Cloning of the monocarboxylate transporter isoform MCT2 from rat testis provides evidence that expression in tissues is species-specific and may involve post-transcriptional regulation

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The cDNA for the monocarboxylate transporter MCT2 from rat testis has been cloned and sequenced. The derived protein sequence shows 82% identity with that from hamster. Rat MCT2 has a relative insertion of five amino acids in the N-terminal sequence preceding the first predicted transmembrane segment. MCT2 appears to be less highly conserved between species than MCT1. Using Northern blotting of RNA from rat and mouse tissues, MCT2 message was demonstrated to be abundant in the testis where a smaller, less abundant MCT2 transcript was also present. Low levels of a slightly different-sized transcript were found in rat and mouse liver, and mouse kidney. In hamster, only one-size transcript was detected at relatively high abundance in all the tissues examined. Antibodies were raised against a peptide derived from the extreme C-terminus of rat MCT2, and Western blotting with these detected MCT2 in membrane fractions prepared from rat testis, liver and brain but not those from heart or skeletal muscle. In hamster, MCT2 was detected in liver, heart and testis but not in brain [Garcia, Brown, Pathak, and Goldstein (1995) J. Biol. Chem. **270**, 1843–1849]. For both rat MCT1 and MCT2 there were marked differences between the relative abundance of their respective messages and the amount of protein in membrane fractions from different tissues. This suggests that expression of both of these transporters in different tissues may be species-specific and regulated post-transcriptionally. The different-sized MCT2 transcripts may arise from alternative splicing. Starvation of rats for up to 48 h did not lead to any change in MCT1 or MCT2 expression in the liver, as determined by either Northern or Western blotting.

INTRODUCTION

Evidence for a family of *m*ono*c*arboxylate *t*ransporters (MCTs) came initially from kinetic studies of lactate transport in various tissues [1] and has been confirmed by the cloning and expression of two MCT isoforms. MCT1 was cloned from Chinese hamster ovary (CHO) cells by Garcia et al. [2], and cDNA for MCT2 was subsequently isolated from a Syrian hamster liver library [3]. Since the majority of kinetic and functional studies of lactate transport have been performed in rat tissues, we have cloned MCT1 from rat [4], and used the sequence to generate anti-peptide antibodies [5] with which to investigate its topology [5], tissue distribution and regulation (see for example [6]). In the course of these studies we found that rat liver expressed a large amount of MCT1. This is in marked contrast with hamster liver where the absence of MCT1 provided the rationale for using hamster liver libraries to screen for a new isoform [3]. The presence of MCT1 in rat liver was of particular interest since we had recently performed a detailed kinetic characterization of monocarboxylate transport in the rat liver [7], and found that some characteristics of transport differed from those described for hamster MCT2 expressed in insect cells. This prompted us to investigate the tissue expression of MCT2 in rat using anti-peptide antibodies.

We raised antibodies against three different peptides derived from the sequence of the C-terminus and the loop between transmembrane segments 11 and 12 of hamster MCT2. The latter sequence was chosen because the equivalent region is absolutely conserved in all of the MCT1 sequences published thus far, namely Chinese hamster [8], human [9], mouse [10] and rat [4]. Thus it was predicted that this region might also be conserved in MCT2 between species. However, these antibodies all failed to cross-react with rat MCT2 in Western blots of plasma-membrane preparations from any rat tissue, including liver, heart, erythrocytes and testis, despite giving a strong 43 kDa band with hamster liver membranes. This suggested that either MCT2 is not expressed in rat or, more likely, that the sequence is sufficiently different to prevent recognition by the antibodies raised against peptides derived from the hamster sequence. The former possibility seemed unlikely since we used a cDNA probe derived from hamster MCT2 to demonstrate by Northern blotting the presence of abundant MCT2 mRNA in rat testes. Thus we set about cloning and sequencing cDNA for rat MCT2 by screening a rat testis cDNA library.

In this paper we report the rat MCT2 sequence and present data on its tissue distribution as determined by both Northern blotting in rat, hamster and mouse tissues and by Western immunoblotting in rat and hamster. Our data show that MCT2 distribution in hamster is quite different from that in rats and mice. Furthermore, in tissues from the latter two species, different-sized MCT2 transcripts were observed, whereas only one size was seen in the hamster tissues examined. The relative

Abbreviations used: MCT, monocarboxylate transporter; KpNPPase, K^+-activated *p*-nitrophenyl phosphatase; poly(A)$^+$, polyadenylated.

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The nucleotide sequence of rat MCT2 cDNA has been deposited with EMBL, GenBank and DDBJ Sequence Databases under the accession number X97445.

levels of mRNA and protein for both MCT1 and MCT2 in different rat tissues were found to show little correlation, suggesting that, in rat, expression of both of these transporters may be subject to post-transcriptional regulation.

EXPERIMENTAL

Materials

Materials were obtained from the sources described previously [4,5], with the following additions. The rat testes cDNA library was from Stratagene (La Jolla, CA, U.S.A.). TRIzol reagent for total RNA preparation was from Gibco–BRL (Paisley, Scotland U.K.), and PolyATract for isolation of the polyadenylated [poly(A)$^+$] fraction was from Promega (Southampton, Hants., U.K.). The mouse multitissue Northern blot was from Clontech. The probe used for screening the cDNA library was the coding region of hamster MCT2 [3] labelled with [α-^{32}P]dCTP by random priming using a Multiprime DNA-labelling kit (Amersham International, Aylesbury, Bucks, U.K.) according to the manufacturer's instructions.

Molecular biology

Screening of the cDNA library, isolation and sequencing of clones were performed as described by Jackson et al. [4]. Total RNA was prepared from rapidly frozen tissues, powdered under liquid nitrogen, using TRIzol reagent (1 ml per 50–100 mg of tissue) according to the manufacturer's instructions. The integrity and concentration of the RNA was determined by agarose-gel electrophoresis of a small aliquot. Poly(A)$^+$ mRNA was extracted from total RNA using the PolyATtract mRNA isolation system according to the manufacturer's instructions. Northern blotting of mRNA was performed using standard procedures as outlined in the Figure legends. Antibodies against keyhole limpet haemocyanin-conjugated peptides corresponding to a C-terminal region of MCT1 (PQQNSSGDPAEEESPV) or MCT2 (NTHNPPSDRDKESSI) were raised in New Zealand White rabbits and purified by affinity chromatography as described by Poole et al. [5].

Preparation of plasma-membrane fractions from various rat tissues

A rapid Percoll gradient technique based on that described previously was used to prepare crude plasma membranes [11,12]. The desired tissue was dissected from the animal, rinsed in PBS and then placed into 50 ml of ice-cold homogenization buffer (0.3 M sucrose, 2 mM EGTA, 10 mM Tris/HCl, pH 7.2) supplemented with 0.5% (w/v) BSA and the following protease inhibitors: 0.5 mM benzamidine, leupeptin (4 μg/ml), pepstatin (4 μg/ml), antipain (4 μg/ml), 0.5 mM PMSF. The tissue was then homogenized using either a Potter homogenizer (most tissues) or Polytron (skeletal muscle). After centrifugation at low speed (2000 ***g*** for 10 min at 4 °C), the supernatant was transferred to a clean tube and centrifuged at 39000 ***g*** for 15 min at 4 °C. The resultant pellet was resuspended in ice-cold homogenization buffer (final volume 10 ml) and then mixed with 2.33 ml of Percoll before centrifugation at 17000 ***g*** for 10 min at 4 °C to separate out the plasma membranes. These were present in the upper layer which was transferred to a fresh tube and, after addition of 50 ml of homogenization buffer, sedimented by centrifugation (48000 ***g*** for 30 min at 4 °C) to remove Percoll. The membranes were then washed twice with 40 ml of homogenization buffer and finally resuspended in a small volume (typically 0.5–1.0 ml) of the same buffer. The protein concentration was determined using the method of Bradford [13]. The extent of purification of the plasma-membrane fraction was determined from measurements of the ouabain-sensitive K^+-activated *p*-nitrophenyl phosphatase (KpNPPase) activity as described by Ploug et al. [14].

RESULTS AND DISCUSSION

In order to identify a suitable source from which to isolate the cDNA for rat MCT2, Northern blotting was performed using poly(A)$^+$ RNA prepared from a number of tissues for which cDNA libraries were available.

Northern blotting of MCT1 and MCT2 RNA from rat, hamster and mouse tissues

The Northern blot of RNA from various rat tissues was probed with ^{32}P-labelled DNA corresponding to the coding region of hamster MCT2 and data are shown in Figure 1 (centre). It should be noted that, for each tissue, the mRNA loaded was that corresponding to 5 μg of total RNA from the tissue. MCT2 transcript is abundant in the testis, where the signal appears as a doublet, with the more slowly migrating species much more abundant than the other form. A weaker signal could also be detected in liver. A very faint band (indicated by an arrow) was also just visible for brain. The brain and liver messages appear to be slightly different in size from one another, and from those in testis. MCT2 transcripts could not be detected in rat heart or skeletal muscle. The size of the major rat MCT2 transcript in

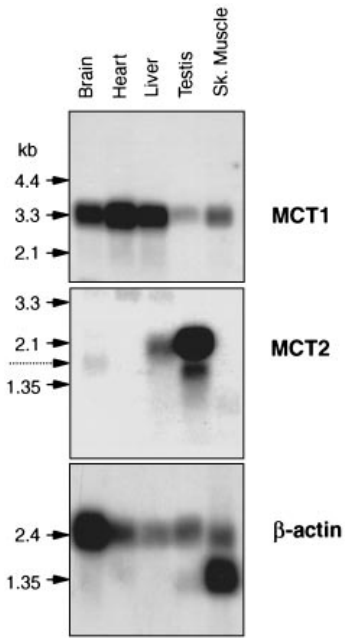

Figure 1 Northern blotting of MCT1 and MCT2 in various rat tissues

Total RNA was prepared from the indicated tissues using TRIzol reagent. Poly(A)$^+$ RNA was prepared from equal amounts of total RNA (as judged by staining of rRNA bands after electrophoresis), and the denatured poly(A)$^+$ RNA (equivalent to that derived from 5 μg of total RNA) was subjected to electrophoresis on a 1.1% (w/v) agarose/formaldehyde gel. The RNA was then transferred to a nylon membrane. The blot was probed with ^{32}P-labelled MCT2 (the coding region of hamster MCT2) (centre). After washing, the blot was subject to autoradiography. After stripping off of the probe, the blot was reprobed with a β-actin probe (bottom panel), and finally with a probe for MCT1 (the coding region of CHO MCT1) (top). Quantitative anlaysis of the data is given in Table 1. Sk., skeletal.

Table 1 Quantification of Northern and Western blots of rat MCT1 and MCT2

The Northern blots of Figure 1 and the Western blots of Figure 6 were analysed using a phosphorimager and quantitative densitometry respectively. Ouabain-sensitive KpNPPase activity was measured in crude homogenates and purified membrane fractions as described in the Experimental section (each fraction in triplicate). The relative MCT expression per g wet weight of tissue was calculated from the KpNPPase activity in the two fractions. Where two bands were visible on the Northern blots the intensities of both are given separated by a slash. Values in parentheses represent expression relative to that seen in liver which is set to one.

	Northern-blot intensities (arbitrary scanner units)			MCT/actin intensity ratios		Western-blot intensities (arbitrary scanner units)		KpNPPase activity in membranes	MCT intensity/KpNPPase ratios		Calculated MCT intensity/mg of tissue	
Tissue	MCT1	MCT2	Actin	MCT1	MCT2	MCT1	MCT2	(nmol/h per mg)	MCT1	MCT2	MCT1	MCT2
Heart	69241	0	10929	6.33 (1.1)	0	62194	0	403	154	0	10.8 (0.19)	0
Skeletal muscle	16007	0	10218/30069	1.57 (0.27)	0	0	0	533	0	0	0	0
Liver	47104	3461	8189	5.75 (1.0)	0.42 (1.0)	102307	67851	533	185	123	58.3 (1.0)	38.6 (1.0)
Brain	36628	1391	37915	0.97 (0.17)	0.04 (0.10)	0	58221	13230	0	4.4	0	28.6 (0.74)
Testis	12223	82899/4845	9990	1.22 (0.21)	8.11/0.48 (19.3/1.14)	98863	59989	557	178	108	33.9 (0.58)	20.6 (0.53)

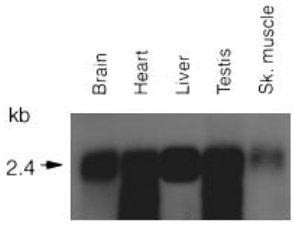

Figure 2 Northern blotting of MCT2 in Syrian hamster tissues

Poly(A)$^+$ RNA was prepared from the same tissues as examined in rat (Figure 1). A blot was prepared as described in the legend of Figure 1, and probed with the same labelled MCT2 probe. The blot was verified to contain approximately equal loadings relative to the signal obtained by reprobing with labelled β-actin probe (not shown). Sk., skeletal.

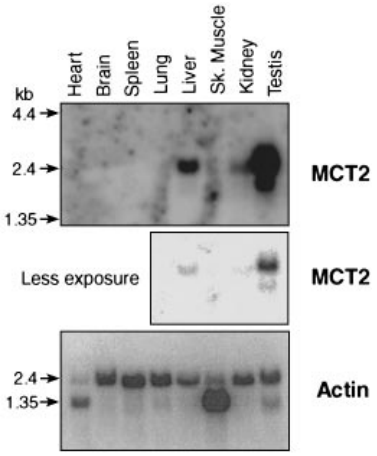

Figure 3 Northern blotting of MCT2 in mouse tissues

A blot containing approx. 2 μg per lane of poly(A)$^+$ RNA from the indicated mouse tissues was obtained from Clontech and probed with ^{32}P-labelled MCT2 as described in the legend to Figure 1. The top panel shows an overexposed autoradiograph to enable visualization of weak bands, whereas the middle panel shows the data from the phosphorimager which allows better discrimination of band mobilities. The blot was stripped and reprobed with labelled β-actin probe (bottom) as described in Figure 1. Sk., skeletal.

testis is approx. 2.3 kb as judged by the migration off RNA standards in the non-denaturing gel (results not shown).

After removal of the MCT2 signal, the blot was reprobed with a labelled fragment of β-actin cDNA (Figure 1, bottom). Expression of β-actin has been shown to be a good marker for total tissue RNA [15]. The blots with the actin probe showed that mRNA loading was similar for all tissue samples except for the brain sample which appeared to contain considerably more mRNA. Despite this, MCT2 message was only just detectable. The data also confirmed that the RNAs extracted from brain, heart and muscle were intact and that the low level of MCT2 in rat liver was not the result of insufficient loading of liver RNA.

The same blot was again stripped and probed for MCT1 message using a probe corresponding to the coding region of rat MCT1 (Figure 1, top). The MCT1 message is present in all five tissues, being particularly abundant in heart, and of low abundance in testis and skeletal muscle. The transcript is of the same size in all tissues examined. Similar results were found by Kim et al. [8] in rat tissues, but in hamster they found MCT1 transcript to be absent from both liver and kidney, whereas in rat high expression was found in both tissues. In Table 1 we have analysed the data of Figure 1 using a phosphorimager to allow the differences in expression between the tissues to be quantified.

Our results suggest that MCT1 and MCT2 may have a different tissue distribution in hamsters and rats. To our knowledge the relative level of MCT2 message in hamster tissues has not been examined previously. Thus Northern blotting of RNA from brain, heart, liver, skeletal muscle and testis of the Syrian hamster was performed, again using the hamster cDNA probe. MCT2 message was found to be abundant in all of these tissues, and was detected as a single band of same size in all of the tissues examined, including testis (Figure 2). The reason for the smearing of the signal from heart and testes is unclear. The same phenomenon was seen with RNA from an independent preparation (not shown), and thus may represent the presence of partially degraded message, perhaps caused by rapid turnover of the MCT2 transcript in these tissues. Alternatively this may simply be due to degradation that occurred during isolation of the poly(A)$^+$ RNA. To explore further this difference in expression of MCT2, a multitissue Northern blot (Clontech) prepared from mouse tissues was probed (see Figure 3). As in rat, a doublet was detected in the testis. MCT2 message was also present in liver and a transcript of the same size was detected at

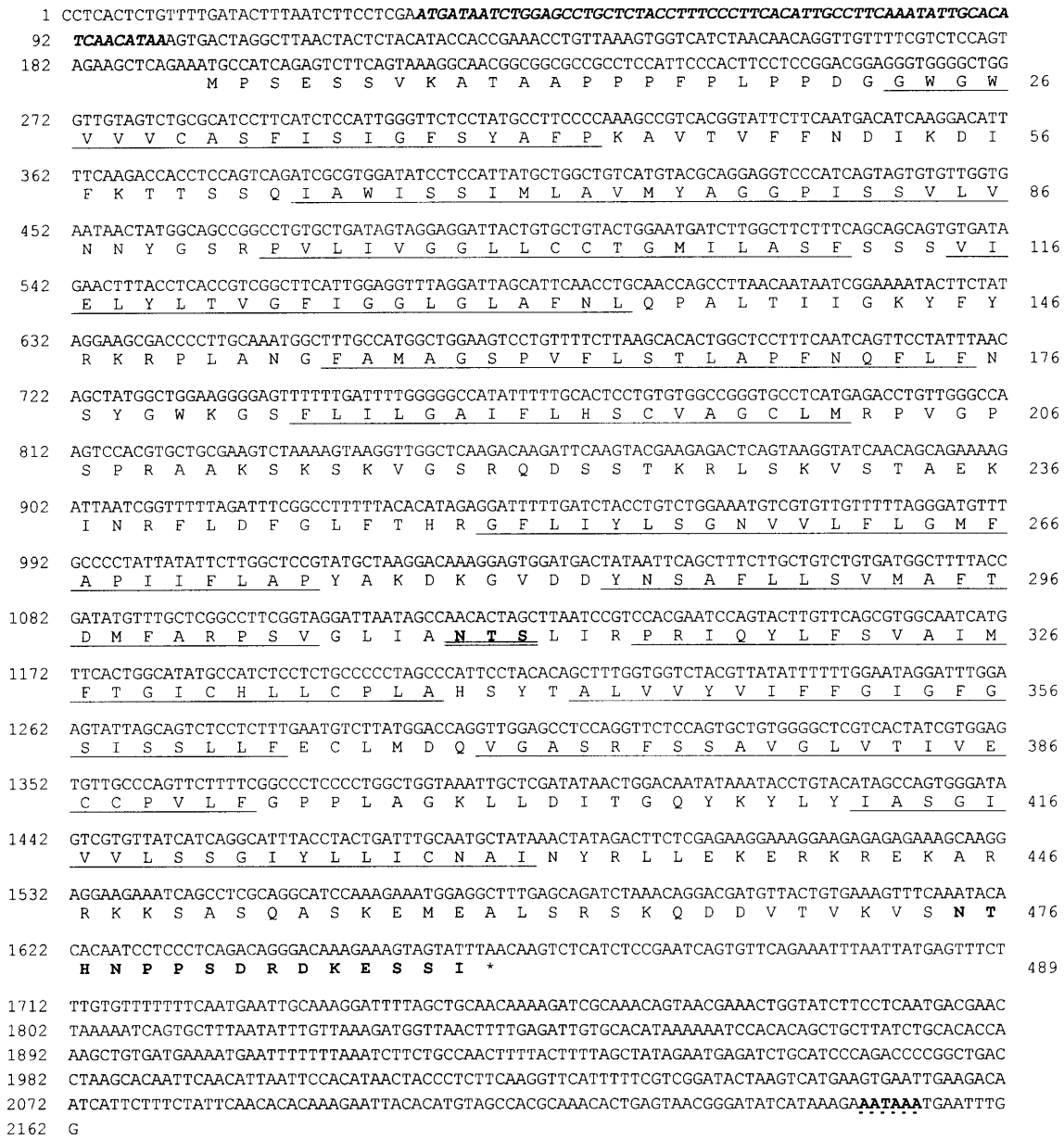

Figure 4 Nucleotide and deduced amino acid sequences of rat MCT2 cDNA

Numbers on the left refer to nucleotides, and those on the right to the amino acids. Underlined regions of the protein sequence denote predicted transmembrane regions, assigned on the basis of hydrophobicity [16]. The putative poly(A)$^+$ signal (AATAAA) is indicated by the dotted line. The putative site for N-linked glycosylation (NTS) is indicated by double underlining. The upstream open reading frame in the 5′ untranslated region is shown in *italics*. The C-terminal sequence on which the synthetic peptide used to raise antibodies was based is shown in bold.

a low level in kidney. The liver and kidney transcripts differed in size from both those present in testis. As in rat, but unlike hamster, MCT2 message was not detected in mouse heart or skeletal muscle. Neither was a signal seen in mouse brain.

The data presented in Figures 1–3 suggest that MCT2 may be differentially transcribed in rat, mouse and hamster tissues, and that the high levels of MCT2 transcript in many tissues of the hamster may be atypical. Furthermore different-sized MCT2 transcripts are seen in rat and mouse tissues, whereas only one-sized message is seen in the hamster tissues examined. Although Northern blots for all three species were probed with the hamster MCT2 cDNA, it is unlikely that some of the minor bands of different sizes seen in rat and mouse represent cross-hybridizing MCT isoforms, since additional higher stringency washes did not alter the relative signal from the different bands (results not shown).

Cloning of cDNA for rat MCT2

Since the rat MCT2 message was found to be most abundant in the testis, a rat testis cDNA library (Stratagene) was screened using a probe corresponding to the coding region of the hamster cDNA. A large number of positive clones were identified and a number of these were plaque purified. Insert sizes were determined by PCR using T3 and T7 primers, and the largest were excised into the pBluescript plasmid using helper phage. The

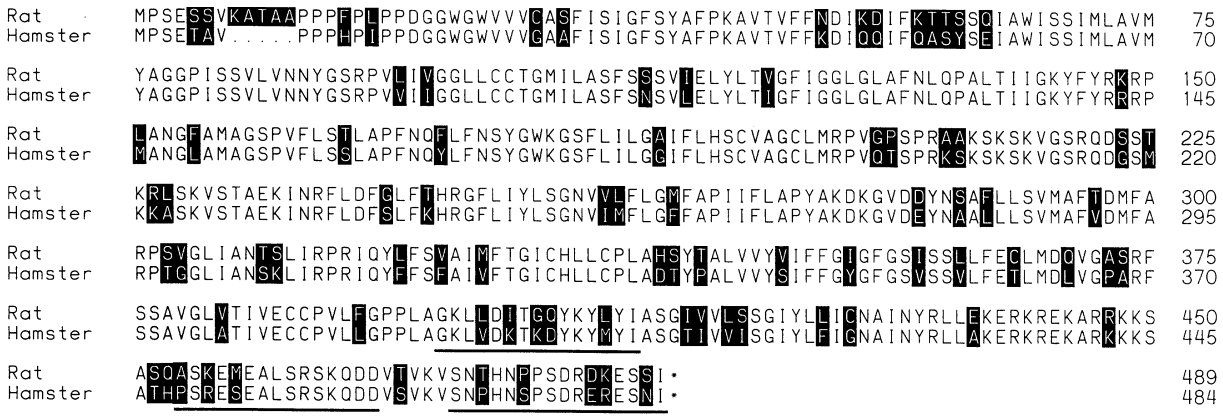

Figure 5 Alignment of the protein sequences of rat and hamster MCT2

Residues that are non-identical in both sequences are highlighted. Regions of the hamster protein sequence from which synthetic peptides were derived for generation of anti-peptide antibodies are indicated.

inserts were sequenced with flanking T3 and T7 primers. A single cDNA that appeared to encode rat MCT2 was sequenced on both strands using custom primers.

The complete nucleotide sequence of rat MCT2 cDNA and its deduced amino acid sequence are presented in Figure 4. This sequence has been deposited in the EMBL DNA sequence database with accession number X97445. The sequence contains an open reading frame encoding 489 amino acids which predicts a polypeptide of 53 kDa. The 2162 bp-long cDNA contains 193 bp of 5′ non-coding sequence and 499 bp of 3′ non-coding sequence. There is an upstream open reading frame (nucleotides 35–100) encoding a predicted 21-amino acid peptide within the 5′ sequence. This open reading frame is in-frame with the proposed coding sequence for MCT2. However, the two sequences are separated by four termination codons. There is a consensus poly(A)$^+$ signal adjacent to the poly(A) tail (AATAAA, nucleotides 2148–2153; see Figure 4), which is also present in the same position in the aligned hamster nucleotide sequence (not shown). The rat MCT2 nucleotide sequence has 84% identity with that from hamster. This identity extends throughout the sequence, with the aligned cDNA sequences having 3′ ends of similar length. The rat MCT2 sequence has an additional 75 bp at the 5′ end compared with the hamster sequence, which suggests that the published hamster cDNA sequence may not be full-length. This region of the rat sequence contains the start codon for the upstream open reading frame.

Comparison of the rat and hamster protein sequences (Figure 5) reveals that the proteins are 82% identical (90% similar). This degree of identity is lower than that between rat and (Chinese) hamster MCT1 (94% identity; 97% similarity). The MCT2 sequences are identical in length, with the exception of a five-residue insertion in the N-terminus of the rat transporter. MCT2 actually shows higher conservation at the nucleotide compared with the protein level, whereas for MCT1 the converse is true. This, together with the lower degree of protein sequence conservation between species, may indicate that MCT2 is subject to less functional constraint by sequence variations than is MCT1. In the C-terminal region and the loop region between transmembrane segments 11 and 12 region the rat and hamster sequences show significant differences, and this explains the failure of anti-peptide antibodies raised against the hamster sequence to cross-react with rat MCT2. In contrast, the MCT1 sequence in the loop region is conserved between species (see the Introduction).

A search of the EST database for sequences closely related to MCT2 revealed a single EST clone isolated from a human skeletal-muscle library (accession number F01173), which is likely to derive from a truncated human MCT2 cDNA. The derived protein sequence corresponds to transmembrane helices 5 and 6, and most of the large loop between transmembrane segments 6 and 7. Although this sequence is derived from a single sequence run on one strand only, it again suggests that MCT2 is less highly conserved than MCT1 (65% identity and 79% similarity between rat and human MCT2 protein sequences compared with 83% identity and 87% similarity in the same region for MCT1 in these species; alignment not shown). It may also be significant that a parallel search of the EST database for sequences closely related to MCT1 revealed 29 sequences from 11 different tissues. This suggests that human MCT2, unlike MCT1, may not be expressed abundantly in many tissues, and provides further evidence that hamster may be atypical in its wide tissue distribution of MCT2.

The rat MCT2 protein sequence contains a putative N-linked glycosylation site (Asn-Thr-Ser; see Figure 4). However, based on hydrophathy plots, this region is predicted to be in a small loop between transmembrane helices 8 and 9 on the cytoplasmic face. N-linked glycosylation typically occurs on residues that are exposed to the extracellular fluid at least 12 amino acids away from the membrane and within loop regions of 30 amino acids or more [17]. Thus is appears that this asparagine residue is a poor candidate for glycosylation, a conclusion strengthened by the absence of this motif in the hamster sequence (see Figure 5).

Examination of the tissue distribution of MCT1 and MCT2 in rat by Western blotting

Garcia et al. [3] have measured the distribution of MCT1 and MCT2 in membrane fractions prepared from various hamster tissues using Western blotting. The tissues expressing MCT2 in significant amounts were heart, liver, kidney, testis, epididymis, stomach and skin, while MCT1 was expressed in lung, heart, epididymis, caecum, eye and erythrocytes. These workers also performed a more detailed immunohistochemical study of the

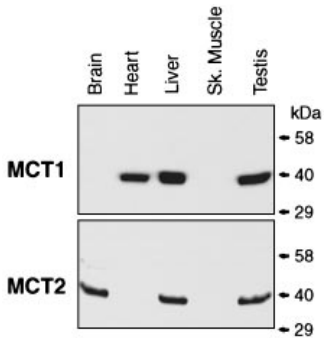

Figure 6 Expression of MCT1 and MCT2 in various rat tissues as determined by Western immunoblotting

Membrane extracts were prepared from homogenized rat tissues in the presence of protease inhibitors. Aliquots of the membrane proteins (20 μg) were electrophoresed on an SDS/10% polyacrylamide gel, transferred to PVDF membrane and probed with affinity-purified anti-MCT1 (top) or anti-MCT2 IgG (bottom). The antibody was detected by enhanced chemiluminescence and the blots exposed to autoradiography film for 2 min as described [5]. Quantitative analysis of the data is given in Table 1. Sk., skeletal muscle.

distribution of the isoforms within these tissues. We have raised and affinity-purified antibodies to both MCT1 and MCT2 (see the Experimental section) in order to perform similar studies of the two isoforms in rat tissues (Figure 6). We used an alternative membrane preparation protocol to that of Garcia et al. [3] since we found their methodology often led to the formation of MCT dimers. This is apparent in the blots shown by these authors and something that we have observed previously for MCT1 from rat erythrocytes under some conditions [5]. However, we confirmed that both methods gave the same relative tissue abundances of the two MCT isoforms (results not shown).

The anti-peptide antibody raised against residues 475–489 of the rat MCT2 sequence (see Figure 5) detected a band of 40 kDa on immunoblots of plasma-membrane fractions prepared from several rat tissues (Figure 6). This band is specifically diminished by immunoblotting in the presence of peptide (not shown). The predicted molecular mass of MCT2 is 53 kDa, but aberrant migration of membrane proteins on SDS/PAGE is a common feature. Indeed rat MCT1 has the same predicted molecular mass of 53 kDa, yet is detected as a 43 kDa protein in rat tissues (Figure 6). MCT2 could be detected in membrane fractions prepared from rat testis, liver and brain but not those from heart or skeletal muscle (Figure 6). In hamster, Garcia et al. [3] detected MCT2 in liver and testis as we have in rat, but unlike the rat it was not present in brain. In contrast, these workers found MCT2 to be abundant in the heart, but we were unable to detect this isoform in rat heart. Differences are also seen between rat and hamster for the distribution of MCT1 within these tissues. MCT1 is expressed in the heart and testis of both rat and hamster, but in liver only in the rat (Figure 6 and [3]). Western blots show no evidence for MCT1 or MCT2 in skeletal muscle of either species, although immunohistochemistry shows the presence of small amounts of both isoforms in oxidative fibres of hamster gastrocnemius muscle [3]. Our Western blots show the presence of MCT2 in brain plasma membranes, but not MCT1. In contrast, Garcia et al. [3] detected neither isoform in plasma membranes from hamster brain by Western blotting.

The different patterns of expression of MCT1 and MCT2 in hamster and rat tissues may be due to metabolic differences between these two animals, especially if the properties of MCT1 and MCT2 are adapted to distinct metabolic roles. Detailed kinetic characterization of monocarboxylate transport in erythrocytes and tumour cells has been carried out and probably represents transport mediated by MCT1 alone [1,18]. We had assumed that detailed studies of the kinetics of monocarboxylate transport into isolated rat liver cells provided sufficient characterization of MCT2 kinetics [7], but the data of Figure 6 show this is not the case since these liver cells express both MCT1 and MCT2. Accurate kinetic characterization of both isoforms will require heterologous expression of the individual cDNAs in cells that possess a low background of monocarboxylate transport activity. To date such characterization of MCT1 and MCT2 has been limited to a rudimentary demonstration of monocarboxylate transport by the expressed MCT and its inhibition by a range of inhibitors [2,3].

Correlation of relative levels of mRNA and protein for MCT2 and MCT1 in different tissues

Quantification of the expression of MCT1 and MCT2 in the plasma-membrane fractions of different rat tissues was performed by densitometric analysis of the blots shown in Figure 6. In parallel the KpNPPase activity of the membrane fractions was measured and results of both are summarized in Table 1. These data, in conjunction with measurements of the KpNPPase activity in crude homogenates, allowed an estimate to be made of the relative expression of the two MCT isoforms per g wet weight in the different tissues. Data are summarized in Table 1 where data for the Northern blots of Figure 1 are also summarized. In both cases, for ease of comparison, data are also given in parentheses for MCT1 and MCT2 mRNA and protein expression relative to that in liver (set to 1). It was somewhat surprising to find similar amounts of the MCT2 protein in plasma membranes prepared from brain and liver to that found in plasma membranes prepared from testis, when the relative amounts of message detected by Northern blotting were very different. Thus MCT2 mRNA was highly abundant in testis, at a relatively low level in liver, and barely detectable in the brain (see above and Figure 1). Since the MCT2 message appears to be a slightly different size in these three tissues, with two sizes of mRNA detectable in testis, it is possible that alternatively spliced forms of the mRNA may be present. As the protein detected is of the same size in all tissues, the splicing must either occur outside the coding region or, if within the coding region, produce proteins with very similar migration on SDS/PAGE. It is possible that the more abundant mRNA species in testis encodes MCT2 that lacks the C-terminal epitope, and thus only the protein resulting from the smaller message with similar abundance to that in liver is detected by Western blotting. However, this seems unlikely since we sequenced eight MCT2 clones from the testis cDNA library, and all had the same 3′ ends (which included the region encoding the epitope to which the antibody was raised). It is perhaps more likely that different variants are transcribed in different tissues and allow some differential regulation of expression of the active transporter. Thus the different MCT2 messages may be translated with different efficiencies, or may be subject to different post-transcriptional regulation in different tissues. For example, the upstream open reading frame present in the testis mRNA (see above) could result in a lowered translational efficiency [19]. This open reading frame may be absent in the MCT2 message from other tissues, or its inhibitory properties may be overcome by some mechanism.

In the Syrian hamster only a single size of transcript was detected on Northern blots (Figure 2) and here the level of transcript (Figure 2) correlates better with the amount of protein detectable in the corresponding tissue [3]. However, there remain

apparent discrepancies. For example, the amount of protein detected in testis relative to that in heart and liver is considerably greater than would be predicted from the amount of RNA detected, which is similar for the three tissues. Conversely very little MCT2 protein is seen in hamster brain, whereas the message appears to be relatively abundant.

There are also clear differences between the relative amounts of MCT1 mRNA and detectable protein in different rat tissues as summarized in Table 1. For example, much lower levels of message are present in testes than in heart, and yet the proteins show approximately the same relative abundance. In contrast, MCT1 message is readily detectable in skeletal muscle and brain, yet no protein was detected in these tissues. It is possible that with relatively complex tissues such as the brain, regions rich in MCT1 were present in the total homogenate used to prepare RNA, but poorly represented in the crude membrane preparation used for Western Blotting. Alternatively, it may be that pools of MCT1 mRNA are stored in mRNPs, allowing rapid translation when more MCT1 transporter is needed. Indeed, the extremely long 3′ untranslated region of MCT1 [2,4] may be involved in translational repression. In contrast with MCT2, our data do not suggest alternative splicing of MCT1 mRNA, since, on Northern blots, only one size of MCT1 transcript was detected. In addition, for the rat, cDNA sequences are available for MCT1 from skeletal muscle [4] and intestine [20] and these are almost identical. Furthermore the structure of the human MCT1 gene has been determined [9], and again there is no evidence for differential splicing.

Starvation does not increase expression of either MCT1 or MCT2 in rat liver

It has been reported by other groups that the maximal rate of lactate transport into rat hepatocytes increases on starvation [21–23], although our own studies have failed to detect this (V. N. Jackson and A. P. Halestrap, unpublished work). Indeed the prevailing evidence suggests that lactate transport exerts minimal control over lactate metabolism in the liver [1,24,25]. We have also been unable to detect any consistent increase in either MCT1 or MCT2 mRNA in the liver after either 24 or 48 h starvation of rats or hamsters (results not shown). In case there was some form of translational control of MCT1 or MCT2 expression, we also performed quantitative Western blots of partially purified plasma-membrane fractions of livers from control and 48 h-starved rats. For four separate preparations of liver plasma membranes, each from a different 48 h-starved rat, the values (means $\pm$ S.E.M.) of the integrated densities of the scanned MCT1 and MCT2 bands were 7224 ± 478 and 6200 ± 264 scanner units/μg of protein. The equivalent values for four control fed rats were 6179 ± 457 and 5822 ± 330 scanner units/μg of protein. Values (means $\pm$ S.E.M.) for the KpNPPase activity in the same samples of membranes from starved and fed rats were 1.61 ± 0.08 and 1.60 ± 0.17 mmol/h per mg of protein respectively, which demonstrates that there were no significant differences in membrane recovery. Thus our data confirm that there is no significant increase in expression of either MCT1 or MCT2 during starvation.

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