Expression of the Sodium-Coupled Monocarboxylate Transporters SMCT1 (SLC5A8) and SMCT2 (SLC5A12) in Retina

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PURPOSE. Monocarboxylates are primary energy substrates in the retina. Recently, the authors identified two sodium-coupled monocarboxylate transporters (SMCTs), SMCT1 (a high-affinity transporter) and SMCT2 (a low-affinity transporter). Expression of SMCT1 and SMCT2 has been studied in several tissues; however, little is known about their expression in retina. In the present study, the authors asked whether SMCT1 and SMCT2 are also expressed in retina and, if so, in which particular retinal cell types.

METHODS. SMCT1 and SMCT2 expression was analyzed in intact mouse retina and cultured retinal cells (ganglion, Müller, RPE) by RT-PCR, in situ hybridization, and immunofluorescence. Uptake assays were performed to demonstrate SMCT1 (RGC-5 and ARPE-19 cells) and SMCT2 (rMC-1 cells) expression at the functional level.

RESULTS. SMCT1 mRNA and protein were detected in the ganglion cell layer, inner nuclear layer, inner/outer plexiform layers, photoreceptor inner segments, and RPE. In RPE, the expression of SMCT1 was restricted to the basolateral membrane. SMCT2 mRNA and protein were detected only in neural retina, with a pattern of protein localization consistent with labeling of Müller cells. In vitro studies confirmed the cell type–specific expression of SMCT1 and SMCT2. Uptake assays demonstrated Na+-coupled monocarboxylate transport in RGC-5, ARPE-19, and rMC-1 cells.

CONCLUSIONS. These data provide the first evidence for the expression of SMCT1 and SMCT2 in the retina and for the cell-type-specific distribution of these transporters within the retina. These studies suggest that SMCT1 and SMCT2 play a differential role in monocarboxylate transport in the retina in a cell type–specific manner. (Invest Ophthalmol Vis Sci. 2007; 48:3356–3363) DOI:10.1167/iovs.06-0888

The metabolic demands of the retina are very high. Although glucose is thought to be the preferred energy substrate in retina, lactate and ketone bodies also play important roles. Lactate is quantitatively abundant in the retina,1,2 and photoreceptor cells preferentially metabolize lactate.2 Recent studies suggest that lactate may also serve as an important energy source for other retinal neurons.3 Thus, though lactate was once viewed simply as a byproduct of glucose metabolism, it is becoming increasingly evident that this metabolite plays an important role in retinal energy metabolism. Metabolic use of glucose in the retina is increased during neuronal activity, but the uptake of glucose in Müller cells rather than in neurons accounts for most of this activity-associated glucose use.4 Müller cells convert glucose to lactate and release it into the extracellular medium to be taken up subsequently by retinal neurons. Thus, the energy needs of active retinal neurons are not met directly by the oxidation of glucose but rather by the oxidation of lactate supplied by Müller cells. Lactate represents a primary metabolic fuel to retinal neurons under normal physiological conditions.

Ketone bodies also represent important metabolic substrates for neuronal cells in the brain and the retina.5,6 The importance of ketone bodies as metabolic fuels becomes increasingly apparent when glucose availability is limited, such as during the suckling period in mammals,7,8 during times of prolonged starvation, and in patients with uncontrolled diabetes.9,10 Plasma concentrations of β-hydroxybutyrate, a monocarboxylate and the principal ketone body, are usually low (less than 3 mg/dL). Under normal circumstances, these substrates are of little physiological relevance as metabolic substrates. However, in conditions such as suckling, starvation, and uncontrolled diabetes, the circulating levels of β-hydroxybutyrate increase substantially (greater than 90 mg/dL).11 Even though it is generally assumed that liver is the sole producer of ketone bodies, recent evidence suggests that astrocytes may also serve as a significant source of ketone bodies to support neuronal cell function.12 As with the interaction between glial cells and neurons in lactate production and use, ketone bodies are thought to be shuttled between astrocytes and neurons.12 In addition to lactate, ketone bodies serve as an important energy source for neurons under certain physiological and pathologic conditions.

Mammalian cells express monocarboxylate transporters (MCTs), which mediate transmembrane transport of lactate and ketone bodies. Because of the importance of lactate and ketone bodies as metabolic fuel for retinal neurons, numerous studies have focused on the expression of MCTs in retina.3,4,13–21 MCTs, which belong to the SLC16 family of H+-dependent monocarboxylate transporters, are thought to be primarily responsible for the transport of monocarboxylates in the retina; however, recent studies have indicated the existence of Na+-dependent and H+-dependent monocarboxylate transporters in retina.15–17 Though the molecular identity of the transporter(s) responsible for Na+-dependent uptake of monocarboxylates was unknown. We recently identified two novel Na+-coupled monocarboxylate transporters that mediate active transport of monocarboxylates such as lactate and ketone bodies.22–30
be described only in kidney.28 Because monocarboxylates
are transported 1:1, SMCT1 and SMCT2 transport substrates through an Na⁺-dependent and
electrogenic process.32–34 SMCT1 is a high-affinity transporter, whereas SMCT2 is a low-affinity transporter. To date, SMCT1 expression has been described in the colon, small intestine, kidney, thyroid gland, and brain.29,30 SMCT2 expression has been described only in kidney.28 Because monocarboxylates such as lactate and ketone bodies represent primary energy substrates for retinal neurons, we sought to determine whether SMCT1 and SMCT2 are expressed in the retina. Our studies demonstrate for the first time the differential and cell type-specific expression of these transporters in retina.

MATERIALS AND METHODS

Reagents

Rabbit polyclonal antibodies against SMCT1 and SMCT2 were generated at a commercial firm (Biosynthesis, Inc., Lewisville, TX); the details of the antigenic peptide and the specificity of the SMCT1 antibody have already been published.25,27 A rabbit polyclonal antibody against mouse SMCT2 was generated using the same commercial firm (Biosynthesis, Inc.). To determine the specificity of the anti-SMCT2 antibody, the vaccinia virus expression system was used to express the cloned mouse SMCT2 heterologously in HRPE cells, and immunofluorescence experiments were performed in parallel in vector-transfected cells and in SMCT2 cDNA-transfected cells. HRPE cells do not express SMCT2; therefore, vector-transfected cells served as a negative control. Normal rabbit preimmune serum was used as an additional negative control. Furthermore, the antibody was neutralized with an excess of the antigenic peptide and was used as a negative control in immunofluorescence studies with mouse retina cryosections and cultured cells. We have used this antibody to localize SMCT2 protein in mouse kidney.31

Other reagents used in these studies were as follows: mouse monoclonal anti–cellular retinaldehyde-binding protein (CRALBP; AbCam, Cambridge, MA); mouse monoclonal anti–vimentin (Chemicon International, Temecula, CA); goat anti–rabbit IgG and goat anti–mouse IgG coupled to fluorescent dyes (Alexa Fluor 568 and Alexa Fluor 488, respectively; Molecular Probes, Carlsbad, CA); and [14C]nicotinate (American Radiolabeled Chemicals, St. Louis, MO).

Animals

C57BL/6 mice were used for collection of retinal tissues for RT-PCR and in situ hybridization and for the preparation of primary cultures of retinal ganglion cells and Müller cells. Balb/c mice were used for the preparation of retinal sections used for immunofluorescence analyses. Care and use of all animals adhered to the institutional guidelines for the humane treatment of animals and to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Cell Lines

RGC-5 and ARPE-19 cells were cultured in Dulbecco modified Eagle medium (DMEM)/F12 medium; rMC-1 cells were cultured in DMEM. All media were supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. For studies of RPE polarization, ARPE-19 cells were cultured on permeable transwell filters for 4 weeks to promote differentiation into a polarized phenotype according to our previously published method.32

Primary Cultures of Retinal Ganglion Cells and Müller Cells

Primary cultures of retinal ganglion cells and Müller cells were prepared from retinas of C57Bl/6 mice according to our previously published methods.33,34 Purity of the cultures was verified by immunodetection of known markers of ganglion cells and Müller cells.

RT-PCR

Total RNA was prepared from neural retina, RPE/eyecup, and cultured retinal cells, as described previously,35 and were used for RT-PCR. PCR was performed with primers specific for mouse SMCT1 (neural retina, RPE/eyecup, RGC-5, and rMC-1 cells) or human SMCT1 (ARPE-19 cells). RGC-5 and rMC-1 cell lines were of rat origin; however, primers specific for mouse SMCT1 were used to detect SMCT1 mRNA in these cell lines because rat SMCT1 has not yet been cloned. RNA from kidney was used as a positive control. Sense and antisense primers for mouse SMCT1 were 5'-TTATTGGGCGGTGCGAAGTA-3' and 5'-CAGAGGCCCACAGGTTGACAT-3', respectively (product size, 727 bp). Sense and antisense primers for human SMCT1 were 5'-CCCTACCGCTAGCTTC-3' and 5'-ATCTTCACCAATGAGCA-3', respectively (product size, 500 bp). 18s ribosomal RNA was used as an internal control.

In Situ Hybridization

To localize SMCT1 mRNA in retina, in situ hybridization was performed on sections of mouse retina according to our previously described method.25,28 Preparation of the antisense and sense riboprobes for mouse SMCT1 has been detailed.25

Immunofluorescence Detection of SMCT1 and SMCT2

Sections of albino mouse eyes were fixed in ice-cold acetone, washed with 0.1 M PBS, and blocked with 1× power block for 10 minutes. Sections were incubated overnight at 4°C with the polyclonal antibodies against SMCT1 or SMCT2 at a dilution of 1:1000 each. For dual-labeling experiments, SMCT1 or SMCT2 polyclonal antibodies were used in combination with monoclonal anti–vimentin (1:100), a cellular marker for Müller cells, or monoclonal anti–CRALBP (1:100), a cytoplasmic marker for RPE and Müller cells. Negative control sections were treated with SMCT1 or SMCT2 antibody that had been preincubated with excess antigenic peptide. Sections were incubated for 1 hour with goat anti–rabbit IgG or goat anti–mouse IgG coupled to fluorescent dye (Alexa Fluor 568 or Alexa Fluor 488; Molecular Probes), at a dilution of 1:1000. Coverslips were mounted with mounting media (Vectashield Hardset; Vector Laboratories, Burlingame, CA) with DAPI nuclear stain, and sections were examined by epifluorescence under a microscope (AxioPlan 2; Carl Zeiss, Oberkochen, Germany) equipped with a slider module (ApoTome; Carl Zeiss). Similar methods were used to localize SMCT1 and SMCT2 in cultured cells.

Uptake Measurements

SMCT1 and SMCT2 are Na⁺-coupled transporters used not only for lactate, ketone bodies, and short-chain fatty acids but also for nicotinate, a B-complex vitamin.23 Therefore, we monitored the transport function of these transporters by Na⁺-dependent uptake of [14C]nicotinate, RGC-5 and ARPE-19 (SMCT1) and rMC-1 (SMCT2) cells were seeded in 24-well culture plates and grown to confluence. The uptake buffer was 25 mM Hepes/Tris (pH 7.5), containing 140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄, and 5 mM glucose. Na⁺-free buffer was prepared by replacing NaCl with an equimolar concentration of N-methyl-D-glucamine (NMDG) chloride. Uptake measurements were performed as described previously.23 Each uptake experiment was performed in duplicate or triplicate and repeated 2 to 4 times; data are presented as mean ± SE.

RESULTS

Analysis of SMCT1 mRNA Expression in Intact Retina

RT-PCR with RNA samples from mouse neural retina and RPE/eyecup yielded an expected product (727 bp) similar to that
amplified from mouse kidney (positive control), indicating SMCT1 expression in these retinal tissues (Fig. 1A). The molecular identity of the PCR products from neural retina and RPE/eyecup was confirmed by sequencing. In situ hybridization was performed on sections of adult C57BL/6 mouse eyes to determine which cells of the retina expressed SMCT1 mRNA (Fig. 1B). SMCT1 mRNA was detected in the ganglion cell layer, the inner nuclear layer, and the inner segments of the photoreceptor cells. Expression of SMCT1 mRNA in RPE was not clear in this experiment because of the presence of pigment in RPE. Therefore, throughout the remainder of the study, we used retinal sections obtained from albino (Balb/c) mice. No signals were detected anywhere in the retina in the presence of a sense probe.

**Analysis of SMCT1 Protein in Intact Retina**

To localize SMCT1 protein by immunofluorescence within the mouse retina, we used a polyclonal antibody against SMCT1 (Fig. 1C). SMCT1 (red) was detected in the cells of the ganglion cell layer, in the inner and outer plexiform layers, and in the inner nuclear layer, where intense SMCT1 labeling was particularly notable in the outer margin (Fig. 1Ca). Strong signals were also detected in the inner segments of the photoreceptor cells and in the RPE. An image showing the merging of positive signals for SMCT1 and DAPI (a nuclear stain) is presented in Figure 1Cc. Examination at a higher magnification revealed the presence of intense labeling in the axonal processes of ganglion cells (Fig. 1Cd). In the outer retina, labeling of SMCT1 in the RPE and in the inner segments of the photoreceptor cells is clearly notable at a higher magnification (Fig. 1Ce). Immunopositive signals were specific because antibody that had been neutralized with excess antigenic peptide gave no signals (Fig. 1 Cf).

The labeling pattern indicated that SMCT1 was expressed specifically in retinal neurons and RPE. However, areas such as the inner nuclear layer and the nerve fiber layer, where prom-
ient labeling of SMCT1 is observed, also contain the processes of Müller cells. Thus, to confirm that the expression of SMCT1 in these regions is indeed specific to retinal neurons, we used double-labeling techniques using vimentin, a Müller cell–specific marker, along with the anti–SMCT1 antibody. Figure 2A shows immunolabeling for SMCT1 (red), depicting positive signal for SMCT1 in the nerve fiber layer. Figure 2A\alpha shows an image of the same retinal section labeled with vimentin (green). Merging of the two signals with DAPI nuclear stain (blue) demonstrated no overlap of the signals for SMCT1 and vimentin (Fig. 2A\alpha).

Analysis of SMCT1 Expression in RGC-5, rMC-1, and ARPE-19 Cells

Immunofluorescence analysis of intact retina indicated that SMCT1 is expressed specifically in retinal neurons and RPE but not in Müller cells. To corroborate these findings, we monitored the expression of SMCT1 in three retinal cell lines: RGC-5 (ganglion), rMC-1 (Müller), and ARPE-19 (RPE). RT-PCR and immunofluorescence analysis detected mRNA specific for SMCT1 in RGC-5 and ARPE-19 cells but not in rMC-1 cells (Fig. 2B). Results of mRNA expression were confirmed by immunofluorescence analysis of SMCT1 protein, which revealed strong positive labeling for SMCT1 (red) in RGC-5 and ARPE-19 cells but not in rMC-1 cells (Fig. 2C).

Because RPE is a polarized epithelial cell layer with distinct apical and basolateral compartments, we performed immuno-histochemical studies in polarized, well-differentiated ARPE-19 cells. Optical sections of cells labeled with SMCT1 are shown in Figure 2D. Cells that were scanned horizontally (xy plane) revealed a cobbledstone pattern of immunopositive signals suggestive of basolateral distribution of SMCT1 (Fig. 2D\alpha, 2D\beta). Vertical scanning (xz plane) of the same cells showed a pattern of SMCT1 expression consistent with labeling of basolateral membranes (Fig. 2Dc, 2Dd). Cells incubated with peptide-blocked SMCT1 antibody showed no immunolabeling (data not shown).

Analysis of Na\(^{+}\)-Coupled Nicotinate Uptake in RGC-5 and ARPE-19 Cells

Immunofluorescence and RT-PCR demonstrated SMCT1 expression in RGC-5 and ARPE-19 cells. These data are supported by uptake measurements. Na\(^{+}\)-dependent uptake of nicotinate was evident in RGC-5 (Fig. 3A) and ARPE-19 (Fig. 3B) cells. In both cell lines, the uptake of \( [\text{\textsuperscript{14}}\text{C}] \) nicotinate was Na\(^{+}\)-dependent to a significant extent and inhibitable (greater than 60%) by excess unlabeled nicotinate.

Analysis of SMCT1 Expression in Primary Ganglion and Müller Cell Cultures

RT-PCR and immunofluorescence analysis of retinal cell lines provided evidence for the expression of SMCT1 in retinal neurons and for the lack of expression in Müller cells. To confirm these results further, we analyzed SMCT1 expression in primary cultures of ganglion (immunofluorescence) and Müller (RT-PCR and immunofluorescence) cells. SMCT1 mRNA was not detectable in primary Müller cells (Fig. 4A), as confirmed by immunofluorescence (Fig. 4B). However, these cells were positive for CRALBP, a marker for Müller cells. In contrast to Müller cells, primary ganglion cells were positive for SMCT1 as monitored by immunofluorescence (Fig. 4C). Positive signals were detectable in the cell body and in axonal processes of these neuronal cells.

Analysis of SMCT2 Expression in Intact Mouse Retina

RT-PCR with RNA samples from mouse neural retina and RPE/eyecup detected SMCT2 mRNA in neural retina but not in RPE/eyecup (Fig. 5A). The molecular identity of the PCR products from neural retina and RPE/eyecup was confirmed by sequencing. To determine in which cells of the neural retina SMCT2 protein is expressed, we analyzed the localization of
SMCT2 protein using a polyclonal antibody specific for SMCT2. We confirmed the specificity of the antibody by two approaches. HRPE cells do not express SMCT2 constitutively. Accordingly, there was no immunopositive signal with the antibody when the cells were transfected with vector alone (Fig. 5B). However, the antibody gave a positive signal in cells transfected with mouse SMCT2 cDNA (Fig. 5B). We also assessed the specificity of the antibody by neutralization with the antigenic peptide. The positive signal detected with the antibody in SMCT2 cDNA–transfected cells disappeared when the antibody was neutralized with an excess of the antigenic peptide (data not shown). Immunofluorescence analysis of SMCT2 protein in retinal cryosections demonstrated SMCT2 expression only in neural retina; positive signals were detected in the inner nuclear layer and in what appeared to be retinal Müller cells, as indicated by the labeling of filamentous-type processes that extended from just above the ganglion cell layer down to the outer nuclear layer (Fig. 5CA). A view of the inner retina of a cryosection incubated with SMCT2 antibody (red) and counterstained with DAPI (blue) at higher magnification is given in Figure 5CB. Positive signals for SMCT2 were not detected in the outer retina (Fig. 5CC). To substantiate the Müller cell–specific localization of SMCT2 further, we performed colocalization studies with the Müller cell–specific marker vimentin (Fig. 5CD–F). In sections incubated with SMCT2 along with vimentin antibody, there was significant overlap between positive signals for SMCT2 (red) and positive signals for vimentin (green). To corroborate these findings with respect to the Müller cell–specific expression of the transporter in intact mouse retina, we analyzed the expression of SMCT1 and SMCT2 in primary cultures of retinal Müller cells and ganglion cells. These studies have shown that SMCT1 is expressed predominantly in retinal neurons and in RPE, whereas SMCT2 is expressed exclusively in Müller cells. RT-PCR provided evidence for the expression of SMCT1 mRNA in neural retina and RPE/eyecup. This was confirmed by in situ hybridization. SMCT1 mRNA was expressed in a variety of retinal cell types, including ganglion cells, cells of the inner nuclear layer, photoreceptor cells, and RPE. We found the SMCT1 protein to be localized to ganglion cells, inner and outer plexiform layers, soma of cells throughout the inner nuclear layer, inner segments of the photoreceptor cells, and RPE cells. However, because several cell types are present in

**DISCUSSION**

To date, the uptake of monocarboxylates in the retina has been attributed primarily to the action of MCTs. There are several isoforms of MCT, of which only four have been demonstrated unequivocally to function as monocarboxylate transporters (MCTs 1–4). Numerous reports have been published on the expression of MCT isoforms in the eye. Interestingly, some of these reports have provided evidence for the existence of Na+/H+–independent (H+/H+–coupled) and Na+/H+–dependent mechanisms for transport of monocarboxylates in retinal cells; however, the molecular identity of the transporter(s) responsible for Na+/H+–dependent uptake was unknown. Here we have shown that retina expresses two Na+/H+–coupled monocarboxylate transporters, SMCT1 and SMCT2. We have established the expression pattern of SMCT1 and SMCT2 in intact retina by RT-PCR, in situ hybridization, and immunofluorescence. In addition, we have analyzed the expression of SMCT1 and SMCT2 in three well-characterized retinal cell lines (RGC-5, rMC-1, and ARPE-19) and in primary cultures of retinal Müller cells and ganglion cells. These studies have shown that SMCT1 is expressed predominantly in retinal neurons and in Müller cells and ganglion cells, whereas SMCT2 is expressed exclusively in Müller cells.
the inner nuclear layer and the radial processes of retinal Müller cells wrap around the nuclei of the ganglion cells and extend further to form “end feet” close to the axonal processes of the ganglion cells, we used dual-labeling techniques to compare the expression of SMCT1 and vimentin, a marker for Müller cells. Results of these experiments showed that SMCT1 expression in the inner retina is limited primarily to the ganglion cells and their axonal processes and to other neuronal cells in the inner nuclear layer, with no apparent expression in retinal Müller cells. Uptake measurements in RGC-5 cells confirmed the presence of a Na\(^+\)/H\(^+\)-coupled monocarboxylate transporter in this neuronal cell type.

Immunofluorescence analysis also revealed the presence of SMCT1 protein in the RPE. Because RPE is a polarized epithelial cell layer, we determined—with well-differentiated, polarized cultures of ARPE-19 cells—whether the expression of SMCT1 in this cell layer was differentially localized to the apical or the basolateral membrane. These studies indicated that SMCT1 differentially polarizes to the basolateral membrane of the RPE. The present findings demonstrating the Na\(^+\)-coupled, electrogenic, monocarboxylate transporter SMCT1 in the basolateral membrane of the RPE, coupled with the previous findings that the H\(^+\)-coupled, electroneutral, monocarboxylate transporter MCT1 localizes to the apical membrane of the RPE, are relevant to the vectorial transfer of lactate across this cell layer. It is likely that, under conditions of increased demands for lactate by neural retina, RPE can transfer lactate effectively from the choroidal blood to the retina by a process coordinated by SMCT1 in the basolateral membrane and MCT1 in the apical membrane. Studies from other investigators have shown that MCT3, another H\(^+\)-coupled, electroneutral MCT isoform, is also located exclusively in the basolateral membrane of the RPE.\(^{19,21}\) This transporter may also play a role in the transcellular movement of lactate across the RPE. We were able to detect SMCT1 transport function in ARPE-19 cells as evident from Na\(^+\)/H\(^+\)-dependent nicotinate uptake. These uptake studies were performed in cells cultured for 3 days. Under these conditions, ARPE-19 cells do not polarize, explaining why we were able to detect the functional activity of SMCT1, which polarizes to the basolateral membrane of RPE in intact retina.

In neural retina, SMCT1 expression appears to be restricted primarily to neuronal cell types, with no apparent expression in Müller cells. This was confirmed by immunofluorescence studies using the cultured cell lines RGC-5 (ganglion), rMC-1 (Müller), and ARPE-19 (RPE). These cell lines are particularly useful as in vitro model systems to study various aspects of retinal cell biology given that they are phenotypically similar to their respective normal nontransformed retinal cell types in culture.\(^{35-39}\) RT-PCR demonstrated the expression of SMCT1 mRNA in RGC-5 and ARPE-19 cells, but not in rMC-1 cells. Immunofluorescence analysis of SMCT1 confirmed these findings. These data support our conclusion that SMCT1 expression in retina is restricted primarily to neuronal cells and RPE, with no apparent expression in retinal Müller cells. Even
though transformed retinal cell lines are similar to normal retinal cells, some properties that are native to normal retinal cells may be lost or altered in these cell lines. Therefore, we examined the expression of SMCT1 in primary cultures of retinal ganglion and Müller cells. Analysis of SMCT1 mRNA and protein in these cells confirmed the results obtained in transformed cell lines, with SMCT1 expression detectable only in primary cultures of ganglion cells. There was no detectable expression of SMCT1 in primary Müller cells.

In light of the recent discovery of a second Na\(^+\)/H\(^+\)-coupled monocarboxylate transporter, SMCT2, we also analyzed the expression of this transporter in the retina. RT-PCR demonstrated the expression of SMCT2 in neural retina but not in the RPE/eyecup. Immunofluorescence analysis of SMCT2 in intact mouse retina confirmed these findings. We found the protein to be localized primarily to Müller cells, with no apparent expression in retinal neurons. Müller cell–specific localization of SMCT2 was confirmed by a dual-labeling immunofluorescence technique using vimentin, a Müller cell-specific marker. Additionally, we examined the expression of SMCT2 in primary cultures of Müller cells. Positive signals indicative of SMCT2 immunolabeling were indeed localized to retinal Müller cells, double-labeling experiments were performed using vimentin, a Müller cell-specific marker. Immunofluorescence analysis of SMCT2 protein in cultured primary Müller cells showed positive signals for SMCT2 (red) alone. Positive signal for vimentin alone (green). Merging of positive signals for SMCT2 and vimentin (yellow). Original magnifications: ×200 (A); ×400 (C); ×630 (CD—CF).

FIGURE 5. Analysis of SMCT2 expression in intact mouse retina and cultured rMC-1 and primary Müller cells. (A) Total RNA was prepared from mouse neural retina and RPE/eyecup. RT-PCR was carried out with primers specific for mouse SMCT2. 18S ribosomal RNA was used as an internal control. (B) Immunofluorescence testing of SMCT2 antibody specificity in transfected hRPE cells. Immunolabeling for SMCT2 revealed robust positive signal (red) in hRPE cells transfected with SMCT2 cDNA (A) but not in hRPE cells transfected with empty vector (B). (C) Immunofluorescence analysis of SMCT2 expression in intact mouse retina. (Ca) Positive signals (red) indicative of SMCT2 labeling were detected in the inner retina. (Cn) Observation of the inner retina at higher magnification revealed a pattern of SMCT2 labeling consistent with labeling of Müller cells. (Cp) Nuclear counterstain is shown in blue. (Cc) Observation of the outer retina at higher magnification revealed no positive labeling for SMCT2 in the outer nuclear layer (onl) or the retinal pigment epithelium (RPE). To confirm whether positive signals indicative of SMCT2 immunolabeling were indeed localized to retinal Müller cells, double-labeling experiments were performed using vimentin, a Müller cell-specific marker. (Cd) Positive signal for SMCT2 (red) alone. (Cf) Positive signal for vimentin alone (green). (Cg) Merging of positive signals for SMCT2 and vimentin (yellow). (D) Immunofluorescence analysis of SMCT2 protein in cultured primary Müller cells. (Da) Positive signals for SMCT2 (red) were detected in these cells. (Db) Immunolabeling for CRALBP, a Müller cell marker. (Dc) Merging of signals for SMCT1 (red), CRALBP (green) and DAPI nuclear stain (blue). (Dd) Cells treated with excess SMCT2 antibody neutralized with excess antigenic peptide. (E) Time-course for uptake of nicotinate in rMC-1 cells demonstrating the functional expression of a Na\(^+\)-dependent monocarboxylate transport system in these cells.
References