Monocarboxylate transporter expression in mouse brain

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1Experimental Diabetes, Metabolism and Nutrition Section, Diabetes Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892; 2Pennsylvania State University, Hershey Medical Center, Hershey, Pennsylvania 17033; and 3University of Texas, Southwestern Medical Center, Dallas, Texas 75235-8591

Koehler-Stec, E. M., I. A. Simpson, S. J. Vannucci, K. T. Landschulz, and W. H. Landschulz. 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 exhibited 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 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 monocarboxylates (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-ZAPrt 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. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
cells. Approximately 1 x 10^6 plaques were screened by transfer to nitrocellulose-coated positively charged nylon filters (Magna-Plus, MSI). The filters were probed with 32P-labeled oligonucleotide primed fragments of pMCT1 and pMCT2 from Chinese hamster ovary cells and Syrian hamster 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) overnight in 4 h. Filters were washed four times (15 min each) at 55°C in 2x SSPE (1x SSPE is 0.15 M NaCl, 0.01 M Na2HPO4, and 0.001 M EDTA, pH 7.4). For 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 generated 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 sequenator. Probes for mMCT1 and mMCT2 were taken from domains 6 (19, 20, 28, 33, 40), both mouse MCTs (mMCT1 and mMCT2) have twelve putative membrane-spanning regions as predicted by Kyte-Doolittle analysis (Figs. 1 and 2). Consistent with previously cloned hamster MCTs (9, 10) and other members of the same superfamily (4, 19, 20, 28, 33, 40), both mouse MCTs (mMCT1 and mMCT2) have twelve putative membrane-spanning regions as predicted by Kyte-Doolittle analysis (Figs. 1 and 2). There is 67% similarity (58% identity) between the two mouse isoforms in terms of their amino acid sequences (Fig. 3). The putative membrane-spanning regions are the most homologous, whereas the COOH terminus and the sequence between transmembrane domains 6 and 7 are the least. Mouse MCT1, a 493-amino acid protein, is ~88% homologous to previously cloned rat and hamster MCT1 and ~80% homologous to human MCT1. It appears to have the same sequence as the MCT1 recently cloned by Carpenter et al. (4) from Ehrlich Letteur tumor cells. Mouse MCT2 (484-amino acid protein), on the other hand, is 82 and 76% homologous to rat and hamster sequences, respectively.

Northern blot analysis of RNAs obtained from a survey of murine tissues revealed that mMCT1 and mMCT2 have distinct patterns of expression (Figs. 4 and 5). Expression of mMCT1 is ubiquitous in the mouse, with an ~3.0-kb mRNA detectable in every tissue tested (Fig. 4). This size message closely approximates the 3.1-kb mMCT1 cDNA. Some tissue-specific differences in mMCT1 abundance are observed, with higher levels of message in eye, red muscle, large intestine, kidney, spleen, and heart and very low levels in adrenals, white muscle, lung, and liver. Mouse MCT1 message is moderately abundant in brain. In contrast, duplicate blots hybridized with mMCT2 probe revealed a more restricted pattern of expression (Fig.
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. 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.

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.
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. 6A).

Fig. 4. Tissue distribution of mouse MCT1 mRNA. Total RNA (20 µg) from tissues of mouse strain 129 was prepared as described in MATERIALS AND METHODS and subjected to size fractionation on a 1% agarose-2% formaldehyde gel. Top: autoradiogram of blot hybridized with a 380-bp 32P-labeled mouse MCT1 DNA probe. Bottom: autoradiogram of same filter stripped and reprobed with a 32P-end-labeled oligonucleotide corresponding to 28S rRNA. Sm, small; Lg, large.
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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1While 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 cerebellum, MCT1 is highly expressed in the Purkinje cell layer, perhaps including Bergman glia; 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 isoform 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...
and MCT7 are the most highly expressed in human brain, may provide the answer as to which MCT isoform(s) is glial.

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