Abundance and subcellular distribution of MCT1 and MCT4 in heart and fast-twitch skeletal muscles

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1Department of Kinesiology, University of Waterloo, Waterloo, Ontario N2L 3G1; 2Department of Physiology, Lipid Research Unit, Hospital Research Center, Laval University, Quebec, Canada G1V 4G2; and 3Department of Biochemistry, University of Bristol, Bristol BS8 1TD, United Kingdom

Bonen, Arend, Dragana Miskovic, Mio Tonouchi, Kathleen Lemieux, Mariangela C. Wilson, André Marette, and Andrew P. Halestrap. Abundance and subcellular distribution of MCT1 and MCT4 in heart and fast-twitch skeletal muscles. Am J Physiol Endocrinol Metab 278: E1067–E1077, 2000.—The expression of two monocarboxylate transporters (MCTs) was examined in muscle and heart. MCT1 and MCT4 proteins are coexpressed in rat skeletal muscles, but only MCT1 is expressed in rat hearts. Among six rat fast-twitch muscles (red and white gastrocnemius, plantaris, extensor digitorum longus, red and white tibialis anterior) there was an inverse relationship between MCT1 and MCT4 (r = −0.94). MCT1 protein was correlated with MCT1 mRNA (r = 0.94). There was no relationship between MCT4 mRNA and MCT4 protein. MCT1 (r = −0.97) and MCT4 (r = 0.88) protein contents were correlated with percent fast-twitch glycolytic fiber. When normalized for their mRNAs, MCT1 but not MCT4 was still correlated with the percent fast-twitch glycolytic fiber composition of rat muscles (r = −0.98). MCT1 and MCT4 were also measured in plasma membranes (PM), triads (TR), T tubules (TT), sarcoplasmic reticulum (SR), and intracellular membranes (IM). There was an intracellular pool of MCT4 but not of MCT1. The MCT1 subcellular distribution was as follows: PM (100%) > TR (31.6%) > SR (15%) = TT (14%) > IM (1.7%). The MCT4 subcellular distribution was considerably different (PM (100%) > TR (66.5%) > TT (36%) = SR (43%) > IM (24%). These studies have shown that 1) the mechanisms regulating the expression of MCT1 (transcriptional and posttranscriptional) and MCT4 (posttranscriptional) are different and 2) differences in MCT1 and MCT4 expression among muscles, as well as in their subcellular locations, suggest that they may have different roles in muscle.

Support for this is also found in our studies using high-resolution, immunogold immunocytochemistry in the heart. With this technique, we were able to observe MCT1 along the plasma membrane (PM), where myocytes appose capillaries and other myocytes (19). The most dense MCT1 labeling occurred in T tubules (TT) that are in close proximity to mitochondria (19). This further suggests that MCT1 facilitates the delivery of lactate to its site of oxidation.

It is now known that another monocarboxylate transporter, MCT4, is also present in rat and human skeletal muscle (32, 38 and Bonen, unpublished data) and human heart (34), but it is unclear why MCT1 and MCT4 are coexpressed in the same tissue. Rodent and human muscles rich in fast-twitch fibers appeared to express most, if not all, of the MCT4 (32, 38). The very low MCT4 protein content in the oxidative, slow-twitch soleus muscles (38) suggests that this MCT isoform is most likely expressed only in the few fast-twitch oxidative glycolytic (FOG) fibers (2) that are present in this muscle.

Because of the predominant expression of MCT4 in fast-twitch muscles, it has been suggested that MCT4 expression may reflect the muscle's requirement for lactate efflux (38), whereas the MCT1 expression correlates with the requirement for lactic acid influx for oxidation (25, 26). If this is the case, then it may be
expected that there should be a reasonable concordance between MCT4 and the glycolytic capacity of various muscles, analogous to the relationship between MCT1 and indexes of oxidative metabolism that we have observed previously in rat muscles (25, 26). Moreover, there may also be an inverse relationship between MCT1 and MCT4 in the same muscle, depending on the muscle fiber composition. Finally, it may also be possible that the subcellular distribution of MCT1 and MCT4 differs to support different roles of these transporters, as has been shown for the glucose transporters (GLUT-1 and GLUT-4) in skeletal muscle (9, 15, 35, 36).

Except for the slow-twitch soleus muscle, all other rat hindlimb muscles are comprised of fast-twitch fibers. Yet the proportion of fast-twitch glycolytic (FG) and FOG muscle fibers varies quite markedly in a number of rat muscles [i.e., red and white gastrocnemius (RG and WG), red and white tibialis anterior (RTA and WTA), extensor digitorum longus (EDL), and plantaris (PL); see Refs. 2 and 25]. These same fast-twitch muscles (RTA, WTA, RG, WG, EDL, PL) also coexpress MCT1 and MCT4 (38). Thus this organization permits an assessment of the relationship between these two MCT transporters and their glycolytic muscle fiber composition as well as an examination of their subcellular distribution. Therefore, in the present studies, we have compared, in six rat fast-twitch muscles with varying proportions of FG and FOG fibers, 1) the relationship between MCT1 and MCT4 and their mRNAs, 2) the relationship between these MCT transporters and muscle fiber composition and lactate uptake, and 3) the subcellular distribution of MCT1 and MCT4 in rat hindlimb muscles. Finally, we also compared the expression of MCT proteins and their mRNAs in hearts with those in oxidative and glycolytic muscles.

**METHODS**

**Animals**

Male Sprague-Dawley rats weighing 250–300 g were used in the experiments. Animals were housed in an air-conditioned room on a 12:12-h light-dark cycle and were fed a diet of Purina Chow and water ad libitum. Ethical approval was obtained for this work from the animal care committee at the University of Waterloo.

**Comparison of Heart and Skeletal Muscles**

We obtained hearts and metabolically heterogeneous skeletal muscles (PL, EDL, RG, WG, RTA, and WTA) from male rats. We have previously shown that these muscles are composed of FOG and FG fibers in varying proportions (24, 26). In the present studies, we have used the muscle fiber composition that we have determined in our previous studies (25, 26).

**Lactate Uptake in Perfused Rat Hindlimb Muscles**

Hindlimb perfusion of rat muscles was performed as previously described in our laboratory (25, 26, 30). Briefly, rats were anesthetized (60 mg/kg ip Somnotol) and surgically prepared for hindlimb perfusion. After rats were heparinized with 1,000 IU heparin (500 U/ml) injected in the inferior vena cava, catheters were inserted in the inferior vena cava and aorta. The rats were placed inside a Plexiglas cabinet containing the perfusion apparatus and were maintained at 37°C. The rats were killed by injecting 1 ml of 10% (wt/vol) KCl in the heart after the onset of perfusion. A cell-free gassed (95% O2–5% CO2) Krebs-Henseleit buffer containing 4% BSA, pH 7.4, and 10 mM glucose was used as the perfusate. A one-pass system was used; therefore, the venous outflow was discarded. The initial 20 min of perfusion was used as a preperfusion period that allowed the flow rate to be adjusted slowly to the experimental level of 20 ml/min. The flow was estimated by timing the venous effluent collections.

We used a 5-min perfusion time to measure lactate uptake, as we have done previously (25, 26) to determine lactate uptake by rat hindlimb muscles. This time period minimized lactate metabolism while lactate has equilibrated with the extracellular space by 3 min (25, 26). For determinations of lactate uptake, the perfusate was supplemented with 2 mM Na1-lactate and 3 µCi [U-14C]lactate and with 1 µCi [3H]sorbitol to account for the extracellular distribution space of lactate. Immediately after 5 min of perfusion, muscles were rapidly extracted and frozen in liquid nitrogen.

**Subcellular Distribution of MCT1 and MCT4**

We examined the subcellular distribution of MCT1 and MCT4. For these purposes, we fractionated skeletal muscles into a number of subcellular fractions using procedures developed by one of our laboratories (9). Full details of the fractionation procedures and their characterizations have been described in great detail elsewhere by us (9). Only slight modifications have been introduced in the original procedures (35, 36). Briefly, this fractionation technique permits the simultaneous and separate isolation of PM and transverse tubule vesicles from the same muscle homogenate. In addition, the use of a strong salt treatment (LiBr) yields a fraction containing sarcoplasmic reticulum (SR) and triad (TR) membranes (where the TT appose the SR cisternae), as well as an intracellular membrane (IM) fraction that is totally devoid of PM and transverse tubule markers (9, 35, 36). The subcellular muscle fractions obtained have been extensively characterized with immunological and enzymatic markers to ensure purity of these subcellular fractions (9, 35, 36). In the present studies, ~8 g of lower leg muscles were pooled from several rats and fractionated (9, 35, 36). Immunological and enzymatic characterizations of the subcellular fractions corresponded to those reported previously (9, 35, 36 and data not shown).

**MCT mRNA and Protein**

Western blotting. MCT1 and/or MCT4 proteins were determined in muscles and heart homogenates (3, 25, 26) and in the subcellular fractions of skeletal muscles (9, 35, 36). Proteins were separated using SDS-PAGE, and MCT1 and MCT4 were detected using Western blotting procedures that we have described in detail elsewhere (3, 5, 25, 26, 38). Blots were quantified using a scanner attached to a computer with appropriate software, as we have done previously (3, 5, 25, 26, 38).

Northern blotting. MCT1 mRNA and MCT4 mRNA were determined using Northern blotting procedures. Total RNA was isolated from heart and muscle tissues using the guanidine isothiocyanate/cesium chloride centrifugation method (8), with some modifications. The tissues were homogenized in 10 ml of 4 M guanidine isothiocyanate and were layered on top of 3.3 ml of 5.7 M cesium chloride solution. The samples were centrifuged in an SW-41 Ti rotor (Beckman) at 30,000 revolutions/min (rpm) for 23 h. The RNA pellets were recovered and purified by two precipitations in ethanol.
Northern blot analysis. Three micrograms of total RNA were used for electrophoresis on 1.2% formaldehyde agarose gels (37) and then were transferred to a positively charged nylon membrane (Boehringer Mannheim, Laval, Quebec, Canada). The Northern blots were ultraviolet cross-linked with a GS-Genelinker (Bio-Rad).

A short fragment (1.9 kb) including the coding sequence of MCT1 cDNA was digested with EcoRI restriction enzyme from the full-length (3.3 kb) MCT1 cDNA (17) and was subcloned into the EcoRI restriction enzyme site of pBlueScript (KS). The orientation was checked by digestion with HindIII restriction endonuclease. Template DNA was linearized with XbaI restriction enzyme, and DIG-labeled antisense riboprobe was generated by in vitro transcription with T3 RNA polymerase. MCT4 cDNA was originally subcloned into BamHI/ApaI restriction enzyme sites of pBlueScript (38). DIG-labeled antisense riboprobe was generated by digestion of the template DNA with XbaI restriction enzyme and in vitro transcription with T7 RNA polymerase.

The ingredients for RNA transcription included 1–2 µg of DNA template plus the NTP mix [2.5 mM rCTP, 2.5 mM rGTP, 2.5 mM rATP, 1.625 mM UTP (Promega), and 0.875 mM DIG-11 UTP (Boehringer Mannheim)], 20 mM dithiothreitol (Promega), 1 U/1 µg template DNA of RNase inhibitor (Promega), and 1X RNA polymerase buffer [5X buffer: 400 mM Tris·HCl, pH 7.5, 60 mM MgCl₂, and 20 mM spermidine hydrochloride (Promega)] maintained at room temperature. The appropriate RNA polymerase [T3 and T7 RNA polymerases (Boehringer Mannheim)] was added (at least 20 IU/1 µg of DNA template) and incubated for 2 h at 37°C. The DNA template was then digested for 10 min at 37°C with RNase-free DNase (1 IU/1 µg of DNA template; Promega). After precipitation in ethanol and centrifugation at 12,000 rpm for 15 min, the probe was resuspended in 10–20 ml DIG EasyHyb hybridization buffer (Boehringer Mannheim) or standard hybridization buffer with 50% formamide [5X saline sodium citrate, 50% formamide, 0.1% sodium lauroylsarcosine, 0.02% SDS, and 2% blocking reagent (Boehringer Mannheim)].

After prehybridization of the membrane for at least 4 h at 68°C, the prehybridization buffer was replaced with the same buffer containing DIG-labeled antisense RNA probe, and the membrane was incubated with the probe overnight at 68°C. High-stringency washes and chemiluminescent detection were performed in accordance with the protocol supplied by the manufacturer (Boehringer Mannheim), and the membrane was exposed to Kodak BioMax film. After exposure, the film was developed in Kodak developer and fixed in Kodak fixer.

Statistics. The data were analyzed using repeated-measures ANOVA. All data are reported as means ± SE.

RESULTS

MCT in the Heart

MCT1. MCT1 mRNA and protein were most abundant in the heart when compared with RG and WG (Fig. 1), muscles rich in FG or FG fibers, respectively. However, on a relative basis, MCT1 protein in the heart was ~3-fold greater than in RG muscle and ~6- to 7-fold greater than in WG muscles, whereas MCT1 mRNA abundance in the heart was only 1.2- and 1.4-fold greater than in the RG and WG muscles, respectively.

MCT4. MCT4 mRNA was barely detectable in the heart (Fig. 1), whereas this transcript was abundantly present in both RG and WG muscles (Fig. 1). We were not able to detect MCT4 protein in the heart homogenates or in giant vesicles prepared from hearts (data not shown). In contrast, both MCTs were expressed in RG and WG muscles.

MCT in Skeletal Muscles

To examine further the relationship between MCT1 and MCT4, we used rat hindlimb muscles with a wide range of oxidative and glycolytic capacities. MCT1
mRNA and MCT4 mRNA and their respective proteins were present in all six rat hindlimb muscles examined (Figs. 2 and 3). There was a good relationship between MCT1 mRNA and MCT1 protein but not between MCT4 mRNA and MCT4 protein (Fig. 4). There was also a strong inverse relationship between the MCT1 and MCT4 proteins (Fig. 5), despite the rather narrow range of MCT4 protein concentrations among the six muscles compared with the range of MCT1 among these muscles (Figs. 2 and 3).

**MCT and Muscle Fiber Composition**

The MCT1 protein content was inversely associated with the percent FG muscle fibers (Fig. 6A). The MCT4 protein content was positively related to the proportion of FG muscle fibers (Fig. 6B).

To determine whether MCT expression varied independently from the abundance of their transcript in the metabolically heterogeneous rat muscles, we normalized the MCT protein to their mRNAs (i.e., ratio MCT protein/MCT mRNA). This showed that, independent of the MCT1 mRNA, there was a strong inverse relationship between MCT1 protein and muscle fiber composition (Fig. 7). In contrast, there was no relationship between the percent FG muscle fiber composition and the ratio of MCT4 protein/MCT4 mRNA (data not shown). Across all six fast-twitch muscles examined, the MCT4 protein expression in relation to MCT4 mRNA (i.e., MCT4 protein/MCT4 mRNA) was relatively constant (data not shown).

**Lactate Uptake and MCT**

Uptake of lactate by perfused rat hindlimb muscles differed considerably among rat muscles. The highest rates of uptake occurred in the muscles rich in FG fibers (Table 1). Lactate uptake was positively associated with the MCT1 protein content of the muscles (Fig. 8A). In contrast, there was a negative relationship between MCT4 protein and lactate uptake (Fig. 8B).

**Subcellular Distribution of MCT1 and MCT4**

To determine the subcellular locations of MCT1 and MCT4, rat muscles were fractionated into PM, TT, TR, and IM fractions. Purification of the fractions and their characteristics were determined for each preparation (data not shown); these were similar to those reported in previous studies from our laboratories (9, 35, 36). The relative concentrations of MCT1 and MCT4 in the subcellular fractions were examined using Western blotting. For comparison purposes, we expressed MCT1 and MCT4 relative to their respective levels in the PM fraction (set to 100 for each transporter; Fig. 9).

The relative subcellular distribution of MCT1 and MCT4 differed substantially. The largest concentration for both MCT1 and MCT4 was present in the PM (Fig. 9). The MCT1 distribution was as follows: PM (100%) > TR (31.6%) > SR (15%) = TT (14%) > IM (1.7%). The MCT4 distribution was considerably different [PM (100%) > TR (66.5%) > TT (36%) = SR (43%) > IM (24%)].

The relative proportions of MCT1 and MCT4 in the subcellular fractions were also compared (Table 2). In the TT, the relative proportion of MCT4 (36%) was 2.6 times greater than MCT1 (14%; Fig. 9, P < 0.05). In the TR, the proportion of MCT4 (66.5%) was two times greater than MCT1 (32%; P < 0.05). MCT4 content...
(43%) in the SR fraction was almost three times greater than MCT1 (15%; P < 0.05). Little or no MCT1 was present in the IM (1.7%), but a substantial amount of MCT4 was present (24%) in the IM (Fig. 9).

**DISCUSSION**

These are the first studies to compare MCT1 and MCT4 protein expression with the abundance of their mRNAs in rat heart and skeletal muscles, to examine the relationship of MCT1 and MCT4 with muscle fiber composition and with lactate uptake from the circulation, and to determine the subcellular distribution of MCT1 and MCT4 in skeletal muscles.
MCT Proteins and mRNAs

MCT1 and MCT4 in heart and muscle. In the past few years, a family of monocarboxylate transporters MCT1–MCT7 have been cloned (34). Screening of commercially available human tissue Northern blots indicated that MCT1 mRNA and MCT4 mRNA were present in human heart and muscle. In the present study, in agreement with our previous work (3, 25), we found that MCT1 protein was present in the heart in four- to sevenfold greater quantities than in muscle. A new observation is that MCT1 mRNA abundance was only slightly greater (1.2- to 1.4-fold) in heart than in red and white muscles. These data suggest that, in the heart, posttranscriptional mechanisms regulate MCT1 protein expression. It has been proposed that pools of MCT1 mRNA may be in mRNA proteins, thus facilitating rapid translation when more protein is required. The very long 3'-untranslated region of MCT1 (1.6 kb; see Refs. 13 and 17) may be involved in the translational repression.

In contrast to MCT1, MCT4 protein was not detected in rat hearts, but a faint MCT4 mRNA signal was present. However, both MCT4 mRNA and MCT4 protein are detectable in human hearts (34, 38). This indicates that there is a species difference in MCT4 expression in human and rat hearts. Previously, it was observed that there are differences in tissue-specific expression of MCT1 and MCT2 in different rodent species (rats and hamsters). For example, MCT1 is expressed in testis, heart, and muscle of both rats and hamsters (13, 18), whereas MCT1 was expressed in rat liver but not hamster liver (18). Differences in MCT2 expression have also been observed; in rat, MCT2 is present in liver, brain, and testis but not in muscle, whereas in hamster, MCT2 is present in the same tissues (liver, brain, and testis), as well as in heart and oxidative skeletal muscle (18). These species differences in tissue-specific expression of the MCT isoforms will complicate extrapolations from animal models to humans.

MCT1 and MCT4 proteins in fast-twitch skeletal muscles. The MCT1 protein expression in fast-twitch muscles is similar to that reported previously by us (3, 25). In the present study as well as in a previous report (25), we found that MCT1 in rat muscles is negatively correlated with the percent FG muscle fibers (or, positively correlated with the %oxidative muscle fibers). It...
has now also been shown that MCT1 is positively correlated with the oxidative muscle fiber composition of human muscle (32).

Both rat (present study and Refs. 4 and 38) and human muscles express MCT4 (Bonen, unpublished data, and Ref. 32). However, the present study demonstrated that the content of MCT4 protein among rat fast-twitch muscles differed sharply from that of MCT1. Despite the narrow range of MCT4 among muscles, MCT4 protein content was positively correlated with percent FG fibers. Recently, it was observed in human muscle that MCT4 was not correlated with the percent fast-twitch fibers (type II fibers), although MCT4 content was higher in muscles rich in type II fibers than in muscles rich in slow-twitch (type I) fibers (32). In these human studies, MCT4 content was not examined in the type IIa and type IIb fibers. Interestingly, in the present study, we found that MCT1 and MCT4 are reciprocally expressed among rat fast-twitch muscles, but the range of MCT1 concentrations among muscles was greater than for MCT4. Nevertheless, the data from the present experiments support the idea that the expression of MCT4 may be primarily regulated according to the need for lactate efflux from muscle, as has recently been proposed (38), since this MCT isoform is so highly correlated with percent FG fibers that are specifically designed to generate ATP at high rates via anaerobic metabolism.

MCT1 and MCT4 mRNA. There are currently no other reports describing the MCT1 mRNA and MCT4 mRNA abundance among metabolically heterogeneous rat muscles. In the developing rat brain, the progressive divergence of MCT1 mRNA and protein suggested that MCT1 protein expression was initially regulated in neonates by transcriptional mechanisms, whereas in adult life posttranscriptional mechanisms regulated MCT1 expression (23). In the present study, the positive relationship between MCT1 mRNA and MCT1 protein indicates that protein expression in muscle is regulated, in part, by pretranslational processing, but the data also suggest that posttranscriptional processing of the MCT1 protein is occurring, because there is a strong inverse relationship between the percent FG muscle fibers and MCT1, independent of MCT1 mRNA (see Fig. 7). Thus our study suggests that MCT1 expression is regulated both by transcriptional and posttranscriptional mechanisms, but the lack of a relationship between MCT4 protein and MCT4 mRNA indicates that the expression of this protein is regulated by posttranscriptional mechanisms.

Because it is well known that oxidative muscles are spontaneously more active than glycolytic muscles (16, 27, 28), we speculate that the neural innervation pattern imposed on a muscle may serve to regulate MCT1 protein expression via posttranscriptional mechanisms. This is also indicated by chronic electrical muscle stimulation studies in which MCT1 protein was increased when a continuous neural innervation pattern (i.e., an innervation pattern resembling that found in oxidative muscles) was imposed on a glycolytic muscle (26), one that is normally innervated intermittently (16). Alternatively, nerve-derived substances may regulate MCT1 expression, as these appear to influence the expression of GLUT-4 transporters in muscle (29, 31). Definitive evidence that nerve-derived factors can influence gene expression was demonstrated several years ago (10).

**Table 1. Lactate uptake by perfused rat hindlimb muscles**

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<td>Lactate uptake, nmol·mg w t⁻¹·min⁻¹</td>
<td>0.97 ± 0.08*</td>
<td>0.87 ± 0.08</td>
<td>0.85 ± 0.12†</td>
<td>0.78 ± 0.06‡</td>
<td>0.40 ± 0.05</td>
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Data are means ± SE; n = 8 animals. RG, red gastrocnemius; RTA, red tibialis anterior; PL, plantaris; EDL, extensor digitorum longus; WTA, white tibialis anterior; WG, white gastrocnemius. *P < 0.05, RG vs. WTA and WG. †P < 0.05, WG vs. WTA and EDL. ‡P < 0.05, PL vs. WTA and WG. §P < 0.05, EDL vs. WTA and WG.
of muscles was increased (26). This same relationship between MCT1 and lactate uptake was observed in the present experiments. It should be noted that measuring lactate uptake in perfused muscle provides only a rough index of lactate transport across the PM.

In rat fast-twitch muscles, there was a negative relationship between lactate uptake from the circulation and MCT4 protein content. The reason for this is not entirely clear, since it is not reasonable to believe that the MCT4 transporter can only extrude lactate out of the muscle. We have shown previously that both MCT4 and MCT1 can transport lactate into and out of the cell (38). However, in cardiac myocytes, the rates of lactate efflux and influx are asymmetric (see Ref. 14 for review). This asymmetry of lactate transport may be due to the different kinetic properties of the two MCT isoforms that appear to be coexpressed in rat hearts, although, until now, only MCT1 has been shown to be expressed in rat hearts. Based on the relationship of MCT1 and MCT4 with lactate uptake in the present

Table 2. Statistical comparisons of MCT1 and MCT4 subcellular distribution in PM, TT, TR, SR, and IM

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Data are P values. MCT, monocarboxylate transporters; PM, plasma membrane; TT, T-tubules; TR, triads; SR, sarcoplasmic reticulum; IM, intracellular membranes. NS, not significant.
studies, there may also be an asymmetry of lactate influx and efflux in the intact muscle, with MCT1 favoring lactate uptake and MCT4 favoring lactate efflux over influx. This then might account for the negative relationship between MCT4 and lactate uptake. However, in a simpler model (spherical giant sarcolemmal vesicles), lactate transport asymmetry was not observed (20), although influx and efflux rates were determined by somewhat different procedures.

The best estimate for the Michaelis constant ($K_m$) of lactate transport in muscle is 20 mM when data from a number of studies are averaged (cf. Ref. 21), but published data indicate that the $K_m$ for MCT4 is 10.1 mM and for MCT1 the $K_m$ is 6.4 mM when measured in cells that are presumed to express only MCT1 or MCT4 but not MCT2 or MCT5–MCT7 (38). However, such cells may express other as yet unknown MCTs with a lower $K_m$. Indeed, there is one report showing that there is likely another MCT that may be very different from the currently known MCTs (1), but full details of that MCT have not been published yet.

From a physiological perspective, it would seem unusual to have two MCT transport proteins coexpressed with apparently similar transport characteristics. Skeletal muscle is typically faced with lactate concentrations that are quite low, such as those observed at rest or during normal daily activities, or, infrequently, high lactate concentrations occur when muscle contractions are particularly intense. These two situations would seem to require a lactate transport system that is effective when lactate concentrations are low and when lactate concentrations are high. Based on the good correlations between MCT1 expression and rates of lactate uptake, it seems likely that MCT1 fulfills the role of a high-affinity transporter in muscle. Recent studies expressing MCTs in Xenopus oocytes have now shown that MCT1 is a high-affinity transporter ($K_m$ $\approx$ 5 mM; see Ref. 7) and that MCT4 is a low-affinity transporter ($K_m$ $\approx$ 22 mM; S. Broer and A. P. Halestrap, unpublished data). These kinetic characteristics of MCT1 and MCT4 would seem to fulfill the need for a high-affinity/low-capacity and a low-affinity/high-capacity lactate transporter in muscle that can be faced with low lactate concentrations at rest and during mild to moderate intensity exercise and with high lactate concentrations during intense exercise.

Subcellular Distribution of MCT1 and MCT4

Until now, the subcellular distribution of MCT1 and MCT4 in muscle was not known. We fractionated muscle into PM, TT, TR, SR, and IM fractions (9, 35, 36) but not mitochondrial membrane fractions. These fractions have been extensively characterized previously by our laboratories (9, 35, 36) and in this study (data not shown).

It has been proposed that the more easily releasable TT, obtained by mild homogenization in our fractionation procedures, are perhaps nonjunctional TT (9). Such nonjunctional TT are not associated with the SR (11) and likely do not propagate membrane signals for myofiber contraction (11). The junctional TT, which are greatly enriched with the dihydropyridine receptor, are found in the TR fraction (9, 35, 36). More rigorous treatment (high salt, LiBr) is required to obtain this TR fraction, presumably because TR form strong complexes with some junctional proteins (22).

There are marked differences in the subcellular distributions of MCT4 and MCT1 in muscle. Most of the MCT1 and MCT4 is concentrated in the PM. For comparative purposes, we designated the concentration in the PM as 100. Relative to this PM pool, MCT4 is more concentrated in the TT (35 vs. 14%), in the SR (43 vs. 15%), and in the TR (66.5 vs. 32%). Interestingly, there was a negligible amount of intracellular MCT1 (1.7%), but there was a relatively greater intracellular content of MCT4 (24%). These MCT isoform distribution patterns also differ from the known glucose transporters distribution patterns. GLUT-1 is found only in the PM, whereas GLUT-4 is present in the PM, TT, and TR, and most of the GLUT-4 is located in the intracellular pool (data not shown and Refs. 9, 35, 36).

The MCT1 subcellular distribution in skeletal muscle is not entirely consistent with that observed in the heart (19). In the heart, high MCT1 concentrations were present in both the PM and in the TT. The most dense MCT1 immunogold labeling occurred in TT that are in close proximity to mitochondria (19). This suggests that MCT1 facilitates delivery of lactate to its site of oxidation. Whether MCT1 in muscle is more densely concentrated near subsarcolemmal mitochondria is not known, but if so, this would possibly account for the good relationship between MCT1 and the indexes of oxidative metabolism of lactate that we have observed (25, 26).

One major difference between MCT1 and MCT4 distribution is the absence of an intracellular pool of MCT1, whereas there is a considerable intracellular MCT4 pool. This may point to a major functional difference in the roles of MCT1 and MCT4. The intracellular MCT4 pool could represent an intracellular storage compartment that could possibly be recruited to assist with the extrusion of lactate from the muscle. This would be particularly advantageous during intense exercise, when lactate accumulation and reductions in pH limit muscle function. The ability to translocate MCT4, even briefly, may have considerable homeostatic advantages for the contracting myocyte. This would allow for a more effective removal of lactate and protons from the cell. However, whether the intracellular MCT4 pool can be translocated, even briefly, may have considerable homeostatic advantages for the contracting myocyte. The possibility that there may be an intracellular, contrac-
tion-sensitive, translocatable MCT4 pool will require further study.

The present studies have shown that MCT4 and MCT1 transport proteins are coexpressed in muscle but not in rat heart, which expressed only MCT1, but we were able to detect low levels of MCT4 mRNA in the heart. In muscles, MCT1 expression appeared to be regulated by both transcriptional and posttranscriptional mechanisms, and MCT4 appeared to be regulated by posttranscriptional mechanisms. The MCT4 concentrations were positively correlated with percent FG fibers and inversely correlated with MCT1. Furthermore, the subcellular distribution of MCT1 and MCT4 was shown to differ markedly. Although MCT1 was more, the subcellular distribution of MCT1 and MCT4 was confined largely to the PM, MCT4 was present in FG fibers and inversely correlated with MCT1. Furthermore, the subcellular distribution of MCT1 and MCT4 was shown to differ markedly. Although MCT1 was more, the subcellular distribution of MCT1 and MCT4 was confined largely to the PM, MCT4 was present in FG fibers and inversely correlated with MCT1. Further-

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