Expression of monocarboxylate transporters in rat ocular tissues

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Submitted 20 January 2004; accepted in final form 22 September 2004


The aim of the present study was to determine the distribution of monocarboxylate transporter (MCT) subtypes 1–4 in the various structures of the rat eye by using a combination of conventional and real-time RT-PCR, immunoblotting, and immunohistochemistry. Retinal samples expressed mRNAs encoding all four MCTs. MCT1 immunoreactivity was observed in photoreceptor inner segments, Müller cells, retinal capillaries, and the two plexiform layers. MCT2 labeling was concentrated in the inner and outer plexiform layers. MCT4 immunolabeling was present only in the inner retina, particularly in putative Müller cells, and the plexiform layers. No MCT3 labeling could be observed. The retinal pigment epithelium (RPE)/choroid expressed high levels of MCT1 and MCT3 mRNAs but lower levels of MCT2 and MCT4 mRNAs. MCT1 was localized to the apical and MCT3 to the basal membrane of the RPE, whereas MCT2 staining was faint. Although MCT1–MCT4 mRNAs were present in the iris and ciliary body samples, only MCT1 and MCT2 proteins were expressed. These were present in the iris epithelium and the nonpigmented epithelium of the ciliary processes. MCT4 immunolabeling was present only in the inner retina, particularly in putative Müller cells, and the plexiform layers. No MCT3 labeling could be observed. The retinal pigment epithelium (RPE)/choroid expressed high levels of MCT1 and MCT3 mRNAs but lower levels of MCT2 and MCT4 mRNAs. MCT1 was localized to the apical and MCT3 to the basal membrane of the RPE, whereas MCT2 staining was faint. Although MCT1–MCT4 mRNAs were present in the iris and ciliary body samples, only MCT1 and MCT2 proteins were expressed. These were present in the iris epithelium and the nonpigmented epithelium of the ciliary processes. MCT4 was localized to the smooth muscle lining of large vessels in the iris-ciliary body and choroid. In the cornea, MCT1 and MCT2 mRNAs and proteins were detectable in the epithelium and endothelium, whereas evidence was found for the presence of MCT4 and, to a lesser extent, MCT1 in the lens epithelium. The unique distribution of MCT subtypes in the eye is indicative of the pivotal role that these transporters play in the maintenance of ocular function.

Monocarboxylates, which include pyruvate, lactate, and ketone bodies, play a major role in the metabolism of cells. They are thought to be the primary energy substrates for certain cell types and are essential fuels when glucose availability is low. Of all the monocarboxylates, lactate is quantitatively the most important oxidizable substrate and is considered to be the preferred substrate of photoreceptor inner segments (23). Lactate is also the end product of glycolysis, and in cells with high rates of glycolysis, such as photoreceptor outer segments, lactate must be rapidly exported to prevent intracellular acidosis. The retina produces considerable quantities of lactate aerobically, and the retinal pigment epithelium (RPE) performs a vital role in transporting excess lactate from the subretinal space into the choroidal circulation. However, the retina is not the only ocular tissue to produce substantial amounts of lactate. The lens and cornea are avascular tissues that are largely mitochondria free and depend on anaerobic glycolysis to meet the majority of their energy needs. As a consequence, they produce and export significant amounts of lactate, and this explains why lactate levels in the aqueous humor are considerably higher than in plasma (9).

Monocarboxylates are transported across membranes via a family of proton-coupled carrier proteins known as monocarboxylate transporters (MCTs). Multiple MCT-related sequences have been identified, although only MCT1–MCT4 have been functionally characterized (15). To date, MCT1 is the best-characterized isoform and is found in the majority of tissues analyzed in the rat (15). In contrast, MCT2 has a more limited distribution in the rat and may well exist in several alternatively spliced forms (16). Rat MCT3 has been localized exclusively in the RPE (21), whereas rat MCT4 is particularly evident in tissues with high glycolytic rates, such as skeletal muscle (17). For clarification of the energy utilization requirements of the various ocular tissues, information as to the distribution of the different MCT subtypes in the eye is warranted. Despite the fact that lactate plays a central role in the metabolism of various ocular tissues, clear knowledge of the precise locations of the different MCT isoforms in the eye is lacking. Studies carried out to date have focused exclusively on the RPE (21) and retina (3, 12) and, with respect to the retina, have generated results that do not always correlate.

The distribution of the MCT1–MCT4 isoforms in the retina has thus far been studied using immunohistochemistry and immunoblotting. MCT1 is expressed throughout the retina, being particularly abundant in photoreceptor inner segments, Müller cells, and blood vessels (3, 12, 20). In contrast, data generated on MCT2 are contradictory: Gerhart et al. (12) showed MCT2-like staining to be associated with Müller cells and synaptic and nuclear layers of the retina, yet Bergersen et al. (3) found no evidence for MCT2-like immunoreactivity in the retina. Evidence to date indicates that MCT3 is not expressed in the retina (20), but some immunoreactivity for MCT4 has been reported (3).

In the rat RPE, Philp et al. (21) provided convincing data indicating that high levels of MCT 1 and MCT3 are polarized to the apical and basolateral membranes, respectively, of these cells. The localization of MCT1, as well as the absence of MCT2, was subsequently confirmed by the work of both Gerhart and Bergersen (3, 12). The latter group also concluded that a low level of MCT4-like immunoreactivity is associated with the RPE.

To our knowledge, no reports exist on the types of MCTs that are associated with the various structures of the anterior segment of the eye, yet there are valid reasons to warrant an investigation. Although historically it has been assumed that lactate diffuses out of the lens and cornea, it is more likely that

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lens epithelial cells and corneal epithelial or endothelial cells actively extrude lactate via one or more of the MCT isoforms. This idea is given significant weight by the finding in rabbit cornea of lactate transport mechanisms that are both carrier mediated and proton coupled (5, 13). With regard to the iris and ciliary epithelia, these tissues are derived from the same embryological structure as the RPE and, as a consequence, share a number of common properties. Indeed, iris pigment epithelium cells in culture can acquire many RPE properties (26). It is therefore of interest to determine whether a pattern of MCT expression exists in the iris and ciliary epithelium that is similar to that in the RPE.

The aim of the present study was to delineate the types of MCTs associated with the various tissues of the rat eye by using more sensitive technologies than previously employed. In particular, the objective was to provide information on the MCT expression in the iris and ciliary epithelia, these tissues are derived from the same embryological structure as the RPE and, as a consequence, share a number of common properties. Indeed, iris pigment epithelium cells in culture can acquire many RPE properties (26). It is therefore of interest to determine whether a pattern of MCT expression exists in the iris and ciliary epithelium that is similar to that in the RPE.

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Antibodies

Rabbit anti-rat MCT1 (1:1,000) and rabbit anti-rat MCT2 (1:1,000) were both generous gifts from Luc Pellerin (Geneva, Switzerland). For detection of MCT3, two distinct antisera were employed: rabbit anti-rat MCT3 from Chemicon (1:200) and rabbit anti-rat MCT3 from ADI (1:200). Rabbit anti-rat MCT4 (1:200) and mouse anti-chicken actin (1:2,000) were also obtained from Chemicon.

Laser Capture Microdissection

After enucleation, retinas were removed and snap frozen, and 10-μm-thick sections were produced. Slides were fixed in 70% ethanol for 1 min and then dehydrated and cleared in xylene. Cells isolated from the inner and outer nuclear layers of retinal sections were obtained using a PixCell I laser capture microdissection system (Arcturus Engineering, Harpenden, UK). The cells attached to the capture film were removed from the film with lysis buffer, and total RNA was then extracted as previously described.

Immunohistochemistry

Rats were anesthetized with pentobarbital sodium and transcardially perfused with phosphate-buffered saline (PBS) and, subsequently, with Davidson’s fixative. After enucleation, whole eyes were fixed in Davidson’s fixative for 24 h, transferred to industrial methylated alcohol, and 5-μm serial sections were cut using a rotary microtome. Tissue sections were deparaffinized, rehydrated into 70% ethanol, and treated for 10 min with 0.1% (vol/vol) H2O2 and 0.03% (vol/vol) H2O2.

RESULTS

Tissue Specificity of MCT2 and MCT4 Antibodies

Localization of MCT2 has proved problematic in the past. To verify the specificity of the MCT2 and MCT4 antibodies for use with ocular sections, we performed preliminary staining using testis and skeletal muscle sections. These tissues are known to display discrete patterns of MCT2 (testis) and MCT4 (skeletal muscle) staining (14, 28). The MCT2 antibody stained specifically in sperm tails throughout the epididymis (Fig. 1A) but no staining in the testis (Fig. 1B). Localization of MCT4 using testis and skeletal muscle sections. These tissues are known to display discrete patterns of MCT2 (testis) and MCT4 (skeletal muscle) staining (14, 28). The MCT2 antibody stained specifically in sperm tails throughout the epididymis (Fig. 1A) but failed to label skeletal muscle (Fig. 1D), whereas incubation with the MCT4 antibody yielded strong staining in the sarcocollicum of extraocular skeletal muscle (Fig. 1C) but no staining in the testis (Fig. 1B).

Validation of RT-PCR

To detect the presence of MCT1–4 mRNAs in rat samples, we first performed RT-PCR by using primers specific to the published sequence for each of the isoforms. To verify the
efficiency of each of the primer pairs for amplification of the relevant mRNAs, we included a positive control cDNA sample appropriate for each MCT isoform with the ocular samples (see Fig. 2). For each of the mRNAs analyzed, amplification of samples resulted in a single detectable product whose size was equivalent to that predicted from the relevant sequence (see Table 1). Synthesis of each product was cycle dependent, with saturation of signal achieved by ~40 cycles (data not shown), and contamination with genomic DNA was excluded by performing RT-PCR in the absence of reverse transcriptase (negative controls; see Fig. 2). In addition, for each mRNA amplified, nonspecific contamination was excluded by performing RT-PCR with the use of water instead of the cDNA sample (data not shown).

Localization of MCT1–MCT4 in Rat Ocular Tissues

Retina. Initial RT-PCR experiments showed clear signals for all four MCT mRNAs in normal retinal samples (Fig. 2). Real-time PCR experiments confirmed these results and demonstrated that the level of MCT1 mRNA is substantially higher than the levels of MCT2–4 mRNAs in the retina (Fig. 3). The presence of MCT3 mRNA in retinal samples was surprising, and to exclude the possibility of contamination from RPE, we utilized laser capture microdissection (LCM). Cells isolated from the ganglion cell layer and the inner and outer nuclear layers of the retina were pooled, the RNA was extracted, and real-time PCR was performed. The levels of all four MCT transcripts in the LCM sample were found to be similar to those measured in retinas dissected by conventional means (Fig. 4). Further information regarding the subretinal localization of the mRNAs encoding MCT1–MCT4 was provided by selectively killing certain cell types in the retina and examining whether the amounts of the MCT transcripts were altered. In 60-day-old RCS rats and in normal Wistar rats that had undergone exposure to intense light, there were marked losses of photoreceptors, which were verified by substantial reduc-
tions in the mRNA for the photoreceptor-specific marker rhodopsin (Table 3). Comparison of the abundance of the MCT mRNAs in these samples with that in age-matched control retinas revealed that in both groups of photoreceptor-deficient rats, there was a significant decrease in the level of MCT1 mRNA and considerable increases in the levels of MCT2, MCT3, and MCT4 mRNAs (Table 3). Conversely, in the retinas of rats given an intraocular injection of kainate, which kills cells in the inner retina but spares the outer retina, there was a decrease in the levels of MCT2, MCT3, and MCT4 but no change in the level of MCT1 (Table 3). Finally, cDNA samples were prepared from an immortalized rat Müller cell culture. Amplification of these samples resulted in a strong signal for MCT1 mRNA, weak signals for MCT2 and MCT4 mRNAs, and little or no signal for MCT3 mRNA (Fig. 2).

Antibodies to MCT1, MCT2, and MCT4 labeled single bands in immunoblots prepared from rat retina (Fig. 5). The MCT3 antibody failed to give a positive band (Fig. 5). In tissue sections, immunoreactivity for MCT1 was observed in photoreceptor inner segments, putative Müller cells, and retinal capillaries and was also evident in the two plexiform layers of

Fig. 3. Detection of MCT1 (A), MCT2 (B), MCT3 (C), and MCT4 (D) mRNA expression in various ocular tissues using real-time RT-PCR analysis. Each value is the mean ± SE of 3 (RPE, ciliary body), 4 (iris, cornea, lens), or 7 samples (retina) and is normalized to the level of GAPDH mRNA. A positive control sample is included for MCT1 (heart), MCT2 (testis), and MCT4 (skeletal muscle) to provide a comparison with other tissues. Sk mus, skeletal muscle.

Fig. 4. Detection of MCT1–MCT4 mRNA expression in total retina and laser capture microdissected (LCM) retina samples using real-time RT-PCR analysis. Each value is the mean ± SE of 3 (retina) or 1 sample(s) (LCM retina) and is normalized to the level of GAPDH mRNA.
Table 3. Effect of degeneration of either photoreceptors or inner retinal neurons on total retinal levels of mRNAs encoding MCT1–MCT4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MCT1</th>
<th>MCT2</th>
<th>MCT3</th>
<th>MCT4</th>
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<tbody>
<tr>
<td>RCS retinas</td>
<td>0.29 (0.23–0.37)</td>
<td>2.83 (2.30–3.48)</td>
<td>3.13† (2.66–3.71)</td>
<td>1.20 (0.96–1.51)</td>
</tr>
<tr>
<td>Age-matched retinas</td>
<td>1.00 (0.72–1.39)</td>
<td>1.00 (0.56–1.78)</td>
<td>1.00 (0.70–1.42)</td>
<td>1.00 (0.77–1.30)</td>
</tr>
<tr>
<td>Light-destructed retinas</td>
<td>0.56‡ (0.55–0.58)</td>
<td>1.64‡ (1.56–1.72)</td>
<td>1.81 (1.49–2.17)</td>
<td>1.62‡ (1.52–1.74)</td>
</tr>
<tr>
<td>Normal retinas</td>
<td>1.00 (0.97–1.04)</td>
<td>1.00 (0.97–1.04)</td>
<td>1.00 (0.81–1.24)</td>
<td>1.00 (0.93–1.08)</td>
</tr>
<tr>
<td>Kainate-destructed retinas</td>
<td>0.87 (0.72–1.06)</td>
<td>0.69 (0.60–0.80)</td>
<td>0.77 (0.62–0.96)</td>
<td>0.77 (0.73–0.82)</td>
</tr>
<tr>
<td>Normal retinas</td>
<td>1.00 (0.94–1.06)</td>
<td>1.00 (0.90–1.12)</td>
<td>1.00 (0.80–1.25)</td>
<td>1.00 (0.88–1.14)</td>
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In 60-day-old Royal College of Surgeons’ (RCS) rats and in light-damaged Wistar rats, losses of rhodopsin mRNA equivalent to ~95 and 70% were measured. In kainate-injected rats, losses of markers of retinal ganglion cells equivalent to ~50% were measured. Statistical significance (*P < 0.05, †P < 0.01, ‡P < 0.001) was determined using unpaired (light, RCS) or paired (kainate) Student’s t-test, where n = 5–7 (RCS), 6 (kainate), or 6 (light).

The retina (Fig. 6, A and B). MCT2 staining in the retina was less abundant than MCT1 and tended to be concentrated in the inner retina, where immunoreactive bands could be clearly delineated in the plexiform layers (Fig. 6C). MCT4 immunolabeling was present in the inner retina, particularly in putative Müller cells, and in the two plexiform layers, but was absent from the photoreceptors and RPE (Fig. 6E). Strong staining for MCT4 was also apparent in the smooth muscle lining of blood vessels in the choroid (Fig. 6, E and F). No labeling could be observed in the retina with the MCT3 antibody (Fig. 6D).

Immunohistochemical localization of MCTs in photoreceptor-deficient rats and kainate-injected rats correlated well with the PCR results. Immunoreactivities for MCT2 (Fig. 7) and MCT4 (Fig. 8), which are abundant in the inner retina, were reduced in kainate-injected rats but unaffected in photoreceptor-deficient retinas. Relative to total cell number in the retina, therefore, MCT2 and MCT4 immunoreactivities increased in photoreceptor-deficient retinas. In contrast, MCT1 immunoreactivity, which is abundant in photoreceptor inner segments but less concentrated in the inner retina, was largely unaffected by kainate injection (Fig. 7) but was obviously reduced in retinas lacking photoreceptors (Figs. 7 and 8).

Retinal pigment epithelium. Conventional and real-time RT-PCR analysis of freshly dissected RPE/choroid revealed the presence of all four MCT mRNAs (Figs. 2 and 3), although the levels of MCT1 and MCT3 were considerably higher than the levels of MCT2 and MCT4. Immunoblotting (Fig. 5) and immunohistochemistry experiments revealed that the RPE in situ expresses immunodetectable protein for MCT1–MCT3 but not for MCT4. MCT1 (Fig. 9A) was localized to the apical membrane and MCT3 (Fig. 9C) to the basal membrane of this cell layer, whereas MCT2 staining (Fig. 9B) was very faint and did not appear to be polarized.

Iris and ciliary body. Amplification of cDNAs from distinct iris and ciliary body samples with the use of primers specific for the mRNAs encoding MCT1–MCT4 revealed the presence of all four MCT transcripts in both tissues (Fig. 10). The relative abundance was explored by using real-time PCR, and the results are shown in Fig. 3. It can be seen that the iris expresses a similarly high amount of MCT1 mRNA to the retina and RPE, yet unlike these latter tissues, it also expresses a high level of MCT2 mRNA. The ciliary body also expresses appreciable amounts of MCT1 and MCT2 mRNA, although the level of MCT2 is somewhat lower than in the iris. As was the case in the retina, significant levels of MCT3 mRNA were detected in iris and ciliary body samples. With regard to MCT4, the transcript was present at lower levels than the other MCT mRNAs in both tissues. In general, the immunoblot (Fig. 5) and immunohistochemical findings corroborated the PCR results. MCT1 and MCT2 antibodies labeled single bands in immunoblots prepared from iris-ciliary body tissue and stained iris and ciliary body tissue sections specifically. The pattern of labeling in the iris and ciliary body was similar for MCT1 and MCT2, with both antibodies yielding strong immunoreactivity in the epithelium of the iris and the nonpigmented epithelium of the ciliary processes (Fig. 11, A and B, respectively). MCT1, but not MCT2, immunoreactivity also was evident in the remainder of the iris and ciliary body tissue, albeit at a lower intensity. Despite the presence of MCT3 mRNA, no labeling was observed in either tissue with the use of the MCT3 antibody (Fig. 11C). MCT4 immunoreactivity was absent from the iris and ciliary body but, as in the retina, was clearly localized to the smooth muscle of the vasculature in these tissues, particularly to the short posterior ciliary arteries and/or arterioles (Fig. 11, D and E).

Cornea. Positive signals for all four MCT mRNAs were evident in at least one of the cornea samples analyzed using RT-PCR, although the bands obtained for MCT1 and particularly MCT2 appeared to be brighter than for MCT3 and MCT4 (Fig. 10). Real-time PCR experiments substantiated these findings and revealed that the levels of MCT2 were not only dramatically higher than for the other MCTs but also considerably higher than in the other ocular samples analyzed (Fig. 3). The PCR data correlated quite well with the results obtained from immunohistochemistry experiments. MCT1 immunoreac-
Activity was present in numerous cells within the corneal epithelium and also in the monolayer of cells that comprise the corneal endothelium (Fig. 12A). Interestingly, the MCT2 antibody yielded slightly different patterns of staining, depending on the temperature of incubation. At 37°C, staining was apparent throughout the corneal epithelium and endothelium (Fig. 12D). However, at 4°C, labeling was only observed to any significant degree around the margins of the basal cells of the epithelium (Fig. 12C). With regard to MCT3, light staining was detectable in the basolateral membrane of the basal epithelial cells with the use of the MCT3 antibody from Chemicon (Fig. 12E), but no specific labeling was discernible with the use of the MCT3 antibody from ADI (Fig. 12F). Although light staining for MCT4 was observed in the corneal epithelium, this was revealed to be nonspecific in nature, because an identical pattern of staining was apparent in the corresponding negative control section (Fig. 12, G and H). None of the antibodies labeled the stromal tissue.

**Lens.** RT-PCR analysis of lens samples with the use of primers specific for the mRNAs encoding MCT1–MCT3 yielded only faint (MCT1 and MCT3) or undetectable (MCT2) signals (Fig. 10). Real-time PCR confirmed these results and showed that the MCT1–MCT3 transcripts are not expressed to any significant degree in the lens. In contrast, RT-PCR analysis provided clearer evidence for expression of MCT4 mRNA in the lens, and examination of the real-time PCR data allows two quantitative deductions to be made. First, the expression of MCT4 mRNA is indeed higher than for the other MCT RNAs in the lens, and second, the relative level of MCT4 mRNA in the lens is higher than in the retina or RPE (Fig. 2). Immunohistochemical detection of MCT1–MCT4 demonstrated labeling for MCT1 (Fig. 11F) and MCT4 (Fig. 11H) in lens epithelial cells but no specific staining for MCT2 (Fig. 11G) and MCT3 (data not shown).

**DISCUSSION**

**Retina**

The results presented in this study demonstrate that the retina expresses MCT1, MCT2, and MCT4. MCT1 was localized throughout the retina but was particularly abundant in the outer retina, whereas MCT2 and MCT4 were located preferentially in the plexiform layers.

In agreement with the results of previous studies, we found MCT1 immunoreactivity in the rat retina to be strongly associated with photoreceptor inner segments (3, 12). The abundance of MCT1 at this site is illustrated by the substantial reduction of retinal mRNA content in the retinas of photore-
ceptor-deficient rats. Lactate is thought to be an important energy substrate of photoreceptor inner segments (23), and MCT1 probably facilitates lactate uptake for oxidative phosphorylation. Studies performed with retinal preparations in vitro have identified the Müller cells as the source of the lactate used by photoreceptors (23, 29). Astrocytes play a similar role in the brain, exporting lactate for use by neurons (8), and recent work indicated that the MCT1 and MCT4 isoforms facilitate this release of lactate (10). The MCT isoform responsible for lactate efflux from Müller cells has not, however, been definitively established. The current study sheds some light on the subject of which MCT isoforms are expressed by retinal Müller cells. Müller cells are identified by their processes, which insinuate themselves between neuronal cell bodies in the nuclear layers, ramify laterally in the plexiform layers, and terminate proximally and distally as end-feet in the nerve fiber layer and outer limiting membrane, respectively. In retinal sections, MCT1, MCT2, and MCT4 immunoreactivities were all clearly detectable in the nerve fiber layer and in both plexiform layers, and it may be inferred therefore that all three isoforms are localized to Müller cells. However, the presence of MCT isoforms in the nerve fiber layer may reflect expression by astrocytes rather than by Müller cell end-feet, and many classes of neurons extend processes into the plexiform layers. Thus, to state with any real confidence that a protein is expressed by Müller cells, it is important to examine the nuclear layers and outer limiting membrane, as well. MCT1 labeling was apparent throughout both nuclear layers and at the outer limiting membrane, and MCT4 labeling was present throughout both nuclear layers and at the outer limiting membrane but was less abundant than MCT1, whereas MCT2 labeling was not seen within either nuclear layer or distally. In cultured, transformed Müller cells, MCT1 was the only MCT transcript to be strongly expressed. The combined results suggest that MCT1 is the dominant MCT isoform found in Müller cells, with a limited expression of MCT4.

As mentioned in the Introduction, contradictory results have been reported regarding the localization of MCT2 in the retina: Gerhart et al. (12) showed MCT2-like staining to be associated with Müller cells and synaptic and nuclear layers of the retina, yet Bergersen et al. (3) found no evidence for MCT2 immunoreactivity in the tissue. Indeed, in a recent paper, Bergersen et al. (4) suggested that the antibody used by Gerhart may recognize MCT4 rather than MCT2 and, by implication, that MCT2 may not be present in the retina. In this study, we used an MCT2 antibody that was previously successful for localization of MCT2 in mouse (10) and rat tissue (2). To verify its suitability for use in rats, we incubated testis and
skeletal muscle sections with this MCT2 antibody. Specific staining was observed in sperm tails throughout the epididymis, but no immunolabeling was detectable in the sarcolemma of the extraocular skeletal muscle. Under the conditions employed in our study, therefore, the antibody is specific for MCT2. The results of our study show that MCT2 is present on the processes of neurons in the inner retina rather than in Müller cells.

With regard to MCT4, the combined PCR and immunohistochemistry results indicate that this isoform is likewise concentrated in the inner retina, specifically in the two plexiform layers. The lack of MCT4 in the photoreceptors is in agreement with the data of Bergersen et al. (3), whereas the presence of this isoform in the inner retina concurs with the unpublished data of Philp et al. in the mouse (see Ref. 20). We suggest that MCT4 is expressed by Müller cells, albeit at lower levels than MCT1, but that it is also localized to neuronal processes within the inner retina.

The finding in this study that the retina expresses significant amounts of MCT3 mRNA was surprising because previous work has shown this isoform to be absent from the neural retinas of rats (21) and mice (20, 22). Initially, we considered...
that the positive signals might have resulted from amplification of mRNA emanating from a few RPE cells that had inadver-
tently contaminated the retinal samples during the dissection process. However, through the use of LCM, we were able to
eliminate this possibility. In previous studies, detection of
MCT3 mRNA in the retina was not attempted using PCR but
with the very much less sensitive technique of Northern blot
analysis. Because the levels of MCT3 transcript are substi-
tially less in the retina than in the RPE, they may well be below
the level detectable by Northern blot analysis. Nevertheless,
although we detected MCT3 mRNA in the retina, immunohis-
tochemistry experiments failed to identify the corresponding
protein. These results are in agreement with those of Philp and
colleagues (20–22) and point to one of two explanations for the
discrepancy: either the MCT3 antibodies used are not sensitive
even to detect low levels of the protein, or the MCT3 mRNA is,
for some unknown reason, not translated into immunode-
tectable protein.

Retinal Pigment Epithelium

Previous work has shown that two MCT isoforms are ex-
pressed in significant levels by the adult rat RPE: MCT1 is
polarized to the apical membrane, whereas MCT3 is restricted
to the basal membrane (3, 21). The coordinated activity of
these two transporters is suggested to allow the rapid transport
of lactate from the subretinal space across the RPE and into the
blood. The results of the current study are in complete agree-
ment with these reported findings. The present study also
provides tentative evidence that the MCT2 subtype is ex-
pressed at low levels in this cell type. The reason for the
inability of previous investigators (3, 12) to detect MCT2 in the
RPE is unclear but, as previously mentioned, is probably
related to the particular antibodies used in these studies.

Iris and Ciliary Body

The present study shows that the iris expresses the mRNAs
encoding all four MCT isoforms. The levels of the MCT1 and
MCT2 transcripts were higher than those of MCT3 and MCT4,
and immunohistochemistry was only able to show staining for
MCT1 and MCT2 in iris tissue. The most prominent immuno-
labeling for both isoforms was seen in the posterior epithelium.
Although little is known about transport processes across the
iris, experiments have indicated that up to 45% of the total
lactate content of the aqueous humor is lost to the blood vessels
of the surrounding iris (24). Historically, this process has been
considered to occur via passive diffusion, but in the light of
these results, roles for MCT1 and MCT2 in the transport of
lactate across the tissue and into the blood vessels must be
considered.
The results obtained with the ciliary body were broadly
similar to those found in the iris. Ciliary body samples ex-
pressed mRNAs encoding all four MCTs, yet immunolocaliza-
tion experiments were only able to show staining for MCT1
and MCT2. As in the iris, MCT1 and MCT2 immunolabeling
was most intense in the epithelium of the tissue. The ciliary
processes consist of a highly vascularized stromal core and a
specialized double layer of epithelium, which is responsible for
the secretion of aqueous humor. The inner nonpigmented
epithelium is directly adjacent to the posterior chamber,
whereas the outer pigmented epithelium rests on the stromal.
The patterns of MCT1 and MCT2 staining indicate that it is the
nonpigmented cells that express these transporters. Formation
of aqueous humor is a highly active process, and the ciliary
epithelium meets its considerable energy demands partly
through aerobic glycolysis. In fact, the tissue produces lactate
in vitro at a rate similar to that in the cornea (25), and the
majority of the lactate is generated by the more active nonpig-
mented epithelium. Lactate produced by the ciliary epithelium
can be lost directly to the blood, unlike the situation in the
cornea, but experiments have shown that 50% of the lactate
produced by the ciliary body is released into the aqueous
humor (7, 24). Transport of lactate between these cell layers
and the blood and aqueous humor may well be facilitated by
MCT1 and MCT2.
The finding that significant levels of MCT3 mRNA but not
MCT3 protein are present in the iris and ciliary body is curious.
Embryologically, the pigmented ciliary epithelium and the
posterior pigmented iris epithelium are the anterior continua-
tion of the RPE. These cell types share many similarities, and
indeed, the iris epithelium offers potential as a candidate for
transplantation in cases of RPE degeneration (26). Thus it is
not surprising that the cells express a similar complement of
mRNAs. Aside from the RPE, the only other tissue that
expresses functional MCT3 is the structurally and functionally
similar choroid plexus (22). Interestingly, compared with the
RPE, choroid plexus expresses a similarly high level of MCT3
transcript yet substantially lower amounts of MCT3 protein.
Philp et al. (22) suggested that expression of MCT3 is regulated
at the posttranscriptional level, and it appears that this is true
for the iris and ciliary body.

Smooth Muscle

The present study demonstrates that, in the eye, the MCT4
isoform is not only localized to the sarcolemma of extraocular
skeletal muscle but also strongly expressed by the smooth muscle lining of larger blood vessels within the iris, ciliary body, and choroid. Of particular note is the expression by vessels comprising the short posterior ciliary artery system. To our knowledge, this is the first demonstration in any tissue of MCT4 expression by vascular smooth muscle cells. Interestingly, MCT4 was not identified in the dilator or sphincter smooth muscle fibers of the iris or in any of the three types of smooth muscle fibers in the ciliary body. An explanation for the presence of MCT4 in ocular vessels is currently a matter for speculation, but given that such large quantities of lactate are produced by virtually all of the ocular tissues, it would be logical if MCT4 were to facilitate the rapid transfer of lactate from these tissues into the vascular system and thereby its removal from the eye. Future work is needed to determine whether MCT4 is expressed by vascular smooth muscle of nonocular tissues.

Cornea

The corneal epithelium is a highly glycolytic tissue that generates significant quantities of lactate. Generated lactate cannot pass into the ocular tear film because of the presence of numerous tight junctions, and kinetic studies have indicated that proton-coupled facilitated transport mechanisms exist for the passage of lactate from the corneal epithelium to the stroma (5) and from the stroma across the corneal endothelium to the aqueous humor (13). The results presented in this article...
provide evidence for the presence of both the MCT1 and MCT2 isoforms on corneal epithelium and corneal endothelium. The abundance of the MCT2 transcript in the cornea suggests that this isoform plays a key role in the transport of lactate, and it appears to be the dominant isoform for removal of lactate from the basal cells of the corneal epithelium. However, the remarkably high levels of MCT2 may reflect the fact that this isoform has a much lower substrate capacity than the other MCTs (15) and that higher levels are needed to ensure that lactate does not accumulate intracellularly and result in intracellular acidosis, fluid buildup, and light scatter.

The cornea, like the iris, ciliary body, and retina, expressed significant amounts of MCT3 mRNA. Unlike these latter tissues, however, tentative evidence was found to indicate that the mRNA is translated into immunodetectable protein. Using the Chemicon MCT3 antiserum, we detected some specific labeling in the basolateral membrane of the basal epithelial cells with the Chemicon anti-MCT3 antiserum (C), but no labeling was observable after incubation with the ADI anti-MCT3 antiserum (F). Diffuse, low-intensity labeling for MCT4 was detected throughout the corneal epithelium (G), but negative control sections (affinity-purified rabbit IgG) showed a similar pattern of staining, signifying that the immunoreactivity was nonspecific (H). Scale bar, 50 µm.

Fig. 12. Localization of MCT isoforms in rat cornea. MCT1 immunoreactivity was present in the corneal epithelium, where it was concentrated toward the stroma (A), and also in the corneal endothelium (A, arrow and inset). MCT2 immunoreactivity was likewise found in the epithelium (arrowheads) and endothelium (arrows) when the antiserum was applied for 30 min at 37°C (D). However, incubation of sections overnight at 4°C with the same MCT2 antiserum yielded a slightly different pattern of staining in which the posterior layer of columnar cells in the epithelium was particularly prominent but the endothelium was not labeled (C). Negative control labeling (nonimmune rabbit serum) for antisera shown in A, C, and D revealed no staining (B). MCT3 immunoreactivity was detected in the basal membrane of the basal epithelial cells with the Chemicon anti-MCT3 antiserum (E), but no labeling was observable after incubation with the ADI anti-MCT3 antiserum (F).
REFERENCES

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Lens

To fulfill its major function of refracting light, the lens must remain transparent. As a consequence, it is both avascular and devoid of major organelles and meets the bulk of its energy demands through anaerobic metabolism of glucose supplied by the aqueous and vitreous humors. The major end product, lactate, has traditionally been thought to diffuse out of the lens, but indirect evidence generated using rabbit and bovine lenses points toward a hydrogen ion-coupled mechanism of lactate extrusion (1, 27). The combined PCR and immunohistochemistry results generated in this study indicate that MCT1 and MCT4 are located in this tissue. PCR analysis provided clear evidence for expression of MCT4 mRNA by the lens and more ambiguous evidence for expression of MCT1 mRNA. The immunohistochemical results show that both MCT1 and MCT4 immunoreactivities are associated with the membrane of lens epithelial cells. The MCT4 isoform has an extremely high capacity for lactate and has a marked preference for lactate over pyruvate as a physiological substrate (11). These features points toward a hydrogen ion-coupled mechanism of lactate transport in lens epithelial cells. The MCT4 isoform has an extremely high capacity for lactate and has a marked preference for lactate over pyruvate as a physiological substrate (11). These features specialize MCT4 for the release of lactate from highly glycolytic cell types such as skeletal muscle (6). The expression of MCT4 by lens epithelial cells thus would be entirely consistent with this function.