Characterization of the high-affinity monocarboxylate transporter MCT2 in *Xenopus laevis* oocytes

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Observations on lactate transport in brain cells and cardiac myocytes indicate the presence of a high-affinity monocarboxylate transporter. The rat monocarboxylate transporter isoform MCT2 was analysed by expression in *Xenopus laevis* oocytes and the results were compared with the known characteristics of lactate transport in heart and brain. Monocarboxylate transport via MCT2 was driven by the H^+ gradient over the plasma membrane. Uptake of lactate strongly increased with decreasing pH, showing half-maximal stimulation at pH 7.2. A wide variety of monocarboxylates and ketone bodies, including lactate, pyruvate, β-hydroxybutyrate, acetoacetate, 2-oxoisovalerate and 2-oxoisohexanoate, were substrates of MCT2. All substrates had a high affinity for MCT2. For lactate a K_m value of 0.74 ± 0.07 mM was determined at pH 7.0. For the other substrates, K_i values between 100 μM and 1 mM were measured for inhibition of lactate transport, which is about one-tenth of the corresponding values for the ubiquitously expressed monocarboxylate transporter isoform MCT1. Monocarboxylate transport via MCT2 could be inhibited by α-cyano-4-hydroxycinnamate, anion-channel inhibitors and flavonoids. It is suggested that cells which express MCT2 preferentially use lactate and ketone bodies as energy sources.

Key words: brain energy metabolism, expression, ketone bodies, lactate transport, pH regulation.

INTRODUCTION

Monocarboxylate transport into mammalian cells is mediated by monocarboxylate transporters, which have been intensively studied in a number of cell preparations, e.g. erythrocytes [1–3], hepatocytes [4], tumour cells [5], cardiac myocytes [6,7], skeletal-muscle cells [8,9] and brain astrocytes [10]. Comparison of the data from these investigations indicates the presence of different monocarboxylate transporter isoforms, which can be discriminated by substrate K_m values and inhibitor-sensitivity. Most cell types express the erythrocyte-type monocarboxylate transporter, which has a K_m value for lactate of about 4–5 mM [1,11]. By contrast, high-affinity lactate transport has been reported in cardiac myocytes [6,7] and neurons [12], whereas low-affinity monocarboxylate transport was reported in muscle cells [8,13]. A difficulty in the characterization of monocarboxylate transport in cultured cells is the simultaneous expression of multiple isoforms with very similar substrate specificity (e.g. [6,7]). The recent cloning of a family of monocarboxylate transporters, designated MCT1–8, now allows a more detailed analysis of monocarboxylate transport and a comparison with data from cultured cells [14–17]. The erythrocyte-type monocarboxylate transporter has been identified as MCT1 [18], the expression of which can be detected in many tissues and cell types. The analysis of the tissue distribution of MCT2 is complicated by species differences and putative post-transcriptional regulation [19]. A 2.4 kb mRNA has been detected in rat testis and liver; additionally a $\approx$ 9.5 kb mRNA was detected in brain, cultured neurons, heart, spleen, kidney and pancreas [10,19,20]. Western blotting confirms the expression of MCT2 in brain, liver and testis, but not in heart [19]. In hamster tissues the protein was in addition detected in heart and kidney [15]. MCT3 was initially cloned from chicken retinal epithelium [16], but subsequently also identified in the same tissue in rat [21]. The isoform MCT4 has been identified as the monocarboxylate transporter which is responsible for lactate efflux from white skeletal muscle [9]. (The designation of monocarboxylate transporters has recently been revised; MCT3 described by Price et al. [17], which is prevalent in muscle [9], is here referred to as 'MCT4', as suggested by Wilson et al. [9]; this is to avoid confusion with the closely related MCT3 of the basolateral membrane of retinal pigment epithelium [16,21].) Only limited data are available about the tissue distribution of the other isoforms (MCT5–MCT8; [17]), which have not yet been functionally characterized.

Of all isoforms, only MCT1 and MCT4 have been thoroughly characterized, owing to their abundance in certain cell types [1,2,5], whereas MCT2 has only been preliminarily characterized [15,20]. The recent introduction of the *Xenopus laevis* oocyte expression system for the investigation of monocarboxylate transporters [10,22] now allows characterization of the less-abundant isoforms.

We have investigated the properties of the monocarboxylate transporter MCT2 after expression in *Xenopus laevis* oocytes. MCT2 mediates the high-affinity transport of a variety of

Abbreviations used: 4-CIN, α-cyano-4-hydroxycinnamate; DBDS, 4,4′-dibenzamidostilbene-2,2′-disulphonic acid; DIDS, 4,4′-di-isothiocyanostilbene-2,2′-disulphonic acid; DNDS, 4,4′-dinitrostilbene-2,2′-disulphonic acid; NPPB, 5-nitro-2-(3-phenylpropylamino)benzoate; pCMBS, *p*-chloromercuribenzenesulphonic acid; pH_i, intracellular pH; $m^7G(5')ppp(5')G$, P^1-5′-(7-methyl)guanosine-P^3-5′-guanosine triphosphate.

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monocarboxylic acids, including ketone bodies, and therefore generates the basis for a preferential feeding of monocarboxylates into MCT2-expressing cells, which include cultured neurons.

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). 2-Oxoisohexanoate, DL-β-hydroxybutyrate, α-cyano-4-hydroxycinnamate (4-CIN), α-oxoisovalerate and acetoacetate were obtained from Sigma (Deisenhofen, Germany). The cap analogue P^1-5′-(7-methyl)guanosine-P^3-5′-guanosine triphosphate [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 units/mg) 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).

Cloning

Two primers were used to amplify the coding sequence of rat MCT2 [19]: the sense primer 5′ TCGGATCCA GTA GAA GCT CAG AAA TGC CAT C 3′ contained a *Bam*HI site additional to the sequence corresponding to the 5′ end of MCT2 (bases 176–201); the antisense primer 5′ TTTCTAGAT GAG ACT TGT TAA ATA CTA CTT TC 3′ contained a *Xba*I site additional to the sequence corresponding to the 3′ end of MCT2 (bases 1649–1674). The amplified MCT2 cDNA was cut with *Bam*HI and *Xba*I and cloned into the corresponding sites of vector pGEM-He ([23]; kindly provided by Dr. J. Ludwig, Institute of Physiology, University of Tübingen, Tübingen, Germany). This vector contains the 5′- and 3′-untranslated regions of the *Xenopus* β-globin interrupted by a multiple cloning site.

Oocytes and injections

Xenopus laevis females were purchased from the South African *Xenopus* Facility (Knysna, South Africa). Oocytes (stages V and VI) were isolated by collagenase treatment as described [24] and allowed to recover overnight before injection.

Plasmid DNA was linearized with *Not*I and transcribed *in vitro* with T7 RNA polymerase in the presence of the cap analogue m^{7}G(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 5 nl of water or 5 nl of MCT1/MCT2 cRNA in water at a concentration of 1 μg/μl, by using a microinjection device (Bachofer, Reutlingen, Germany).

Recording of intracellular pH values with electrodes

Double-barrelled pH-sensitive microelectrodes to measure intracellular pH (pH_i) and membrane potential in frog oocytes, were prepared as previously described [25]. Briefly, the electrodes were pulled in two stages and silane-treated by placing a drop of 5% tri-n-butylchlorosilane in 99.9% pure carbon tetrachloride 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 silane-treated barrel and the remainder 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 and if they reacted more quickly in the bath during calibration before and after each experiment than the fastest pH_i changes recorded upon lactate addition. On average electrodes responded with a change of 54 mV to a change in pH by one unit.

The recording arrangement was the same as described previously [25,26]. The central and the reference barrel were connected by chloride-treated silver wires to the headstages of an electrometer amplifier.

As described previously [22], optimal pH changes were detected when the electrode was located near the inner surface of the plasma membrane. This was achieved by carefully rotating the oocyte with the impaled electrode. All experiments were carried out at room temperature (22–25 °C). Only oocytes with a membrane potential > 25 mV were used for experiments.

Flux measurements

For each determination, groups of seven cRNA- or water-injected oocytes were washed twice with 4 ml of OR2+ buffer (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 [10,22]. Single oocytes were placed into scintillation vials and lysed by addition of 200 μl of 10% (w/v) SDS. After lysis, 3 ml of scintillation fluid was added and the radioactivity determined by liquid-scintillation counting.

Calculations

For the determination of kinetic parameters, non-linear regression algorithms of Microcal Origin software (Microcal Software, Inc., Northampton, MA, U.S.A.) were used. The initial slopes of pH transients from single oocytes were first fitted to a logistic equation, and then normalized by calculation of v/V_{max}. The final curve was then calculated from v/V_{max} values of the indicated number of experiments. K_m values were determined by non-linear regression of values to the equation:

$$v/V_{max} = [S]/(K_m + [S])$$

Competition experiments were analysed by non-linear regression of data to the equation:

$$v = V_{max}[S]/\{K_m(1 + [I]/K_i) + [S]\}$$

where [S] is the substrate concentration, [I] is the inhibitor concentration and K_i is the inhibition constant. The substrate concentration used in these experiments was well below the K_m value. The pH-dependence of lactate transport was analysed by non-linear regression to the equation:

$$J/J_{max} = 10^{-h\times pH}/(10^{-h\times pK} + 10^{-h\times pH})$$

The flux J is used in this equation to indicate that the maximum flux rate under the conditions of the experiment was used and not V_{max} as defined by the Michaelis–Menten equation; h is the Hill coefficient. The number of investigated oocytes (n) is given in the text. When using pH-sensitive microelectrodes, single oocytes

could be superfused with complete sets of concentrations (e.g. pH-dependence; concentration-dependence). For radioactive flux measurements, each individual data point represents the difference between the mean (±S.D.) uptake activity of n MCT2-expressing and n non-injected oocytes (usually $n = 7$). The S.D. of the difference was calculated by Gauss' law of error propagation.

RESULTS

Characterization of MCT2 in *Xenopus laevis* oocytes

Expression of MCT2 in *Xenopus laevis* oocytes resulted in a strong increase of lactate transport activity at pH 7.0 (Figure 1) which allowed the investigation of kinetic parameters of the transport process. For further experiments, incubation periods of 5–10 min were used to achieve optimal discrimination between MCT2-expressing and control oocytes. In initial experiments we compared lactate transport in oocytes expressing the ubiquitous transporter MCT1 with oocytes expressing MCT2. At low lactate concentration (0.1 mM), uptake of labelled lactate was similar in both preparations. However, at higher concentrations, transport activity in MCT2-expressing oocytes was much lower than in MCT1-expressing oocytes (Figure 2), although both cRNAs contained the same *Xenopus* β-globin 5′- and 3′-untranslated regions to enhance translation in oocytes. A K_m value for L-lactate of 0.74±0.07 mM was determined for MCT2 at pH 7.0 (Figure 2), which is six times lower than the corresponding value for MCT1. The V_{max} value of MCT2 was as much as 20 times lower than that of MCT1.

The pH-dependence of MCT2 was compared with that of MCT1, with which its amino acid sequence shares 55% identity. As was observed for MCT1, the rate of lactate uptake mediated by MCT2 increased greatly with decreasing pH (Figure 3) in a manner that resembled a titration curve. The apparent pK value of the curve depended on the lactate concentration. At a more saturating lactate concentration (1 mM), the curve was shifted to more alkaline pH values, rendering uptake almost unaffected by the proton concentration below pH 7 (Figure 3).

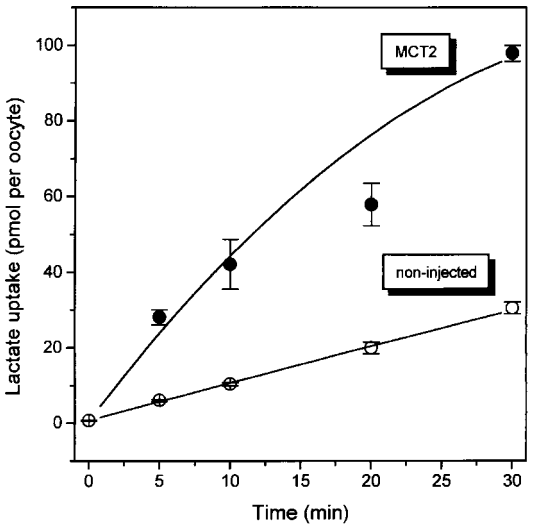

Figure 1 Expression of rat MCT2 in *Xenopus laevis* oocytes

Oocytes were injected with 5 ng of MCT2 cRNA (●) or remained non-injected (○). After an expression period of 5 days, uptake of labelled lactate (0.1 mM) was monitored at pH 7.0 and terminated after different times by washing the oocytes four times with ice cold oocyte–Ringer solution. For each time point the mean uptake activity of seven oocytes is shown.

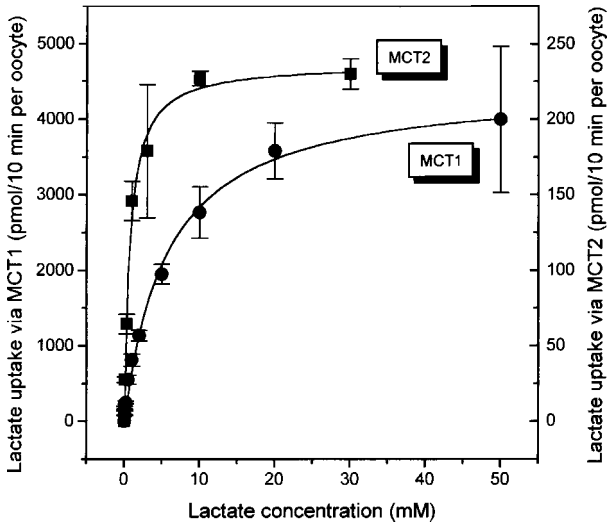

Figure 2 Kinetic parameters of lactate transport in oocytes expressing rat MCT1 and rat MCT2

Oocytes were each injected with 5 ng of MCT1 (●) or MCT2 (■) cRNA. After an expression period of 4 days, uptake of labelled lactate was determined at pH 7.0 over a time period of 10 min. For each concentration the mean uptake activity of seven injected and seven non-injected oocytes was determined. The transport activity of the non-injected oocytes has already been subtracted. Note that the two ordinates have different scales.

In order to investigate the mechanism of lactate transport catalysed by MCT2 in more detail, changes in the intracellular pH of MCT2 expressing oocytes were recorded during superfusion with lactate (Figure 4). In agreement with the pH-dependence of the transporter, a co-transport of protons could be detected, which resulted in an acidification of the cytosol. Owing to the comparatively low expression level of MCT2 in oocytes, pH changes were slow when the substrate was given at an external pH of 7.0. To evaluate the pH changes, the external pH was decreased to 6.0, which increased the driving force for H^+/monocarboxylate$^-$ co-transport. The K_m value, which was derived from these measurements (0.48±0.12 mM), was slightly lower than the value determined by using labelled lactate at pH 7.0 (see above). This shift of the K_m value with pH was also observed for the erythroid lactate transporter (MCT1) and was interpreted as a consequence of an ordered binding mechanism, with H^+ binding first [3]. The slight decrease of the K_m value with increasing H^+ concentration and the partial pH-independence at higher lactate concentration (see above) are in agreement with the notion that MCT2, similar to MCT1, has an ordered binding mechanism.

In current-clamped oocytes, only small deviations of the membrane potential were visible during superfusion (Figure 4), suggesting an electroneutral transport mechanism. Small depolarizations during transport have also been observed in MCT1-expressing oocytes [22]. The magnitude of the corresponding currents, however, was much lower than the expected currents for an electrogenic transport mechanism [22]. In MCT2-expressing oocytes, instantaneous depolarization was only observed during superfusion with 30 mM lactate (Figure 4), whereas lower, but still saturating, concentrations caused only a drift of the membrane potential. Taken together with a Hill coefficient of 1.0, which was derived from the pH-dependence of

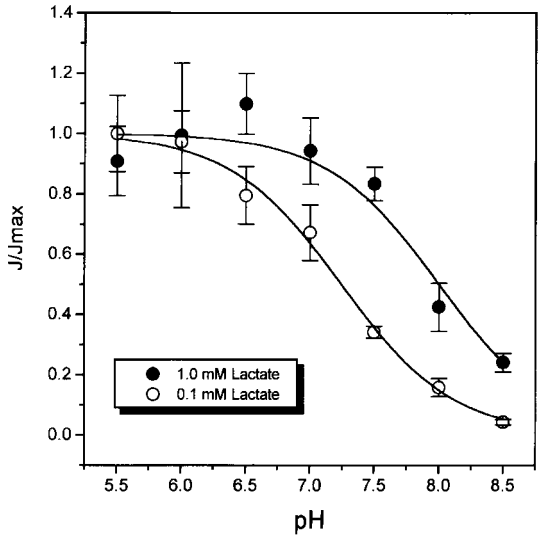

Figure 3 pH-dependence of lactate transport via MCT2

Oocytes were injected with 5 ng of MCT2 cRNA, followed by an expression period of 6 days. The mean uptake activity of seven oocytes was determined at different pH values over a time period of 5 min at substrate concentrations of 1mM (●) or 0.1 mM (○). The data were normalized by dividing all data by the extrapolated value of the titration curve. The corresponding uptake activity of non-injected oocytes has already been subtracted.

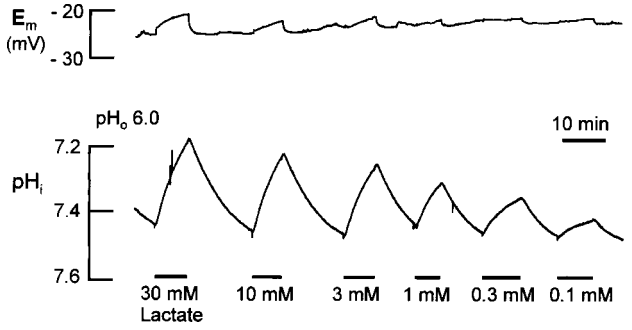

Figure 4 pH_i changes elicited in MCT2-expressing oocytes by superfusion with lactate

Each oocyte was injected with 5 ng of MCT2 cRNA, followed by an expression period of 4–7 days. Cytosolic pH and membrane potential (E_m, in mV) were recorded with a double-barrelled pH-sensitive microelectrode. In the experiment depicted, a representative oocyte was superfused with lactate-containing solutions (pH 6.0) of different concentrations (marked by bars) and lactate-free solutions (pH 7.0; intervals between bars). pH_o is $pH_{outside}$.

lactate transport, the data indicate that MCT2, like MCT1, works as an electroneutral co-transporter with an H^+/monocarboxylate$^-$ stoichiometry of 1:1. Both an increased H^+ gradient and an increased lactate gradient resulted in larger and stronger acidification of the oocytes (results not shown), indicating that both driving forces were equivalent. Transport of lactate via MCT2 was fully reversible. The direction of transport was determined by the gradients of the substrate and the co-transported proton (Figure 4). The ratio of extracellular to cytosolic lactate concentrations in the equilibrium is therefore equal to the inverse of the ratio of the proton concentrations in these compartments.

Table 1 Comparison of kinetic constants of MCT1 and MCT2

Oocytes were each injected with 5 ng of MCT2 cRNA. After an expression period of 4 days, uptake of labelled lactate (0.1 mM) was determined over a time period of 10 min in the presence of different concentrations of unlabelled monocarboxylates. For each concentration the mean uptake activity of seven injected and seven non-injected oocytes was determined. The transport activity of the non-injected oocytes has already been subtracted. Values for MCT1 are from [5] and [22]. Values in this and the other Tables are means ± S.D.

	K_m or K_i (mM)	
Substrate	MCTI	MCT2
Lactate	3.5	0.74 ± 0.07
DL-β-Hydroxybutyrate	12.5	1.2 ± 0.2*
Pyruvate	1.0	0.08 ± 0.01*
2-Oxoisovalerate	1.25	0.30 ± 0.02*
2-Oxoisohexanoate	0.67	0.10 ± 0.01*
Acetoacetate	5.5	0.8 ± 0.1*

* K_i values were determined by assuming competitive inhibition.

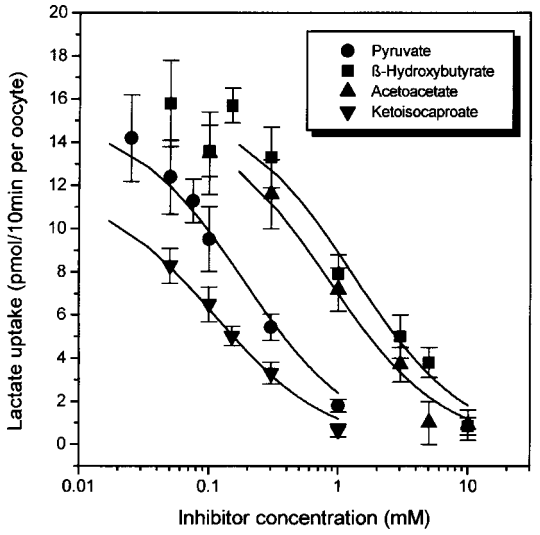

Figure 5 Determination of K_i values of different MCT2 substrates

Oocytes were each injected with 5 ng of MCT2 cRNA. After an expression period of 4 days, uptake of labelled lactate (0.1 mM) was determined over a time period of 10 min in the presence of different concentrations of pyruvate (●), β-hydroxybutyrate (■), acetoacetate (▲) or 2-oxoisohexanoate (▼). For each concentration, the mean uptake activity of seven injected and seven non-injected oocytes was determined. The transport activity of the non-injected oocytes has already been subtracted. 'Ketoisocaproate' is 2-oxoisohexanoate.

Substrate specificity of MCT2

The low V_{max} value of lactate transport in MCT2-expressing oocytes in contrast with MCT1-expressing oocytes excluded an investigation of the transport kinetics of monocarboxylates other than lactate by changes in cytosolic pH. We therefore determined the affinity of other monocarboxylates by measuring the K_i values for inhibition of lactate transport, as has been done previously [10]. The substrate specificity of MCT2 was remarkably similar to that of MCT1. A wide variety of nutritionally important monocarboxylates, such as pyruvate, β-hydroxybutyrate, acetoacetate (3-oxobutyrate), 2-oxoisovalerate (2-oxoisopentanoate) and 2-oxoisohexanoate, strongly inhibited lactate uptake into MCT2-expressing oocytes, with K_i values ranging from 100 μM to 1.2 mM (Table 1; Figure 5). Similar to the K_m

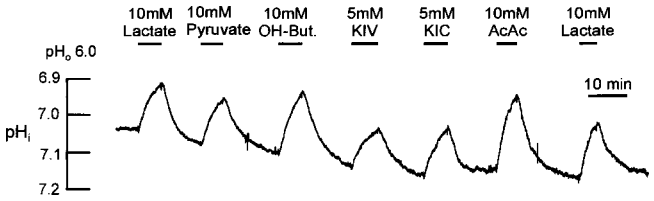

Figure 6 Substrate specificity of MCT2

Each oocyte was injected with 5 ng of MCT2 cRNA, followed by an expression period of 4–7 days. Cytosolic pH was recorded with a double-barrelled pH-sensitive microelectrode. In the experiment depicted a representative oocyte (out of $n = 4$) was superfused with solutions containing different monocarboxylates (pH 6.0, marked by bars) and monocarboxylate-free solutions (pH 7.0; intervals between bars). Abbreviations used: OH-But., β-hydroxybutyrate; KIV, 'α-ketoisovalerate' (2-oxoisovalerate); KIC, 'α-ketoisocaproate' (2-oxoisohexanoate); AcAc, acetoacetate.

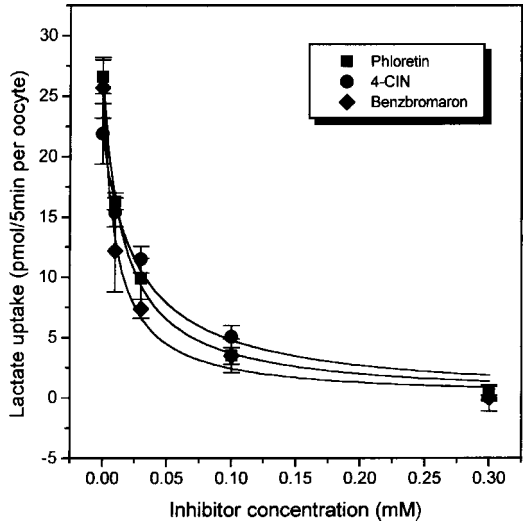

Figure 7 Inhibitor-sensitivity of MCT2

Oocytes were each injected with 5 ng of MCT2 cRNA. After an expression period of 4 days, uptake of labelled lactate (0.05 mM) was determined over a time period of 5 min in the presence of different concentrations of unlabelled phloretin (■), 4-CIN (●) or benzbromaron (◆). For each concentration the mean uptake activity of seven injected and seven non-injected oocytes was determined. The transport activity of the non-injected oocytes has already been subtracted.

value for lactate, the K_i values for the other monocarboxylates were all about tenfold lower than the corresponding values for MCT1 (Table 1). The 2-oxoacids are the preferred substrates of MCT2, having, with the exception of 2-oxoisovalerate, K_i values of about 100 μM. Therefore, MCT2 can be defined as a high-affinity monocarboxylate transporter isoform. The examined monocarboxylates were not only inhibitors of lactate transport, but also substrates of the transporter, as judged from the changes in cytosolic pH induced during superfusion of MCT2-expressing oocytes (Figure 6). The K_i values presented above and in Table 1 were therefore calculated under the assumption of a competitive inhibitory action. The assumption was supported by experiments in which pH changes were recorded at different concentrations of β-hydroxybutyrate. The K_m value of 1.2 ± 0.5 mM ($n = 5$) that was derived from these measurements was identical with the K_i value for inhibition of lactate transport by β-hydroxybutyrate (Table 1).

Table 2 Inhibitor constants of MCT1 and MCT2

Oocytes were each injected with 5 ng of MCT2 cRNA. After an expression period of 4 days, uptake of labelled lactate (0.05 mM) was determined over a time period of 10 min in the presence of different concentrations of inhibitors. The concentration which is required for 50% inhibition (IC_{50}) is given for each substance. For each concentration the mean uptake activity of seven injected and seven non-injected oocytes was determined. The transport activity of the non-injected oocytes has already been subtracted.

	IC_{50} (μM)	
Inhibitor	MCT1	MCT2
4-CIN	425 ± 155	24 ± 4
Phloretin	28 ± 8	14 ± 2
Benzbromaron	22 ± 7	9 ± 1
Quercetin	14 ± 5	5 ± 2

Inhibitor specificity of MCT2

It would be desirable to determine the contribution made to lactate transport by different MCT isoforms within a single cell. For this purpose, selective inhibitors of MCT1 and MCT2 would be helpful. A number of analogues and structurally unrelated inhibitors were tested for their inhibitory potential on both MCT1 and MCT2 (Figure 7; Tables 2 and 3). The similarity in substrate specificity between MCT1 and MCT2 was also apparent in their sensitivity towards different inhibitors. In general, MCT2 was much more sensitive to most inhibitors than MCT1. Three types of inhibitors were found to be most effective (Table 3): (i) hydrophobic substrate analogues, as exemplified by α-cyano-4-

Table 3 Inhibitor-sensitivity of MCT1 and MCT2

Oocytes were each injected with 5 ng of MCT2 cRNA. After an expression period of 4 days, uptake of labelled lactate (0.05 mM) was determined over a time period of 10 min in the presence of 0.1 mM inhibitor (with the exception indicated). For each concentration the mean uptake activity of seven injected and seven non-injected oocytes was determined. The transport activity of the non-injected oocytes has already been subtracted. Inhibitors were dissolved in transport buffer, ethanol or DMSO (final concentrations given in parentheses). Controls were performed in the presence of the same solvent concentrations. DNDS, 4,4'-dinitrostilbene-2-2'-disulphonic acid.

	Uptake rate (% of control)	
Inhibitor	MCT1	MCT2
None	100	100
4-CIN (0.1% DMSO)	74 ± 4	34 ± 7
2-Hydroxyphenylacetate (0.1% DMSO)	92 ± 5	105 ± 8
2-Hydroxy-2-phenylbenzeneacetic acid (0.1% ethanol)	97 ± 8	117 ± 10
Adamantaneacetic acid (1% DMSO)	96 ± 4	113 ± 11
Biphenyl-4-carboxylic acid (1% DMSO)	88 ± 7	74 ± 13
NPPB (1% DMSO)	54 ± 1	25 ± 5
Anthracene-9-carboxylic acid (1% DMSO)	102 ± 7	104 ± 8
Niflumate (1% DMSO)	36 ± 8	14 ± 3
Eicosa-5,8,11,14-tetraynoic acid (30 μM, 1% ethanol)	117 ± 11	125 ± 13
Probenecid (0.1% DMSO)	110 ± 6	88 ± 10
Benzbromaron (0.1% DMSO)	19 ± 7	12 ± 2
Phloretin (0.1% DMSO)	14 ± 4	3 ± 1
Quercetin (1% EtOH)	11 ± 1	17 ± 15
DNDS (1% DMSO)	112 ± 12	119 ± 4
DBDS (1% DMSO)	99 ± 11	44 ± 5
$HgCl_2$ (Buffer)	37 ± 7	54 ± 5
pCMBS (Buffer)	9 ± 2	118 ± 13

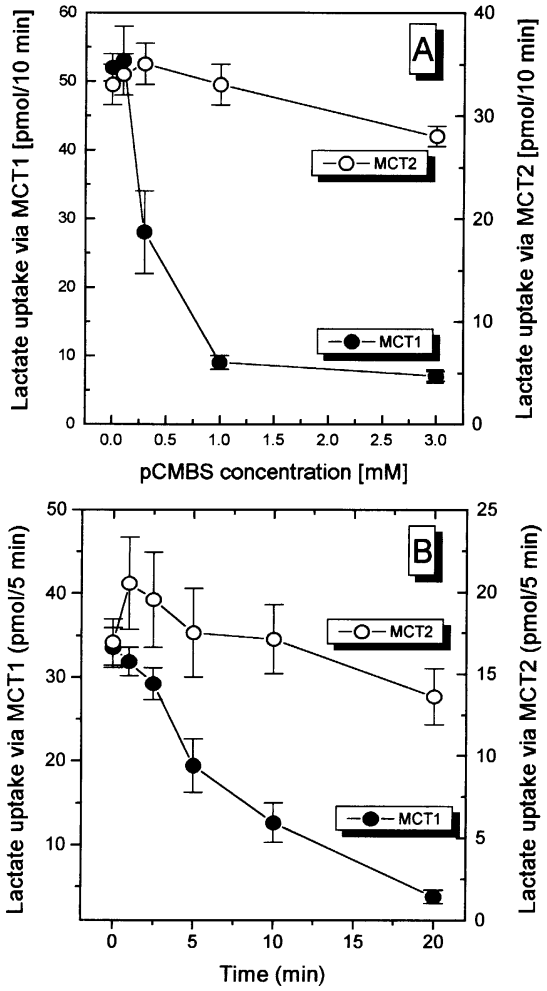

Figure 8 Sensitivity of MCT1 and MCT2 to pCMBS

Oocytes were each injected with either 5 ng of MCT1 (●) or MCT2 (○) cRNA. After an expression period of 4 days, uptake of labelled lactate (0.1 mM) was determined over a time period of 10 min (**A**) or 5 min (**B**). For the concentration- dependence (**A**) experiments, oocytes were preincubated for 10 min with solutions containing the indicated pCMBS concentration. Subsequently, oocytes were washed three times and uptake was investigated in the absence of the inhibitor. The inactivation kinetic (**B**) was measured during incubation of the oocytes with 0.3 mM pCMBS. After the indicated incubation period, oocytes were washed three times and the uptake activity was subsequently determined in the absence of the inhibitor. For each concentration or time point the mean uptake activity of seven injected and seven non-injected oocytes was determined. The transport activity of the non-injected oocytes has already been subtracted.

hydroxy-cinnamate; (ii) general inhibitors of anion transport, such as niflumate, 5-nitro-2-(3-phenylpropylamino)benzoate (NPPB), 4,4′-di-isothiocyanostilbene-2,2′-disulphonic acid (DIDS) and its derivative 4,4′-dibenzamidostilbene-2,2′-disulphonic acid (DBDS); and (iii) flavonoids, such as quercetin, phloretin and benzbromaron. The differences in sensitivity between MCT1 and MCT2 were most significant for 4-CIN and the DIDS derivative DBDS. A complete discrimination between the two isoforms was only possible by using *p*-chloromercuribenzenesulphonic acid (pCMBS). Treatment of MCT1-expressing oocytes with 0.3 mM pCMBS resulted in a complete inactivation of the transporter in 20 min (Figure 8B). By contrast, MCT2 retained more than 90 % activity after this time. A concentration-dependence was determined using an incubation time of 10 min (Figure 8A).

DISCUSSION

High-affinity transport of lactate has been described in neurons [12] and cardiac myocytes [6,7]. In contrast with lactate transport in other tissues, uptake into neurons was found to be insensitive against pCMBS and was kinetically characterized as H^+ co-transport with a K_m value of 0.4 mM for lactate. These data are in agreement with the characteristics of MCT2 expressed in *Xenopus laevis* oocytes. Taken together with the established presence of MCT2 mRNA in cultured neurons [10], it can be concluded that MCT2 is the neuronal lactate transporter. There is increasing evidence that neurons derive a significant part of their energy demands under physiological conditions by the consumption of lactate [27,28]. This view is strongly supported by the transport properties of MCT2. Although both isoforms, MCT1 as well as MCT2, allow uptake and efflux of lactate, the kinetic characteristics of MCT2 support a role in lactate uptake. At similar expression levels and low extracellular lactate concentrations, MCT2-expressing cells will take up lactate at higher rates than MCT1-expressing cells. The expression of MCT1 in astrocytes and of MCT2 in neurons supports the model of a transfer of lactate between astrocytes and neurons [10,28,29,30].

In the Cori cycle, a transfer of lactate occurs between muscle and liver. Similar to the situation in the brain, low-affinity transporters (MCT1, MCT4) are found in the source tissue, whereas the high-affinity transporter (MCT2) is expressed in the sink tissue liver [15,17]. Monocarboxylate transport has been investigated in cultured liver cells [4]; the transport properties, however, resembled more closely those of MCT1. Culturing of liver cells obviously favours expression of MCT1, which renders MCT2 hardly detectable.

The difficulty of detecting high-affinity monocarboxylate transport in cultured cells might also be related to the low V_{max} value of MCT2 when expressed in oocytes. To compare the expression levels of both transporters, surface proteins of intact oocytes expressing either MCT1 or MCT2 were biotinylated. Subsequently, biotinylated proteins were solubilized and bound to streptavidin–agarose. Western blots of these samples clearly indicated the presence of MCT1 in the membranes by a colorimetric method, whereas MCT2 was not detectable (results not shown). Quantification, however, is difficult, because the MCT2-specific antibody offers less sensitivity in Western blots than the MCT1-specific antibody. We would nevertheless tentatively suggest that the low V_{max} values which we determined in MCT2-expressing oocytes resulted from a low surface expression.

Owing to the identical substrate specificity of MCT1 and MCT2, the search for selective inhibitors turned out to be very difficult. So far only pCMBS discriminates between both isoforms. Nevertheless, 4-CIN and DBDS, when used at concentrations of 200 μM and 100 μM respectively, exert a much stronger effect on MCT2 than on MCT1. However, care has to be taken when using these inhibitors, because kinetic constants vary with the experimental conditions. As shown above, K_m (K_i) values decrease with decreasing pH and also may vary with the temperature [11].

Although MCT2 displays high affinity for monocarboyxlates, the DBDS-sensitive monocarboxylate transporter of cardiac myocytes does not share all characteristics of MCT2 [6,7]. Whereas the K_m value for pyruvate is close to the value for MCT2, the affinity for lactate is fourfold lower than that of MCT2. Most obviously, MCT2 is sensitive to inhibition by NPPB, whereas lactate uptake into cardiac myoctes was com-

pletely resistant to this compound [7]. It is tempting to speculate that this cell type might express a MCT2-related, but as yet unidentified, monocarboxylate transporter.

This work was supported by grants from the Deutsche Forschunggemeinschaft to J.W.D. (De231/13-1) and S.B. (Br1318/2-2) and by a grant of the Wellcome Trust to A.P.H.

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Received 8 February 1999/12 April 1999; accepted 14 May 1999