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Research report

Monocarboxylate transporter (MCT1) abundance in brains of suckling and adult rats: a quantitative electron microscopic immunogold study

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Abstract

Transcellular transport of energy substrates across the vascular endothelial cells of the brain is accomplished by integral membrane carrier proteins, such as the glucose transporter GLUT1 and the monocarboxylic acid transporter MCT1. The abundance of these proteins may vary depending on age and nutritional status. In this study we compared the expression of MCT1 in cerebral cortex of suckling and adult rats to determine whether the former, which use considerably more monocarboxylates such as lactate and ketone bodies as fuel than do older rats, correspondingly express more MCT1 than adults. Using electron microscopic immunogold methods, we found that 17-day old suckling rat pups had 25 times more MCT1 labeling in the membranes of capillary endothelial cells than adults. This transporter was nearly equally distributed in luminal and abluminal membranes with less than 10% of the immunogold particles in the endothelial cytoplasmic compartment. The suckling rats also had 15 times more immunogold particles associated with pericyte membranes and 19 times heavier labeling of membranes associated with astrocytic end feet adjacent to microvessels. Neuropil and choroid plexus were lightly labeled. Some MCT1-positive astrocyte and neuron cell bodies were observed, suggesting active synthesis of MCT1 by these cells. The potential for regulation of expression of MCTs by dietary or other factors may have important consequences for the progression and treatment of cerebrovascular disorders and other diseases.

Keywords: Monocarboxylate transporter (MCT1); Brain; Endothelium; Pericyte; Astrocyte; Neuron

1. Introduction

The role of lactate in brain energy metabolism has received renewed attention with 1) the emergence of a controversial hypothesis that neurons require lactate as an energy substrate during periods of neuronal activation and that this lactate is supplied by astrocytes via metabolic coupling [3,28]; and 2) the cloning of two monocarboxylate transporters, MCT1 and MCT2 [11,12], that appear to be members of a family of such transporters [30,31]. The cloning of MCT1 and MCT2 has allowed the exploration of their possible functions regarding brain lactate fluxes among astrocytes, neurons, and vascular endothelial cells.

In addition to its possible role in supplying energy for neurons during activation, lactate (among other monocarboxylates) is a major energy substrate for the brain during the neonatal period [6,25,27,32]. In very young presuckling rat pups, lactate rather than glucose is the primary fuel molecule for the brain [6,8,24,36]. In 14-day old pups, lactate and other monocarboxylates still supply more than 40% of the brain's energy requirement [8]. As the animals mature, their brains rely increasingly on glucose for energy, but retain the ability to transfer and utilize monocarboxylates as major fuels, and do so under certain conditions such as diabetes, starvation, and high fat consumption.

The ability of the brain to switch between monocarboxylates and glucose as major fuels suggests that the MCTs may be subject to up- and downregulation in response to altered metabolic states related to factors such as age, nutrition, or disease. For example, using light microscopic immunocytochemical techniques, Gerhart et al. [13] showed that brain microvessels of suckling rats appear to stain more intensely for MCT1 than those of adults. Similar differences were observed in vessels of adult rats fed a ketogenic diet, as compared to a traditional diet, for one month [7]. The regulation of MCT1 may be comparable to

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that of glucose transporters in the brain, for which there is evidence of altered expression in a variety of stressful conditions and diseases including ischemia and seizures [5,16,17,19–23,26,34,35].

One possible mode of regulation of endothelial membrane transport proteins has been proposed [10]. Evidence was presented, using electron microscopic immunogold methods, that in rat endothelial cells the glucose transporter GLUT1 is asymmetrically distributed: the luminal membrane contains about 12% of the labeled transporter, the cytoplasm 48%, and the abluminal membrane 40%. These investigators hypothesized that the cytoplasmic GLUT1 could serve as reservoir for the upregulation of this transporter via its recruitment to the endothelial plasma membranes.

One goal of the present study was to determine by electron microscopic immunogold methods the relative levels of expression of MCT1 in the luminal, abluminal, and cytoplasmic 'compartments' of endothelial cells. If there is an asymmetric distribution of MCT1 in these compartments, it may suggest that this transport protein is available for rapid recruitment to the appropriate membrane to meet changing metabolic needs of the brain, similar to that proposed for GLUT1. A second goal of the study was to compare in adult versus suckling rats the relative levels of expression of MCT1 in various compartments: endothelia, pericytes, astrocytic foot processes, neuropil and neuron cell bodies. High levels of MCT1 expression in suckling rats would suggest that this transporter is vital for the shuttling of monocarboxylates between the blood and parenchymal cells of the brain of these animals.

2. Materials and methods

2.1. MCT1 antibody

The antibody to MCT1 was characterized previously by light and electron microscopy [13]. For its preparation, a peptide consisting of the 15 carboxyl-terminal amino acids of rat MCT1 (LQNSSGDPAEEESPV) was synthesized with an amino-terminal cysteine, conjugated to keyhole limpet hemocyanin, and used for immunization. IgY was purified as previously described [13]. The affinity purified MCT1 antibody recognized a protein migrating with an apparent molecular weight of approximately 48 kDa (range 46–50 kDa) on immunoblots of brain membrane proteins from adult and suckling rats [13]. This band was not observed when the antibody was preabsorbed with 1 μg/ml of the MCT1 peptide.

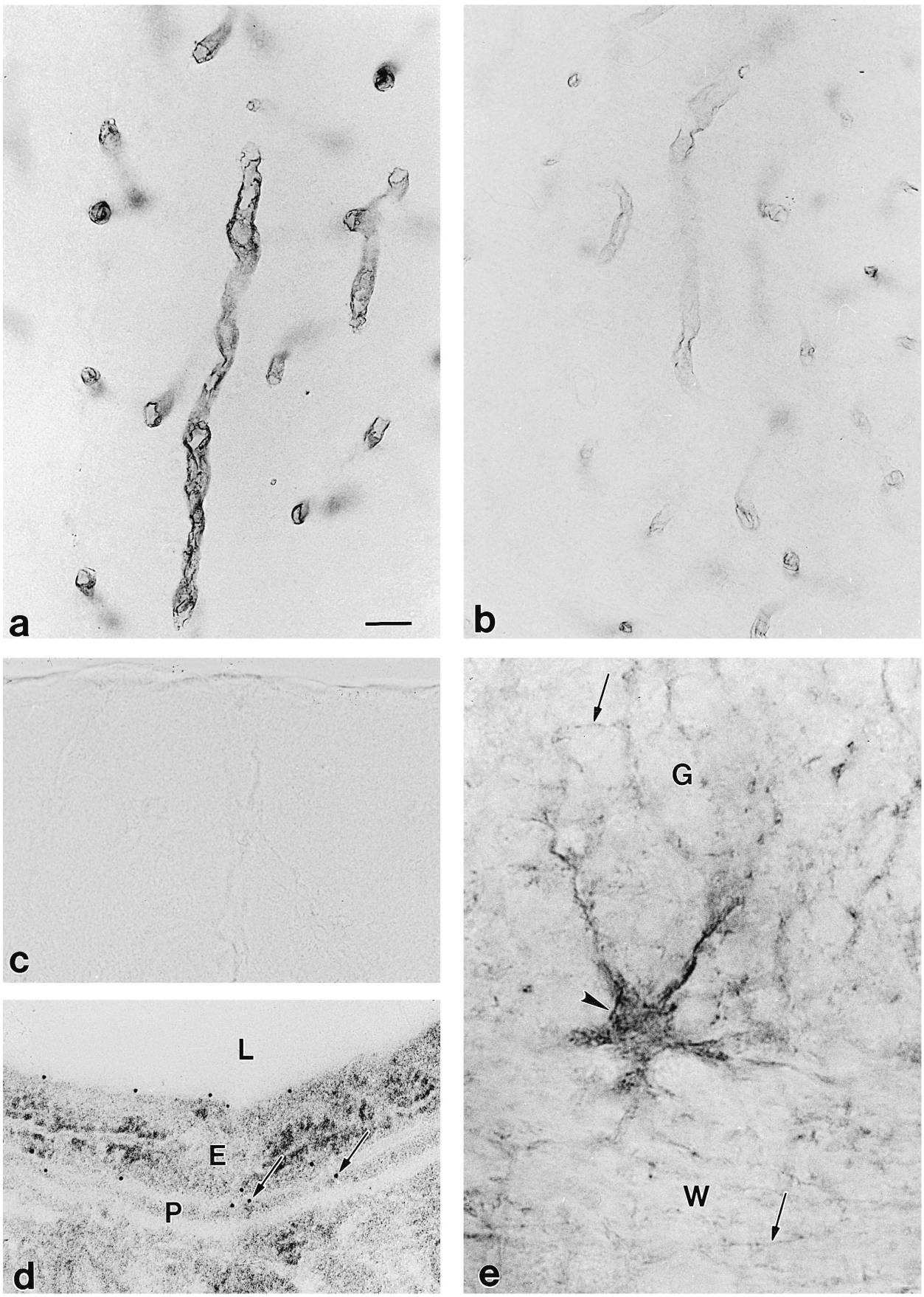
2.2. Light microscopic immunocytochemistry

Rats were anesthetized with 5% halothane prior to cardiac puncture perfusions with formal-acetic fixative (4% formaldehyde from paraformaldehyde, 2% acetic acid). Perfusion time was 12 min, and brains were removed and stored at 4°C in fixative overnight before processing. Ten brains of adults and three of 14- and three of 17-day old pups were examined using either 50-μm vibratome sections or 8-μm paraffin sections. (Vibratome sections were better for visualizing blood vessels, which were immunolabeled throughout the sections' full thickness, whereas parenchymal structures were only superficially labeled. Paraffin sections were better for visualizing parenchymal structures due to superior penetration of the immunoreagents.) Coronal sections were cut at the level of the anterior hippocampus and sagittal sections within 1 mm of the central sulcus. Tissue sections were blocked with phosphate buffered saline containing 0.1% bovine serum albumin (BSA) and 1.5% normal goat serum. The primary antibody was diluted 1:1000 in 0.1% BSA and applied to the sections for 1.5 h at room temperature or overnight at 4°C. For negative controls, MCT1 peptide (10–20 μg/ml) was added to the antibody solution. Sections were then incubated 30 min with biotinylated goat anti-chicken IgG (5 μg/ml in blocking solution) and 30 min with avidin–biotin–peroxidase complex (ABC) reagent (both reagents from Vector Laboratories, Burlingame, CA). Color development was from 1 to 6 min in 0.6 mg/ml 3,3′-diaminobenzidine (Dako, Carpinteria, CA). Astrocytes were identified in some preparations by examining serial sections immunostained for glial fibrillary acidic protein (GFAP).

2.3. Immunogold electron microscopy

The brains of three adult and three 17-day old suckling rats were examined. Rats were anesthetized and perfused as for light microscopy, but with 4% formaldehyde (from paraformaldehyde) in 0.15 M Sorensen's phosphate buffer, pH 7.4. Small pieces of cerebral cortex (1–3 mm^3) were excised dorsal to the hippocampus (approximately 4 mm caudal to bregma). The pieces were washed in Sorensen's, partially dehydrated in 50% and 75% ethanol, and trans-

Fig. 1. MCT1 immunoreactivity in cerebral cortex of adult and suckling rats (light micrographs are from vibratome sections). (a) Microvessels of 14-day old pups are heavily labeled (×425). (b) Microvessels of adults are lightly labeled (×425). (c) Peptide preabsorption control sections, in this case from a 14-day old pup, are unlabeled (×425). (d) Electron micrograph of a capillary wall of a 17-day old pup showing immunogold labeling of luminal and abluminal endothelium (E) and pericyte (P). Arrows point to gold particles; clumps of electron dense particles within the endothelial cell cytoplasm are ribosomes and polyribosomes. L, capillary lumen (×71,975). (e) Besides being MCT1-positive in the glial limiting membrane, astrocytes (arrowhead) occasionally exhibited strong immunoreactivity in other cortical regions, especially at the junction of gray and white matter, as in this micrograph. Note the fine immunolabeled processes suggestive of astrocyte processes (arrows), in both the gray (G) and white (W) matter. Adult, cerebral cortex-corpus callosum (×1360). Bar shown in $a = 20$ μm for (a), (b), and (c); 0.12 μm for (d), and 6.25 μm for (e).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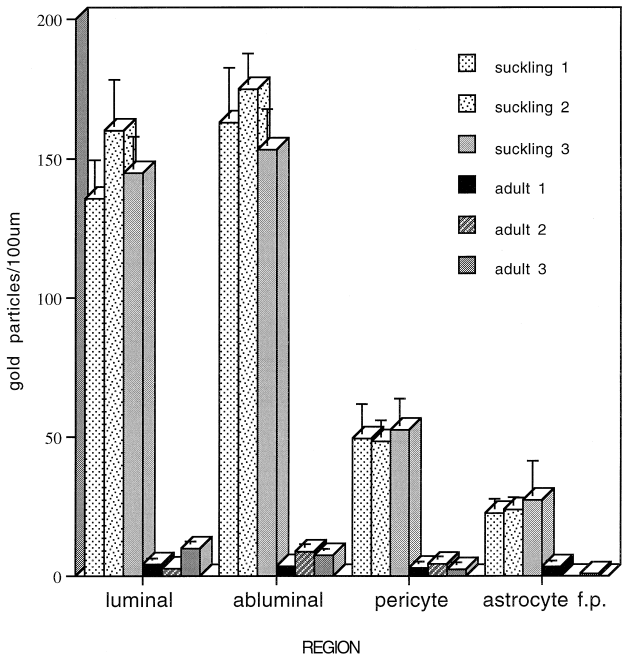

Fig. 2. Comparison of MCT1 labeling of different cell types in the microvascular region of cerebral cortex of adult ($n = 3$) and suckling ($n = 3$) rats by immunogold electron microscopy (16–29 micrographs of same region of cortex for each animal). Suckling rats have much higher levels of MCT1 reflecting a much greater capacity to transfer monocarboxylates across the blood brain barrier. Luminal, abluminal: luminal and abluminal endothelial plasma membrane; astrocyte f.p.: membranes of astrocytic foot processes. Error bars represent the S.E.M. of normalized particle counts for each electron micrograph.

ferred to 2:1 L.R. White resin (Ted Pella, Redding, CA):75% ethanol. After three changes in 100% L.R. White, including an overnight infiltration, the pieces were embedded in gelatin capsules and the resin was cured at 53°C for 24 h. Thin sections were collected on nickel electron microscope grids. The sections were blocked in 0.5 M Tris, pH 8.1, containing 1% BSA, 10% normal rabbit serum, and 0.1% Tween 20. Sections were then incubated for 1.5 h in primary antibody diluted 1:200 or 1:100 in 0.5 M Tris containing 1% BSA, 1% normal rabbit serum, and 0.1% Tween 20. This buffer was also used as a rinse buffer between incubations. For negative controls, MCT1 peptide (10 μg/ml) was added to the primary antibody solution. Sections were then incubated 1 h in rabbit anti-chicken IgY-10 nm gold (Goldmark Biologicals, Phillipsburg, NJ) diluted 1:100 in wash buffer. Finally, the grids were immersed for 10 min in 2% glutaraldehyde in Sorensen's and stained with 2% aqueous uranyl acetate and with lead citrate.

The grids were coded so that the evaluator (RLL) had no knowledge of the age group they represented. For counting immunogold particles, 16–29 electron micrographs of capillary profiles (approximately 150–175 μm of capillary endothelial length) were obtained for each brain and the negatives were examined at a final magnification of 500,000×. Immunogold particle densities were evaluated by two methods: 1) particles per unit length of membrane and 2) particles per unit area of specific compartments. The first method was used for determining densities of labeled membranes in the blood brain barrier. Particles were counted on luminal and abluminal endothelial membranes, the pericyte membranes, and the membranes in the astrocytic foot process region. Membrane lengths were then measured (on prints) with a Jandel digitizer. In the second method, particles were counted in the endothelial, pericyte, and neuropil compartments (membranes plus cytoplasm) of the same electron micrographs and then the volume density of these compartments was computed using a superimposed grid screen and point count morphometry [37]. After the data were decoded, differences in particle density between pups and adults were analyzed using *t*-tests.

3. Results

Cerebral microvessels of suckling rats were more densely stained for MCT1 than were those of adults (Fig. 1a,b). Staining was absent in sections pretreated with the MCT1 peptide (Fig. 1c). The strong labeling of rat pup microvessels observed by light microscopy was confirmed by immunogold electron microscopy (Fig. 1d). Plasma membranes of capillary endothelial cells of pups had about 25 times as many gold particles per 100 μm of membrane than did the endothelia of adults (Fig. 2). The level of expression of MCT1 in pup microvessels was remarkably consistent from animal to animal (Fig. 2).

In 17-day old rats, over 70% of the total immunogold particles were associated with endothelial cells. Of these particles, 43.1% were on the luminal membrane, 8.9% cytoplasmic, and 48.0% on the abluminal membrane. The slightly greater labeling ratio (1:1.1) of the abluminal versus the luminal membrane was statistically significant for this age group (*t*-test, $p < 0.05$). Adult rat brains exhibited only a small fraction of the endothelial labeling of brains of suckling rats (Fig. 2). However, the endothe-

Table 1
Mean immunogold particle densities[a] [particles per 100 μm^2 (S.E.M.)] in various compartments in adult and suckling rats; MCT1, cerebral cortex

Compartment	Adult	Suckling	Percentage of adult vs. suckling
Vascular endothelium	23.0 (4.0)	327.4 (16.3)	7.0
Pericyte	14.1 (3.4)	117.8 (15.0)	12.0
Neuropil[b]	3.7 (< 0.1)	9.1 (0.2)	40.7

[a] Mean particle density in peptide preabsorption control electron micrographs was 0.63 (0.3) particles per 100 μm^2. Particle densities were significantly different between adult and suckling rats in all compartments, *t*-tests, $p < 0.001$.
[b] Includes labeling of astrocytic foot processes adjacent to blood vessels.

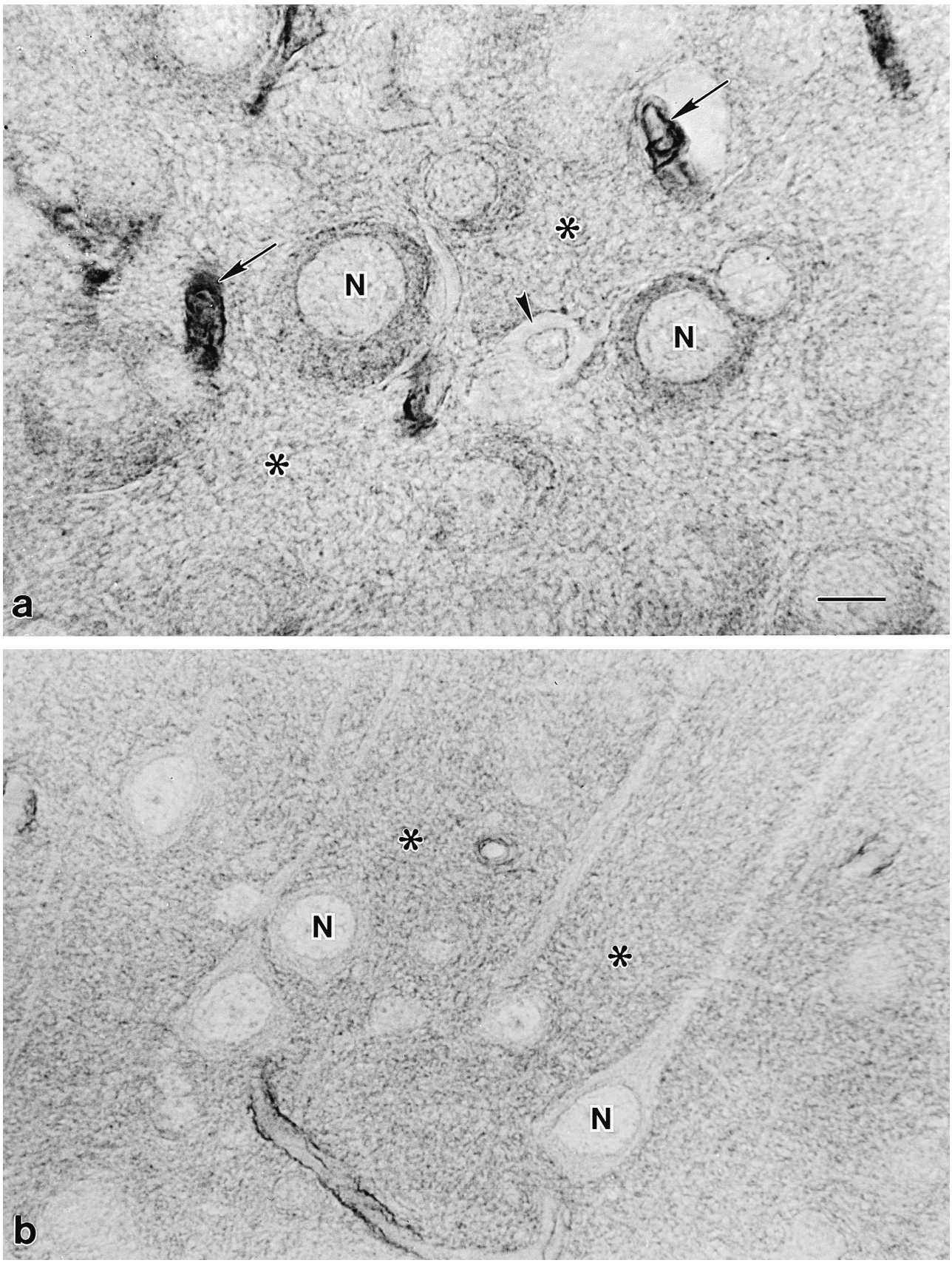

Fig. 3. MCT1 immunoreactivity in rat cerebral cortex (paraffin sections). (a) In 17-day old pups, neuropil (*) and cell bodies of neurons (N) are MCT1-positive, but lightly labeled compared to microvessels (arrows). Note that the cell body of a glial cell (arrowhead) is unlabeled (×1215). (b) In adults, neuropil is lightly MCT1 positive but neuronal cell bodies are mostly unlabeled (×1215). Bar shown in $a = 10$ μm for (a) and (b).

lial staining pattern was similar, with most gold particles associated with the luminal and abluminal membranes and few particles in the cytoplasm. Immunolabeling was not as consistent from animal to animal in adults (Fig. 2), and differences in labeling density between luminal and abluminal membranes were not statistically significant.

Pericytes were the second most heavily labeled cells in brains of both pups and adults (36 and 61% as densely labeled as capillaries, respectively; Fig. 1d, Fig. 2, Table 1). The pericytes of 17-day old rats had about 15 times as many gold particles per 100 μm of membrane as those of adults (Fig. 2). Nearly all labeling was associated with the plasma membrane and 94% of those gold particles were on the luminal-facing membranes.

The neuropil was lightly labeled in both suckling and adult rats (Fig. 3a,b), although suckling rats had about 2.5 times the labeling density (particles per 100 μm^2, Table 1) of adults. Many of the immunogold particles in neuropil were on astrocyte foot processes adjacent to capillaries (Fig. 2), and some were on membranes of small astrocyte and neuron-like processes located throughout the neuropil. In addition, pial cells and astrocytes forming the glial limiting membrane were heavily labeled. The epithelium of the choroid plexus was lightly labeled.

In some areas of the brain (thalamus, cortex near the corpus callosum—Fig. 1e), MCT1-labeled astrocytes were consistently observed. Their identity was confirmed with serial sections immunostained for GFAP. Overall, however, except in certain brain regions, the expression of MCT1 in astrocyte cell bodies was very low, and GFAP-positive astrocytes vastly outnumbered the discernable MCT1-positive cells.

In pups, many pyramidal cells and other neuronal cell bodies throughout the brain were moderately MCT1-positive (Fig. 3a). In adults, a few scattered neurons were weakly MCT1-positive, with the exception of the lateral septal nucleus which contained many moderately-positive neurons. Electron micrographs revealed that most of the immunogold particles in neuronal cell bodies were associated with the rough endoplasmic reticulum.

4. Discussion

The vascular endothelium of suckling rats contains many more MCT1 transporters than that of adults. This finding is consistent with the observations that the brains of rat pups use large quantities of monocarboxylates for energy substrate and also for synthesis of amino acids and lipids (see Ref. [27]). Blood levels of one monocarboxylate, lactate, are very high just after birth (the presuckling period) and lactate is the primary fuel for the brain at this time [6,8,24,36]. When the animals begin to suckle, the mother's milk provides them with high levels of lipids which are converted into ketone bodies that the brain avidly consumes [18]. Blood levels of monocarboxylates fall dramatically after weaning. For example, Hawkins et al. [18] reported arterial concentrations of ketone bodies (3-hydroxybutyrate plus acetoacetate) of 2.20 mM in 18-day old rats and 0.23 mM in adults. After weaning, the brain relies almost entirely on glucose for fuel and may be a net exporter of small amounts of lactate. Correspondingly, the levels of MCT1 in the cerebrovasculature are much lower in older rats. In the present study endothelial MCT1 immunogold staining in adults was only 7% of that of suckling rats.

The high levels of MCT1 present in the brain capillaries of rat pups, where blood concentrations of monocarboxylates are elevated, suggest that vascular MCT1 levels in adult brains may also be upregulated when blood concentrations of monocarboxylates rise, as in starvation, diabetes, and during high fat diets. For example, Drewes et al. [7], using light microscopic immunocytochemistry, reported that microvessels in brains of adult Long–Evans rats fed a ketogenic diet (90% fat, 10% protein; [1]) for one month stained more strongly for MCT1 than microvessels of control rats. This apparent MCT1 upregulation is probably not the result of recruitment of MCT1 to endothelial plasma membranes from cytoplasmic stores as has been postulated to occur for GLUT1 in brain microvessels [10]. The latter investigators reported that 48% of GLUT1 was located in the cytoplasmic compartment of rat brain endothelium whereas the luminal membrane contained only 12% of the transporter. Presumably, recruitment of GLUT1 from the cytoplasmic compartment to the luminal membrane could result in a rapid increase in glucose transport from blood to brain. For MCT1, however, our present results show that this transporter is much more symmetrically distributed on the luminal and abluminal membranes with little MCT1 in the endothelial cytoplasmic compartment. MCT1 upregulation would therefore be more likely to involve mechanisms such as enhanced transcription and/or translation.

The high levels of MCT1 that we observed in brain microvessels of suckling rats versus the low levels found in adults conform with studies of MCT1 mRNA levels in brains of suckling and adult rats and mice [9,29]. In these studies, it was shown that MCT1 mRNA levels peak postnatally (by day 15 for both rats and mice) and then begin to decline. Similarly, an in situ hybridization study has demonstrated much lower MCT1 mRNA levels in brain microvessels of adult than of suckling mice [29].

The presence of significant MCT1 on the *luminally oriented* plasma membranes of pericytes of suckling rats indicates that monocarboxylate transport is important in pericyte metabolism in these animals. Furthermore, it suggests that this transport is associated with exchanges involving the blood rather than the brain parenchyma. The metabolically active pericytes of young rats may be importing monocarboxylates for use as an energy source or as a precursor of amino acids for basement membrane production or other protein synthesis (see Refs. [27,33]).

Alternatively, pericytes are thought to be closely related to vascular smooth muscle cells [33] which also express MCT1. These cells typically take up glucose and metabolize it to lactate even under aerobic conditions [2]. Like vascular smooth muscle, pericytes may perform aerobic glycolysis with the production of lactic acid that is transported out of the cell via MCT1.

Several studies have addressed the possible sites of expression of MCT1 in brain parenchyma. We have shown that astrocytes are the source of at least part of the parenchymal MCT1 staining in our rat brain preparations. Indeed, some astrocytic cell bodies stained positively for this transporter (present study, [13]). Evidence that MCT1 mRNA is expressed in cultured astrocytes but not highly expressed in cultured neurons was presented by Broer et al. [4]. However, in situ hybridization studies have demonstrated apparently high levels of MCT1 mRNA in neurons, particularly in Purkinje and hippocampal neurons, of suckling and adult mice [29]. Our present work also reveals that neurons express MCT1. In addition, a highly specialized neuron in rats, the retinal rod cell, has been shown to express this transporter [15].

Additional MCTs may be involved in the transport of monocarboxylates in neurons and astroglia of the brain parenchyma. This may be especially true for the peri- and postnatal periods when consumption of monocarboxylates is greatest. One could also predict a significant expression of monocarboxylate transporters in the neuropil if there is metabolic coupling [28] involving lactate exchanges between astrocytes and neurons. Indeed, Gerhart et al. [14] showed that a second MCT (MCT2) not associated with the cerebral microvessels is present in astrocytes. At least six additional members of the MCT family, MCT3 (REMP) and MCTs 3–7, have been reported [30,31], and additional members with highly specific spatial and temporal expression patterns may exist. The roles, if any, of these transporters in the CNS remain to be explored.

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References

[1] A.S. Al-Mudallal, J.C. Lamanna, W.D. Lust, S.I. Harik, Diet-induced ketosis does not cause cerebral acidosis, Epilepsia 37 (1996) 258–261.

[2] J.T. Barron, S.J. Kopp, J. Tow, J.E. Parrillo, Substrate-dependent alteration in O_2 consumption and energy metabolism in vascular smooth muscle, Am. J. Physiol. 270 (1996) H1869–H1877.

[3] P.G. Bittar, Y. Charnay, L. Pellerin, C. Bouras, P.J. Magistretti, Selective distribution of lactate dehydrogenase isoenzymes in neurons and astrocytes of human brain, J. Cereb. Blood Flow Metab. 16 (1996) 1079–1089.

[4] S. Broer, B. Rahman, G. Pellegri, L. Pellerin, J.L. Martin, S. Verleysdonk, B. Hamprecht, P.J. Magistretti, Comparison of lactate transport in astroglial cells and monocarboxylate transporter 1 (MCT 1) expressing *Xenopus laevis* oocytes, J. Biol. Chem. 272 (1997) 30096–30102.

[5] E.M. Cornford, S. Hyman, M.E. Cornford, E.M. Landaw, A.V. Delgado-Escueta, Interictal seizure resections show two configurations of endothelial Glut1 glucose transporter in the human blood–brain barrier, J. Cereb. Blood Flow Metab. 18 (1998) 26–42.

[6] J.E. Cremer, V.J. Cunningham, W.M. Pardridge, L.D. Braun, W.H. Oldendorf, Kinetics of blood–brain barrier transport of pyruvate, lactate, and glucose in suckling, weanling and adult rats, J. Neurochem. 33 (1979) 439–445.

[7] L.R. Drewes, D.Z. Gerhart, R.L. Leino, B.E. Enerson, Astrocyte foot processes and blood–brain transport, in: T.J.F. Lee, W.J. Pearce (Eds.), XVIIIth International Symposium on Cerebral Blood Flow and Metabolism. Williamsburg, VA, USA, 1997, p. 40.

[8] G.J. Dombrowski Jr., K.R. Swiatek, K.-L. Chao, Lactate, 3-hydroxybutyrate and glucose as substrates for early postnatal rat brain, Neurochem. Res. 14 (1989) 667–675.

[9] B.E. Enerson, O. Zhdankina, D.Z. Gerhart, L.R. Drewes, Monocarboxylic acid transporter 1 (MCT1) expression in developing rat brain, FASEB J. 12 (1998) A1032.

[10] C.L. Farrell, W.M. Pardridge, Blood–brain barrier glucose transporter is asymmetrically distributed on brain capillary endothelial lumenal and ablumenal membranes: an electron microscopic immunogold study, Proc. Natl. Acad. Sci. USA 88 (1991) 5779–5783.

[11] C.K. Garcia, J.L. Goldstein, R.K. Pathak, R.G.W. Anderson, M.S. Brown, Molecular characterization of a membrane transporter for lactate, pyruvate, and other monocarboxylates: implications for the Cori cycle, Cell 76 (1994) 865–873.

[12] C.K. Garcia, J.L. Goldstein, R.K. Pathak, J.L. Goldstein, cDNA cloning of MCT2, a second monocarboxylate transporter expressed in different cells than MCT1, J. Biol. Chem. 270 (1995) 1843–1849.

[13] D.Z. Gerhart, B.E. Enerson, O.Y. Zhdankina, R.L. Leino, L.R. Drewes, Expression of monocarboxylate transporter MCT1 by brain endothelium and glia in adult and suckling rats, Am. J. Physiol. 273 (1997) E207–E213.

[14] D.Z. Gerhart, B.E. Enerson, O.Y. Zhdankina, R.L. Leino, L.R. Drewes, Expression of the monocarboxylate transporter MCT2 by rat brain glia, Glia 22 (1998) 272–281.

[15] D.Z. Gerhart, R.L. Leino, L.R. Drewes, Monocarboxylic acid transporters MCT1 and MCT2 in retina, FASEB J. 12 (1998) A1032.

[16] D.Z. Gerhart, R.L. Leino, W.E. Taylor, N.D. Borson, L.R. Drewes, GLUT1 and GLUT3 gene expression in gerbil brain following brief ischemia: an in situ hybridization study, Mol. Brain Res. 25 (1994) 313–322.

[17] K.M. Gronlund, D.Z. Gerhart, R.L. Leino, A.L. McCall, L.R. Drewes, Chronic seizures increase glucose transporter abundance in rat brain, J. Neuropath. Exp. Neurol. 55 (1996) 832–840.

[18] R.A. Hawkins, D.H. Williamson, H.A. Krebs, Ketone-body utilization by adult and suckling rat brain in vivo, Biochem. J. 122 (1971) 13–18.

[19] N. Horwood, D.C. Davis, Immunolabelling of hippocampal microvessel glucose transporter protein is reduced in Alzheimer's disease, Virchows Archiv. 425 (1994) 69–72.

[20] A.K. Kumagai, S.A. Vinores, W.M. Pardridge, Pathological upregulation of inner blood–retinal barrier Glut1 glucose transporter expression in diabetes mellitus, Brain Res. 706 (1996) 313–317.

[21] W.-H. Lee, C.A. Bondy, Ischemic injury induces brain glucose transporter gene expression, Endocrinology 133 (1993) 2540–2544.

[22] F. Maher, I.A. Simpson, Modulation of expression of glucose transporters GLUT3 and GLUT1 by potassium and *N*-methyl-D-aspartate in cultured cerebellar granule neurons, Molec. Cell. Neurosci. 5 (1994) 369–375.

[23] A.L. McCall, A.M. Van Bueren, V. Nipper, M. Moholt-Siebert, H. Downes, N. Lessov, Forebrain ischemia increases Glut1 protein in brain microvessels and parenchyma, J. Cereb. Blood Flow Metab. 16 (1996) 69–76.
[24] J.M. Medina, E. Fernandez, J.P. Bolanos, C. Vicario, C. Arizmendi, Fuel supply to the brain during the early postnatal period, in: J.M. Cuezva, A.M. Pascual-Leone, M.S. Patel (Eds.), Biochemical Development of the Fetus and Neonate, Plenum Press, New York, 1990, pp. 175–194.
[25] A. Nehlig, Respective roles of glucose and ketone bodies as substrates for cerebral energy metabolism in the suckling rat, Dev. Neurosci. 18 (1996) 426–433.
[26] A. Nehlig, Cerebral energy metabolism, glucose transport and blood flow: changes with maturation and adaptation to hypoglycaemia, Diabetes Metab. 23 (1997) 18–29.
[27] A. Nehlig, A.P. Vasconcelos, Glucose and ketone body utilization by the brain of neonatal rats, Prog. Neurobiol. 40 (1993) 163–221.
[28] L. Pellerin, P.J. Magistretti, Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization, Proc. Natl. Acad. Sci. USA 91 (1994) 10625–10629.
[29] L. Pellerin, G. Pellegri, J.L. Martin, P.J. Magistretti, Expression of monocarboxylate 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. USA 95 (1998) 3990–3995.
[30] N. Philp, P. Chu, T.C. Pan, R.Z. Zhang, M.L. Chu, K. Stark, D. Boettinger, H. Yoon, T. Kieber-Emmons, Developmental expression and molecular cloning of REMP, a novel retinal epithelial membrane protein, Exp. Cell Res. 219 (1995) 64–73.
[31] N.T. Price, V.J. Jackson, A.P. Halestrup, Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past, Biochem. J. 329 (1998) 321–328.
[32] L. Sokoloff, Circulation and energy metabolism of the brain, in: G. Siegel, B. Agranoff, R.W. Albers, P. Molinoff (Eds.), Basic Neurochemistry, Raven Press, New York, 1989, pp. 565–590.
[33] R.G. Tilton, Capillary pericytes: perspectives and future trends, J. Electron Micros. Tech. 19 (1991) 327–344.
[34] S.J. Vannucci, F. Maher, E. Koehler, I.A. Simpson, Altered expression of GLUT-1 and GLUT-3 glucose transporters in neurohypophysis of water-deprived or diabetic rats, Am. J. Physiol. 267 (1994) E605–E611.
[35] S.J. Vannucci, R. Reinhart, F. Maher, C.A. Bondy, W.-H. Lee, R.C. Vannucci, I.A. Simpson, Alterations in GLUT1 and GLUT3 glucose transporter gene expression following unilateral hypoxia–ischemia in the immature rat brain, Dev. Brain Res. 107 (1998) 255–264.
[36] C. Vicario, C. Arizmendi, G. Malloch, J.B. Clark, J.M. Medina, Lactate utilization by isolated cells from early neonatal rat brain, J. Neurochem. 57 (1991) 1700–1707.
[37] M.A. Williams, Stereological techniques, in: A.M. Glarert (Ed.), Practical Methods in Electron Microscopy, Vol. 6. Elsevier, Amsterdam, 1977, pp. 5–84.