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Histochemical demonstration of a monocarboxylate transporter in the mouse perineurium with special reference to GLUT1

Kumiko TAKEBE, Junko NIO-KOBAYASHI, Hiromi TAKAHASHI-IWANAGA and Toshihiko IWANAGA
Laboratory of Histology and Cytology, Graduate School of Medicine, Hokkaido University, Kita 15-Nishi 7, Sapporo 060-8638, Japan
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ABSTRACT

Peripheral nerves express GLUT1 in both endoneurial blood vessels and the perineurium and utilize glucose as a major energy substrate, as does the brain. However, under conditions of a reduced utilization of glucose, the brain is dependent upon monocarboxylates such as ketone bodies and lactate, being accompanied by an elevated expression of a monocarboxylate transporter (MCT1) in the blood-brain barrier. The present immunohistochemical study aimed to examine the expression of MCT1 in the peripheral nerves of mice. MCT1 immunoreactivity was found in the perineurial sheath and colocalized with GLUT1, while the endoneurial blood vessels expressed GLUT1 only. An intense expression of MCT1 in the perineurium was confirmed by Western blot and *in situ* hybridization analyses. Ultrastructurally, the MCT1 and GLUT1 immunoreactivities in the thick perineurium showed an intensity gradient decreasing towards the innermost layer. In neonates, the MCT1 immunoreactivity in the perineurium was intense, while the GLUT1 immunoreactivity was faint or absent. These findings suggest that peripheral nerves depend on monocarboxylates as a major energy source and that MCT1 in the perineurium is responsible for the supply of monocarboxylates to nerve fibers and Schwann cells.

Peripheral nerves are ensheathed by one or more layers of flattened fibroblast-like cells which make up the structure known as the perineurium (30, 36, 37). The cellular elements of the perineurium possess the basement membrane along the entire length of their cell surfaces and are individually connected by tight junctions to form a diffusion barrier against surrounding tissues. Some researchers believe that a potential space exists between the perineurium and endoneurium and it contains a small amount of fluids derived from the cerebrospinal fluid. Therefore, there is a possibility that the perineurial cells mimic endothelial cells of the blood and lymphatic vessels. The perineurium as well as the vascular endothelium in the endoneurium form a blood-nerve barrier which regulates the endoneurial microenvironment by restricting the passage of ions, water-soluble non-electrolytes, and macromolecules (24, 28). The blood-nerve barrier is also viewed as a nutrient delivery system essential to the peripheral nerve metabolism (7). D-glucose is a major fuel for peripheral nerves, as in the brain (9, 10, 29).

GLUT (facilitated-diffusion glucose transporter)-1 constitutes a key factor in the transcellular passage of glucose through the barrier cell layer (34), thereby supplying glucose to neurons and other cells located inside the barrier. GLUT1 is abundantly expressed in both the perineurium and the endothelium of endoneurial blood vessels running among nerve fibers (4, 7, 12, 13, 22, 32, 38). GLUT1 in nerves functions to mediate an adequate supply of glucose to nerve fibers and Schwann cells through the perineurium and through the blood vessels in the endoneurium.

Monocarboxylates such as lactate and ketone bod-

Address correspondence to: Dr. Toshihiko Iwanaga, Laboratory of Histology and Cytology, Graduate School of Medicine, Hokkaido University, Kita 15-Nishi 7, Sapporo 060-8638, Japan
Tel: +81-11-7065033, Fax: +81-11-7067151
E-mail: tiwanaga@med.hokudai.ac.jp

ies represent alternative energy substrates under particular conditions with a decreased utility of glucose, including diabetes and prolonged starvation. The contribution of monocarboxylates to energy supply is also important in neonatal periods, as represented by the neonatal brain (2, 14, 21). Animal cells take up and excrete the monocarboxylate anions by means of proton-coupled monocarboxylate transporters (MCTs) in an electroneutral manner (5, 6). Although fourteen MCT isoforms—each having a unique distribution and different sequence homology—have been identified in mammals, only the first four (MCT1-MCT4) have been demonstrated experimentally to catalyse the proton-linked transport of metabolically important monocarboxylates (11, 27). Among the MCT subtypes, MCT1 and MCT2 are rich in the brain, where MCT1 is localized in blood vessels as well as some glial and neural elements, while MCT2 expression is primarily neuronal (8, 18, 26, 31). MCT1 expression in the brain of neonates is more intense than that in the post-weaning period, in contrast to GLUT1, which increases in the expression level after weaning (39). This is parallel to a switching of metabolized substrates from lactate and ketone bodies to glucose during developmental stages. Similar to GLUT1 expression in the blood-brain barrier, the expression of GLUT1 in the perineurium is low in the developing peripheral nerves after birth and increases up to adult levels until weaning (32), suggesting that monocarboxylates also serve as a major energy source for peripheral nerves in neonatal stages. The present study aimed to demonstrate the expression of MCTs in the perineurium and compare the expression patterns with those of GLUTs in mouse neonates and adults.

MATERIALS AND METHODS

Tissue samples. Adult male (8 weeks old) ddY mice and neonates (5 and 10 days old) were used in this study. Fresh tissues were collected from the sciatic nerves, upper lips, vertebrae, and the crania including the brain. These were immersed in Bouin's fluid for 12 h and processed to paraffin sections for conventional immunohistochemistry. The vertebrae and carnia from adult mice were decalcified in 5% ethylenediamine-tetraacetic acid (EDTA) solution for 7 days at 4°C after fixation. For immunohistochemistry at the electron microscopic level, another three male mice were deeply anesthetized by an intraperitoneal injection of pentobarbital and then perfused through the left ventricle of the heart with a physiological saline and subsequently with 4% paraformaldehyde plus 0.2% picric acid in 0.1 M phosphate buffer, pH 7.4. The lips and sciatic nerves with femoral muscles were dissected out and immersed in the same fixative for an additional 6 h. For *in situ* hybridization analyses, the sciatic nerves with surrounding muscles from adults and neonates were directly embedded in a freezing medium (OCT compound; Sakura FineTechnical Co. Ltd., Tokyo, Japan) and quickly frozen in liquid nitrogen. All experiments using animals were performed under protocols following the Guidelines for Animal Experimentation, Hokkaido University Graduate School of Medicine.

Immunohistochemistry. Bouin-fixed paraffin sections were dewaxed and immersed in water and 0.01 M phosphate buffered saline (PBS, pH 7.2). After preincubation with normal goat serum, the sections were incubated with a chicken anti-rat MCT1 antibody (AB1286; Chemicon International, Temecula, CA) at a concentration of 0.5 μg/mL or a rabbit anti-rat GLUT1 antibody (1 : 2,000 in dilution; AB1340, Chemicon International) overnight. The sites of the antigen-antibody reaction were detected by incubation with biotin-conjugated goat anti-chicken IgY (Santa Cruz Biotechnology, Santa Cruz, CA) or goat anti-rabbit IgG (Nichirei, Tokyo, Japan), followed by incubation with the avidin-peroxidase complex (Vectastain ABC kit; Vector, Burlimgame, CA). The reactions were visualized by incubation in 0.01 M Tris-HCl buffer (pH 7.6) containing 0.01% 3,3'-diaminobenzidine and 0.001% H_2O_2. The specificity of immunoreactions on sections was confirmed according to conventional procedures, including absorption tests. The immunoreactivities were completely abolished using the primary antibodies preabsorbed with the corresponding antigens (human MCT1 and GLUT1 from Santa Cruz Biotechnology).

Silver-intensified immunogold method for electron microscopy. The paraformaldehyde-fixed tissues (sciatic nerves and lips) were dipped in 30% sucrose solution overnight at 4°C, embedded in OCT compound, and quickly frozen in liquid nitrogen. Frozen sections of some 15 μm in thickness were mounted on poly-L-lysine-coated glass slides and incubated with a rabbit anti-human MCT1 antibody (3 μg/mL; Biogenesis Ltd, Poole, UK) or the rabbit anti-rat GLUT1 antiserum mentioned above (1 : 800 in dilution) overnight and subsequently reacted with goat anti-rabbit IgG covalently linked with 1-nm gold particles (1 : 200; Nanoprobes, Yaphank, NY). Fol-

lowing silver enhancement using HQ silver (Nanoprobes), the sections were osmificated, dehydrated, and directly embedded in Epon (Nisshin EM, Tokyo, Japan). Ultrathin sections were prepared and stained with both uranyl acetate and lead citrate for observation under an electron microscope (H-7100; Hitachi, Tokyo, Japan).

Western blot analysis. The sciatic nerves and mammary gland at the 5th day after parturition were obtained from Wistar rats and homogenized with an ice-cold 10 mM Tris-HCl buffer (pH 7.0) containing 1 mM EDTA, 20 mM KCl, and a protease inhibitor cocktail (Complete Mini; Roche, Mannheim, Germany). Soluble fractions were processed according to Garcia *et al*. (6), and proteins of 50 μg were subjected to 12% sodium dodecyl sulfate polyacrilamide gel electrophoresis under reducing conditions. The proteins were then transferred to polyvinylidine difluoride membranes (Hypond-P; GE Healthcare Biosciences, Buckinghamshire, UK) and incubated with the chicken antibody against MCT1 (0.1 μg/mL) or the rabbit anti-GLUT1 antibody (1: 20,000). The bound antibodies were visualized using peroxidase-labeled anti-chicken IgY (1 : 2,000 in dilution; Santa Cruz Biotechnology) or anti-rabbit IgG (1 : 10,000; DAKO, Tokyo, Japan) and an enhanced chemiluminescence system (ECL-plus; GE Healthcare Biosciences) according to the manufacturer's instructions.

In situ hybridization. Two non-overlapping antisense oligonucleotide DNA probes (45 mer in length) were designed for each mRNA of mouse MCT1 and GLUT1, as described in our previous study (15, 35). The probes were labeled with ^{33}P-dATP using terminal deoxynucleotidyl transferase (Invitrogen, Carlsbad, CA). Fresh frozen sections, 14-μm-thick, were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer for 15 min and then acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine-HCl (pH 8.0) for 10 min. Hybridization was performed at 42°C for 10 h with a hybridization buffer containing ^{33}P-labeled oligonucleotide probes (10,000 cpm/μL). The sections were rinsed at room temperature for 30 min in 2 × SSC (1 × SSC: 150 mM sodium chloride, 15 mM sodium citrate) containing 0.1% *N*-lauroylsarcosine sodium, then rinsed twice at 55°C for 40 min in 0.1 × SSC containing 0.1% *N*-lauroylsarcosine sodium, dehydrated through a graded series of ethanol, and air-dried. Sections were dipped in an autoradiographic emulsion (NTB-2; Kodak) at 4°C for 8–10 weeks. The hybridized sections used for autoradiography were counterstained with hematoxylin after development.

An *in situ* hybridization technique using the two non-overlapping antisense probes for each mRNA exhibited identical labeling in all the tissues examined. The specificity of the hybridization was also confirmed by the disappearance of signals upon the addition of an excess of an unlabeled antisense probe.

RESULTS

An intense MCT1 immunoreactivity was found in the perineurium of the sciatic nerves and nerves with various diameters in the lips and skeletal muscles of adult mice (Fig. 1). The thick perineurium in the sciatic nerve and other nerves of large diameter appeared at a light microscopic level to be diffusely immunolabeled throughout the cell layers (Fig. 1c). The staining patterns were essentially identical to those of GLUT1 immunoreactivity when compared in adjacent sections (Fig. 1a–d). However, in thin nerves located close to effector cells such as muscles and the sensory apparatus, the perineurial MCT1 immunoreactivity became faint in contrast to GLUT1, maintaining a considerably intense immunoreactivity (Fig. 1e, f). The epineurium, the connective tissue covering the thick nerve bundles (*e.g.* sciatic nerve), was negative in reactions for MCT1 and GLUT1. Blood vessels in the loose connective tissues of the epineurium did not immunostain with either antibody except for the vascular smooth muscles which were moderately immunolabeled with the MCT1 antibody. Small blood vessels in the endoneurium of the sciatic nerves exhibited an intense immunoreactivity for GLUT1 but lacked any immunoreactivity for MCT1 (Fig. 1a, b). When sections from the decalcified vertebrae containing the spinal cord were stained, the spinal pia mater and dura mater were largely immunonegative for both transporters, although the dura mater in neonates exhibited a significant immunoreactivity for MCT1, as mentioned below. The cellular sheath totally enveloping the dorsal root ganglion was positive for both transporters in adults. At the inside of the ganglion, another immnuoreactivity for MCT1 was found in satellite cells, while GLUT1 immunoreactivity was also localized in the blood vessels (data not shown).

At the electron microscopic level, an accumulation of gold particles showing the existence of MCT1 immunoreactivity was confirmed in the multi-layered or single-layered perineurium. The immunoreactivity in the multi-layered perineurium of

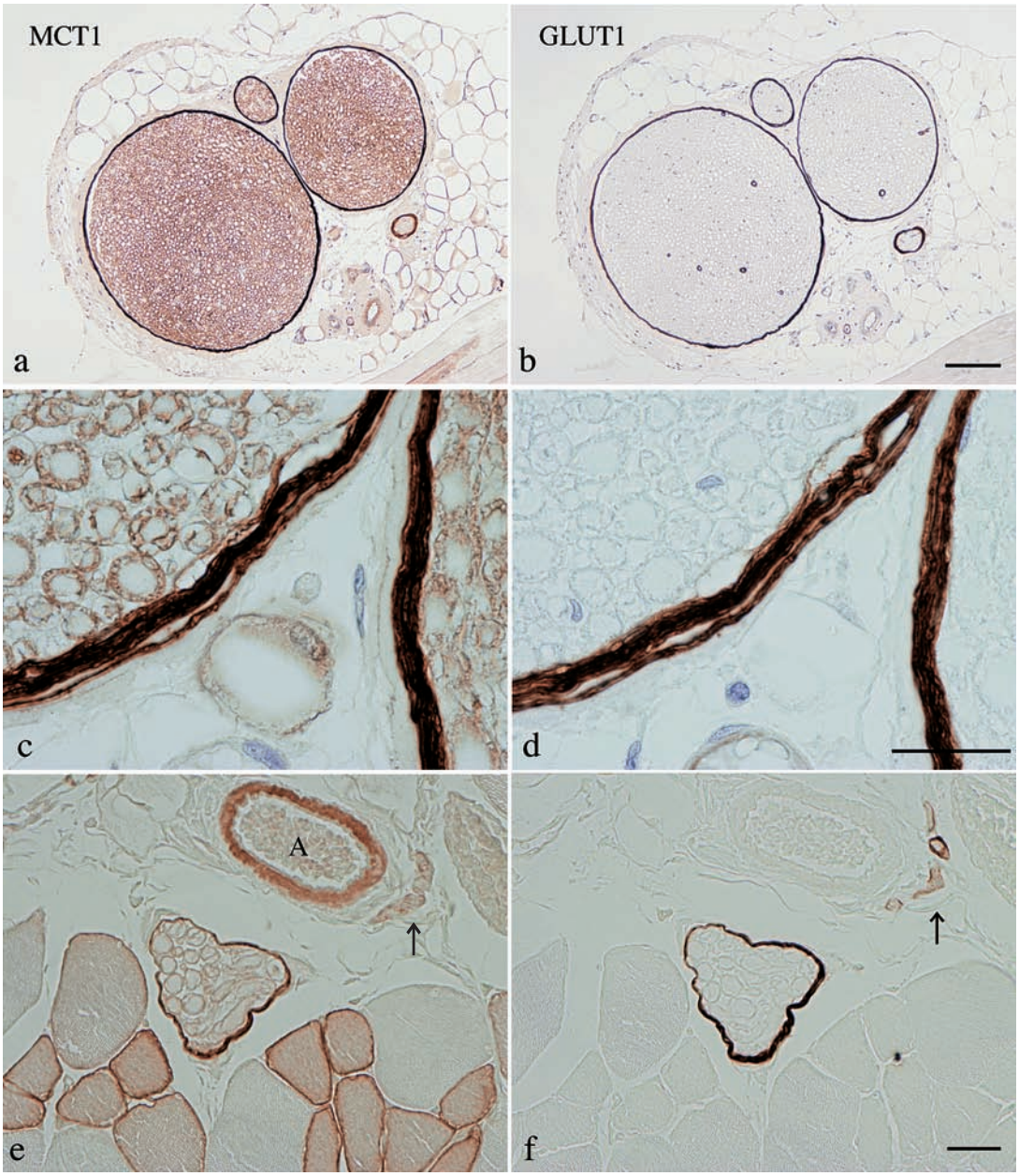

Fig. 1 MCT1 (a, c, and e) and GLUT1 immunoreactivities (b, d, and f) in the perineurium of adult mice. The perineurium of the sciatic nerve is equally immunoreactive for both MCT1 and GLUT1 in adjacent sections (a, b). Myelin sheaths are non-specifically labeled brown with the MCT antibody. Specific GLUT1 immunoreactivity is also seen in several small blood vessels in the endoneurium but not MCT1. At high magnification (c, d), all layers of the perineurium appear to be diffusely immunolabeled for both transporters. The perineurium of a small nerve in the skeletal muscle is immunoreactive for MCT1 and GLUT1 (e, f), but that of very thin nerves indicated by an arrow is faint in reaction with MCT1 antibody. Another MCT1 immunoreactivity is found in oxidative (red) skeletal muscle fibers and the smooth muscle of an arterial wall (A). Bars a, b:100 μm, c-f: 20 μm

the sciatic nerve and other nerves of large caliber tended to decrease in intensity towards the innermost cell layer (Fig. 2a). Both luminal and contraluminal basement membranes in the thin processes of perineurial cells were equally immunolabeled with the MCT1 antibody in outer layers of the perineurium. The immunoreactivity of GLUT1 was essentially identical to that of MCT1 and tended to show more dense labeling with gold particles than MCT1 (Fig. 2b).

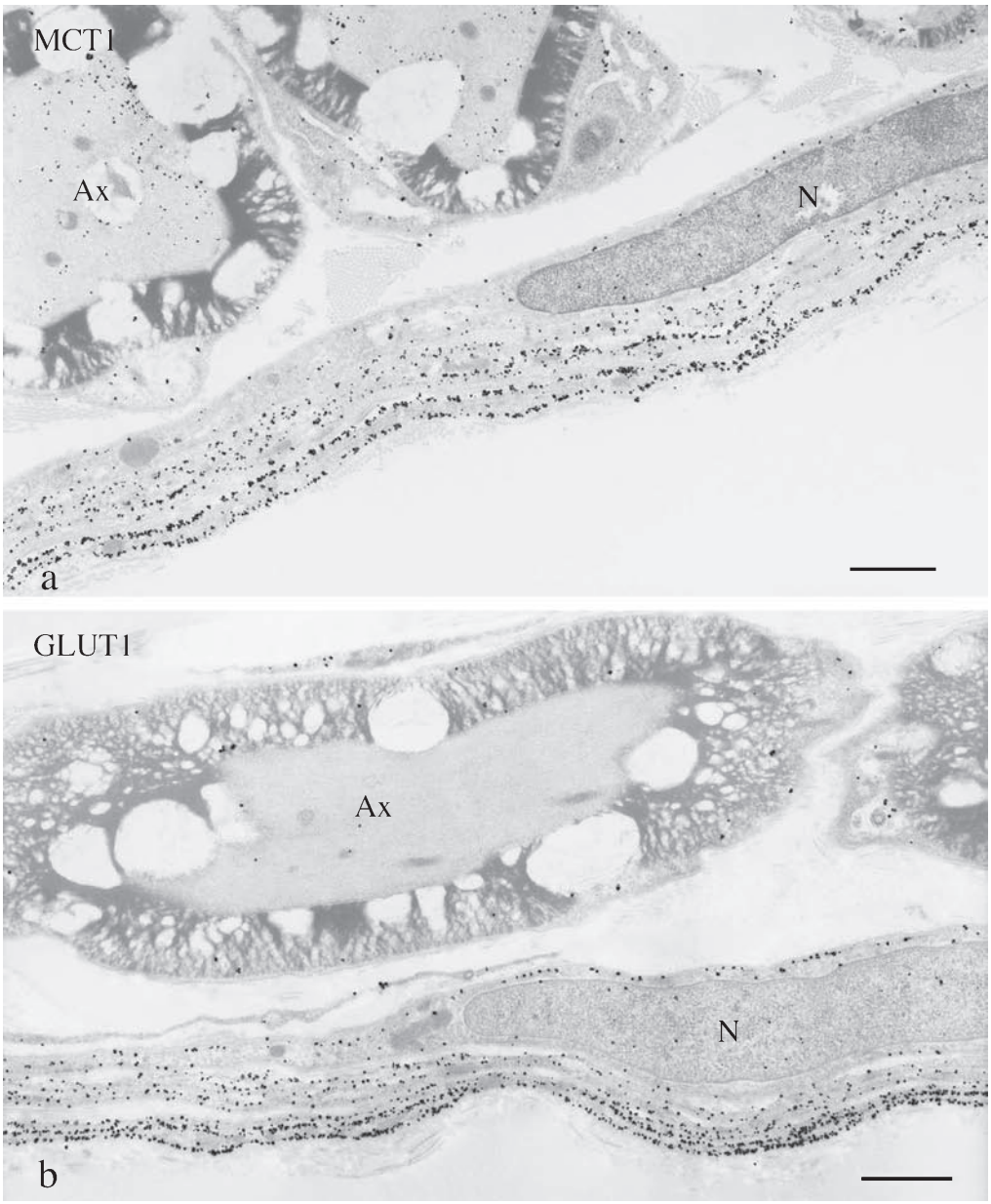

Fig. 2 Electron microscopy of MCT1 (a) and GLUT1 immunoreactivities (b) in nerves present in the subcutaneous tissue of lip skin in an adult mouse. Silver-intensified gold particles are deposited along the plasma membrane of thin processes of perineurial cells with a denser aggregation of particles in the outer layers. Ax axons, N nucleus of perineurial cells, Bars 1 μm

In 5-day-old neonates, the perineurium of sciatic nerves and other nerves exhibited an intense immunoreactivity for MCT1, while the GLUT1 immunoreactivity was negative or faint in all types of nerves (Fig. 3a–d). The perineurium became positive for GLUT1 in 10-day-old neonates, but the reactivity was still weak as compared with adults. The sciatic nerve in the neonates did not contain any endoneurial blood vessels with the intense GLUT1 immunoreactivity (Fig. 3b). Blood vessels in the parenchyma of the spinal cord were intensely labeled with the MCT1 antibody and moderately immunoreactive for GLUT1 (Fig. 3e, f). Unlike the adults, the spinal dura mater of neonates showed a significant immunoreactivity for MCT1 but not for GLUT1.

Western blot analysis

We first confirmed the same immunostaining pat-

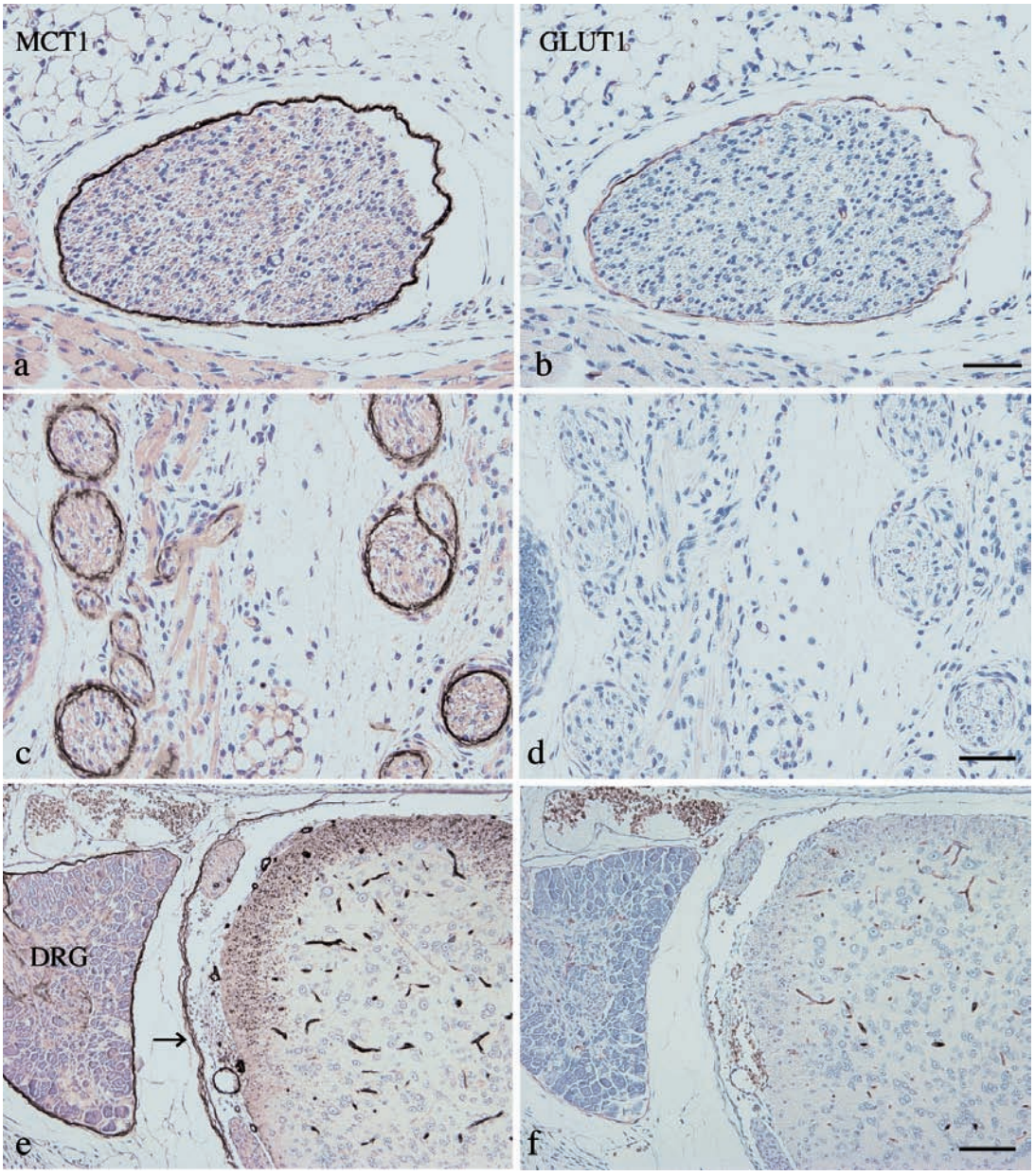

Fig. 3 Immunohistochemistry of MCT1 (a, c, and e) and GLUT1 (b, d, and f) in nerve tissues of 5-day-old neonates. The perineurium of the sciatic nerve (a, b) and small nerves in the lip skin (c, d) is intensely immunoreactive for MCT1. In the vertebra (e, f), MCT1 immunoreactivity is seen in the dura mater (arrow) and sheath of the dorsal root ganglion (DRG) as well as blood vessels in the parenchyma of the spinal cord, while only a weak immunoreactivity of GLUT1 is seen in the blood vessels in the spinal cord. Bars a-d: 50 μm, e, f: 100 μm

terns for MCT1 and GLUT1 on paraffin sections of the rat sciatic nerves as observed in the mouse (data not shown). The rat sciatic nerves were used in the present immunoblot analysis since we could obtain a sufficient volume of tissues necessary for blotting and there is available information on immunoblot analyses of rat MCT1 and GLUT1.

A single immunoreactive band for MCT1 appeared at 41–43 kDa in the lanes of the sciatic nerve and mammary gland, the latter being used as a positive control (Fig. 4a). Previous immunoblot analyses for rat MCT1 have detected a major band at 41 kDa in the renal cortex (3) or 43 kDa in the heart, liver, and testis (16), although the predicted molecular weight of MCT1 is 53 kDa.

Immunoblotting with the GLUT1 antibody detect-

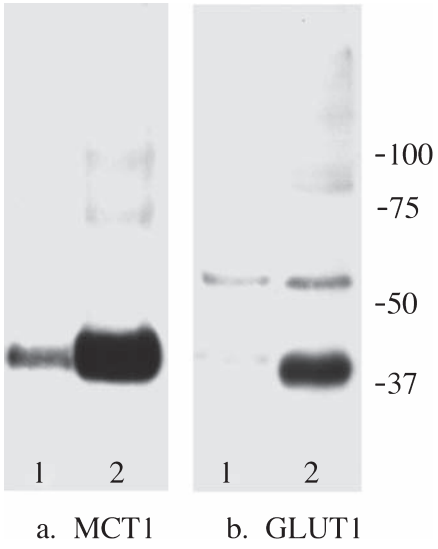

Fig. 4 Immunoblot analysis of MCT1 (a) and GLUT1 (b) in extracts of the sciatic nerve (lane 1) and mammary gland (lane 2) of a rat. The antibody against MCT1 detects an intense single band at 41–43 kDa. An immunoreactive band of GLUT1 is localized around 55 kDa in both tissues, but another band around 40 kDa appears only in the mammary gland.

ed a major band around 55 kDa in sciatic nerves, while two bands appeared around 55 kDa and 40 kDa in the mammary gland (Fig. 4b). The protein band of 40 kDa was faint in the samples of sciatic nerves. Previous immunoblottings of the rat sciatic nerves for GLUT1 have recognized a single band of 55 kDa (4) and of 55–60 kDa (22). Another immunoreactive band of GLUT1 existing at the lower position corresponded to the 43-kDa protein detected in the rat mammary gland by Takata *et al*. (33).

In situ hybridization

In situ hybridization using DNA probes was carried out in the femoral muscles containing the sciatic nerves in 5-day-old and adult mice. Significant signals for MCT1 mRNA were found in the perineurium of sciatic nerves in neonates (Fig. 5). Those for GLUT1 mRNA were detectable but very weak. In adult sciatic nerves, the transcripts of both MCT1 and GLUT1 genes were under the detectable level.

DISCUSSION

Under normal conditions, glucose is a major metabolic fuel for the brain and peripheral nerves. However, monocarboxylates such as lactate and ketone bodies can serve as alternative energy sources for neural functions when the availability of glucose decreases, *i.e*., in starvation, diabetes, and during the early postnatal period. The present immunohistochemical study revealed a concomitant expression of MCT1 and GLUT1 in the perineurium of nerve fasciculi in adult animals and a switching of the predominant transporter from MCT1 to GLUT1 during the developmental stage. The expressions of MCT1 and GLUT1 in the perineurium were confirmed by immunoblot and *in situ* hybridization analyses. That the mRNA expressions of MCT1 and GLUT1 in the perineurium were under a detectable level in adult animals may be explained by a low *de novo* production of the transporters in adults in normal conditions.

Expression of MCT1 in the peripheral nerves of adults

This study is the first to report the expression of MCT1 in the perineurium of peripheral nerves. The present immunohistochemical study thoroughly confirmed the localization of GLUT1 in the peripheral nervous system (4, 7, 12, 13, 22, 32, 38) and was able to compare it with the distribution of MCT1. MCT1 and GLUT1 in the perineurium coexisted at a cellular level—although the MCT1 immunoreactivity was less intense in the peripheral end of nerve fibers with the smallest diameters. In contrast to GLUT1, which was also expressed in the vascular endothelium of the endoneurium, the MCT1 immunoreactivity was absent in these blood vessels. The localization of GLUT1 in nerve bundles indicates the dual supply of glucose to individual nerve fibers via endoneurial blood vessels and via the perineurium. The endoneurial capillaries have been recognized a main route for the uni-directional transport of glucose by some researchers (29). However, this idea is refused by the following morphological findings. First, the endoneurial blood vessels are only rarely positive for GLUT1 in the adult human nerves in contrast to the intense and consistent immunoreactivity of GLUT1 in the perineurium (22). Second, nerves fasciculi of smaller diameters do not contain any GLUT1-expressing blood vessels in the endoneurium, as confirmed by the present study. On the other hand, MCT1 with a localization limited to the perineurium suggests that this supplies monocarboxylates to nerve fibers and Schwann cells only via a trans-perineurial route. Monocarboxylates to be transported to the endoneurium may be derived from blood vessels running in the epineurium and surrounding tissues. If the monocarboxylates are directly supplied by adjacent non-vascular tissues, they may be lactate produced by glycolysis in skele-

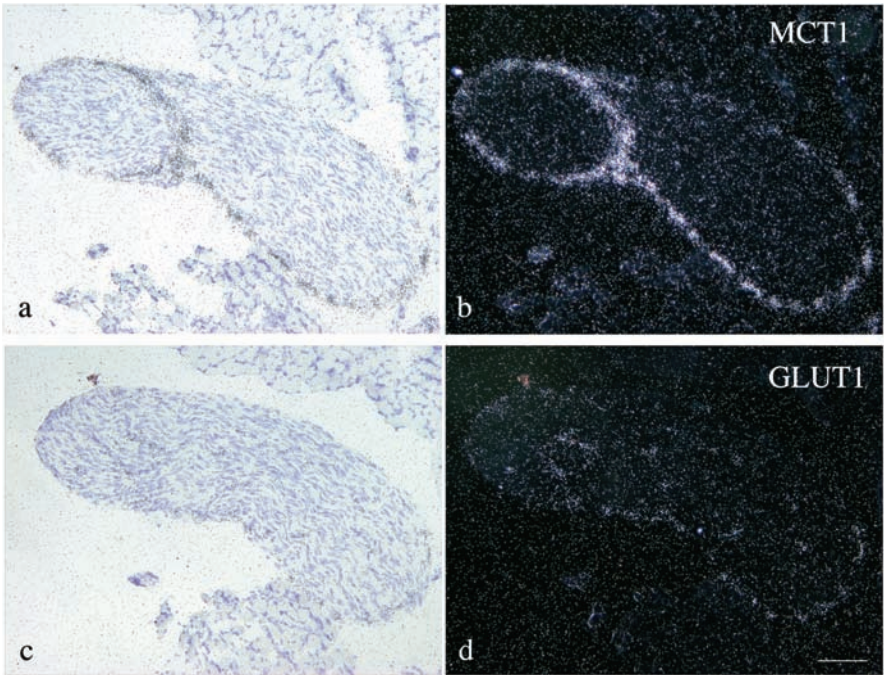

Fig. 5 *In situ* hybridization analysis for MCT1 (a, b) and GLUT1 (c, d) in the sciatic nerve of neonates (5 days-old). Figures **b** and **d** are dark-field images of figures **a** and **c**, respectively. Intense signals of MCT1 mRNA are located along the perineurium, while those of GLUT1 mRNA are weak. Bar 100 μm

tal muscles and ketone bodies released from adipose tissues, which are rich around nerve fasciculi.

The cellular localization of MCT in the brain is a controversial topic among researchers, in part due to species difference. MCT1 in adult mouse and rat brains is localized in the surface glial limiting membranes and ependyma as well as the vascular endothelium (8, 26). Epithelium of the choroid plexus in the mouse brain expressed MCT1 but not that in the rat brain. We confirmed the intense immunoreactivity of MCT1 in the blood vessels, glial limiting membrane, and some regions of the ependyma in the brain of adult mice, but we failed to obtain any significant reactivity in the epithelium of the choroid plexus (our unpublished data). The present study found an intense immunoreactivity for MCT1 in the spinal dura mater in neonates, while this was less so in adults. In the dura mater of the brain, the MCT1 immunoreactivity was not significant in either neonatal or adult mice. These findings suggest that the dura mater of the spinal cord is situated in a functional aspect between the dura mater of the brain and the perineurium of nerves. As for GLUT1, no immunoreactivity was reported to be detectable in the meninges of the spinal cord in young and adult rats, while an *in situ* hybridization technique detected intense signals of GLUT1 mRNA in the pia mater of the spinal cord in adult rats (32). The present study confirmed the lack of GLUT1 immunoreactivity in the spinal meninges of both neonatal and adult mice.

Subcellular localization of MCT1 in the peripheral nerves

The perineurium is composed of concentric layers of flattened cells with basement membrane on the cell surface and collagen fibers intervening between the cellular laminae (36). The cell layers are connected with developed tight junctions, which, in sealing off the extracellular space, force metabolites to traverse a cytoplasmic pathway. The GLUT1 immunoreactivity in perineurial cells of the canine tibial and rat sciatic nerves is mainly associated with the plasma membrane along its entire length (7, 38). We confirmed the condensed localization of GLUT1 in the luminal and contraluminal plasma membranes of perineurial cells in the peripheral nerves of mice. Similar to GLUT1, MCT1 in perineurial cells was present essentially in the plasma membrane on both sides. These morphological findings support the idea of a transcellular passage of glucose and monocarboxylates in the perineurium.

In the multi-layered perineurium such as that of sciatic nerves, the GLUT1 immunoreactivity is evenly distributed throughout the cell layers (7, 22, 38) or more intense in the inner perineurial laminae (20). The present study detected diffuse immunoreactivities for GLUT1 and MCT1 in all cell layers of the perineurium at a light microscopic level. In our electron microscopy, however, both immunoreactivi-

ties were found to decrease in intensity towards the innermost layer of the thick perineurium. The uneven distribution of MCT1 and GLUT1 under the electron microscope clashes with the staining pattern obtained by light microscopic immunostaining, and may be caused by the difference in sensitivity between the indirect immunostaining (two steps) and avidin-biotin complex methods (three steps). This also indicates the decreasing gradient of transporters from the outside to the inside of the perineurium. Interestingly, a similar staining pattern with an intense expression of MCT1 only in the basal (contraluminal) part of stratified cell layers was reported in the stratified squamous epithelium in the ruminant forestomach, which transports a great amount of monocarboxylates (17). The apical (luminal) part of the stratified squamous epithelium, which lacks the MCT1 expression, does not develop a junctional apparatus between cells. Similarly, the inner layer of the perineurium is leaky, for when peroxidase was injected into the endoneurium, it passed the first or second innermost cell layers and stopped abruptly at a deeper cell layer (24). Therefore, the inner (luminal) cell layers of the perineurium may not be conducive for the MCT1 transporter to convey monocarboxylates trans-cellularly.

Switching of transporters from MCT1 to GLUT1 during developing stages

After birth, lactate present in high amounts in the blood circulation provides an important energy source for the brain (2, 40). In addition, ketone bodies formed by the hepatic oxidation of milk-derived fat are also significant energy substrates for the brain during the preweaning period in rodents (23, 39). Lactate and ketone body utilization in the brain is very high during the suckling period but decreases after weaning to reach adult values (1, 2, 14, 21, 23). In parallel with this energy utility, a higher expression of MCT1 in the microvessels of the brain is observed during suckling in rodents (8, 19, 25) and declines with weaning, being accompanied by the predominant expression of GLUT1 in the postweaning period (39). The present study confirmed the dynamic switching of energy transporters from MCT1 to GLUT1 in the perineurium of peripheral nerves during developmental stages. Stark *et al.* (32) reported the lack of GLUT1 in the perineurium of peripheral nerves in P0 and P14 rats, in contrast to its intense expression in adult rats. In accordance with this, the present study showed that nerves in the murine neonates at P5 and P10 were respectively negative and faintly immunoreactive for GLUT1 but intensely positive for MCT1. The MCT1 immunoreactivity in the perineurium still remained at a significant level in adults as compared with the expression of MCT1 in the blood-brain barrier, suggesting that peripheral nerves are dependent upon monocarboxylates at a higher level than the brain.

Finally, the present study cannot rule out the possibility that MCT1 functions in the excretion of monocarboxylate metabolites from an endoneurial circumference. Because of the bi-directional transporting ability of MCT, it is not easy to determine this. Further study, including an *in vitro* analysis using perineurial tubes, will be required to investigate the possibility.

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