Monocarboxylate transporter expression in mouse brain


Monocarboxylate transporter expression in mouse brain. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E516–E524, 1998.—Although glucose is the major metabolic fuel needed for normal brain function, monocarboxylic acids, i.e., lactate, pyruvate, and ketone bodies, can also be utilized by the brain as alternative energy substrates. In most mammalian cells, these substrates are transported either into or out of the cell by a family of monocarboxylate transporters (MCTs), first cloned and sequenced in the hamster. We have recently cloned two MCT isoforms (MCT1 and MCT2) from a mouse kidney cDNA library. Northern blot analysis revealed that MCT1 mRNA is ubiquitous and can be detected in most tissues at a relatively constant level. MCT2 expression is more limited, with high levels of expression confined to testes, kidney, stomach, and liver and lower levels in lung, brain, and epididymal fat. Both MCT1 mRNA and MCT2 mRNA are detected in mouse brain using antisense riboprobes and in situ hybridization. MCT1 mRNA is found throughout the cortex, with higher levels of hybridization in hippocampus and cerebellum. MCT2 mRNA was detected in the same areas, but the pattern of expression was more specific. In addition, MCT1 mRNA, but not MCT2, is localized to the choroid plexus, ependyma, microvessels, and white matter structures such as the corpus callosum. These results suggest a differential expression of the two MCTs at the cellular level.

Monocarboxylate transporter-1; monocarboxylate transporter-2; cDNA sequences; in situ hybridization

The transport and metabolism of monocarboxylic acids such as lactate, pyruvate, and ketone bodies have been extensively studied (for review, see Ref. 27). Early studies indicated that lactate and other monocarboxylic acids are transported out of muscle and into liver (the so-called Cori cycle) by proton-coupled transporters (monocarboxylate transporters; MCTs) that exhibit distinct substrate specificities and could be differentially inhibited by α-cyanocinnamates (27). However, it was only very recently that any members of this family were cloned. MCT1, originally isolated from a Chinese hamster ovary cell cDNA library, is found in cardiac muscle, erythrocytes, basolateral intestinal epithelium, and skeletal muscle, where it is localized to myocytes containing high levels of mitochondria (10). MCT2, cloned from hamster liver, is functionally similar to MCT1 in terms of transport capabilities, but its tissue distribution is significantly different (9). For example, MCT2 is found in hepatocytes but not erythrocytes or intestinal epithelium (9). In addition, MCT1 and MCT2 exhibit distinct cellular localization within structures such as stomach, kidney, testes, and muscle (9), suggesting that the two transporters have different functions. Since the initial studies in hamster, these two transporters have also been cloned from rat (MCT1, skeletal muscle and intestine; MCT2, testes), mouse (MCT1, Ehrlich Lettre’s tumor cells), and humans (MCT1) (4, 11, 19, 20, 33). Recently, a third transporter isoform, MCT3, was identified in chick retinal pigment epithelium (40), and even more recently, four new MCT homologs have been cloned and sequenced in human tissue (MCT3–MCT7; Ref. 28). These reports bring this newly emerging family of membrane nutrient transporters to a total of seven members, similar to the facilitative glucose transporters.

Under normal situations, glucose is the major metabolic fuel for the brain. However, other metabolic substrates such as lactate and ketone bodies can serve as alternative energy sources for cerebral function when available in significant quantities, i.e., in starvation, diabetes, and during the early postnatal period, when milk is rich in fatty acids (3, 16, 17, 23, 25, 32, 35, 38, 39). Initial studies by Cremer et al. (6) and by Nemoto and Severinghaus (24) suggested that the blood-brain barrier has a saturable and stereospecific monocarboxylate transport system, of which the capacity for transporting monocarboxylic acids (i.e., lactate) is six times greater in the neonate compared with adult animals. Recently, Gerhart et al. (12), using immunocytochemical techniques, demonstrated the presence of MCT1 in rat brain, in particular, in microvessels, ependymocytes, glial-limiting membranes, and neuropil. In this study, we report the cloning and sequencing of two MCT isoforms (MCT1 and MCT2) from mouse. Using these sequences, we have generated specific antisense riboprobes to further define the regional and cellular expression of both MCT1 and MCT2 in adult mouse brain.

Materials and methods

cDNA cloning of mouse MCT1 and MCT2. Strain 129 mouse kidney poly(A)+ RNA was isolated, and cDNA was prepared by using the protocol and materials in the Stratagene ZAP-cDNA kit. The cDNA was ligated into the Uni-ZAPt XR vector, and the ligation products were packaged using Stratagene Gigapack Gold packaging extract. The primary library was plated out on XL1-Blue MRF Escherichia coli

1

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cells. Approximately $1 \times 10^6$ plaques were screened by
transfer to nitrocellulose-coated positively charged nylon filters (Magna-Plus, MSI). The filters were probed with
$^{32}$P-labeled oligonucleotide primed fragments of pMCT1 and
pMCT2 from Chinese hamster ovary cells and Syrian ham-
ster liver, respectively (gift from C. Garcia, M. Brown, and J.
Goldstein; see Refs. 9, 10). Initial probing was done at 42°C
with Rapid-Hyb (Amersham) over 4 h. Filters were washed
twice (15 min each) at 55°C in 2×SSPE (1×SSPE is 0.15
M NaCl, 0.01 M Na$_3$PO$_4$, and 0.001 M EDTA, pH 7.4).
MCT1, the longest hybridizing clone contained a 3.1-kb insert
and was designated mMCT1. For MCT2, the longest strongly
hybridizing clone contained an insert of 2.4 kb and was
designated mMCT2. A series of deletion clones were gener-
ated using the Exo Mung Bean Deletion kit (Stratagene) from
both 3′ and 5′ ends of both clones. Overlapping deletion clones
were sequenced using an Applied Biosystems 373A DNA
sequencer. Probes for mMCT1 and mMCT2 were taken from
5′ clones of the deletion series. By using the T3 or T7
promoters, sense and antisense riboprobes were generated.

Northern blot analysis. Total cellular RNA was extracted from
mice injected with liquid nitrogen immediately after harvesting and stored at
from pulverized mouse tissue that had been frozen in liquid
agarose, 2% (vol/vol) formaldehyde gel. Duplicate gels were
processed using the modification of this method, described by
Mannheim) were used to isolate the radiolabeled cRNA. After
precipitation of the cRNA, the samples were subjected to
precipitation of the cRNA, the samples were subjected to
precipitation of the cRNA, the samples were subjected to
dithiothreitol, 20 units of the appropriate polymerase, and 40
mM NaCl, 0.01 M NaH$_2$PO$_4$, and 0.001 M EDTA, pH 7.4). For

RESULTS

cDNA cloning and Northern blot analysis of MCT1
and MCT2. Mouse MCT1 and MCT2 were cloned and
sequenced from a mouse kidney cDNA library, and the
time resolution is shown in Figs. 1 and 2, respectively.

In situ hybridization. Adult male C57/BL6 mice were
anesthetized with halothane and decapitated, and the brains
were rapidly frozen in −40°C isopentane. Sixteen-microme-
eter sections were then mounted onto slides coated with
poly-l-lysine and gelatin-chrom-alum. Slides were stored at
−70°C until analysis.

$^{35}$S-labeled cRNA riboprobes for MCT1 and MCT2 were
prepared as previously described by Bondy et al. (2). In
general, the riboprobes were synthesized in 10-µl reactions
containing 100 µCi $^{35}$S-labeled CTP and 100 µCi $^{35}$S-labeled
UTP (NEN), 10 mM NaCl, 6 mM MgCl$_2$, 40 mM Tris (pH 7.9),
2 mM spermidine, 500 ng linearized DNA template (1.9 kb in
length for MCT1, 1.4 kb for MCT2), 500 µM each unlabeled
ATP and GTP, 25 µM each unlabeled CTP and UTP, 10 mM
dithiothreitol, 20 units of the appropriate polymerase, and 40
units RNasin. The reaction was incubated at 37°C for 1 h,
after which time a 50 µl sample was added to remove the DNA
template (all molecular biology reagents and enzymes were obtained from Promega). Quickspin columns (Boehringer
Mannheim) were used to isolate the radiolabeled cRNA. After
precipitation of the cRNA, the samples were subjected to
alkaline hydrolysis (MCT1, 17.9 min; MCT2, 16.2 min) to
achieve a riboprobe ~400 bp in length. Sense and antisense
riboprobes were synthesized for both MCT1 and MCT2.

In situ hybridization was performed as previously de-
scribed by Vannucci et al. (36) with some changes in incuba-
tion times to increase stringency. Before hybridization, sec-
tions were fixed in 4% paraformaldehyde (0.1 M PBS), rinsed
in 0.1 M PBS, and then immersed in triethanolamine
(0.1 M)-NaCl (0.9%)-acetic anhydride (0.2%). Brain sections
were then washed with 2× SSC (1× SSC is 0.15 M NaCl
and 0.015 M sodium citrate, pH 7.0) and dehydrated, delipitated,
rehydrated, and allowed to dry. The hybridization solution
contained the $^{35}$S-labeled riboprobe (1× 10$^7$ counts·min$^{-1}$·
ml$^{-1}$), hybridization buffer (50% formamide, 50 mM Tris, 2.5
mM EDTA, 0.2 M NaCl, 10% dextran sulfate, 1× Denhardt
solution (1× Denhardt is 0.02% polyvinylpyrrolidone, 0.02%
Ficoll, and 0.02% bovine serum albumin), and 250 µg/ml
tRNA), and 50 mM dithiothreitol. The slides were cov-
slipped after the hybridization solution was added and were
placed in a 55°C humidified incubator overnight.

The next day, the slides were washed in 4× SSC to remove
coverslips and hybridization solution. The sections were then
immersed in 50% formamide, 0.6 M NaCl, 40 mM Tris·HCl,
and 2 mM EDTA at 60°C for 30 min (an increase of 10–15
min), followed by treatment with RNase A (20 µg/ml). Sec-
tions were washed through graded salt solutions and washed
in 0.1× SSC at 65°C for 40 min (an increase of 10 min). Slides
were then dehydrated and air dried before being exposed to
Hyperfilm®-max (Amersham) for 3 wk. After exposure to the
film, the slides were dipped in Kodak NTB3 photographic
emulsion and stored at 4°C for 6 wk. The slides were devel-
opened and stained with hematoxylin for evaluation.
5. Mouse MCT2 is highly abundant in testes, stomach, kidney, and liver, whereas lower levels were detected in epididymal fat, brain, and lung. Its expression pattern is also more complicated in that three message species, approximately 3, 9, and 14 kb in size, are present in most tissues. An RNA species at least 2.4 kb in length is predicted from the mMCT2 cDNA. The ratio of the 3- to the 14-kb message varies in a tissue-specific fashion.

Fig. 1. Mouse monocarboxylate transporter (MCT)-1 cDNA sequence and deduced protein structure. Nucleotide sequence of mouse MCT1 and corresponding single letter deduced amino acid sequence are shown. Doubly underlined nucleotide sequence corresponds to region of cDNA used for riboprobe synthesis. Singly underlined amino acid sequences correspond to putative intramembrane helical regions. This sequence has been deposited in GenBank data bank under accession no. AF058055.

Fig. 2. Mouse MCT2 cDNA sequence and deduced protein structure. Nucleotide sequence of mouse MCT2 and corresponding single letter deduced amino acid sequence are shown. Doubly underlined nucleotide sequence corresponds to region of cDNA used for riboprobe synthesis. Singly underlined amino acid sequences correspond to putative intramembrane helical regions. This sequence has been deposited in GenBank data bank under accession no. AF058054.
Whereas liver, testes, and epididymal fat predominantly express equal amounts of the 3-kb species, tissues like brain, lung, stomach, and kidney express equal amounts of the 3- and 14-kb messages. The significance of these differences awaits further investigation.

In situ hybridization. To more specifically examine the regional and cell-specific expression of MCT1 and MCT2 in the brain, the 5' portion of each nucleotide sequence shown in Figs. 1 and 2 was used to generate riboprobes for in situ hybridization. The expression of MCT1 and MCT2 in several brain regions is illustrated in Fig. 6, which shows adjacent coronal sections at the level of the striatum (Fig. 6, A and B), anterior hippocampus (Fig. 6, C and D), and cerebellum (Fig. 6, E and F); control sense hybridizations to cerebellar sections are also shown (Fig. 6, G and H).

Both MCT1 mRNA and MCT2 mRNA are detected in the cortex (Fig. 6, A–D); MCT2 is expressed uniformly, whereas MCT1 shows lower levels of hybridization in the first layer compared with the other cortical layers. This pattern exists regardless of cortical region (i.e., frontal or temporal cortices). There is, however, a very strong hybridization signal in the piriform cortex for both transporters, especially MCT2. Hybridization of both MCT1 and MCT2 in the cortex appears to be primarily neuronal.

The regional differences between MCT1 and MCT2 mRNA expression are most striking in white matter areas, e.g., corpus callosum, where MCT1 mRNA is detected but MCT2 is not (Fig. 6, A and B). This differential expression is further apparent in the choroid plexus and ependymal lining of the cerebral ventricles, which only express MCT1 (Fig. 6, A and B).

Differences in the regional localization of the two transporter isoforms are also present at the level of the hippocampus, as illustrated in Fig. 6, C and D. Higher levels of MCT1 mRNA are found in the CA fields of the hippocampus.

Fig. 3. Alignment of protein sequences of mouse MCT1 and MCT2. Alignments were performed using PileUp program of Genetics Computer Group (Wisconsin Package version 9.1, Madison, Wisconsin). Black regions, areas of identity; gray regions, areas of similarity; open regions, areas where there is neither identity nor similarity. Deduced amino acid sequence of MCT1 has 58% identity to MCT2.

Whereas liver, testes, and epididymal fat predominantly express equal amounts of the 3-kb species, tissues like brain, lung, stomach, and kidney express equal amounts of the 3- and 14-kb messages. The regional differences between MCT1 and MCT2 mRNA expression are most striking in white matter areas, e.g., corpus callosum, where MCT1 mRNA is detected but MCT2 is not (Fig. 6, A and B). This differential expression is further apparent in the choroid plexus and ependymal lining of the cerebral ventricles, which only express MCT1 (Fig. 6A).

Differences in the regional localization of the two transporter isoforms are also present at the level of the hippocampus, as illustrated in Fig. 6, C and D. Higher levels of MCT1 mRNA are found in the CA fields of the hippocampus.
Ammon's horn, with lower levels in the dentate gyrus (Fig. 6C). Conversely, MCT2 expression is highest in the dentate gyrus and is lower in the CA1–4 fields of Ammon's horn (Fig. 6D).

Both MCT1 mRNA and MCT2 mRNA are detected in the cerebellum, in particular, in the Purkinje and granule cell layers (Fig. 6, E and F). MCT1 mRNA is highly expressed throughout the Purkinje cell layer of the cerebellum and, to a lesser extent, in the granule cell layer (Fig. 7C). MCT2 expression is similar, except that there is an equivalent level of hybridization in both the granule cell and Purkinje cell layers (Fig. 6F). However, no expression of either transporter isoform was evident in the molecular layer.

To more clearly define the cell-specific expression of both MCT1 and MCT2, regions of interest from Fig. 6 were examined at higher magnification, as shown in the bright-field micrographs of Fig. 7. The apparent punctate signal seen for MCT1 in the cortex (Fig. 6, A and C) is indeed localized to microvessels (Fig. 7A). Figure 7B confirms that the MCT2 expression in the dentate gyrus of the hippocampus is localized to granule cells, whereas, in the cerebellum, most of the MCT1 expression is in the Purkinje cells (Fig. 7C).

DISCUSSION

The results of our study clearly demonstrate the presence of mouse MCT isoforms, mMCT1 and mMCT2, in the brain as well as in peripheral tissues. Initial studies by Garcia and colleagues (9, 10) describing the cloning and localization of MCT1 in the hamster reported protein expression of the transporter isoform in erythrocytes, heart, red skeletal muscle, kidney, gastrointestinal system, lung, eye, sperm, epididymis, and, albeit at low levels, liver. Similar results were found in the rat by Northern blot analysis (33). Using our cloned mouse sequence, which is ~88% homologous to hamster and rat sequences, we detected MCT1 in these same tissues as well as in brain. Low but detectable levels of MCT1 mRNA are also found in the adrenal, white muscle, lung, and liver, indicating that MCT1 probably is not the major MCT in these tissues. Interestingly, MCT1 is detected in the eye both in this study and others (9). This may have clinical implications in that MCT1 was recently mapped to a region of chromosome 1 (p13.2-p12) near to where a retinal dystrophy was assigned (1p21-p13; Ref. 11).

In agreement with the studies of Garcia et al. (9) regarding MCT2 in hamster, we also detected this isoform in a variety of peripheral tissues, including testes, stomach, kidney, liver, epididymal fat, and lung. Recently, Jackson et al. (19) reported MCT2 expression in rat brain, liver, and testes but not in heart or skeletal muscle. These researchers also reported that MCT2 is expressed as different-sized transcripts, similar to those seen in the Northern blots presented here (Fig. 5). What is clear from the results of this study in the mouse, as well as those of others (4, 9, 10, 19, 20, 33), is that certain tissues preferentially express one transporter isoform, i.e., erythrocytes (MCT1), eye (MCT1),...
and liver (MCT2), whereas others express both at relatively high levels.

The primary focus of this study was, however, the investigation of this potential differential expression of MCT1 and MCT2 mRNAs in mouse brain. Whereas MCT1 mRNA has been detected in mouse (26), rat, and human brain (19, 28, 33), MCT2 mRNA is barely detectable in rat brain (19) and not at all in human brain (28). We report here that MCT1 is significantly expressed in mouse brain, in neurons, as well as in choroid plexus and the ependymal lining of the cerebral ventricles, cerebral microvessels, and white matter tracts such as the corpus callosum. These results are consistent with studies in the rat by Takanaga et al. (33), who described the presence of MCT1 mRNA in whole brain and isolated brain capillaries, and a preliminary report of MCT1 in mouse brain, with a primarily neuronal location (26). With the use of immunohistochemical methodologies in rat brain, MCT1 protein has been detected in brain microvessels, neuropil, ependyma, and glial-limiting membranes (12). In the pres-

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1 While this article was under review, a paper was published describing the expression of MCT mRNAs in mouse brain (L. Pellerin, G. Pellegri, J.-L. Martin, and P. J. Magistretti. Proc. Natl. Acad. Sci. USA 95: 3990–3995, 1998).
In the choroid plexus of adult animals and glial elements of the corpus callosum in adult brain, we also detected MCT1. MCT2 appears to be equally expressed by both Purkinje and granule cells. These results suggest that, whereas both transporter isoforms may be present in the same region and perhaps even in the same cells, they likely subserve distinct functions.

Glucose is the major metabolic fuel used by the brain, but alternative substrates, such as ketone bodies and monocarboxylates, can be utilized when present in sufficient quantities. Circulating levels of ketone bodies in the adult animal are low; however, they may become greatly increased during starvation, diabetes, prolonged exercise, and high fat intake (13–16, 25, 39). In the postnatal suckling animal, circulating levels of ketone bodies are extremely high and provide up to 50% of the cerebral energy requirements (15, 17, 35). Despite the ability of the brain to metabolize ketone bodies, early studies demonstrated regional specificity in ketone body and glucose utilization. In particular, although glucose utilization in the cortex is relatively uniform, uptake of 3-hydroxybutyrate is greater in the lower cortical layers (14, 15), a pattern consistent with the cortical layering of MCT1.

The other substrates transported by this family of proteins (MCTs) are lactate and pyruvate. Both of these substrates are capable of maintaining synaptic potentials and hence neuronal function in vitro hippocampal slice preparations under conditions of glucose deprivation (18, 30, 31). Additionally, cultured neurons, astrocytes, and oligodendrocytes have the capacity to transport and utilize lactate, pyruvate, and ketone bodies (8, 32, 38). These studies have also indicated that lactate transport is saturable, proton dependent, and inhibited by cinnamates (1, 7, 22, 34), all of which are characteristics of the MCT (reviewed in Ref. 27).

It is important to consider that the MCTs are responsible for the transport of these different substrates, which may serve different metabolic functions. The delivery of ketone bodies from the circulation to the brain provides a critical alternative substrate during development and starvation and relies on the expression of the appropriate transporter in the microvessels of the blood-brain barrier. It is of interest that the highest expression of MCT1 in the microvessels has been reported during suckling in the rats (12, 26, 37) and declines with weaning. This observation, combined with the cortical specificity of MCT1 expression, supports the notion that this isofrom may be primarily responsible for ketone body transport during times of active utilization. The relatively constant detection of both MCT1 and MCT2 in the neural elements on the other side of the blood-brain barrier would suggest that, in these cells, the transporters may indeed be more involved with the recycling of lactate between glia and neurons, as has been suggested by Magistretti et al. (21). However, this level of intracellular trafficking would require significant expression of a glial MCT, and our studies do not support sufficient glial expression for either MCT1 or MCT2. The recent report of Price et al. (28) of four additional MCT isoforms, of which MCT6...


