate fluxes, however, the observed currents were small. When determined with [^{14}C]lactate (10 mM), uptake rates were in the range 0.3–0.5 nmol/min per oocyte [9]. In an electrogenic transport mechanism, on the basis of the transfer of one charge per transport cycle, a current of 500–800 nA would be expected, whereas the observed lactate-induced currents (in the range of 5–10 nA) were two orders of magnitude lower. The model of MCT1 as an electroneutral 1 H^+/1 monocarboxylate co-transporter cannot therefore be refuted by our experiments. The experiment depicted in Figure 3 also shows that acidification was independent of the applied membrane potential. Lactate-induced currents might nevertheless give an insight into the transport pore of MCT1. In contrast a secondary effect, resulting from the change in pH_i, for example on membrane permeability, cannot be excluded.

A limitation of the oocyte expression system is the variability of the equilibration time of the internal pH. When pH values were recorded near the membrane of the oocyte, pH transients were very fast, whereas insertion of the microelectrode into the egg yolk led to a delayed response of the electrodes. Diffusion of lactic acid into the egg yolk might be the rate-limiting step for the acidification of the cytosol. Secondly, the response time of the pH-sensitive microelectrodes could have limited the recording of pH_i changes. This might particularly apply at high lactate concentrations and low external pH values, when the transporter is assumed to be maximally challenged. The possibility exists that pH microelectrodes respond more slowly intracellularly than in the bath. Therefore only electrodes were used that responded clearly more rapidly in the bath during calibration before and after each experiment than the fastest pH_i changes recorded on the addition of lactate. Another limitation is that cytosolic pH and lactate concentration cannot easily be varied independently. Preloading of the oocytes with lactate is always coupled to changes in pH_i. The K_m for lactate efflux was therefore determined under conditions in which the proton concentrations were not identical for the different lactate concentrations; nevertheless the value was compatible with published K_m values [11,21].

Expression of MCTs in *X. laevis* oocytes combined with H^+ flux measurements with pH-sensitive microelectrodes permits an investigation of the mechanistic properties of cloned MCTs and a comparison of different MCT isoforms. Present work in our laboratory shows that the isoform MCT2 can be similarly expressed and investigated by using the approach described above (S. Bröer, A. P. Halestrap and J. W. Deitmer; unpublished work).

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