THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 272, No. 48, Issue of November 28, pp. 30096–30102, 1997
Printed in U.S.A.

Comparison of Lactate Transport in Astroglial Cells and Monocarboxylate Transporter 1 (MCT 1) Expressing *Xenopus laevis* Oocytes

EXPRESSION OF TWO DIFFERENT MONOCARBOXYLATE TRANSPORTERS IN ASTROGLIAL CELLS AND NEURONS*

(Received for publication, July 25, 1997, and in revised form, September 21, 1997)

Stefan Bröer‡§, Basim Rahman‡, Gioranni Pellegri¶, Luc Pellerin¶, Jean-Luc Martin¶, Stephan Verleysdonk‡, Bernd Hamprecht‡, and Pierre J. Magistretti¶

From the ‡Physiologisch-chemisches Institut der Universität, Hoppe-Seyler Str. 4, D-72076 Tübingen, Germany and the ¶Institut de Physiologie, Faculté de Médecine, Université de Lausanne, Rue du Bugnon 7, CH-1005 Lausanne, Switzerland

The transport of lactate is an essential part of the concept of metabolic coupling between neurons and glia. Lactate transport in primary cultures of astroglial cells was shown to be mediated by a single saturable transport system with a K_m value for lactate of 7.7 mM and a V_{max} value of 250 nmol/(min × mg of protein). Transport was inhibited by a variety of monocarboxylates and by compounds known to inhibit monocarboxylate transport in other cell types, such as α-cyano-4-hydroxycinnamate and *p*-chloromercurbenzenesulfonate. Using reverse transcriptase-polymerase chain reaction and Northern blotting, the presence of mRNA coding for the monocarboxylate transporter 1 (MCT1) was demonstrated in primary cultures of astroglial cells. In contrast, neuron-rich primary cultures were found to contain the mRNA coding for the monocarboxylate transporter 2 (MCT2). MCT1 was cloned and expressed in *Xenopus laevis* oocytes. Comparison of lactate transport in MCT1 expressing oocytes with lactate transport in glial cells revealed that MCT1 can account for all characteristics of lactate transport in glial cells. These data provide further molecular support for the existence of a lactate shuttle between astrocytes and neurons.

The transport of lactate is an essential part of the concept of metabolic coupling between neurons and glia (1, 2). It has been demonstrated that glutamate at concentrations around 200 μM strongly increases the rates of glycolysis and lactate release in cultured astroglial cells (3). It has further been shown that neurons are able to take up lactate and to use this compound as an energy substrate (1, 4, 5). In the mammalian retina, direct evidence has been provided for a transfer of lactate between Müller glial cells and photoreceptors (6). Besides its role as an exchangeable metabolic fuel, lactate also interferes with pH and volume regulation in neural cells (7).

There is a considerable debate over the types of transporters involved in the uptake and release of lactate by astroglial cells. Nedergaard and Goldman (8) characterized lactate transport in cultured astrocytes and determined a low K_m value of 0.4 mM. The carrier-mediated transport could not be inhibited by α-cyano-3-hydroxycinnamate or pCMBS,[1] both being typical inhibitors of monocarboxylate transport in other cell types. The transport process was reversible and accompanied by a cotransport of protons. Diffusion of protonated lactate could not be detected. In contrast to these results, Tildon *et al.* (9) identified two carrier-mediated processes for lactate uptake, characterized by K_m values of 0.5 mM and 11 mM, respectively. The maximum velocity of the low-affinity transporter was 170 nmol/(min × mg of protein), whereas only 10% of this value was found for the high affinity component. Transport was only partially inhibited by α-cyano-4-hydroxycinnamate and mersalyl. Acidic pH strongly increased transport activity, a finding consistent with a lactate/proton cotransport mechanism. Dringen *et al.* (10) detected solely non-saturable lactate transport in primary cultures of astroglial cells which could not be inhibited by α-cyano-3-hydroxycinnamate or α-cyano-4-hydroxycinnamate and strongly increased when the pH value was lowered. Volk *et al.* (11) showed that lactate release from astroglial cells could be inhibited by quercetin but not by α-cyano-4-hydroxycinnamate.

Recently three different cDNAs were identified which encode H^+/monocarboxylate cotransporters of mammalian cells (12–16) and were designated MCT1, MCT2, and MCT3. MCT1 is expressed in erythrocytes, lung, heart, skeletal muscle, and the basolateral membranes of the intestinal epithelium; MCT2 predominates in heart, liver, kidney, and testis. MCT3 was isolated from retinal pigment epithelium, its tissue distribution has not been established. Contrasting results have been reported concerning expression in the brain; thus neither MCT1 nor MCT2 were detected in brain in two studies (12, 14), whereas MCT1 mRNA was detected in rat brain in two other studies. (16, 17). The physiological properties of MCT1 have been investigated extensively (18, 19). Transport of pyruvate by MCT2 has been investigated in transfected cells (14). The well characterized lactate transport in liver cells (20) might reflect the action of both MCT1 and MCT2 (17).

In this report we present functional and molecular evidence that MCT1 is the only monocarboxylate transporter of astroglial cells, whereas cultured neurons express the transporter isoform MCT2. High level expression of MCT1 in *Xenopus laevis* oocytes allowed the investigation of transport properties

* This work was supported by Fonds National de la Recherche Scientifique Grant 31-40565.94 (to P. J. M.) and Deutsche Forschungsgemeinschaft Grant Br 1318/2-1 (to S. B.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence should be addressed: Physiologisch-chemisches Institut der Universität, Hoppe-Seyler Str. 4, D-72076 Tübingen, Germany. Tel.: 49-7071-2973331; Fax: 49-7071-295360; E-mail: stefan.broeer@uni-tuebingen.de.

[1] The abbreviations used are: pCMBS, *p*-chloromercuribenzenesulfonate; HBSS, Hank's buffered salt solution; MCT, monocarboxylate transporter; bp, base pair(s).

of MCT1 using [^{14}C]lactate as a substrate. The transport properties of MCT1 expressed in oocytes are in agreement with transport data gained with astroglia-rich primary cultures.

EXPERIMENTAL PROCEDURES

Materials—Radiochemicals were purchased from Amersham Buchler (Braunschweig, Germany) or from DuPont (Regensdorf, Switzerland). Fetal calf serum was supplied by Boehringer (Mannheim, Germany) or Fakola AG (Basel, Switzerland). Superscript reverse transcriptase was from Life Technologies, Eggenstein (Germany) and Dulbecco's modified Eagle's medium was obtained from Life Technologies or Sigma (Buchs, Switzerland). The Cap-analogue m^{7}G(5′)ppp(5′)G was purchased from New England Biolabs, Schwalbach (Germany). GeneScreen membranes were obtained from Life Science Products, Regensdorf (Switzerland), RNasin from Promega, Mannheim (Germany), and Ultima Gold scintillation mixture from Canberra Packard, Frankfurt (Germany). All other chemicals were of analytical grade and supplied by E. Merck, Darmstadt, Germany; Roth, Karlsruhe, Germany; Boehringer, Mannheim, Germany; or Sigma, Buchs, Switzerland; or Deisenhofen, Germany.

Cell Culture—Astroglia-rich primary cultures were prepared from brains of neonatal Wistar rats and cultured as described by Hamprecht and Löffler (21). Primary cultures of mouse cerebral cortical astrocytes were prepared from Swiss albino newborn mice as described by Sorg and Magistretti (22). Cortical neurons devoid of glial cells were prepared as described previously (23).

Transport Assay—For uptake experiments rat astroglial cells were grown to a density of 4×10^6 per 60-mm culture dish in a humidified atmosphere of 10% CO_2 in air at 37 °C in 90% Dulbecco's modified Eagle's medium, 10% fetal calf serum containing 44 mM $NaHCO_3$. All experiments were performed at 21 °C. Growth medium was aspirated, and cells were washed three times with 3 ml of HBSS (136.6 mM NaCl, 5.4 mM KCl, 4.0 mM HEPES, 2.7 mM Na_2HPO_4, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.44 mM KH_2PO_4, 0.41 mM $MgSO_4$, pH 7.8). To reduce metabolism of lactate, cells were preincubated for 5 min with 1 mM aminooxyacetate in HBSS. To initiate transport, the preincubation medium was aspirated and replaced by 3 ml of HBSS containing 1 mM aminooxyacetate, [^{14}C]lactate, and unlabeled lactate at different concentrations resulting in a specific activity of 500 dpm/nmol. After 15 s, transport was stopped by aspirating the transport buffer followed by three washing cycles with 3 ml of ice-cold HBSS. Cells were lysed by addition of 1 ml of 0.1 M HCl. Of the resulting suspension an aliquot portion of 900 μl was mixed with 3 ml of scintillation mixture, and radioactivity was determined in a scintillation counter. An aliquot portion of 100 μl was used for protein determination using the Bio-Rad Protein assay (Bio-Rad Laboratories, München, Germany).

Northern Blotting—Total RNA was extracted from primary cultures of cortical neurons or astroglial cells using the CsCl centrifugation procedure according to Chirgwin and collaborators (24). Total RNA (10 μg) was electrophoresed on a 1.2% agarose, 2 M formaldehyde gel and transferred onto GeneScreen nylon membrane. Hybridization was performed overnight at 65 °C in 50% formamide, 5 × SSC (0.75 M NaCl, 75 mM sodium citrate, pH 7.0), 1 × PE (50 mM Tris-HCl, pH 7.5, 0.1% sodium pyrophosphate, 1% SDS, 0.2% polyvinylpyrrolidone, 0.2% Ficoll, 5 mM EDTA, 0.2% bovine serum albumin), 150 μg/ml salmon sperm DNA with a ^{32}P-antisense MCT1 or MCT2 riboprobe. Filters were then washed under high stringency conditions, first with 2 × SSC, 0.1% SDS at 65 °C for 15 min, followed by two washes of 15 min each in 0.1 × SSC, 0.1% SDS at 65 °C. Filters were dried and apposed to Kodak AR film at −70 °C with an intensifying screen.

The ^{32}P-antisense MCT1 and MCT2 riboprobes were generated using T7 RNA polymerase and [α-^{32}P]UTP from a linearized pT7Blue(R)T-vector (Novagen) containing a 505-bp MCT1 cDNA fragment (nucleotides 1128–1635; Ref. 13) or a 581-bp MCT2 cDNA fragment (nucleotides 979–1559; Ref. 14).

The 505-bp MCT1 cDNA fragment was obtained by reverse transcription and polymerase chain reaction amplification of poly(A) RNA isolated from cultured cortical astrocytes with a set of oligonucleotide primers (5′-CAAGTGGATCAGACCTCGG-3′ and 5′-GGAGCTATTCTGCTGCG-3′) located at 1128–1146 and 1619–1635 bp in the coding region of the hamster MCT1 sequence (13). The amplified MCT1 cDNA fragment was shown to be identical to the mouse MCT1 cDNA sequence (25) and to share 89% nucleotide identity with the hamster MCT1 cDNA sequence (13). The 581-bp MCT2 cDNA fragment was obtained by reverse transcription and polymerase chain reaction amplification of poly(A)$^+$ mRNA isolated from mouse liver with oligonucleotide primers 5′-GATGGCTTTTGTTGATATG-3′ and 5′-CTCTTTCTCTGTCTGAGGG-3′ located at 979–997 and 1541–1559 bp, respectively, in the coding region of the hamster MCT2 cDNA sequence (14). The amplified MCT2 cDNA fragment shared 84% nucleotide identity with the hamster MCT2 cDNA sequence (14).

Cloning of MCT1—A 3.3-kilobase cDNA clone of MCT1 was isolated from a size-selected C6-BU-1 cDNA library (26). The identity with rat MCT1 (27) was confirmed by sequencing. The 1.9-kilobase *Eco*RI fragment of MCT1 containing the complete coding sequence was cloned into the *Eco*RI site of the oocyte expression vector pGEM-He (Ref. 28; kindly provided by Dr. Jost Ludwig, Hamburg). This vector contains the 5′- and 3′-untranslated regions of the *Xenopus* β-globin interrupted by a multiple cloning site. For expression, plasmid DNA was linearized with *Not*I and transcribed *in vitro* with T7 RNA polymerase in the presence of the cap analog m^{7}G(5′)ppp(5′)G at a concentration of 1 mM.

Oocytes and Injections—*X. laevis* females were generously supplied by Dr. P. Hausen (Max-Planck-Institut für Entwicklungsbiologie, Tübingen). Oocytes (stages V and VI) were isolated as described (29) and allowed to recover overnight. They 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).

Hybrid Depletion Experiments—For each experiment, 1.5 μl of poly(A) RNA (2 mg/ml) was heated to 65 °C for 3 min and then mixed with 3 μl of oligonucleotide solution (0.6 mg/ml) followed by incubation at 42 °C for 10 min. After cooling on ice, 50 nl of the mixture were immediately injected into each oocyte to avoid degradation of the cRNA. The following oligonucleotides were used in the experiments: (i) antisense oligonucleotide MCT3a, TGCCATAGCCAGGCCATTGGC (corresponding to bases 647–667 of the rat MCT1 cDNA sequence; Ref. 27); (ii) antisense oligonucleotide MCT 6a, CATGATGGATGATATCCATG (corresponding to bases 387–406); and (iii) antisense oligonucleotide MCT9a, TCAGTAAATAAATGAGCTAT (corresponding to bases 2761–2780).

Uptake Measurements—For each determination, groups of 7 oocytes injected with cRNA or water were washed twice with 4 ml of 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, final pH was 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 [^{14}C]lactate and different amounts of unlabeled lactate. Transport was stopped after different times by washing oocytes three times with 4 ml of ice-cold OR2+ buffer. Single oocytes were placed into scintillation vials and lysed by addition of 200 μl of 10% SDS. After lysis, 3 ml of scintillation fluid was added and radioactivity determined in a liquid scintillation counter.

Calculations—Standard deviations are given for all values. Gausses law of error propagation was applied when values had to be subtracted. Each experiment presented was performed at least twice with similar results. Data were analyzed by non-linear regression using commercially available software (Fig. P, Biosoft, Cambridge, United Kingdom).

RESULTS

Uptake of Radioactive Lactate by Primary Cultures of Glial Cells—It is well recognized that lactate is rapidly transported and metabolized in mammalian cells. After oxidation to pyruvate, this metabolite is rapidly transaminated to alanine, a reaction which is fast in glial cells (30). In preliminary experiments accumulation of radioactivity derived from labeled lactate in primary cultures of rat glial cells at pH 7.0 was found to be much higher than expected for an equilibration of lactate between the medium and the cytosol of the cells. A 5-min preincubation of the cells with 1 mM aminooxyacetic acid greatly reduced the accumulation of radioactivity by inhibiting the transamination of pyruvate to alanine. The decrease of lactate accumulation did not result from inhibition of lactate transport. When the preincubation step was omitted and aminooxyacetic acid (1 mM) was added together with labeled lactate (0.1 mM) to initiate transport, no inhibition of lactate uptake was observed (data not shown). To reduce the effects of metabolism and trans-effects further, short incubation times and low temperature were used in all experiments. Uptake of lactate was almost linearly correlated with time up to 20 s at 21 °C. By using these optimized conditions, the basic transport parameters were determined for lactate uptake in primary cultures of rat astrocytes. A K_m value of 7.7 ± 0.7 mM and a V_{max} value of 250 nmol/(min × mg of protein) was determined (Fig. 1). When the data were transformed and plotted according

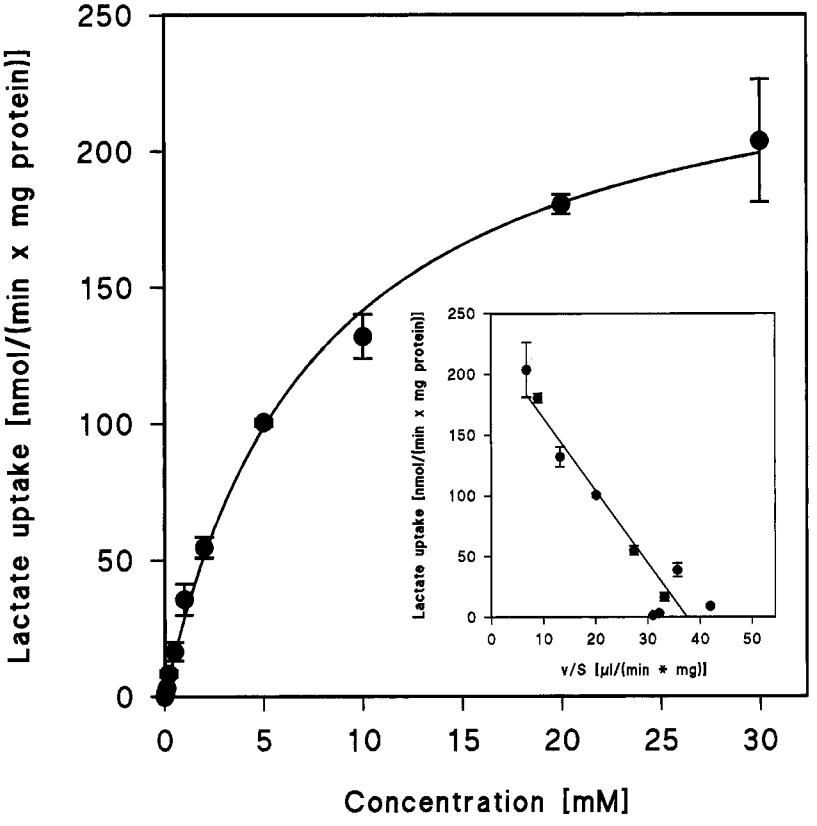

FIG. 1. **Determination of the kinetic parameters for lactate transport in rat astroglia-rich primary cultures.** Cells were washed three times with HBSS and then incubated for 5 min in HBSS with 1 mM aminooxyacetate. After preincubation, HBSS was replaced by the same buffer containing [^{14}C]lactate and unlabeled lactate of varying concentrations. Transport was stopped after 15 s by aspirating the transport buffer followed by washing the plates three times with ice-cold HBSS. The experiment was performed at pH 7.0. Data were plotted according to Michaelis-Menten (*main figure*) or Eadie-Hofstee (*inset*). The mean ± S.D. was calculated from the transport activity of three culture dishes. The experiment was performed at pH 7.0.

to Eadie-Hofstee only one component was visible (Fig. 1, *inset*).

Uptake of lactate by primary cultures of rat glial cells increased with increasing H^+ concentration. At a substrate concentration of 0.1 mM, lactate uptake at pH 6.0 was more than three times faster than at pH 8.0 (Fig. 2). To determine if other monocarboxylates are also substrates of the lactate transporter in glial cells, competition experiments were performed. At an extracellular concentration of 0.1 mM lactate, transport was strongly inhibited by a 50-fold excess of α-ketoisocaproate or acetoacetate and to a lesser extent by pyruvate and DL-3-hydroxybutyrate (Table I). Typical inhibitors of monocarboxylate transport such as α-cyano-4-hydroxycinnamate and pCMBS strongly decreased lactate uptake into astroglial cells (Table I).

Similar results were obtained with mouse astroglia-rich cultures. At pH 6.0, K_m values of 3.5 mM and 8 mM and V_{max} values of 573 nmol/(min × mg of protein), respectively, 576 nmol/(min × mg of protein) were determined in two independent experiments. These V_{max} values, determined at pH 6.0, are more than twice the V_{max} value of lactate transport in rat astroglia-rich cultures, which was determined at pH 7.0. Due to the strong pH dependence of lactate transport both types of culture will most likely have quite similar capacities of lactate transport at identical pH values.

Expression of MCT1 and MCT2 mRNAs in Astroglial Cells and Cortical Neurons—Northern blot analysis of MCT1 expression in primary cultures of cortical neurons and astroglial cells revealed that MCT1 mRNA was predominantly expressed in astroglial cells with a very faint expression in neuronal cultures (Fig. 3). The size of the transcript corresponded to that previously reported for rat and hamster tissues (12). In contrast, MCT2 mRNA appeared to be highly abundant in cortical neurons with a barely detectable level of expression in cortical astroglial cells (Fig. 3). It is worth noting the presence of three different transcripts with sizes of 2.7, 6.3, and 9.3 kilobases in neuronal cultures.

Comparison of Lactate Transport in Glial Cells and MCT1

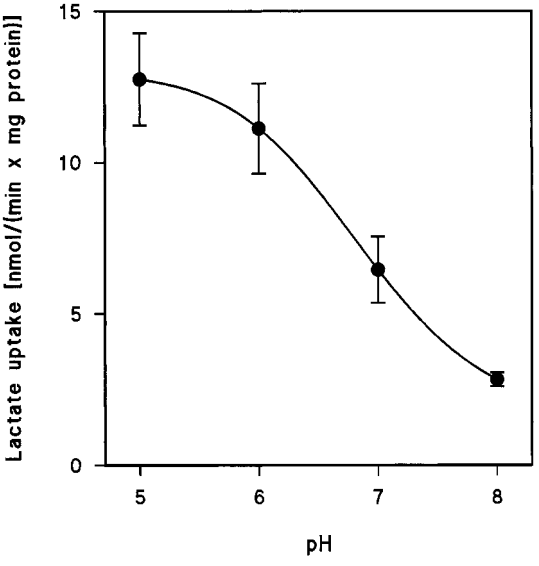

FIG. 2. **Dependence of lactate uptake in rat astroglia-rich primary cultures on the pH value.** Transport activity was determined as described in the legend to Fig. 1, but at different pH values and lactate at a final concentration of 1 mM. The mean ± S.D. was calculated from the transport activity of three culture dishes.

Expressing Oocytes—To investigate the properties of glial lactate transport in more detail, the MCT1 cDNA was isolated from a cDNA library of C6-BU-1 rat glioma cells and cloned into the oocyte expression vector pGEM-He. High expression of MCT1 could be achieved in oocytes. Oocytes which were injected with cRNA showed a 10–20-fold higher [^{14}C]lactate uptake than water-injected oocytes (Fig. 4). Although *X. laevis* oocytes have a diameter of 1 mm, lactate uptake was proportional to time for only 5 min. Transport parameters were therefore determined using a time scale of 2.5 min. To investigate

TABLE I

Inhibitors of lactate transport in astroglial cells and in Xenopus oocytes expressing MCT1

Cells were preincubated with aminooxyacetate (1 mM) for 5 min in Hank's buffered salt solution at pH 7.0. Uptake was determined in the same buffer at a lactate concentration of 0.1 mM in the presence or absence of inhibitors at 21 °C for 15 s. Acetoacetate was tested in a separate experiment with a separate control. Oocytes were injected with 12.5 ng of MCT1 cRNA each. Four days later uptake of [^{14}C]lactate was determined at a concentration of 0.1 mM in the presence or absence of inhibitors at 21 °C for 2.5 min.

Inhibitor	Concentration	Transport rate	Transport rate	Relative transport rate in	
				Cells	Oocytes
	mM	*nmol·min^{-1}·mg protein^{-1}*	*pmol/2.5 min/oocyte*	*% of control*	
None	—	6.5 ± 0.2	35.7 ± 2.3	100	100
4-CIN	5	0.38 ± 0.02	3.3 ± 0.3	6	9
pCMBS	1	1.31 ± 0.07	6.7 ± 1.1	20	18
Pyruvate	5	5.7 ± 0.2	8.3 ± 0.4	88	23
	50	1.7 ± 0.1		26	
α-Ketoisocaproate	5	1.4 ± 0.1	4.8 ± 0.1	22	13
D,L-3-Hydroxybutyrate	5	6.3 ± 0.1	5.1 ± 0.3	97	14
	50	2.2 ± 0.2	2.6 ± 0.1	34	7
None		5.1 ± 0.4	35.7 ± 2.3	100	100
Acetoacetate	5	4.3 ± 0.2	16.2 ± 1.3	84	43
	50	1.6 ± 0.2	4.7 ± 0.4	31	13

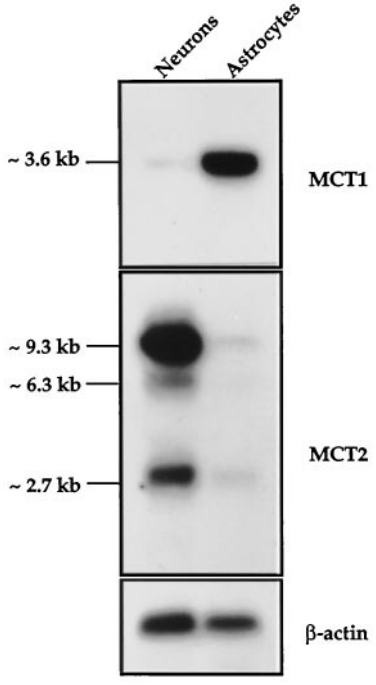

FIG. 3. **Detection of MCT1 and MCT2 mRNAs by Northern blot analysis.** Total RNA was isolated from mouse astroglia-rich and mouse neuron-rich primary cultures. Samples of 20 μg were separated by denaturing agarose gel electrophoresis and blotted onto nylon membranes. MCT1 and MCT2-specific ^{32}P-riboprobes were hybridized under stringent conditions. As a loading control, the same blot was subsequently analyzed using a β-actin probe. *kb*, kilobase.

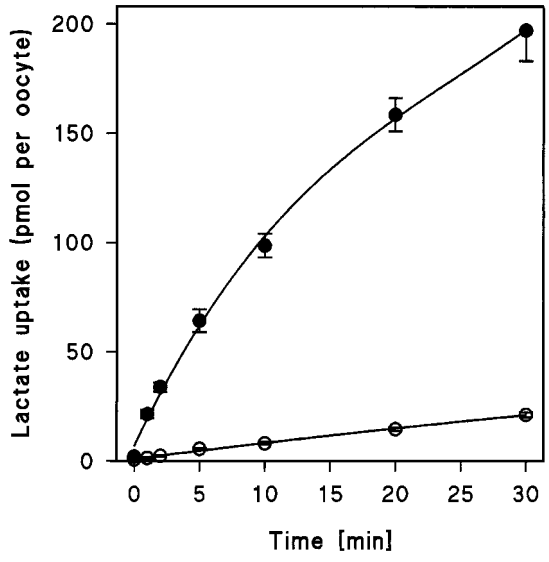

FIG. 4. **Expression of MCT1 in *X. laevis* oocytes.** MCT1 was cloned into the oocyte expression vector pGEM-He and cRNA was synthesized by *in vitro* transcription from the T7 promotor. Each oocyte was injected with 12.5 ng of cRNA. After 4 days of expression uptake of [^{14}C]lactate (0.1 mM) from a solution was determined at different time intervals (●) and compared with the uptake of water-injected oocytes (○).

whether MCT1 could account for all characteristics of lactate transport in glial cells, the basic kinetic parameters of glial cells and oocytes expressing MCT1 were compared. Using [^{14}C]lactate a K_m value of 5.6 mM was determined in oocytes which was in good agreement with the data obtained with glial cells (Fig. 5). To compare both data sets the V_{max} value of lactate transport in astroglia-rich cultures was adjusted and is shown in Fig. 5 as a *dashed line*. In MCT1 expressing oocytes a second kinetic component with a K_m value of 1.1 mM was visible. Not only the K_m value, but also the pH dependence of lactate transport in MCT1 expressing oocytes and glial cells was very similar. When the data were fitted as a titration curve, a pK value of 6.6 was calculated for MCT1 expressing oocytes and a value of 6.9 for glial cells (Fig. 6). From a Hill plot of the data (Fig. 6, *inset*), slopes of 0.74 and 0.72 were determined for glial cells and MCT1 expressing oocytes, respectively. Therefore, a 1:1 stoichiometry of lactate/proton symport was assumed in both systems.

MCT1 is a nonspecific monocarboxylate transporter, which transports a wide variety of substrates with different kinetic constants. The inhibitory action of different monocarboxylic acids on MCT1-mediated lactate transport was therefore compared with the data obtained from glial cells (Table I). Lactate transport in MCT1 expressing oocytes was strongly inhibited by α-cyano-4-hydroxycinnamate, α-ketoisocaproate, DL-3-hydroxybutyrate, pyruvate, and acetoacetate. While α-cyano-4-hydroxycinnamate and α-ketoisocaproate inhibited lactate transport in glial cells and MCT1 expressing oocytes to a similar extent, large discrepancies were found in the case of DL-3-hydroxybutyrate, pyruvate, and acetoacetate. To further investigate the inhibitory potency of pyruvate on lactate transport in MCT1 expressing oocytes, the concentration of pyruvate competing with lactate (0.1 mM) for uptake was varied. At concentrations below 0.2 mM, pyruvate did not exert significant inhibition on lactate uptake, whereas at concentrations higher than 0.2 mM it strongly inhibited lactate transport (Fig. 7). A

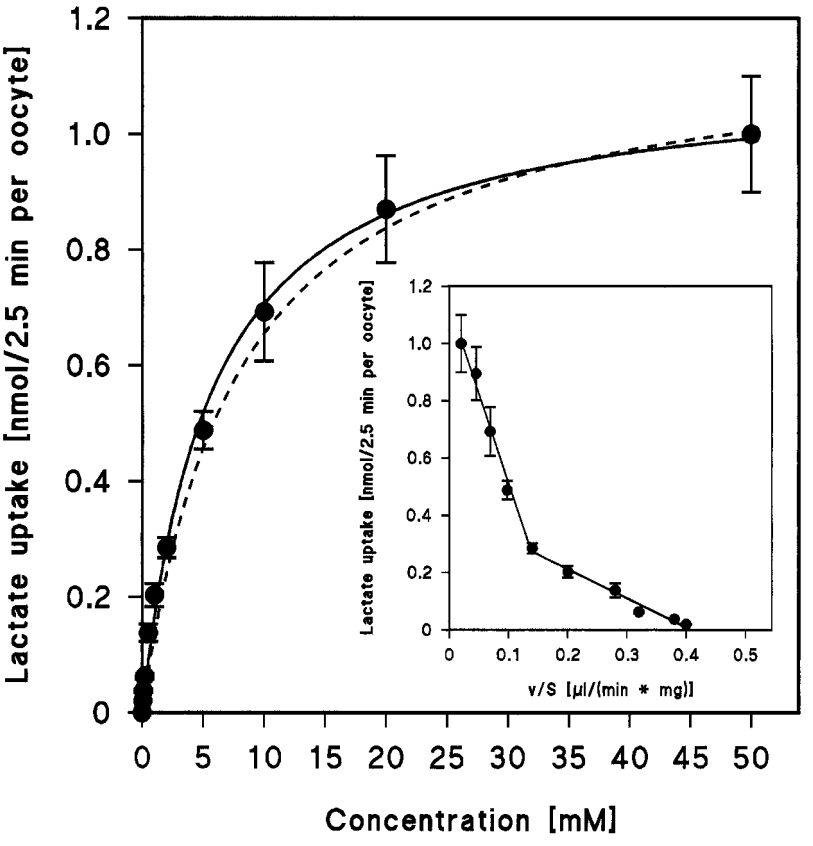

FIG. 5. **Determination of the kinetic parameters for lactate transport in MCT1 expressing oocytes.** Oocytes were injected with 12.5 ng of MCT1 cRNA each. Four days later uptake of [^{14}C]lactate was determined at different concentrations of unlabeled lactate after 2.5 min of incubation. The experiment was performed at pH 7.0. To allow a comparison with the data from Fig. 1, the maximum velocity of lactate uptake in astroglia-rich cultures was adjusted and the resulting curve is shown as a *dashed line*. *Inset*, Eadie-Hofstee transformation of the data gained from oocytes.

pyruvate concentration of 0.7 mM was necessary for half-maximal inhibition of lactate transport. The thiol reagent pCMBS inhibited lactate transport in MCT1 expressing oocytes to an extent similar to that encountered in glial cells (Table I).

Involvement of Other Possible Transport Pathways in Lactate Uptake in Glial Cells—To investigate the possible involvement of other lactate transport pathways in glial cells, hybrid depletion experiments were performed. When poly(A) RNA was isolated from rat astroglia-rich primary cultures and injected into *X. laevis* oocytes, a 2-fold induction of lactate uptake could be determined in poly(A) RNA-injected oocytes in comparison to water-injected oocytes (Table II). Whereas injection of 12.5 ng of MCT1 cRNA resulted in the uptake of 200 pmol of lactate in 30 min at pH 7.0 (Fig. 4), injection of 50 ng of poly(A) RNA induced a lactate transport capacity of 17 pmol in 30 min at pH 6.0. When poly(A) RNA was coinjected with antisense-oligonucleotides corresponding to sequences of the MCT1 mRNA, the lactate uptake activity, which resulted from the expression of glial poly(A) RNA, was completely suppressed (Table II).

DISCUSSION

Lactate transport in primary cultures of astroglial cells has been investigated by several groups (8–10). The results of these studies are contradictory. Several typical features of lactate transport as described in other cell types were not detected in glial cells (*e.g.* lactate transport was only weakly or not inhibited by cinnamic acid derivatives and very low K_m values or non-saturability were reported). Together, these results suggested the presence of an unusual lactate transporter in astroglial cells.

Reduction of incubation temperatures, depletion of cytosolic lactate by preincubation in glucose-free medium, and inhibition of lactate metabolism by aminooxyacetate resulted in experimental conditions which allowed the determination of kinetic parameters using [^{14}C]lactate. Although external pH was controlled in all experiments, variations of the internal pH could not be avoided during experiments. In bicarbonate-buffered culture medium (pH 7.4) the internal pH of astroglial cells can be expected to be about pH 7.0 (Ref. 31).[2] In astroglia-rich primary cultures prepared from rat cerebellum, replacement of bicarbonate containing buffer with bicarbonate-free buffer resulted in a fast alkalinization followed by a slower acidification and regulation back to pH 7.0 (31). Treatment of the same cell cultures with buffer of pH 6.6 in the absence of bicarbonate resulted in a decrease of the internal pH to 6.6 (31). Similar results have been reported with astroglia-rich primary cultures from rat forebrain (32) and rat cerebral cortex (33). During the 5-min preincubation with aminooxyacetic acid in our experiments, pH transients will be mostly completed and the pH can be expected to be close to 7.0 or a little lower. All experiments were therefore performed at low pH gradients. The kinetic parameters of lactate transport in the present study were in almost complete agreement with the expected characteristics of the monocarboxylate transporter 1 (MCT1) as determined by Carpenter and Halestrap (18) in Ehrlich-Lettre tumor cells. The K_m value of 7.7 mM determined in astroglial-rich primary cultures is close to the values determined in erythrocytes (34), Ehrlich-Lettre tumor cells (4.5 mM; Ref. 18), and in MCT1-transfected cells (8.3 mM; Ref. 14). Although V_{max} values are high in primary cultures of rat or mouse astroglial cells, they are within the range determined for other cell types (34). The apparent pK value of the proton-binding site and the derived stoichiometry of 1:1 for lactate:proton cotransport were identical to the values determined for lactate transport in erythrocytes (19, 34). α-Ketoisocaproate, acetoacetate, pyruvate, and DL-3-hydroxybutyrate were found to be inhibitors of lactate transport in astroglial cells. Lactate uptake was also strongly inhibited by α-cyano-4-hydroxycinnamate and pCMBS. Taken together these data strongly indicated the presence of a typical

[2] J. W. Deitmer personal communication.

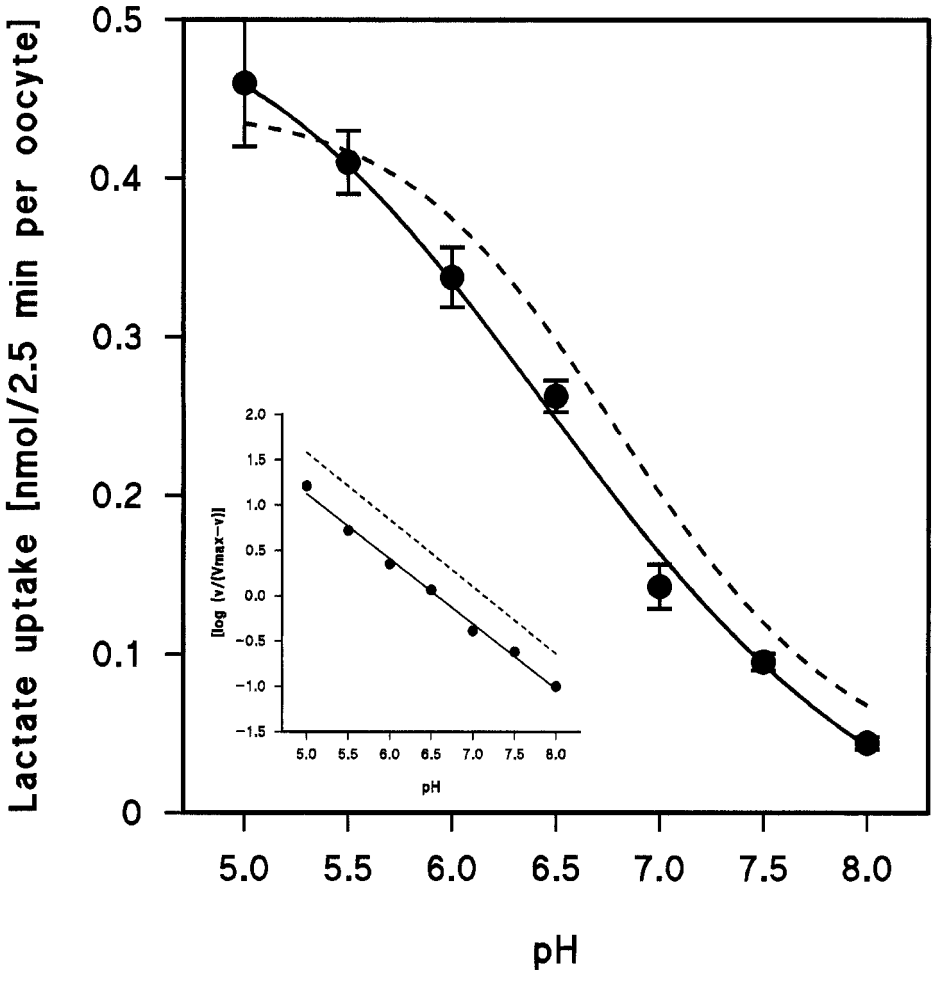

FIG. 6. **Dependence of lactate transport in MCT1 expressing oocytes on the pH value.** Oocytes were injected with 12.5 ng of MCT1 cRNA. Four days later uptake of [^{14}C]lactate during 2.5 min was determined at a concentration of 1 mM as a function of pH. Data from Fig. 2 are shown as a *dashed line* for comparison. In the *inset* the transformation of the data into a Hill plot is shown.

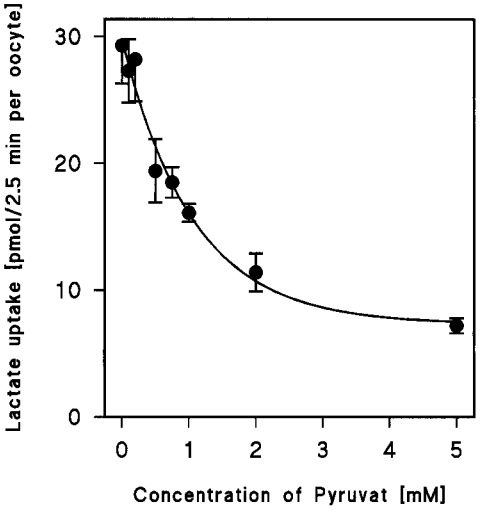

FIG. 7. **Concentration-dependent inhibition of lactate transport in MCT1 expressing oocytes by pyruvate.** Oocytes were injected with 12.5 ng of MCT1 cRNA each. Four days later uptake of [^{14}C]lactate was determined at a concentration of 0.1 mM in the presence of increasing concentrations of pyruvate. The experiment was performed at pH 7.0.

monocarboxylate transporter in astroglial cells similar to MCT1 of Ehrlich-Lettre tumor cells and erythrocytes. Although pyruvate displays a lower K_m value for MCT1 than lactate (14, 18), inhibition by pyruvate was weak in experiments with glial cells. To elucidate the inhibitory action of pyruvate, MCT1 was expressed in *X. laevis* oocytes. Expression of MCT1 in oocytes has been reported previously, however, expression was too low to characterize the transport process (16). By using an oocyte expression vector, MCT1 expression levels could be raised severalfold (not shown) and allowed the investigation of transport properties. Oocyte experiments were performed in buffer of pH 7.0. The intracellular pH of the oocytes was about 7.3 under these conditions as determined with microelectrodes.[3] This would allow a 2-fold accumulation of lactate in the oocyte. Assuming an oocyte volume of 500 nl, 2-fold accumulation should be reached at about 100 pmol. Although 200 pmol of lactate were accumulated in the oocyte after 30 min, the curve

TABLE II

Hybrid depletion of MCT1 mRNA in poly(A)RNA using antisense oligonucleotides directed against MCT1

Oocytes were either injected with 30 ng of poly(A) RNA, isolated from rat astroglia-rich primary cultures, or 20 ng of different antisense oligonucleotides directed against rat MCT1, or a mixture of 30 ng of poly(A) RNA and 20 ng of antisense oligonucleotide. Five days later transport activity was determined in both groups of oocytes, by measuring the uptake of [^{14}C]lactate (100 μM, pH 6.0) for 30 min. Transport activity is given as picomole of lactate/30 min/oocyte. The mean ± S.D. of seven oocytes is given for each experiment.

Oligonucleotide (1)	Poly(A) RNA + oligonucleotide injected (2)	H_2O + oligonucleotide injected (3)	Column 2 minus column 3 (4)
None (control)	37.7 ± 4.4	20.7 ± 1.7	17.0 ± 4.7
MCT3a	24.0 ± 1.5	21.5 ± 1.0	2.5 ± 1.8
MCT6a	19.5 ± 1.1	20.8 ± 0.7	−1.0 ± 2.7
MCT9a	21.8 ± 1.3	25.6 ± 2.6	−3.2 ± 3.5

[3] S. Bröer, B. Rahman, J. W. Deitmer, and B. Hamprecht, unpublished results.

flattened after reaching 100 pmol. No attempts were made to inhibit metabolism, because transamination is virtually absent in oocytes (29). At 2.5 min only about 30 pmol of lactate were transported into the oocyte, which was well below the equilibration level. A comparison of lactate transport in MCT1 expressing oocytes and glial cells revealed that lactate transport in glial cells was likely to be mediated by MCT1. The K_m value, the pH dependence, as well as the inhibition by α-cyano-4-hydroxycinnamate and pCMBS, were similar in both systems. An additional kinetic component with a lower K_m value was detected in MCT1 expressing oocytes. Three explanations are available for this observation: (i) up-regulation of an oocyte endogenous transport system due to overexpression of foreign protein (*e.g.* Refs. 35 and 36); (ii) two binding sites are present on one transporter (*e.g.* Ref. 37); or (iii) experimental conditions resulting in overestimation of transport at low concentrations or underestimation at high concentrations. Two kinetic components have been observed with other expressed transporters in oocytes as well (26, 38), which might indicate a still unresolved experimental problem in this system. A comparison of the competition experiments revealed that α-cyano-4-hydroxycinnamate and α-ketoisocaproate exerted similar inhibitory potential in MCT1 expressing oocytes and in glial cells, whereas the inhibitory potential of DL-3-hydroxybutyrate, pyruvate, and acetoacetate was strongly underestimated in transport experiments in glial cells. Only at very high concentrations was inhibition prevailed. A second component of lactate transport has been detected in astrocytes by Tildon *et al.* (9). The calculation of this component mostly relied on one data point at low concentration (about 0.1 mM). Regression analysis of the remaining points results in a K_m value of about 8 mM, which is close to the K_m value determined in this work. Hybrid depletion experiments were used to investigate the participation of other pathways in lactate transport. Injection of poly(A) RNA isolated from astroglia-rich primary cultures induced an increased lactate transport capacity. This additional transport activity was completely suppressed by a set of antisense oligonucleotides targeted to different regions of MCT1 mRNA. Also, diffusion of lactic acid does not seem to be a significant route of lactate transport. The dependence of lactate transport on substrate concentration could be optimally fitted by using a simple Michaelis-Menten equation. Inclusion of a non-saturable component did not improve the regression curve. This is in agreement with results of Nedergaard and Goldman (8), who also detected only saturable transport of lactate in astrocytes. These results further support the predominant if not exclusive role of MCT1 in monocarboxylate transport in astroglial cells.

In agreement with the kinetic data, MCT1 mRNA was detected mainly in astroglia-rich primary cultures while MCT2 mRNA could be detected only faintly. In contrast, neuron-rich primary cultures expressed MCT2 mRNA at a much higher level than MCT1. Previous observations suggest that MCT2 is expressed in tissues, such as liver or heart, which mainly consume lactate, whereas MCT1 is expressed, for example, in muscle, which preferentially release lactate (20). The data on cellular distribution of MCTs fit well with the distribution of lactate dehydrogenase isoforms in the brain. Thus, lactate dehydrogenase isoform 1, which is predominantly expressed in tissues which can oxidize lactate for energy production, is the only LDH isoform found in neurons (39). In contrast, lactate dehydrogenase isoform 5, which is found in tissues displaying anaerobic glycolysis, is expressed in astrocytes (39). Taken together, the distribution of lactate transporter and lactate dehydrogenase isoforms in astrocytes and neurons support the view that brain astrocytes may supply neurons with the energy substrate lactate under conditions of increased neuronal energy demand (40, 41).

Acknowledgments—We are indebted to Angelika Bröer for technical assistance. We also thank Dr. Jan Tytgat (University of Leuven) for permission to use the oocyte vector pGEM-He in this study. A modified version of this vector was kindly provided by Dr. Jost Ludwig (ZMNH, Hamburg).

REFERENCES

1. Dringen, R., Wiesinger, H., and Hamprecht, B. (1993) *Neurosci. Lett.* **163,** 5–7
2. Tsacopoulos, M., and Magistretti, P. J. (1996) *J. Neurosci.* **16,** 877–885
3. Pellerin, L., and Magistretti, P. J. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 10625–10629
4. Schurr, A., and Rigor, B. M. (1988) *Science* **240,** 1326–1328
5. Schneider, U., Poole, R. C., Halestrap, A. P., and Grafe, P. (1993) *Neuroscience* **53,** 1153–1162
6. Poitry-Yamate, C. L., Poitry, S., and Tsacopoulos, M. (1995) *J. Neurosci.* **15,** 5179–5191
7. Siesjö, B. K. (1988) *Neurochem. Pathol.* **9,** 31–88
8. Nedergaard, M., and Goldman, S. A. (1993) *Am. J. Physiol.* **265,** R282–R289
9. Tildon, J. T., McKenna, M. C., Stevenson, J., and Couto, R. (1993) *Neurochem. Res.* **18,** 177–184
10. Dringen, R., Peters, H., Wiesinger, H., and Hamprecht, B. (1995) *Dev. Neurosci.* **17,** 63–69
11. Volk, C., Kempski, B., and Kempski, O. (1997) *Neurosci. Lett.* **223,** 121–124
12. Kim, C. M., Goldstein, J. L., and Brown, M. S. (1992) *J. Biol. Chem.* **267,** 23113–23121
13. Kim-Garcia, C., Goldstein, J. L., Pathak, R. K., Anderson, R. G. W., and Brown, M. S. (1994) *Cell* **76,** 865–873
14. Garcia, C. K., Brown, M. S., Pathak, R. K., and Goldstein, J. L. (1995) *J. Biol. Chem.* **270,** 1843–1849
15. Yoon, H., Fanelli, A., Grollmann, E. F., and Philp, N. J. (1997) *Biochem. Biophys. Res. Commun.* **234,** 90–94
16. Takanaga, H., Tamai, I., Inaba, S., Sai, Y., Higashida, H., Yamamoto, H., and Tsuji, A. (1995) *Biochem. Biophys. Res. Commun.* **217,** 370–377
17. Jackson, V. N., Price N. T., Carpenter, L., and Halestrap, A. P. (1997) *Biochem. J.* **324,** 447–453
18. Carpenter, L., and Halestrap, A. P. (1994) *Biochem. J.* **304,** 751–760
19. Deuticke, B. (1982) *J. Membr. Biol.* **70,** 89–103
20. Jackson, V. N., and Halestrap, A. P. (1996) *J. Biol. Chem.* **271,** 861–868
21. Hamprecht, B., and Löffler, F. (1985) *Meth. Enzymol.* **109,** 341–345
22. Sorg, O., and Magistretti, P. J. (1991) *Brain Res.* **563,** 227–233
23. Stella, N., Pellerin, L., and Magistretti, P. J. (1995) *J. Neurosci.* **15,** 3307–3317
24. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) *Biochemistry* **18** 5294–5299
25. Carpenter, L., Poole, R. C., and Halestrap, A. P. (1996) *Biochim. Biophys. Acta* **1279,** 157–163
26. Bröer, S., Bröer, A., and Hamprecht, B. (1995) *Biochem. J.* **312,** 863–870
27. Jackson, V. N., Price N. T., and Halestrap, A. P. (1995) *Biochim. Biophys. Acta* **1238,** 193–196
28. Liman, E. R., Tytgat, J., and Hess, P. (1992) *Neuron* **9,** 861–871
29. Bröer, S., Bröer, A., and Hamprecht, B. (1994) *Biochim. Biophys. Acta* **1192,** 95–100
30. Yudkoff, M., Nissim, I., Hummeler, K., Medow, M., and Pleasure, D. (1986) *Biochem. J.* **234,** 185–192
31. Brune, T, Fetzer, S., Backus, K. H., and Deitmer, J. W. (1994) *Pflügers Arch. Eur. J. Physiol.* **429,** 64–71
32. Boyarsky, G., Ransom, B., Schlue, W.-R., Davis, M. B. E., and Boron, W. F. (1993) *Glia* **8,** 241–248
33. Shrode, L. D., and Putnam, R. W. (1994) *Glia* **12,** 196–210
34. Poole, R. C., and Halestrap, A. P. (1993) *Am. J. Physiol.* **264,** C761-C782
35. Shimbo, K., Brassard, D. L., Lamb, R. A., and Pinto, L. H. (1995) *Biophys. J.* **69,** 1819–1829
36. Tzounopoulos, T., Maylie, J., and Adelman, J. P. (1995) *Biophys. J.* **69,** 904–908
37. Lolkema, J. S., Carrasco, N., and Kaback, H. R. (1991) *Biochemistry* **30,** 1284–1290
38. Van Winkle, L. J., Kakuda, D. K., and MacLeod, C. L. (1995) *Biochim. Biophys. Acta* **1233,** 213–216
39. Bittar, P. G., Charnay, Y., Pellerin, L., Bouras, C., and Magistretti, P. J. (1996) *J. Cerebr. Blood Flow Metab.* **16,** 1079–1089
40. Dringen, R., Gebhardt, R., and Hamprecht, B. (1993) *Brain Res.* **623,** 208–214
41. Magistretti, P. J., and Pellerin L. (1996) *Cerebral Cortex* **6,** 50–61