Monocarboxylic acid transporters, MCT1 and MCT2, in cortical astrocytes in vitro and in vivo

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Hanu, Rekha, Mary McKenna, Andrea O'Neill, Wendy G. Resneck, and Robert J. Bloch. Monocarboxylic acid transporters, MCT1 and MCT2, in cortical astrocytes in vitro and in vivo. Am J Physiol Cell Physiol 278: C921–C930, 2000.—We used sequence-specific antibodies to characterize two monocarboxylic acid transporters, MCT1 and MCT2, in astrocytes. Both proteins are expressed in primary cultures of cortical astrocytes, as indicated by immunoblotting and immunofluorescence. Both MCT1 and MCT2 are present in small, punctate structures in the cytoplasm and at the cell membrane. Cells showing very low levels of labeling for glial fibrillary acidic protein (GFAP) also label more dimly for MCT2, but not for MCT1. In vivo, double-label immunofluorescence studies coupled with confocal microscopy indicate that MCT1 and MCT2 are rare in astrocytes in the cortex. However, they are specifically labeled in astrocytes of the glial limiting membrane and in white matter tracts. Both transporters are also present in the microvasculature. Comparison of labeling for MCT1 and MCT2 with markers of the blood-brain barrier shows that the transporters are not always limited to the astrocytic endfeet in vivo. Our results suggest that the level of expression of monocarboxylic acid transporters MCT1 and MCT2 by cortical astrocytes in vivo is significantly lower than in vitro but that astrocytes in some other regions of the brain can express one or both proteins in significant amounts.

blood-brain barrier; immunofluorescence; plasma membrane; lactate

THE METABOLISM OF DIFFERENT compounds by cells in the brain requires that each of these compounds, or their precursors, be transported across the plasma membranes of neurons or glial cells. Glucose, the most important source of metabolic energy in the adult brain, is transported into the brain and then into individual cells by glucose transporters. Developing brain, however, can effectively utilize monocarboxylic acids such as 3-hydroxybutyrate, acetoacetate, lactate, and pyruvate for energy (7, 10, 23, 24, 39, 40). Indeed, there is strong evidence that these substrates, rather than glucose, are preferentially utilized for energy and the biosynthesis of lipids and amino acids during the early neonatal period (7, 33, 40). The role of these molecules in brain metabolism is generally thought to decrease as the brain matures. Nevertheless, cells in the adult brain continue to produce large amounts of monocarboxylic acids through normal metabolism (8, 17, 23, 34, 37). Furthermore, lactate and ketone bodies released by astrocytes (3) may serve as important carbon sources for other brain cells, especially neurons (2, 8, 27, 34, 38). These observations suggest that cells in the adult brain express significant levels of the transporters that mediate the uptake and release of monocarboxylic acids.

This study focuses on the monocarboxylic acid transporters (MCT) in astrocytes in vitro and in vivo. There is now considerable evidence that the carrier-mediated transport of lactate by astrocytes (4, 15, 27, 31, 38), synaptic terminals (28), and neurons (9, 31) is mediated by multiple transporters that are subject to differential regulation. The kinetics and susceptibility to mercurials of lactate uptake in vitro are consistent with a possible role for MCT1 and MCT2 (13, 14, 20, 28, 38). MCT1 and MCT2 are the first members to be doned and sequenced of what has recently been recognized as a family of at least seven homologous proteins that mediate the transport of monocarboxylic acids in a wide variety of cells (36). The proteins in this family have molecular masses of 40–60 kDa and contain 12 putative transmembrane domains that, together with large regions of the NH2-terminal domain, share a high degree of homology. Significantly, however, the COOH-terminal sequences of each of the MCT are different, allowing the preparation of sequence-specific antibodies that can distinguish among the different family members. Using sequence-specific antibodies to MCT1 and MCT2 and ultrastructural approaches, Gerhart et al. (15, 16) showed that in the brain these transporters are selectively enriched in astrocytes in the glial limitans, in ependymocytes of the lateral ventricle, and in the microvasculature. With the exception of the localization of MCT1 in astrocytic endfeet, however, these in vivo studies did not unambiguously identify these or other labeled structures as astrocytes.

These immunocytochemical results have recently been complemented by in situ hybridization studies (33, 34). Koehler-Stec et al. (22) also found evidence of high levels of expression of MCT1 in the microvasculature and in the ependymal lining of the cerebral ventricles in mouse brain. In addition, they reported the presence of MCT1 mRNA in white matter tracts, such as the corpus callosum. Using RT-PCR and North-
ern blotting, Broer et al. (4) showed that cultures of astroglial cells contain mRNA encoding MCT1, but little mRNA encoding MCT2. Thus studies of cells in vitro and in vivo have yielded somewhat different results.

We have now reinvestigated the question of the localization of MCT1 and MCT2 in astrocytes both in vitro and in vivo, using double-label immunofluorescence techniques coupled with confocal laser scanning microscopy. Here we report that neonatal rat astrocytes in primary culture express significant levels of both MCT1 and MCT2. Using the same techniques, we confirm previous results indicating that the expression of MCT1 and MCT2 is restricted to glial endfeet.

MATERIALS AND METHODS

Materials. Tissue-culture dishes (Nunc) were purchased from Vanguard International (Neptune, NJ). Culture media (MEM with Earle’s salts and nonessential amino acids), and Dulbecco’s PBS were purchased from BioWhittaker (Walkersville, MD). Fetal bovine serum was from HyClone (Logan, UT). Nylon screening cloth (Nitex) was from Teko (Elmsford, NY). Timed-pregnant female rats were purchased from Zivic Miller Laboratories (Zelienople, PA).

Affinity-purified chicken antibodies to the COOH-terminal sequences of MCT1 and MCT2 were from Chemicon International (Temecula, CA). The antigenic peptides were a gift from Dr. L. Drewes (Univ. of Minnesota, Duluth, MN). Mouse monoclonal antibodies to a blood-brain barrier antigen (SNI 71) and to glial fibrillary acidic protein (GFAP) were from Sternberger Monoclonals (Baltimore, MD). Rabbit antibodies to GFAP and to the erythroid isoform of the glucose transporter, GLUT1, were from East Acres Biologicals (Southbridge, MA). A rabbit antibody against S-100 (S2644) and a mouse antibody to a 58-kDa protein of the Golgi apparatus (G2404) were from Sigma Chemical (St. Louis, MO). Mouse antibody against vimentin was from ICN Biomedicals (Costa Mesa, CA).

Cell culture. Primary cultures of cortical astrocytes were prepared as described (27, 29). Briefly, cerebral hemispheres were removed, placed in medium, cleaned of meninges, and trimmed to retain the neopallium. The isolated neopallia were removed, placed in medium, cleaned of meninges, and trimmed to retain the neopallium. The isolated neopallia were dissected from cerebral hemispheres from newborn rat brain, minced, mechanically disrupted by vortexing, and filtered through sterile nylon screening cloth. The cell suspension, enriched in astrocytes, was seeded in medium (MEM with Earle’s salts and nonessential amino acids and 10% fetal bovine serum, 1 ml/brain) in plastic culture flasks for Western blotting, or on glass coverslips for immunofluorescence studies. The cells were incubated at 37°C in an atmosphere of 95% air-5% CO2 with 90–95% humidity. The culture medium was replaced after 3 days and twice weekly thereafter. All experiments were done after 10–11 days, when cultures were usually ~50% confluent.

Western blotting. Cultures in tissue-culture flasks were tapped sharply three times and then rinsed twice with PBS to loosen and remove oligodendrocytes (29). The remaining astrocytes were scraped with a rubber policeman into a minimal volume of PBS supplemented with a mixture of protease inhibitors (0.15 mM phenylmethylsulfonyl fluoride, 0.22 μM aprotonin, 1 mM benzamidine, 10 μg/ml leupeptin, 10 μg/ml antipain, and 200 μg/ml soybean trypsin inhibitor). The samples were then homogenized briefly with a Dounce homogenizer in hypotonic buffer (20 mM Tris·HCl, 40 mM NaCl, and 1 mM dithiothreitol, pH 7.4) (15). Protein concentrations were determined with the Bio-Rad protein assay reagent (Hercules, CA). Equal amounts of protein were incubated for 15 min at 37°C in SDS-PAGE sample buffer and loaded onto 4–15% polyacrylamide gradient gels. After electrophoresis, samples were transferred to nitrocellulose membranes (Hybond enhanced chemiluminescence, Amersham, Arlington Heights, IL). The membranes were blocked in 3% milk-PTA (PTA consists of the following: 10 mM sodium phosphate, 10 mM sodium azide, 145 mM sodium chloride, and 0.5% Tween-20, pH 7.2) for 3 h. Samples were incubated overnight with the primary antibody to MCT1 or MCT2, or with a nonimmune chicken IgY, diluted to 100–400 ng/ml in 3% milk-PTA. The membranes were washed repeatedly with 3% milk-PTA for 1 h and then incubated with goat anti-chicken IgY conjugated to alkaline phosphate (J. Jackson Immunoresearch, West Grove, PA), diluted 1:10,000 in 3% milk-PTA. After repeated washes with 3% milk-PTA, bound antibody was visualized by chemiluminescence (Hyperfilm ECL, Amersham).

Specificity of the signals was confirmed by probing blots with antibodies to MCT1 or MCT2 that had been preabsorbed with the appropriate and inappropriate MCT peptide. Antibodies were preincubated with 20 μg/ml of the peptide, diluted in 3% milk-PTA, and the antibody-peptide mixture was then added to the blot. Secondary antibodies and visualization of bound antibodies were as above.

The molecular masses of the bands that labeled specifically with antibodies to MCT1 and MCT2 were determined by comparison with a mixture of prestained standard proteins, purchased from Gibco (Gaithersburg, MD).

Immunocytochemistry. Astrocytes plated on coverslips were fixed with 2% paraformaldehyde in PBS for 15 min at room temperature. Cultures were rinsed three times with PBS/azide (PBS containing 10 mM sodium azide) and permeabilized with 0.25% Triton X-100 in PBS/azide for 5 min. The cultures were then incubated with 10% BSA in PBS/azide for 30 min, followed by incubation for 1 h with the primary antibodies diluted in the same solution. After several washes in PBS/azide, bound antibodies were labeled for 1 h with the appropriate fluorescein- or tetramethylrhodamine-conjugated goat antibodies to rabbit anti-IgG, mouse anti-IgG, or chicken IgY (J. Jackson Immunoresearch). After being washed, the coverslips were mounted in 9 parts glycerol, 1 part 1 M Tris·HCl, pH 8.0, containing 1 mg/ml p-phenylenediamine, to reduce photobleaching (21).

Antibodies to MCT1 and MCT2 were used at dilutions of 1:100, rabbit polyclonal anti-GFAP at 1:200, and nonimmune IgY at 2 μg/ml. All dilutions were in 3% BSA in PBS. Peptide blocking studies were done using 50 μg/ml of the haptenic peptides.

For studies of the MCT1 in frozen sections, adult Sprague-Dawley rats (Zivic Miller) were anesthetized with ketamine and Rompun and perfused with PBS. The brains were removed, fixed in 4% paraformaldehyde in PBS for 30 min, placed in a solution of 18% sucrose in PBS, and incubated overnight at 4°C. The next day the brains were frozen in isopentane on dry ice. Sagittal sections, 10 μm in thickness, were prepared on a Reichert-Jung model 2800 Frigocut E cryostat (Reichert-Jung, Cambridge Instruments, Deerfield, IL), collected on gelatin-coated glass slides, and stored with dessicant at ~70°C. Before immunolabeling, sections were rehydrated for 5 min in PBS containing 0.1 M glycine, fixed...
and permeabilized in methanol at −20°C for 5 min, and then incubated for 30 min in PBS containing 1 mg/ml BSA (PBS/BSA). Sections were labeled with primary antibodies overnight at 4°C, rinsed three times with PBS/BSA, and incubated with secondary antibodies and mounted, as above. In some experiments, we used identical procedures to examine sections of brains from younger animals (postnatal days 1 and 7).

Some frozen sections were double labeled with antibodies to other astroglial (S-100, vimentin) markers (30) together with anti-GFAP, anti-MCT1, or anti-MCT2. Antibodies to the latter proteins were used at the same concentrations mentioned above. When necessary for double-labeling protocols, we also used rabbit antibodies to GFAP. Antibodies to S-100 and vimentin were used at dilutions of 1:200 (40 μg/ml) and 1:500, respectively.

Microscopy and imaging. Samples were observed and imaged with a Zeiss 410 confocal laser scanning microscope (Carl Zeiss, Tarrytown, NY), with pinholes set for maximum resolution in both tetramethylrhodamine and fluorescein channels. For comparisons of different samples labeled with the same antibodies, the contrast and brightness scales were first established for the brightest positive control, and the same settings were then used for all other samples. Images were obtained with software provided by Zeiss and were assembled with CorelDraw (Corel, Ottawa, Canada). Quantitative comparisons of labeling intensities were made with MetaMorph software (Universal Imaging, West Chester, PA).

Intensities of MCT1 and MCT2 immunofluorescence were the average of measurements from three to six astrocytes from each group. The area of the cell body sampled each time was constant for all measurements. Background immunofluorescence was subtracted from each of the experimental readings.

**RESULTS**

We used immunoblotting and immunofluorescence to study the presence and distribution of MCT1 and MCT2 in rat astrocytes grown in vitro and in astrocytes in frozen sections of adult rat brain. Antibodies generated in chickens against the unique COOH-terminal sequences of MCT1 and MCT2 were used in all experiments. As controls, we used nonimmune chicken IgY antibodies. We also routinely used the peptide antigens as competitive inhibitors of the anti-MCT antibodies. Our experiments demonstrate that all astrocytes in vitro, but only limited populations of astrocytes in the brain, express significant levels of MCT1 and MCT2.

MCT1 and MCT2 in astrocytes in vitro. We grew neonatal rat astrocytes in tissue culture under well-established conditions (27, 29) and used them as sources of protein and cells for immunoblotting and immunofluorescence studies. Immunoblotting tended to give variable results, depending on the method used to prepare and process the samples. In particular, nondenaturing labeling increased significantly when rat astrocyte proteins were boiled in SDS-PAGE sample buffer (data not shown). We therefore used sample buffer at 37°C, which we found in other experiments to be more useful for immunoblotting of integral membrane proteins. We found that antibodies to MCT1 generated a single, major band with an apparent molecular mass of ~40 kDa (Fig. 1, lane A1). Similarly, anti-MCT2 labeled a single band at ~50 kDa (Fig. 1, lane B1). Nonimmune chicken IgY failed to label a band in this range (Fig. 1, lane C), suggesting that the labeling by anti-MCT1 and anti-MCT2 was specific. As an additional control, we used the antigenic peptides as competitive inhibitors. The COOH-terminal peptide antigen of MCT1 blocked the labeling by anti-MCT1 antibodies of the ~40-kDa band (Fig. 1, lane A2) but had no effect on labeling by anti-MCT2 (Fig. 1, lane B2). Similarly, the MCT2 peptide blocked the ability of anti-MCT2 antibodies to label the ~50-kDa band (Fig. 1, lane B3) but had no effect on labeling by anti-MCT1 (Fig. 1, lane A3). These results suggest that the antibodies to MCT1 and MCT2 react specifically with polypeptides with apparent polypeptide masses of ~40 and ~50 kDa, respectively. These values are in reasonable agreement with the values of Gerhart et al. (15, 16), who reported values of 48 and 46 kDa for MCT1 and MCT2, respectively. The small differences in apparent molecular mass are probably due to differences in sample processing and, in particular, to our use of SDS-PAGE sample buffer at 37°C, rather than 100°C, to dissolve astrocyte proteins.

In immunofluorescence studies, antibodies to both MCT1 and MCT2 labeled every astrocyte in our primary cultures at moderate-to-bright intensities (Fig. 2, a and e). In double-label experiments with antibodies to GFAP, astrocytes showed strong, punctate MCT1 immunolabeling throughout the cell. In most cells, anti-MCT1 antibodies also labeled bright clusters of small spots that presented as asterlike structures near the nuclei (Fig. 3C, arrow). Although much of the labeling appeared to be intracellular, the anti-MCT1 antibody also labeled punctate structures at the cell membrane (e.g., Fig. 3A, arrow). All labeling was specific, because it was not mimicked by nonimmune chicken antibodies.
Immunofluorescence also showed that all cultured cortical astrocytes express MCT2. As with MCT1, antibodies to MCT2 labeled punctate structures in the cytoplasm and at the plasma membrane (Fig. 3B, arrow). In addition, anti-MCT2 antibodies gave dim staining of the nuclei and brighter staining of long, linear structures surrounding nuclei, which we identified as the trans-Golgi network with appropriate antibodies (data not shown). The immunolabeling of all these structures by anti-MCT2 was specific, by the same criteria used for anti-MCT1 (Fig. 2, e–g).

In the course of our studies, we observed that some cells in the culture labeled with MCT1 or MCT2 more intensely than others, consistent with previous observations on the microheterogeneity of astrocytes in primary culture (6, 19, 26). All of the labeled cells were astrocytes, because they also reacted with antibodies to GFAP in double-label protocols (see below). In the case of MCT2, the intensity of labeling tended to correlate with that of GFAP. In 59 of 81 cell pairs in which anti-GFAP antibodies labeled one cell of the pair brightly and the other dimly, the former tended to display more MCT2 ($P < 0.00001$ by the rank sum test). This correlation did not extend to MCT1, however. In 24 cell pairs examined similarly, only 3 of the cells that labeled brightly for GFAP displayed significantly higher levels of MCT1 than the dimly labeled cell ($P > 0.1$ by the rank sum test). Thus the level of expression of MCT2, but not of MCT1, may be governed in part by the same factors that control the expression of GFAP by cortical astrocytes in culture.

We used digital imaging software to estimate the relative intensities of immunofluorescence labeling for MCT1 and MCT2 in the cultured astrocytes (see MATERIALS AND METHODS, Fig. 2). Rat astrocytes in tissue culture were fixed and immunolabeled with antibodies to MCT1 or MCT2, followed by a fluorescein-conjugated secondary antibody. Confocal images show labeling for MCT1 (a) and MCT2 (d). This labeling is specific, since it is abolished in the presence of the appropriate peptides (b and f, respectively) but not in the presence of the inappropriate peptides (c and e, respectively). Nonimmune IgY did not show any labeling (g). Bar, 25 µm.

![Fig. 2. Rat cortical astrocytes in culture express MCT1 and MCT2.](image)

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![Fig. 3. Surface and intracellular labeling of MCT in rat astrocytes in culture.](image)

Fig. 3. Surface and intracellular labeling of MCT in rat astrocytes in culture. Anti-MCT1 (A) and anti-MCT2 (B) labeling in the cytoplasm and at the plasma membrane is punctate (arrows). Anti-MCT1 also labels clusters near the nucleus (C, arrow). Bars: A and B, 10 µm; C, 25 µm.
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RIALS AND METHODS). All confocal images were collected identically so that the intensities could be compared reliably. All readings for anti-MCT1 and anti-MCT2 labeling were significantly higher than those obtained with nonimmune chicken IgY (\( P < 0.007 \)) and with the specific antibodies in the presence of their appropriate peptide antigens (\( P < 0.005 \)), which generated 6- to 10-fold lower values than the mean values obtained with anti-MCT1 and anti-MCT2 (data not shown). We conclude from these studies and our immunoblotting experiments that cortical astrocytes in culture express significant amounts of both MCT1 and MCT2.

MCT1 and MCT2 in astrocytes in vivo. We used the anti-MCT1 and anti-MCT2 antibodies to study the distribution of these transporters in the brains of adult rats. Labeling by anti-MCT1 and anti-MCT2 was reproducible and specific by the same criteria summarized above; it was not generated by nonimmune chicken IgY (data not shown), and it was blocked by the appropriate but not by the inappropriate peptide (e.g., Fig. 4) for white matter tracts. Similar results were obtained in all other areas of the brain discussed below. Much of the labeling by anti-MCT1 and anti-MCT2 was in the cerebrovasculature and neuropil, but labeling of individual astrocytes was seen in some regions of the brain. Labeling of neuronal populations by anti-MCT1 and anti-MCT2 will be discussed elsewhere (unpublished data).

Labeling of astrocytes in situ by anti-MCT1 was apparent in the glial limiting membrane of the cerebral cortex (Fig. 5a, arrowheads), in astrocytes in the corpus callosum (Fig. 5m), and in glial processes radiating off the surface of the brain stem (Fig. 5g). Antibodies to MCT1 also labeled intensely ependymocytes lining the lateral ventricle (data not shown), and, somewhat less intensely, cerebral microvessels (see below). The intensity of immunolabeling of different structures in the brain, from brightest to dimmest, was ependymocytes > microvessels > glial limiting membrane > astrocytes > neuropil.

We obtained similar but distinct results with antibodies to MCT2. Like anti-MCT1, antibodies to MCT2 labeled the glial limiting membrane (Fig. 5d, arrowheads), and glial processes radiating off the surface of the brain stem (Fig. 5j), as well as cerebral microvessels (see below). While anti-MCT1 labeled scattered astrocytes in white matter (Fig. 5, m–o), anti-MCT2 labeled more cells in these regions (Fig. 5, p–r). All these labeled cells were identified as astrocytes, because they could be immunolabeled with antibodies to GFAP (Figs. 5 and 6) but not with antibodies to a neuronal marker, calcium/calmodulin-dependent protein kinase II (data not shown). The order of intensity of labeling by anti-MCT2, from brightest to dimmest, was microvessels > glial limiting membrane > neuropil > astrocytes.

Given the proximity of many astrocytes to blood vessels and the presence of MCT in the cerebrovasculature (Fig. 6, g and g’; see below), we were concerned that many of the structures we showed to contain the MCT might in fact be blood vessels and not astrocytes. This is possible if much of the labeling obtained with anti-GFAP is concentrated in glial endfeet, which cover most of the surface of the cerebrovasculature (18). We therefore carried out several double-labeling experiments with anti-MCT and antibodies to GLUT1, which is highly enriched in the cerebrovasculature (32). Antibodies to MCT1 and MCT2 labeled cerebral blood vessels (Fig. 6, g and g’, respectively). Occasionally, small differences in labeling could be detected between anti-MCT and anti-GLUT1 antibodies (e.g., Fig. 6, g’ and h’). This may reflect the asymmetric distribution of GLUT1 between abluminal and luminal surfaces, since the amount of GLUT1 is fourfold greater on the abluminal than on the luminal membrane (11), whereas MCT1 is present on both membranes of endothelial cells (15).

Occasionally, capillary-like structures also appeared to label for GFAP (data not shown). This is probably due to the presence of MCT in astrocytic endfeet (16), which
are closely apposed to and enclose most of the capillary surface (18).

We also observed labeling for the MCT in GFAP-positive cells that were in close association with blood vessels in the region proximal to the lateral ventricle (e.g., Fig. 6, d'–f'). These cells were identified as astrocytes, because they were double labeled by antibodies to the MCT and to GFAP, but not to GLUT1 (data not shown) or the blood-brain barrier antigen (e.g., Fig. 6, e' and f'). In some astrocytic processes (e.g., Fig. 6, a, a', and d', arrowheads), labeling by antibodies to the MCT was clearly punctate, as we also noted for astrocytes in vitro, but this pattern was hard to capture, even under confocal optics.

Although these experiments showed relatively high levels of labeling for MCT1 and MCT2 in some astrocytes, especially those in the glial limiting membrane and in white matter tracts, MCT1 and MCT2 were...
difficult to identify in astrocytes in other areas of the brain. In particular, neither MCT1 nor MCT2 could be detected by double-label immunofluorescence methods in astrocytes in the cortex (Fig. 5, long arrows), the hippocampus, or the cerebellum (data not shown). However, the microvessels in these regions were positive for both transporters (Fig. 5, short arrows), as shown by double-labeling protocols with an antibody against GLUT1 (data not shown).

Our observations using double-label immunofluorescence definitively identified populations of astrocytes in the adult brain that express MCT1 and MCT2 through the use of antibodies to GFAP. We were concerned, however, about the apparently low densities of astrocytes that were labeled for GFAP in the cortex. We therefore used antibodies to two other astroglial markers, vimentin and S-100, and obtained similar results (data not shown). These results suggest that astrocytes in large areas of the adult rat brain, recognized by a variety of markers, do not express significant levels of MCT1 or MCT2. Thus, although astrocytes in some brain regions can express high levels of MCT1, MCT2, or both transporters, astrocytes in many regions of the adult brain fail to express these proteins at detectable levels.

This is in sharp contrast to the results of our studies with astrocytes in vitro (see above). Because our cultures are prepared from neonatal rat brains, we considered the possibility that MCT1 and MCT2 may be expressed at higher levels immediately after birth and at more limited levels in adulthood. We therefore examined frozen sections of rat brains collected at 1 and 7 days after birth. Labeling by MCT1 was present at significant levels at 1 day after birth (Fig. 7A) but did not codistribute with vimentin (Fig. 7B), a glial marker that is expressed early in development (35). Labeling by MCT2 was extremely dim in postnatal day 1 brain (Fig. 7). A preliminary examination of early developmental stages suggests that the expression of MCT2 lags behind the expression of MCT1. Strong signals for MCT2 are not visible until after day 7 (data not shown). These results suggest that the high levels of expression of MCT1 and MCT2 by astrocytes in culture are not matched by the astrocyte populations in neonatal brain.

**DISCUSSION**

We used immunological approaches to study the cellular expression of two monocarboxylic acid trans-

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**Fig. 6. MCT expression in astrocytes outside the endfeet and in individual capillary endothelial cells.** Cryosections were double labeled as in Fig. 5, with antibodies to MCT1 (a, d, g) or MCT2 (a', d', g') and to either GFAP (b, b'), a blood-brain barrier antigen (e, e'), or GLUT1 (h, h') to mark the capillary endothelium. In the color composite images (c, f, c', f'), anti-MCT labeling is shown in green, the other antibodies in red, and structures containing both labels in yellow. Anti-MCT labeling is found in cells that also label with GFAP but not with antibodies to the blood-brain barrier antigen, even when the MCT is expressed in astrocytes that are in close association with blood vessels (e.g., f', arrow). Both MCT are present in capillaries (g, h, g', h'). Bars: a–f, a’–f’, 10 µm; g, h, g’, h’, 100 µm.
porters, MCT1 and MCT2, in astrocytes in vivo and in vitro. Our results establish that astrocytes express significant levels of both transporters in vitro but that the expression of these proteins in vivo is restricted to astrocytic populations in only specific areas of the brain.

Astrocytes in vitro. The major new finding of the present study is that primary cortical astrocytes in vitro express significant amounts of both MCT1 and MCT2, whether assayed by immunofluorescence or by immunoblotting. Both transporters are readily apparent in primary astrocytes both in the cytoplasm and at the astrocytic cell membrane. These data contrast with studies of cortical rat astrocytes in situ (15, 16; this paper) and with those of Broer et al. (4), who found high levels of mRNA for MCT1 but not for MCT2 in astroglial cells cultured from mouse brain.

The presence of both MCT1 and MCT2 at the cell membrane is consistent with earlier studies that suggested that rat astrocytes in vitro use at least two distinct carrier mechanisms to transport lactate (38). The activities of these transporters, which share several features with MCT1 and MCT2, including substrate affinities and susceptibility to mercurials (28, 38), are also consistent with the rates of lactate oxidation in astrocytes cultured from brain (27, 28, 40). Thus MCT1 and MCT2, or closely related transporters, are likely to mediate the uptake of lactate into astrocytes in vitro.

The labeling of intracellular vesicles and other intracellular structures with the anti-MCT antibodies was specific by our two criteria: inhibition by the appropriate peptide and lack of labeling by nonimmune IgY. Some of this labeling may have been due to the presence of other proteins that share sequence homology with the antigenic peptides of MCT1 and MCT2. In this case, however, antibodies should have reacted specifically with multiple bands in immunoblots. In our

Fig. 7. Double labeling of neonatal rat brain sections with anti-MCT antibodies and anti-vimentin. Frozen sections from newborn (P1) rat brain were labeled as described in previous figures. In color composite images (C and F), MCT labeling is shown in green and vimentin labeling in red, and structures containing both labels are shown in yellow. A–C: glial processes in P1 cortex that are positive for vimentin (B and E) do not label with anti-MCT1 (A) or anti-MCT2 (D). Color composites (C and F) show that none of the labeled structures contains both vimentin and MCT. Bars: A–C, 50 µm; D–F, 25 µm.
hands, each anti-MCT antibody labeled only one band specifically. We therefore favor the idea that MCT1 and MCT2 are present in significant amounts in other structures, including intracellular vesicles.

These results therefore raise the possibility that MCT1 and MCT2 are involved in the transport of monocarboxylic acids across the intracellular membranes of astrocytes. Studies by our laboratory (25) and others (1), demonstrating the presence of two discrete compartments of pyruvate in the astrocytic cytosol, are consistent with such a role. Because the transport of monocarboxylic acids such as lactate and ketone bodies can facilitate the net movement of protons across membranes, the present data suggest that MCT1 and MCT2 may also participate in the maintenance of the pH gradients that exist between the cytoplasm and the lumen of the Golgi apparatus and secretory vesicles (5).

We are currently examining different subcellular fractions of astrocytes in an attempt to identify the intracellular vesicles that are labeled by antibodies to MCT1 and MCT2.

Astrocytes in the brain. Our results in vivo agree in many respects with those of Gerhart et al. (15, 16), who used similar antibodies and immunohistochemical approaches to study the distribution of MCT1 and MCT2 in rat brain. These authors observed significant levels of labeling of MCT1 in the glial limiting membrane of the cerebral cortex, in ependymocytes lining the lateral ventricle, in thalamic astrocytes, and in the prepositus hypoglossal nucleus of the medulla, as well as in the microvasculature. However, in contrast to our results, which suggest higher levels of labeling in ependymocytes and cerebral microvessels than in glial cells, Gerhart et al. (15) reported the most prominent staining in the glial limiting membrane. These authors (16) also report significant labeling of MCT2 in the glial limiting membrane, ependymocytes, and the astrocytes of the white matter, and the innermost layers of cerebral cortex. Unlike our findings, however, they did not detect MCT2 in the cerebrovasculature. These differences are probably attributable to differences in the techniques used.

The conclusions of the immunological studies described here and by Gerhart et al. (15, 16) agree substantially with results obtained with Northern blotting and in situ hybridization (22). In that study, MCT1 expression was seen in the ependymal lining of the ventricles, in the choroid plexus, and in white matter tracts, as well as in cerebral microvessels. In contrast, there was no significant expression of mRNA encoding MCT2 in the white matter, ependyma, or microvessels, suggesting that MCT2 was primarily expressed by neurons. This is in agreement with the data of Pellerin et al. (33, 34), who reported significant levels of expression of both MCT in the microvasculature in developing but not adult brain. In view of the well-documented differences in resolution, sensitivity, and interpretation between immunohistochemistry and in situ hybridization, further studies to compare these techniques in the same samples will be required to resolve the apparent discrepancy.

Nevertheless, all the currently available results suggest that some astrocytes in vivo are capable of expressing MCT1 and probably MCT2 but that astrocytes in wide areas of the brain fail to do so (e.g., cerebral cortex, Fig. 5, long arrows). We speculate that the predominant transporter of monocarboxylic acids in cortical astrocytes in vivo is one of the recently identified but as yet uncharacterized MCT that are found in the brain (e.g., MCT5 or MCT6, Ref. 36).

Differences in vitro and in vivo. The discrepancies between the results we obtain in vitro and in vivo suggest that the expression of MCT1 and MCT2 is upregulated in primary cultures of cortical astrocytes. There are several possible explanations for this upregulation. 1) Developmental regulation: primary cultures of astrocytes are prepared from neonatal rat brain, and although they differentiate in culture, they may never reach the state of maturity of astrocytes in vivo. Our results with neonatal brain (Fig. 7) argue against this possibility. 2) Metabolic regulation: our culture medium contains 5 mM glucose, which is significantly higher than the concentration in vivo (~1 mM, Ref. 11). The increased production of lactate from glucose may regulate MCT1 and MCT2 synthesis by specific feedback mechanisms, possibly similar to that which governs the expression of the glucose transporter, GLUT1, in astrocytes (39). 3) Communication with other cells: cultured astrocytes are deprived of interactions with the neurons and capillary endothelial cells with which they interact closely in the adult brain. Communication, either through direct cell-cell contact or paracrine mechanisms, may be required to downregulate the expression of MCT1 and MCT2 in most astrocytes in vivo. Currently ongoing experiments will test these and other hypotheses.

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