Journal of Neuroscience Research 81:644–652 (2005)

Transfer of Glycogen-Derived Lactate From Astrocytes to Axons Via Specific Monocarboxylate Transporters Supports Mouse Optic Nerve Activity

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It is hypothesized that L-lactate derived from astrocyte glycogen sustains axon excitability in mouse optic nerve (MON). This theory was tested by using a competitive antagonist of L-lactate transport and immunocytochemistry to determine whether transport proteins are appropriately distributed in adult MON. L-lactate sustained the compound action potential (CAP), indicating that exogenous L-lactate was an effective energy substrate. During 60 min of aglycemia, the CAP persisted for 30 min, surviving on a glycogen-derived substrate (probably lactate), before failing. After failing, the CAP could be partially rescued by restoring 10 mM glucose or 20 mM L-lactate. Aglycemia in the presence of 20 mM D-lactate, a metabolically inert but transportable monocarboxylate, resulted in accelerated CAP decline compared with aglycemia alone, suggesting that D-lactate blocked the axonal uptake of glycogen-derived L-lactate, speeding the onset of energy failure and loss of the CAP. The CAP was maintained for up to 2 hr when exposed to 20% of normal bath glucose (i.e., 2 mM). To test whether glycogen-derived L-lactate "supplemented" available glucose (2 mM) in supporting metabolism, L-lactate uptake into axons was reduced by the competitive inhibitor D-lactate. Indeed, in the presence of 20 mM D-lactate, the CAP was lost more rapidly in MONs bathed in 2 mM glucose artificial cerebrospinal fluid. Immunocytochemical staining demonstrated cell-specific expression of monocarboxylate transporter (MCT) subtypes, localizing MCT2 predominantly to axons and MCT1 predominantly to astrocytes, supporting the idea that L-lactate is released from astrocytes and taken up by axons as an energy source for sustaining axon excitability. © 2005 Wiley-Liss, Inc.

Key words: glycogen; mouse optic nerve; L-lactate; glucose

It is universally acknowledged that glucose is the main energy support of the mammalian brain (McIlwain and Bachelard, 1985), but recent studies have suggested that the pathway of glucose from blood to neural elements may not be as straightforward as previously thought (Magistretti et al., 1995; Brown et al., 2004b). It was noted over 100 years ago that neurons do not directly contact capillary endothelial cells (Andriezen, 1893); rather, astrocyte end feet tightly and uniformly surround capillary endothelial cells (Nedergaard et al., 2003) and constitute the first cellular barrier encountered by glucose entering the brain parenchyma (Bachelard et al., 1973). Virtually every neuron in the brain has a substantial portion of its surface membrane bordered by astrocytes, which are thus ideally positioned to act as intermediaries in the transfer of energy substrates from blood to neurons.

The first evidence of glial cell–neuron energy substrate transfer emerged from work on the structurally and functionally compartmentalized honeybee retina (Tsacopoulos and Magistretti, 1996), demonstrating that glial cells released alanine, which was taken up and oxidatively metabolized by the neuronal photoreceptors (Tsacopoulos et al., 1994). Analogous substrate transfer has since been implicated in the mammalian CNS, where the transferred substrate appears to be the monocarboxylate L-lactate, rather than alanine (Dringen et al., 1993; Pellerin et al., 1998b; Wender et al., 2000; Brown et al., 2003). The evidence for this intercellular metabolic transfer is based not only on the ability of astrocytes to release L-lactate (Dringen et al., 1993) and for

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Received 31 March 2005; Revised 20 April 2005; Accepted 28 April 2005

Published online 13 July 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.20573

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neurons to take it up (Bouzier-Sore et al., 2003) but also on the cellular distribution of the isozymes of lactate dehydrogenase, the enzyme that interconverts pyruvate and L-lactate (Bittar et al., 1996), and on the cellular localization of the monocarboxylate transporters (MCTs) that transport monocarboxylates into and out of cells (Koehler-Stec et al., 1998; Pierre et al., 2000, 2002; Debernardi et al., 2003). Although glucose remains an important energy substrate for neurons, especially under resting conditions, accumulating evidence suggests that, upon activation, or in the face of glucose deprivation, neurons take up astrocyte-derived L-lactate as a preferred oxidative substrate (Larrabee, 1983, 1996; Bouzier-Sore et al., 2002, 2003; Brown et al., 2003; Pellerin and Magistretti, 2003). The advantages of this metabolic flexibility with regard to energy substrate are obvious in the setting of glucose deprivation, where astrocyte glycogen can stave off catastrophic neural energy failure (Wender et al., 2000). It is also appealing as a mechanism to compensate for the temporary glucose shortfalls that may occur with intense metabolic demand.

The experiments described here provide further evidence that, in white matter, L-lactate arising from glycogen mobilization in astrocytes is shuttled to axons via distinct MCT isoforms found on astrocytes and axons and that this mechanism sustains action potential firing in the face of either decreased glucose availability or enhanced firing rates. A preliminary account of this work has been published previously (Baltan Tekkök et al., 2003).

MATERIALS AND METHODS

Preparation of Optic Nerves

All experiments were carried out in accordance with the guidelines for Animal Care of the University of Washington. Adult male Swiss Webster mice (20–25 g) were deeply anesthetized with CO_2, then decapitated. The optic nerves were exposed, gently freed from their dural sheaths, and placed in an interface perfusion chamber (Medical Systems Corp, Greenvale, NY). Nerves were maintained at 37°C and perfused with control artificial cerebrospinal fluid (aCSF) that contained (in mM): 126 NaCl, 3 KCl, 2 $MgSO_4$, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 2 $CaCl_2$, and 10 glucose. Osmotic compensation for aCSF containing 20 mM D- or L-lactate was achieved by equimolar reduction in NaCl. The aCSF was bubbled with a 5% CO_2-containing gas mixture (95% O_2, 5% CO_2) to maintain pH at 7.45. The tissue was continuously exposed to a moisturized gas mixture containing 95% O_2, 5% CO_2. Suction electrodes filled with aCSF (of the same composition as the test perfusate for each experiment) were attached to the nerve for stimulation and recording of the compound action potential (CAP) after the nerves had been equilibrated for 60 min in control aCSF. CAPs were evoked every 30 sec, and stimulus strength was adjusted to evoke the maximal-amplitude CAP and then increased another 25% to ensure that stimulus strength was always supramaximal (Stys et al., 1991).

Data were acquired online (Digidata 1320; Axon Instruments, Foster City, CA) with proprietary software (Clampex 8.1; Axon Instruments). CAP area was calculated in Clampfit (Axon Instruments). Curve-fitting procedures to determine latency to CAP fall and percentage CAP decline were carried out as previously described (Wender et al., 2000). Data are presented as means and SD. Significance was determined by ANOVA with Tukey's posttest or the Students *t*-test, where appropriate, and $P < 0.05$ was taken to indicate statistical significance.

Immunocytochemistry

Antibodies that specifically recognize the monocarboxylate transporters MCT1 and MCT2 were used in this study. The generation, purification, and characterization of the MCT antibodies used in this study have been reported previously (Pellerin et al., 1998a; Pierre et al., 2000, 2002; Debernardi et al., 2003). Adult mice were anesthetized with Nembutal and perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer. The optic nerves were removed immediately, postfixed for 2 hr, and then cryoprotected in 10% (w/v) sucrose for 12 hr and 30% sucrose for 48 hr.

Staining of Optic Nerves

Sections (20 μm) were cut on a cryostat, thaw-mounted onto superfrost slides (Fischer, Fair Lawn, NJ), and stored at –80°C. Sections were then warmed at room temperature or in a 37°C oven for 30 min before being processed for immunocytochemistry. Tissue sections were rinsed in 0.1 M phosphate buffer for 5 min, fixed in 4% paraformaldehyde for 20 min, rinsed in 0.1 M phosphate buffer for 5 min, rinsed in 0.1 M Tris buffer (TB) for 15 min, rinsed in 0.1 M Tris-buffered saline (TBS) for 15 min, and then blocked in TBS containing 10% nonfat milk for 30 min. The tissue sections were then incubated in appropriate antibody (diluted 1:200) overnight at 4°C. The antibodies were diluted in a solution containing 0.02% Triton X-100 and 10% nonfat milk in 0.1 M TBS. The tissue sections were rinsed for 30 min in TBS and incubated in biotinylated goat anti-rabbit IgG diluted 1:300 for 2 hr at room temperature. The tissue sections were then rinsed with TBS for 30 min and then incubated in avidin Texas red diluted 1:300 for 2 hr at room temperature. The sections were rinsed in TBS for 10 min, rinsed in TB for 20 min, coverslipped with Vectashield, sealed with nail polish, and viewed with a Bio-Rad MRC 600 microscope located in the W.M. Keck Imaging Facility at the University of Washington. Control sections were incubated in normal rabbit serum, or the primary antibody was omitted, and no specific staining was observed.

RESULTS

Ability of Glucose and L-Lactate To Support MON Function

MONs superfused with control aCSF containing 10 mM glucose displayed robust, stable CAPs for several hours without decay or alteration in CAP shape, as previously described (data not shown; Brown et al., 2001). Substituting 10 mM glucose with 20 mM L-lactate

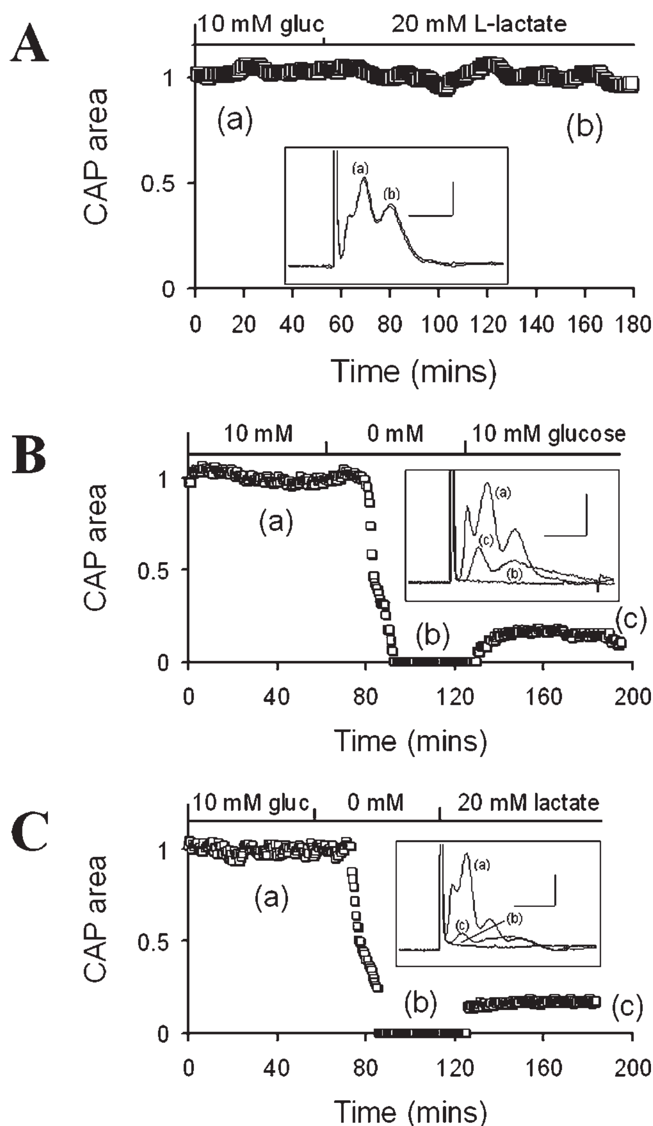

Fig. 1. L-lactate-supported MON function. **A:** In the absence of glucose, 20 mM L-lactate sustained axon excitability, measured as CAP area, for at least 2 hr (n = 3). **Inset** shows superimposed CAPs at the indicated time points. **B:** One hour of aglycemia resulted in delayed CAP failure that partially recovered when glucose was restored (n = 8). **Insets** show representative CAPs at the indicated time points. **C:** One hour of aglycemia resulted in delayed CAP failure that partially recovered when 20 mM L-lactate was introduced (n = 3). **Insets** show representative CAPs at the indicated time points. Scale bars = 1 msec and 1 mV.

(20 mM L-lactate is the carbon equivalent of 10 mM glucose) sustained the CAP for at least 2 hr (Fig. 1A; Brown et al., 2001, 2003; Abi-Saab et al., 2002), indicating that L-lactate supported MON function in the absence of glucose. As with glucose, L-lactate "rescued" the CAP after it was lost following 60 min of aglycemia (Fig. 1B,C). The CAP recovered to 20.4% ± 6.3% of control (n = 8; Fig. 1B) after 1 hr of aglycemia, followed by 1 hr of recovery in 10 mM glucose, indicating that, although irreversible injury had occurred to a large percentage of the MON axons, at least some of the axons recovered (Stys et al., 1991; Ransom, 1995). MONs allowed to recover in 20 mM L-lactate, as opposed to glucose, after a 60-min period of aglycemia showed a similar level of CAP recovery (20.2% ± 4.8% of the baseline value, n = 3; Fig. 1C).

D-Lactate Is Metabolically Inert and Accelerates CAP Failure During Aglycemia

The ability of D-lactate to support function in the MON was tested. Mammalian cells do not effectively convert D-lactate into pyruvate (Flick and Konieczny, 2002), so D-lactate is not oxidatively metabolized to a significant degree. In the presence of 10 mM glucose, addition of 20 mM D-lactate had no effect on CAP area or shape, indicating that D-lactate had no nonspecific toxic effects (Fig. 2A; n = 4), although intracellular accumulation of D-lactate would be expected to acidify the cell. In MONs exposed to aglycemia for 1 hr, subsequent addition of 20 mM D-lactate did not rescue function (Fig. 2B; n = 4; compare with Fig 1C), indicating that in this preparation it is metabolically inert.

MCTs exhibit a certain degree of stereospecificity, preferring the L isomer, but they will significantly transport D-lactate, allowing the D isomer to act as a competitive inhibitor for L-lactate transport (Jackson and Halestrap, 1996; Cassady et al., 2001). We tested this possibility. Prior studies demonstrated that, during aglycemia in the MON, astrocytic glycogen is degraded and apparently exported as L-lactate that is taken up by neighboring axons as an energy substrate. Once the astrocytic glycogen reserves are exhausted, function fails (Dringen et al., 1993; Pellerin et al., 1998b; Wender et al., 2000; Brown et al., 2003). Glucose was withdrawn in the presence or absence of 20 mM D-lactate. In the presence of D-lactate, CAP failure was accelerated to 16.9 ± 6.8 min (n = 6) compared with 29.4 ± 9.0 min (n = 8; $P < 0.05$) in the absence of D-lactate (Fig. 2C). The acceleration of CAP failure during aglycemia in the presence of D-lactate suggested that D-lactate was interfering with the transfer of glycogen-derived L-lactate from the astrocyte into the axon.

To demonstrate further the ability of D-lactate to block glycogen-derived L-lactate transport into axons, experiments were carried out in which MONs were superfused with 30 mM glucose to increase glycogen content (Dringen et al., 1993; Pellerin et al., 1998b; Wender et al., 2000; Brown et al., 2003). Withdrawal of glucose after preincubation in 30 mM glucose for 30 min resulted in delayed CAP failure (40.1 ± 6.3 min, n = 5), and the CAP did not completely fall to zero even after 1 hr of aglycemia. Moreover, after aglycemia, CAP recovery in 10 mM glucose was enhanced (72.6% ± 16.1% of baseline, n = 5; Fig. 2D). In parallel experiments, 20 mM D-lactate was included during the 1-hr period of aglycemia. Under these conditions, CAP failure was significantly accelerated (28.3 ± 3.6 min, n

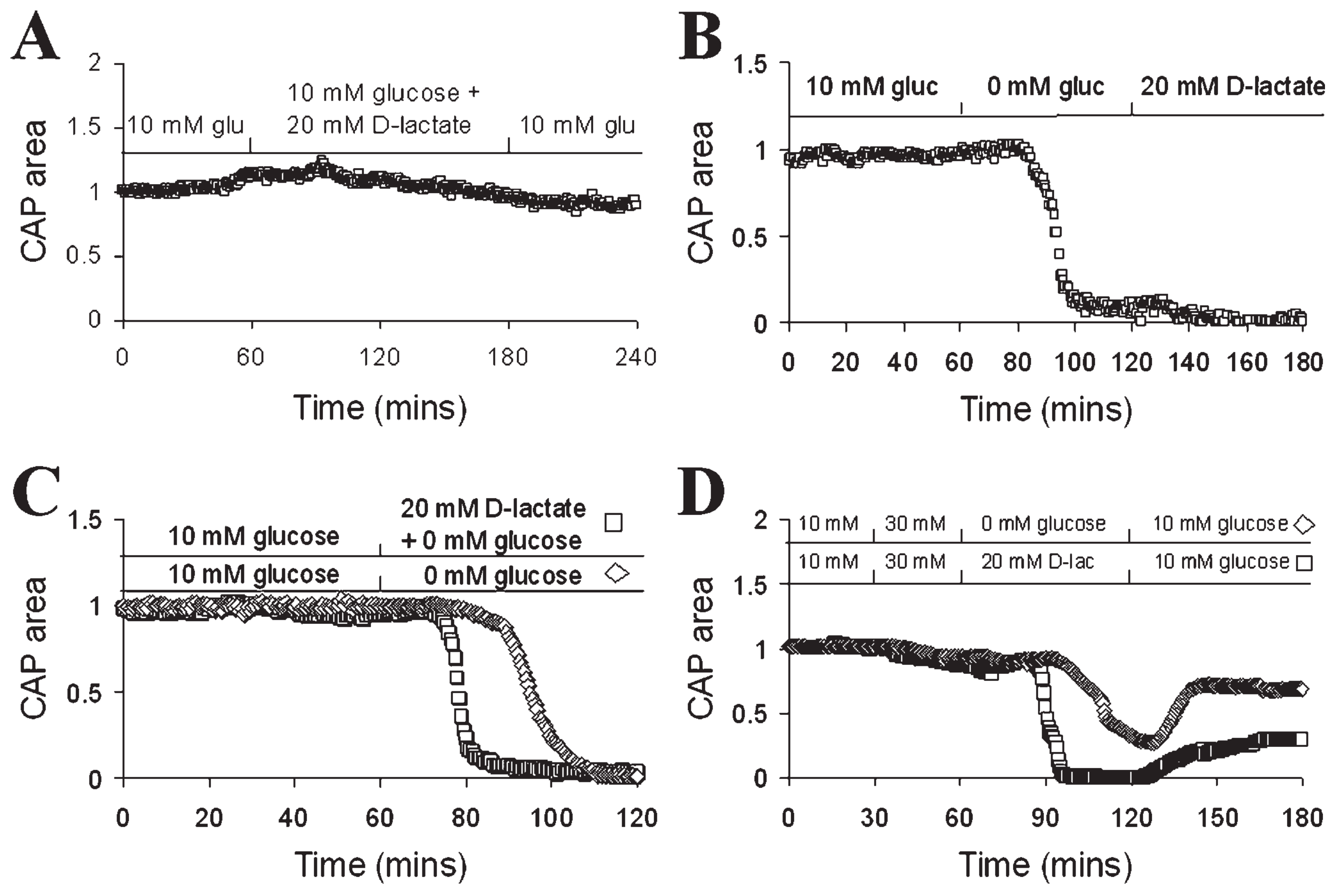

Fig. 2. D-lactate did not support CNS axon excitability and accelerated CAP failure during aglycemia. **A:** MONs perfused with 10 mM glucose exhibited stable CAPs that were unaffected by 20 mM D-lactate (n = 4). **B:** One hour of aglycemia resulted in delayed CAP failure, and the CAP showed no recovery in the presence of 20 mM D-lactate (n = 4). **C:** Comparison of CAP failure during aglycemia in the absence (n = 8) or presence (n = 6) of 20 mM D-lactate. D-lactate accelerated CAP failure during aglycemia. **D:** MONs preincubated in 30 mM glucose for 30 min prior to aglycemia (n = 5) did not exhibit complete CAP failure during subsequent aglycemia (1 hr) because of higher glycogen content (see text). This effect was negated in the presence of 20 mM D-lactate (n = 3); during aglycemia in the presence of D-lactate CAP failure was accelerated, and CAP area fell to zero.

= 3; $P < 0.05$), and the CAP did fall to zero. The CAP also recovered poorly in the presence of D-lactate (29.8% ± 8.5% of baseline, n = 3, vs. 72.6% ± 12.4%; $P < 0.005$; Fig. 2D). To demonstrate the ability of D-lactate to inhibit L-lactate uptake into axons, we incubated MONs with 5 mM L-lactate and 20 mM D-lactate, similar to their K_m values (Jackson and Halestrap, 1996) at the MCT1 (no information for the MCT2 is available), and found that D-lactate does block L-lactate-supported function (data not shown).

D-Lactate Inhibits Axon Uptake of Glycogen-Derived L-Lactate Under Physiological Conditions

D-lactate was used to investigate further the involvement of glycogen-derived L-lactate transport in sustaining the CAP under physiological conditions. MON function can be fully supported for up to 2 hr in the presence of 2 mM glucose (Brown et al., 2003), a glucose concentration closer to values normally found in the extracellular space of brain (Abi-Saab et al., 2002). Because glycogen content gradually falls in the presence of 2 mM glucose, it is suspected that a glycogen-derived substrate (probably L-lactate) supplements the 2 mM bath glucose in maintaining axon function. This is supported by the observation that, after depletion of glycogen by preexposure to 15 min of aglycemia, 2 mM glucose can no longer fully support MON excitability, implying that glycogen was indeed providing energy substrate to supplement ambient glucose (Brown et al., 2003).

These data prompted us to test directly the ability of D-lactate to block glycogen-derived L-lactate transport. MONs perfused with 2 mM glucose were able to support function fully, as expected (Fig. 3A; n = 6). In the presence of 20 mM D-lactate, however, MON function gradually failed in the presence of 2 mM glucose; the CAP failed after 18.3 ± 0.7 min (Fig. 3B; n = 3). Similar results were obtained with 150 μM α-cyano-4-hydroxycinnamate, another MCT competitive substrate, which produced CAP failure after 7.9 ± 3.8 min (data not shown).

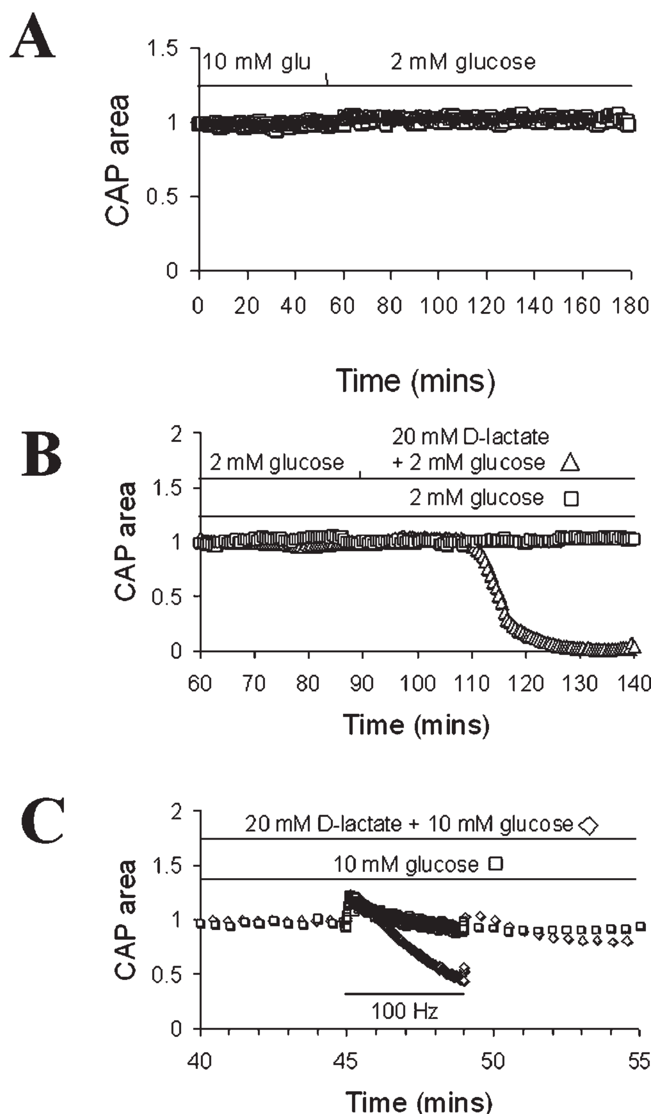

Fig. 3. D-lactate caused CAP failure when bath glucose was reduced to 2 mM. **A:** Switching from 10 mM to 2 mM glucose had no effect on CAP area for at least 2 hr (n = 6). **B:** Addition of 20 mM D-lactate in the presence of 2 mM glucose resulted in CAP failure after about 20 min (n = 3). Compare with the stable CAP area seen during continuous perfusion with 2 mM glucose. **C:** D-lactate caused significantly greater CAP area decline during 100 Hz stimulation for 4 min in 10 mM glucose (n = 4; 0.43 ± 0.08) compared with control CAP area decline under identical conditions in the absence of D-lactate ($P < 0.00001$; Student's *t*-test).

We have previously shown that, in 10 mM glucose, 100-Hz supramaximal stimulation for 4 min results in a significant decrease in glycogen, which is metabolized to L-lactate and transported to the axons (Brown et al., 2003). If D-lactate blocks L-lactate transfer from the extracellular space into axons, it should worsen CAP decline during intense stimulation. This was, in fact, the observed result (Fig. 3C; n = 4).

Localization of the MCTs

Immunocytochemical labellings were carried out to confirm the presence of MCTs on astrocytes and axons. Sections were doubly labelled with either antineurofilament antibodies to reveal the pattern of axonal distribution or antiglial fibrillary acidic protein to highlight the pattern of astrocytic distribution. Double labelling for MCT1 and neurofilaments demonstrated a sparse presence of the MCT1 transporter on axons (Fig. 4A; note the rare occurrence of yellow on the merged image). However, in sections stained with antiglial fibrillary acidic protein (red, Fig. 4B) and the MCT1 transporter (green), there was a greater degree of colocalization as indicated by the regions of yellow or orange on the merged image (Fig. 4B), which appeared to be localized to the membranes (arrows), indicating that the MCT1 transporter was distributed extensively in astrocytes. Similar experiments were carried out with the MCT2 antibody. There was very little colocalization of MCT2 antibody staining and antiglial fibrillary acidic protein staining, indicating sparse astrocytic MCT2 expression (Fig. 4D). There was, however, considerable colocalization of MCT2 antibody and antineurofilament antibody staining, indicating axonal expression of the MCT2 transporter (Fig. 4C).

DISCUSSION

Our results add new evidence supporting the existence of a pathway of L-lactate transfer between astrocytes and axons in the CNS. By using a pure white matter preparation, the MON, we confirmed that L-lactate was equivalent to glucose in supporting axon excitability. Blocking L-lactate transport with the nonmetabolizable isoform, D-lactate, prevented astrocyte glycogen from supporting axon function during aglycemia and hypoglycemia (i.e., 2 mM glucose), which is consistent with the idea that L-lactate generated from astrocyte glycogen is exported and taken up by axons. D-lactate also caused a decline in CAP area during high-frequency stimulation, which is best explained as blockade of L-lactate (derived from astrocyte glycogen) uptake into axons. Finally, immunocytochemical data demonstrated the prerequisite location of the MCT subtypes for optimal transport of L-lactate from astrocyte to axon: the MCT1 transporter, which is expressed predominantly in tissues that release L-lactate (Jackson and Halestrap, 1996; Bröer et al., 1997, 1998, 1999), was located predominantly on astrocytes, and the MCT2 transporter, which is expressed on tissues that take up L-lactate (Pierre et al., 2002), was located on axons. Taken together, these data substantiate the idea that astrocyte glycogen can provide fuel to neighboring axons under emergency circumstances, i.e., during periods of reduced glucose availability or enhanced metabolic demand. These data also represent proof of principle that a monocarboxylate, very likely L-lactate, can shuttle from astrocytes to neurons, or in this case their axons, for the purpose of maintaining energy metabolism (Fig. 5). These

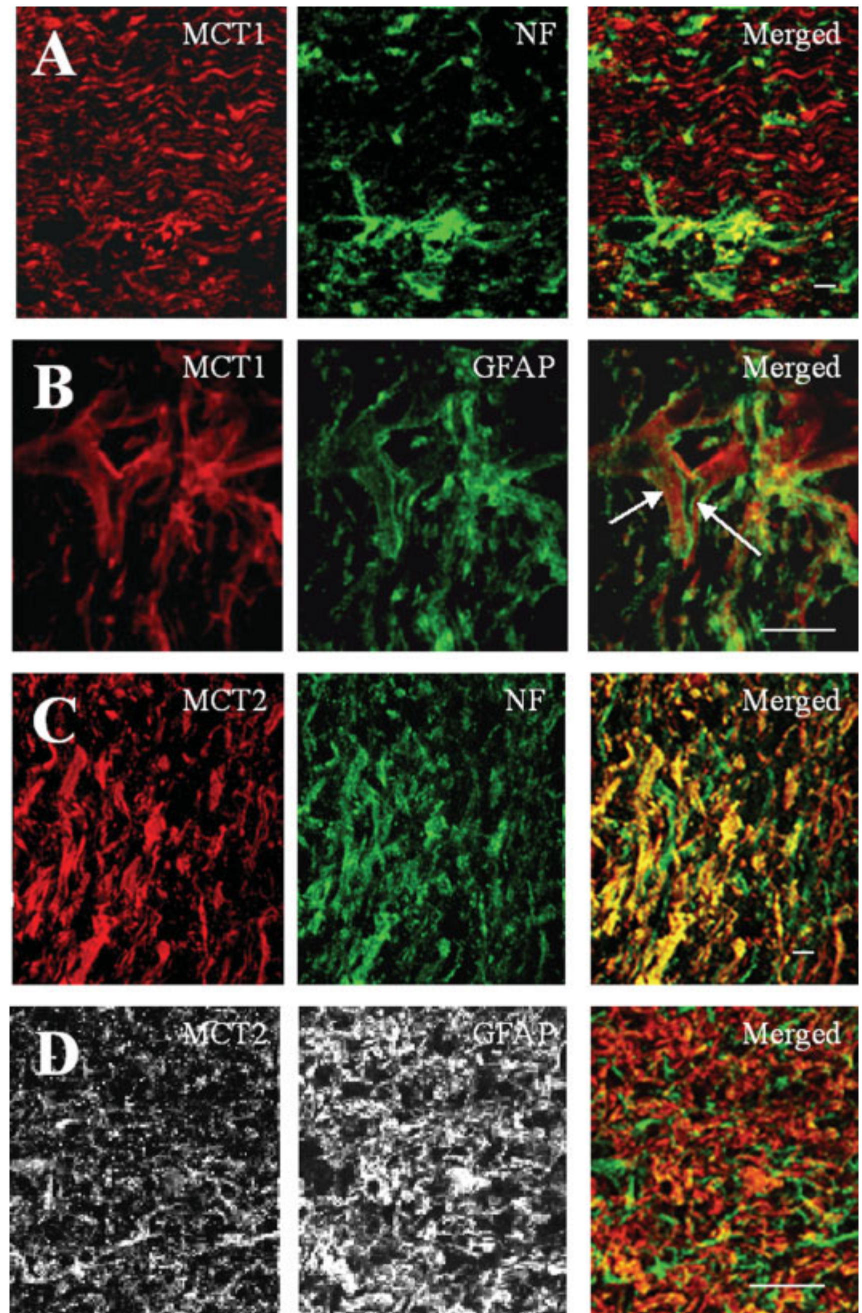

Fig. 4. Immunocytochemistry demonstrates cell-specific localization of MCT subtypes. **A:** MON sections colabelled with antineurofilament antibodies and the MCT1 transporter antibody demonstrated little overlap in merged images, implying that the MCT1 transporter is not located on axons. **B:** However, there was a greater degree of overlap with the anti-GFAP antibody, demonstrating the presence of the MCT1 transporter on astrocytes (arrows). **C:** The MCT2 transporter showed significant colocalization with the antineurofilament antibody, demonstrating a predominantly axonal localization of the MCT2 transporter. **D:** MON sections colabelled with anti-GFAP antibodies and the MCT2 transporter antibody demonstrated little overlap in merged images, implying that the MCT2 transporter is not located on astrocytes. Scale bars = 2 μm in A,C, 10 μm in B,D.

observations do not bear on the controversy regarding whether astrocytes generate lactate that is used by neighboring neurons following bursts of glutamate neurotransmission, although they are certainly compatible with this hypothesis (Pellerin and Magistretti, 2004).

D-Lactate

D-lactate is the stereoisomer of L-lactate and is transported by MCTs with a slightly higher K_m than L-lactate (Nedergaard and Goldman, 1993; Jackson and Halestrap, 1996; Shimozono et al., 1998). However, D-lactate is not metabolized in mammalian cells, because they lack significant expression of lactate dehydrogenase necessary to allow transformation into pyruvate (Flick and Konieczny, 2002). Thus D-lactate is an effective metabolic dead end in mammals, as demonstrated by its inability to rescue function after 1 hr of aglycemia.

We took advantage of its ability to act as a competitive inhibitor of L-lactate transport at MCT2 to block L-lactate transport into axons. We have previously argued that, during aglycemia, glycogen is metabolized to a monocarboxylate, probably L-lactate, which is transported out of astrocytes and into axons (Brown et al.,

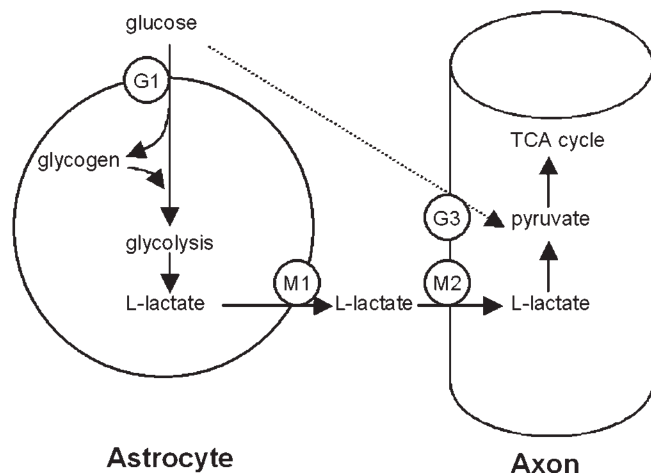

Fig. 5. Schematic illustration of astrocyte–axon intercellular L-lactate transfer in MON. Extracellular glucose is taken up by astrocytes via the GLUT1 glucose transporter (G1). The fate of astrocytic glucose may be glycolysis with L-lactate as the end product or storage in the form of glycogen. Astrocytes lack the enzyme glucose-6-phosphatase, so glycogen cannot be released from astrocytes as glucose but is released as L-lactate. Astrocytic L-lactate is released via the MCT1 transporter (M1) into the extracellular space and is taken up into axons by the MCT2 transporter (M2). L-lactate is then assumed to be oxidatively metabolised. Glucose may also be taken up into axons, presumably via the GLUT3 glucose transporter (G3), although we have no direct evidence for this.

2003). The fact that D-lactate accelerated CAP failure during aglycemia supports this argument, insofar as D-lactate would compete at sites of L-lactate transport but would not be metabolized.

It is theoretically possible that axons might subsist exclusively on L-lactate, even in the presence of glucose (see Discussion in Brown et al., 2004a). Astrocytes might preferentially take up the majority of glucose delivered to the brain and continuously convert a portion of the glucose to lactate for export to axons. Axons, for their part, might prefer lactate over glucose for energy metabolism. This extreme case seems intuitively unlikely, and it was not supported by our experiments. D-lactate exposure in the presence of glucose did not lead to CAP failure, as would be expected if L-lactate was the sole energy substrate for MON axons.

The ability of D-lactate to interfere with L-lactate transport has been demonstrated previously. In cortex, localized application of glutamate results in a lesion, which is attenuated in the presence of L-lactate. However, infusion of D-lactate augments the lesion size implying that the neuroprotective effects of L-lactate are blocked in the presence of D-lactate (Ros et al., 2001). In a similar study, ischemia followed by reperfusion caused an increase in amino acid release, which was attenuated in the presence of L-lactate. However D-lactate enhanced amino acid release (Cassady et al., 2001). In rats, the response to insulin-induced hypoglycemia is an increase in noradrenaline and adrenaline, the so-called hormonal counterregulatory response. Injection of L-lactate into the ventromedial hypothalamus, which contains the glucose-sensitive neurons responsible for the counterregulatory response, results in an attenuation of the response. However, injection of D-lactate had no effect on the response, implying that L-lactate but not D-lactate is a metabolically active substrate (Borg et al., 2003). Thus D-lactate is a valuable tool with which to investigate L-lactate transport, and it has advantages over other MCT blockers, such as cinnemate (Izumi et al., 1997; Schurr et al., 1997, 1999; Wender et al., 2000; Cater et al., 2001; McKenna et al., 2001), which have other actions complicating the interpretation of results. Cinnemate, for example, inhibits mitochondrial pyruvate transport, thereby reducing oxidative metabolism (McKenna et al., 2001).

Role of MCTs in the Brain

MCTs are found both in the CNS and elsewhere in the body. Their function is to facilitate either the export or the import of monocarboxylates and ketone bodies in cells. The mRNA for two types of MCT transporter, MCT1 and MCT2, have been detected in mouse brain, being abundant in cerebral cortex, hippocampus, and cerebellum but also present in the striatum and other subcortical structures (Pellerin et al., 1998a; Pierre et al., 2000, 2002). Recent studies have identified the MCT4 transporter in the brain (Pellerin et al., 2005). Immunocytochemical data have further localized the MCT2 transporter to identified cellular elements throughout the brain (Pierre et al., 2002), and this study extends these findings demonstrating the presence of both MCT1 and MCT2 transporters in the optic nerve, a purely white matter structure.

Lactic acid is a biologically strong acid, and it is fully dissociated at physiological pH. If significant amounts of the lactate anion are to cross cell membranes, a transport mechanism is required. MCTs provide this function (Pellerin and Magistretti, 1994), transporting the lactate anion in conjunction with a proton. The two isoforms of MCT differ in their substrate-binding affinities such that MCT1 is best suited for L-lactate export and MCT2 for lactate uptake. It has been known for 50 years that in vitro brain preparations can survive on nonglucose metabolites such as L-lactate (McIlwain, 1953), implying that cells have an inherent capacity to take up and efficiently metabolize this compound. The question of whether cells *do* take up lactate under normal conditions is a controversial issue beyond the scope of this current paper (but for current views on the topic see Chih et al., 2001; Chih and Roberts, 2003; Pellerin and Magistretti, 2003). Nevertheless, the work presented here provides proof of principle that L-lactate can shuttle from astrocytes to neurons (specifically axons) under certain conditions.

The expression of MCT1 and MCT2 in the mouse brain appears to be cell specific, with MCT2 transporters located on neuronal processes, whereas the MCT1 transporter tends to be located on astrocytes (Koehler-Stec

et al., 1998; Pierre et al., 2000, 2002; Debernardi et al., 2003). This in vivo cellular localization is in agreement with studies reporting that, in primary cortical cultures, astrocytes expressed predominantly MCT1 mRNA, whereas neurons expressed MCT2 mRNA (Debernardi et al., 2003). A subset of neurons also expresses MCT1 mRNA (Debernardi et al., 2003). The data presented here extend the differential cellular localization of MCTs to white matter. This localization bolsters the general hypothesis that astrocytes generate and release L-lactate into the ECS, which can be taken up by neurons or axons and oxidatively metabolized, at least under certain conditions.

ACKNOWLEDGMENTS

This research was supported by National Institutes of Health grants NS15589 (B.R.R.) and DE13531 (R.W.), the Eastern Paralyzed Veterans Association (A.M.B., B.R.R.), and Swiss FNR grant 3100A0-1000679/1 (L.P.).

REFERENCES

Abi-Saab WM, Maggs DG, Jones T, Jacob R, Srihari V, Thompson J, Kerr D, Leone P, Krystal JH, Spencer DD, During MJ, Sherwin RS. 2002. Striking differences in glucose and lactate levels between brain extracellular fluid and plasma in conscious human subjects: effects of hyperglycemia and hypoglycemia. J Cereb Blood Flow Metab 22:271–279.

Andriezen WL. 1893. The neuroglia elements of the brain. Br Med J 2: 227–230.

Bachelard HS, Daniel PM, Love ER, Pratt OE. 1973. The transport of glucose into the brain of the rat in vivo. Proc R Soc Lond B Biol Sci 183: 71–82.

Baltan Tekkök S, Brown AM, Ransom BR. 2003. On the role of D-lactate as a competitive inhibitor for astrocyte to axon transfer of L-lactate in a central white matter pathway. J Physiol 551P:C66.

Bittar PG, Charnay Y, Pellerin L, Bouras C, Magistretti PJ. 1996. Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain. J Cereb Blood Flow Metab 16:1079–1089.

Borg MA, Tamborlane WV, Shulman GI, Sherwin RS. 2003. Local lactate perfusion of the ventromedial hypothalamus suppresses hypoglycemic counterregulation. Diabetes 52:663–666.

Bouzier-Sore AK, Merle M, Magistretti PJ, Pellerin L. 2002. Feeding active neurons: (re)emergence of a nursing role for astrocytes. J Physiol (Paris) 96:273–282.

Bouzier-Sore AK, Voisin P, Canioni P, Magistretti PJ, Pellerin L. 2003. Lactate is a preferential oxidative energy substrate over glucose for neurons in culture. J Cereb Blood Flow Metab 23:1298–1306.

Bröer S, Rahman B, Pellegri G, Pellerin L, Martin JL, Verleysdonk S, Hamprecht B, Magistretti PJ. 1997. 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. J Biol Chem 272:30096–30102.

Bröer S, Schneider HP, Bröer A, Rahman B, Hamprecht B, Deitmer JW. 1998. Characterization of the monocarboxylate transporter 1 expressed in Xenopus laevis oocytes by changes in cytosolic pH. Biochem J 333:167–174.

Bröer S, Bröer A, Schneider HP, Stegen C, Halestrap AP, Deitmer JW. 1999. Characterization of the high-affinity monocarboxylate transporter MCT2 in Xenopus laevis oocytes. Biochem J 341:529–535.

Brown AM, Wender R, Ransom BR. 2001. Metabolic substrates other than glucose support axon function in central white matter. J Neurosci Res 66:839–843.

Brown AM, Baltan Tekkök S, Ransom BR. 2003. Glycogen regulation and functional role in mouse white matter. J Physiol 549.2:501–512.

Brown AM, Baltan Tekkök S, Ransom B. 2004a. Energy transfer from astrocytes to axons: the role of CNS glycogen. Neurochem Int 45:529–536.

Brown AM, Baltan Tekkök S, Ransom BR. 2004b. Metabolic coupling between glia and neurons. In: Hatton GI, Parpura V, editors. Glial neuronal signaling. Norwall, MA: Kluwer. p 279–296.

Cassady CJ, Phillis JW, O'Regan MH. 2001. Further studies on the effects of topical lactate on amino acid efflux from the ischemic rat cortex. Brain Res 901:30–37.

Cater HL, Benham CD, Sundstrom LE. 2001. Neuroprotective role of monocarboxylate transport during glucose deprivation in slice cultures of rat hippocampus. J Physiol 531:459–466.

Chih CP, Roberts EL. 2003. Energy substrates for neurons during neural activity: a critical review of the astrocyte–neuron lactate shuttle hypothesis. J Cereb Blood Flow Metab 23:1263–1281.

Chih C, Lipton P, Roberts EL. 2001. Do active cerebral neurons really use lactate rather than glucose? Trends Neurosci 24:573–578.

Debernardi R, Pierre K, Lengacher S, Magistretti PJ, Pellerin L. 2003. Cell-specific expression pattern of monocarboxylate transporters in astrocytes and neurons observed in different mouse brain cortical cell cultures. J Neurosci Res 73:141–155.

Dringen R, Gebhardt R, Hamprecht B. 1993. Glycogen in astrocytes: possible function as lactate supply for neighboring cells. Brain Res 623: 208–214.

Flick MJ, Konieczny SF. 2002. Identification of putative mammalian D-lactate dehydrogenase enzymes. Biochem Biophys Res Commun 295: 910–916.

Izumi Y, Benz AM, Katsuki H, Zorumski CF. 1997. Endogenous monocarboxylates sustain hippocampal synaptic function and morphological integrity during energy deprivation. J Neurosci 17:9448–9457.

Jackson VN, Halestrap AP. 1996. The kinetics, substrate, and inhibitor specificity of the monocarboxylate (lactate) transporter of rat liver cells determined using the fluorescent intracellular pH indicator, 2′,7′-bis(-carboxyethyl)-5(6)-carboxyfluorescein. J Biol Chem 271:861–868.

Koehler-Stec EM, Simpson IA, Vannucci SJ, Landschulz KT, Landschulz WH. 1998. Monocarboxylate transporter expression in mouse brain. Am J Physiol 275:E516–E524.

Larrabee MG. 1983. Lactate uptake and release in the presence of glucose by sympathetic ganglia of chicken embryos and by neuronal and non-neuronal cultures prepared from these ganglia. J Neurochem 40:1237–1250.

Larrabee MG. 1996. Partitioning of CO_2 production between glucose and lactate in excised sympathetic ganglia, with implications for brain. J Neurochem 67:1726–1734.

Magistretti PJ, Pellerin L, Martin JL. 1995. Brain energy metabolism: an integrated cellular perspective. In: Bloom FE, Kupfer DJ, editors. Psychopharmacology: the fourth generation of progress. New York: Raven Press. p 657–670.

McIlwain H. 1953. Substances which support respiration and metabolic response to electrical impulses in human cerebral tissues. J Neurol Neurosurg Psychiatry 16:257–266.

McIlwain H, Bachelard HS. 1985. Biochemistry and the central nervous system. London: Churchill Livingstone.

McKenna MC, Hopkins IB, Carey A. 2001. α-Cyano-4-hydroxycinnamate decreases both glucose and lactate metabolism in neurons and astrocytes: implications for lactate as an energy substrate for neurons. J Neurosci Res 66:747–754.

Nedergaard M, Goldman SA. 1993. Carrier-mediated transport of lactic acid in cultured neurons and astrocytes. Am J Physiol 265:R282–289.

Nedergaard M, Ransom B, Goldman SA. 2003. New roles for astrocytes: redefining the functional architecture of the brain. Trends Neurosci 26: 523–530.

Pellerin L, Magistretti PJ. 1994. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. Proc Natl Acad Sci U S A 91:10625–10629.

Pellerin L, Magistretti PJ. 2003. Food for thought: challenging the dogmas. J Cereb Blood Flow Metab 23:1282–1286.

Pellerin L, Magistretti PJ. 2004. Neuroenergetics: calling upon astrocytes to satisfy hungry neurons. Neuroscientist 10:53–62.

Pellerin L, Pellegri G, Martin JL, Magistretti PJ. 1998a. Expression of monocarboxylate transporter mRNAs in mouse brain: support for a distinct role of lactate as an energy substrate for the neonatal vs. adult brain. Proc Natl Acad Sci U S A 95:3990–3995.

Pellerin L, Pellegri G, Bittar PG, Charnay Y, Bouras C, Martin JL, Stella N, Magistretti PJ. 1998b. Evidence supporting the existence of an activity-dependent astrocyte–neuron lactate shuttle. Dev Neurosci 20: 291–299.

Pellerin L, Halestrap AP, Pierre K. 2005. Cellular and subcellular distribution of monocarboxylate transporters in cultured brain cells and in the adult brain. J Neurosci Res 79:55–64.

Pierre K, Pellerin L, Debernardi R, Riederer BM, Magistretti PJ. 2000. Cell-specific localization of monocarboxylate transporters, MCT1 and MCT2, in the adult mouse brain revealed by double immunohistochemical labeling and confocal microscopy. Neuroscience 100:617–627.

Pierre K, Magistretti PJ, Pellerin L. 2002. MCT2 is a major neuronal monocarboxylate transporter in the adult mouse brain. J Cereb Blood Flow Metab 22:586–595.

Ransom BR. 1995. Optic nerve injury. In: Drance SM Drance SM, editors. Optic nerve in injury. Amsterdam: Kugler Publications. p 181–198.

Ros J, Pecinska N, Alessandri B, Landolt H, Fillenz M. 2001. Lactate reduces glutamate-induced neurotoxicity in rat cortex. J Neurosci Res 66:790–794.

Schurr A, Payne RS, Miller JJ, Rigor BM. 1997. Brain lactate is an obligatory aerobic energy substrate for functional recovery after hypoxia: further in vitro validation. J Neurochem 69:423–426.

Schurr A, Miller JJ, Payne RS, Rigor BM. 1999. An increase in lactate output by brain tissue serves to meet the energy needs of glutamate-activated neurons. J Neurosci 19:34–39.

Shimozono M, Liu J, Scofield MA, Wangemann P. 1998. Vestibular dark cells contain an H^+/monocarboxylate$^-$ cotransporter in their apical and basolateral membrane. J Membrane Biol 163:37–46.

Stys PK, Ransom BR, Waxman SG. 1991. Compound action potential of nerve recorded by suction electrode: a theoretical and experimental analysis. Brain Res 546:18–32.

Tsacopoulos M, Veuthey AL, Saravelos SG, Perrottet P, Tsoupras G. 1994. Glial cells transform glucose to alanine, which fuels the neurons in the honeybee retina. J Neurosci 14:1339–1351.

Tsacopoulos M, Magistretti PJ. 1996. Metabolic coupling between glia and neurons. J Neurosci 16:877–885.

Wender R, Brown AM, Fern R, Swanson RA, Farrell K, Ransom BR. 2000. Astrocytic glycogen influences axon function and survival during glucose deprivation in central white matter. J Neurosci 20:6804–6810.