Expression and Function of Glutamine Transporters SN1 (SNAT3) and SN2 (SNAT5) in Retinal Müller Cells

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PURPOSE. The expression and function of the glutamine transporters ATA1 and ATA2 (isoforms of system A), SN1 and SN2 (isoforms of system N), and LAT1 and LAT2 (isoforms of system L) were investigated in Müller cells in a rat Müller cell line (rMC1) and primary cultures of mouse Müller cells.

METHODS. Glutamine uptake in rMC1 cells and primary Müller cells was measured. The relative contributions of various transport systems to glutamine uptake were determined based on the differential substrate specificities and Na⁺/H⁺ dependence of individual transport systems. RT-PCR was used to analyze the expression of transporter-specific mRNAs.

RESULTS. Three different transport systems participated in glutamine uptake in rMC1 cells: system L (Na⁺/H⁺ independent), system A (Na⁺/H⁺ dependent and α-(methylamino)isobutyric acid [MeAIB]-sensitive), and system N (Na⁺/H⁺ dependent and MeAIB-insensitive). System N was the principal contributor (~70%); the contributions of systems A and L were relatively lesser (20% and <10%, respectively). The functional features of Na⁺/H⁺ dependent and MeAIB-insensitive glutamine uptake were similar to the known characteristics of clones of SN1 and SN2. Glutamine uptake in primary Müller cells behaved in a manner similar to that in rMC1 cells. mRNA transcripts specific for ATA1, ATA2, SN1, SN2, LAT1, and LAT2 were expressed in Müller cells.

CONCLUSIONS. System N (SN1 as well as SN2) is responsible for most of the glutamine uptake in Müller cells. Because system N is capable of mediating the release of glutamine from the cells, its abundant expression in Müller cells is of importance in the handling of glutamine in the retina. (Invest Ophthalmol Vis Sci. 2005;46:3980–3987) DOI:10.1167/iovs.05-0488

Retinal Müller cells are the principal radial glial cells of the retina and span the entire thickness of the retina, contacting virtually all retinal neurons.¹⁻² The layered arrangement of the retinal neurons is created and maintained by the Müller cell framework.³ Müller cells have numerous functions.⁴ Important among these functions are the uptake of extracellular glutamate, conversion of glutamate to glutamine, and subsequent release of glutamine into the extracellular space. These functions form essential components of the glutamate-glutamine cycle that occurs between Müller cells and glutamatergic neurons within the retina and is critical for the maintenance of glutamatergic neurotransmission.⁵⁻⁶ The glutamate-glutamine cycle is an example of the complex metabolic interactions between neurons and glial cells in the retina. In this cycle, glutamatergic neurons release glutamate into the extracellular space on activation to initiate glutamate signaling in postsynaptic neurons. Neurotransmission is subsequently terminated by effective clearance of glutamate from the synapse by energy-coupled uptake of glutamate via different glutamate transporters expressed in neuronal cells and glial cells. Glutamate uptake by Müller cells is a major contributor to this clearance process in the retina. The glutamate transporter expressed in Müller cells is EAAT1 (excitatory amino acid transporter 1), also known as GLAST (glutamate/aspartate transporter).⁷ Once inside the Müller cell, glutamate is converted to glutamine by glutamine synthetase, an enzyme found exclusively in Müller cells within the retina.⁸ Müller cells release glutamine into the extracellular space, which is then taken up by glutamatergic neurons and used as an immediate precursor of glutamate. There is no information available at present on the molecular identity of the transporter that is responsible for the release of glutamine from Müller cells.

Transfer of glutamine across the plasma membrane of mammalian cells is mediated by a multitude of transporters that have been characterized at the molecular level.⁹⁻¹⁹ Important among these transporters are ATA1/SNAT1 (amino acid transporter A1 or sodium-coupled neutral amino acid transporter 1; SLC38A1), ATA2/SNAT2 (amino acid transporter A2 or sodium-coupled neutral amino acid transporter 2; SLC38A2), SN1/SNAT3 (system N1 or sodium-coupled neutral amino acid transporter 3; SLC38A3), and SN2/SNAT5 (System N2 or sodium-coupled neutral amino acid transporter 5; SLC38A5). ATA1 and ATA2 represent two different isoforms of the amino acid transport system known as system A,¹²⁻¹⁵ whereas SN1 and SN2 represent two different isoforms of the amino acid transport system known as system N.¹⁶⁻¹⁹ ATA1 and ATA2 are Na⁺/H⁺ coupled and function under physiological conditions as influx transporters. SN1 and SN2 are coupled to an Na⁺ gradient as well as an H⁺ gradient and mediate a transport process in which Na⁺ and glutamine move in one direction, and H⁺ moves in the opposite direction.¹⁶⁻¹⁹ Furthermore, the direction of glutamine flux through these transporters can be altered, even under normal physiological conditions. They can mediate the movement of glutamine either into the cells (influx) or out of the cells (efflux). These transporters, however, are by no means specific for glutamine, because they also interact with other amino acids. ATA1/ATA2 and SN1/SN2 can be differentiated easily based on their amino acid selectivity.¹²⁻¹⁹ In addition, there are Na⁺/H⁺ independent transport systems for glutamine, and this category of glutamine transporters includes LAT1/4F2hc and LAT2/4F2hc.⁹⁻¹⁰ These transporters are heterodimeric, consisting of 4F2hc as the heavy chain and either LAT1 or LAT2 as the light chain, and represent two different isoforms of the amino acid transport system known as system L.

In the present study, we investigated the expression and function of ATA1, ATA2, SN1, SN2, LAT1, and LAT2 in Müller cells. The expression and function of ATA1, ATA2, SN1, SN2, LAT1, and LAT2 were measured in rMC1 cells. RT-PCR was used to analyze the expression of transporter-specific mRNAs.
cells because of the known relevance of these cells in the handling of glutamine within the retina. Herein, we provide evidence for the expression of SN1 and SN2 as the principal transporters for glutamine in a rat Müller cell line (rMC1) as well as in primary cultures of mouse Müller cells.

**Methods**

**Materials**

Reagents were obtained from the following sources: \[^{[3]}\Hglutamine (GE Healthcare, Amersham, UK); \[^{[1]}\C]methylaminoisobutyric acid (MeAIB; American Radiolabeled Chemicals, Inc., St. Louis, MO); RNA extraction reagent (TRIZol; Invitrogen-Gibco Corp., Grand Island, NY); fetal bovine serum (FBS) (Atlanta Biologicals, Atlanta, GA); an RNA PCR kit (Applied Biosystems, Inc., [ABI] Foster City, CA); a Taq kit (TaKaRa, Tokyo, Japan); rat Müller cells (rMC1; Vijay P. Sarothy, Northwestern University, Chicago, IL). Antibodies used were obtained from the following sources: rabbit anti-GLAST (EAAT1; Alpha Diagnostics, San Antonio, TX); rabbit anti-glutamine synthetase, goat anti-GFAP, goat anti-neurofilament-L (Santa Cruz Biotechnology Inc., Santa Cruz, CA); Cy3-conjugated secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA); and Alexa Fluor 488-conjugated secondary antibody (Invitrogen, Carlsbad, CA). Anti-CRALBP was a generous gift from John C. Saari (University of Washington, Seattle, WA).

**Animals**

C57BL/6 mice were used to prepare primary cultures of Müller cells. Care and use of the mice adhered to the principles set forth in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**rMC1 Cell Culture**

rMC1 cells were cultured in DMEM, supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 \(\mu\)g/ml streptomycin at 37°C in a humidified chamber with 5% CO\(_2\). The culture medium was replaced every other day. At 80% to 90% confluence, cells were dissociated with 0.05% (wt/vol) trypsin. Trypsin-released cells were seeded in 24-well plates at a density of 1 \(\times\) 10\(^5\) cells/well and allowed to grow as a monolayer. Twenty-four hours after subculturing, the medium was replaced with fresh medium. Uptake measurements were performed at 48 hours after seeding.

**Uptake Measurements in rMC1 Cells**

The culture medium was removed by aspiration, and the cells were washed once with the standard uptake buffer (140 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl\(_2\), 0.8 mM MgSO\(_4\), 5 mM glucose, and 25 mM HEPES, pH 8) and preincubated in 0.25 mM of the same buffer for 10 minutes at 37°C. The medium was then replaced by 0.25 mM of uptake buffer containing \[^{[3]}\Hglutamine (0.5 \mu\)M/assay; final concentration of radiolabeled plus unlabeled glutamine during uptake, 5 \(\mu\)M) in the presence or absence of 5 mM MeAIB, and incubation continued for 15 minutes at 37°C. Uptake was terminated by removing the uptake buffer, followed by washing twice with ice-cold uptake buffer. Cells were then lysed with 0.5 mM of 1% sodium dodecylsulfate in 0.2 N NaOH, and the lysate was used for measurement of radioactivity by liquid scintillation spectrometry.

MeAIB was added to the uptake buffer to suppress any glutamine uptake that may occur via amino acid transport system A (i.e., ATA1 and ATA2). For measurement of Na\(^+-\)independent glutamine uptake, the uptake buffer was modified by substituting NaCl iso-osmotically with N-methyl-D-glucamine (NMDG) chloride. For the analysis of the influence of extracellular pH on glutamine uptake, uptake buffers of different pH (pH range, 6.0–8.5) were prepared by appropriately adjusting the concentrations of HEPES, Mes, and Tris. \[^{[14]}\C]MeAIB was used as a specific substrate for ATA1 and ATA2 and uptake of MeAIB via these transporters was monitored in the presence of Na\(^{-}\). Uptake measurements were made in duplicate or triplicate, and experiments were repeated at least three times with independent cell cultures. Data are given as the mean ± SE of these replicates.

**Determination of Relative Contributions of Specific Transport Systems to Glutamine Uptake**

Glutamine is a substrate for all three transport systems: A, N, and L. The relative contributions of each of these systems to glutamine uptake observed in a given cell can be evaluated because of the differential substrate selectivity and Na\(^+\)-dependence of these systems. Systems A and N are Na\(^+\)-coupled, whereas system L is Na\(^+\)-independent. Therefore, glutamine uptake measured under Na\(^+\)-free conditions represents uptake that occurs via system L. This uptake also consists of any glutamine entry into cells via diffusion. Thus, glutamine uptake that is Na\(^+\)-dependent (i.e., uptake in Na\(^+\)-containing buffer minus uptake in Na\(^+\)-free buffer) represents the combined uptake via systems A and N. MeAIB is a highly specific substrate for system A and this model amino acid does not interact with system N. Therefore, the Na\(^+\)-dependent glutamine uptake that is inhibitable by MeAIB represents the system A-mediated uptake component, whereas the Na\(^+\)-independent glutamine uptake that is insensitive to MeAIB represents the system N-mediated uptake component.

We used this strategy to determine the relative contributions of systems L, A, and N to glutamine uptake in Müller cells.

**Kinetic Analyses**

Saturation kinetics of glutamine uptake via system N was analyzed by measuring the system N-specific uptake in the presence of increasing concentrations of glutamine. The data were then fit to the Michaelis-Menten equation to calculate the kinetic parameters (\(K_m\) and maximum velocity, \(V_{max}\)). The Na\(^+\)-activation kinetics of glutamine uptake via system N was analyzed by measuring the system N-specific uptake in the presence of increasing concentrations of Na\(^+\). The concentration of Na\(^+\) was varied in the uptake buffer by substituting NaCl iso-osmotically with N-methyl-D-glucamine (NMDG) chloride. The Na\(^+\)-dependent uptake data were then analyzed by the Hill equation to determine the Hill coefficient (\(b\), the number of Na\(^+\) ions involved in the activation process). The relative affinities of various amino acids for system N were evaluated by competition studies in which system N-specific uptake of \[^{[3]}\Hglutamine (5 \mu\)M) was measured in the presence of increasing concentrations of amino acids. The \(IC_{50}\) (i.e., concentration of the competitor necessary to cause 50% inhibition of glutamine uptake) was then calculated from the dose-response curves. Because the concentration of glutamine used in these studies was less than 5% of the \(K_m\) for the uptake process, the \(IC_{50}\) was taken as a close approximation of \(K_m\) for the respective amino acids.

**Isolation and Culture of Primary Müller Cells from Mouse Retina**

Müller cells were isolated from 7- to 10-day-old C57BL/6 mice by a method adapted from Hicks and Courtois. Mice were killed by decapitation followed by immediate removal of the eyeballs, which were placed in DMEM with 10 \(\mu\)g/ml gentamicin and soaked overnight at room temperature in the dark. The next morning, the eyeballs were rinsed in phosphate-buffered saline (PBS), incubated 2 to 3 hours at 37°C with 0.05% trypsin in 0.5 mM EDTA and 200 U/ml collagenase. Retinas were removed from the eyeballs (with care taken to avoid contamination by pigmented retinal pigment epithelium) and transferred to a 10-cm culture dish containing DMEM with 5.5 mM glucose, 10% fetal bovine serum, and 1% penicillin-streptomycin. Retinas were gently pipetted out and placed in culture dishes as small aggregates at a density of 10 to 16 retinas/dish. Isolated cells were detectable within 1 to 3 days and by 3 to 5 days, substantial cell growth ensued. Cultures were vigorously washed with medium until only a strongly adherent flat cell population remained. The cells were seeded into culture flasks or dishes at a density of 50,000 cells/cm\(^2\). The culture medium was changed three times a week. Measurements of glutamine uptake via...
system N were made in these primary Müller cell cultures by the procedure described previously for rMC1 cells.

Evaluation of the Purity of Primary Müller Cell Cultures

Primary Müller cells were grown on 12-mm coverslips in DMEM, supplemented with 2% fetal bovine serum and 1% penicillin-streptomycin. Cells were rinsed once with PBS, fixed for 10 minutes in 4% paraformaldehyde, and permeabilized for 5 minutes in 0.25% Triton X-100. Cells were then blocked for 10 minutes in 1× blocking solution (Power Block; BioGenex, San Ramon, CA) and rinsed once in PBS. The following antibodies were used to verify the purity of the Müller cell cultures: glutamine synthetase, cellular retinaldehyde-binding protein (CRALBP), glial fibrillary acidic protein (GFAP), EAAT1, and neurofilament-L (NF-L). NF-L is a neuronal marker and was used as a negative control. The primary antibodies were diluted in normal antibody diluent. The cells were incubated with primary antibodies for 5 hours at room temperature. The cells were rinsed 1× in PBS, covered with PBS, and maintained at 4°C overnight. The next day, the cells were washed twice in PBS for 10 minutes on the shaker. Secondary antibodies were also diluted in normal antibody diluent. The cells were incubated with the appropriate secondary antibody for 30 minutes at room temperature and then rinsed in PBS, followed by a rinse in 0.05 M Tris/HCl (pH 7.3). The nuclei were stained with 1:5000 Hoechst 33342 for 10 minutes. The cells were rinsed in 0.05 M Tris/HCl (pH 7.3). The cells were examined and photographed with a microscope equipped with a digital camera (Axioplan 2 microscope; AxioCam HR Camera and Axiovision 4.2 software; Carl Zeiss Meditec, Dublin, CA).

RT-PCR Analysis of Expression of mRNA Transcripts for ATA1, ATA2, SN1, SN2, LAT1, and LAT2

The presence of specific mRNA transcripts for the amino acid transporters ATA1, ATA2, SN1, SN2, LAT1, and LAT2 in rMC1 cells (rat) and in primary Müller cells (mouse) was evaluated by RT-PCR. Species-specific (rat or mouse) PCR primers were designed based on the published sequences (Table 1). Total RNA was prepared from rMC1 cells and primary Müller cells (TRIzol; Invitrogen-Gibco). RT-PCR was performed with optimal conditions specific for individual primer pairs. The products were subcloned into the pGEM-T vector and sequenced to confirm their molecular identity.

RESULTS

Relative Contributions of Systems N, A, and L to Glutamine Uptake in rMC1 Cells

First, we measured glutamine uptake in the presence and absence of Na⁺ with increasing concentrations of MeAIB as a competitor (Fig. 1A). In the absence of MeAIB, uptake of glutamine in these cells was 15 times higher in Na⁺-containing buffer than in Na⁺-free buffer, indicating that uptake via system L and diffusion constitutes <10% of total uptake. Within the Na⁺-dependent uptake component, MeAIB at the highest concentration used (10 mM) was able to cause only 20% inhibition. This concentration of MeAIB was sufficient to saturate uptake fully, via system A, as evidenced from the complete inhibition of Na⁺-coupled uptake of [14C]MeAIB by 10 mM unlabeled MeAIB (Fig. 1B). These data show that only 20% of Na⁺-coupled glutamine uptake in rMC1 cells occurred via system A. Considering all evidence, we conclude that system N is the major contributor (~70%) to glutamine uptake in rMC1 cells, the remainder (~30%) occurring via systems A and L.

Table 1. Sequences of RT-PCR Primers and Accession Numbers

<table>
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<tr>
<th>Gene Name</th>
<th>NCBI Accession No.</th>
<th>Primers Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAT1</td>
<td>Mouse AB023409</td>
<td>Forward: AGTGGGGCTGGGCTGCTACTTC</td>
</tr>
<tr>
<td></td>
<td>Rat AB015432</td>
<td>Reverse: TGACCAAAATGAGGCTACAAA</td>
</tr>
<tr>
<td>LAT2</td>
<td>Mouse NM_178798</td>
<td>Forward: GCTTCATGGCCTTTATCCGCTCT</td>
</tr>
<tr>
<td></td>
<td>Rat AB024400</td>
<td>Reverse: AATGTCACAGCGACGCGTCA</td>
</tr>
<tr>
<td>ATA1</td>
<td>Mouse BC030578</td>
<td>Forward: GGTATCTTGGCTACAGGATGG</td>
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<tr>
<td></td>
<td>Rat NM_138832</td>
<td>Reverse: ATGTCACCTGCAATCGAA</td>
</tr>
<tr>
<td>ATA2</td>
<td>Mouse NM_175121</td>
<td>Forward: GCTGATCTTTATCCCTCGGCTCT</td>
</tr>
<tr>
<td></td>
<td>Rat AF249673</td>
<td>Reverse: TCTCCAGATGCAGCATGAC</td>
</tr>
<tr>
<td>SN1</td>
<td>Mouse AF159856</td>
<td>Forward: TGGGCTACCTGGGTATGCTCT</td>
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<tr>
<td></td>
<td>Rat AF273025</td>
<td>Reverse: ATGACCAAGAGATGGATG</td>
</tr>
<tr>
<td>SN2</td>
<td>Mouse NM_172479</td>
<td>Forward: TCAAGGGAGATGGCATCC</td>
</tr>
<tr>
<td></td>
<td>Rat AF276870</td>
<td>Reverse: AGTGGGCTGCCTCTTCT</td>
</tr>
</tbody>
</table>

RT-PCR was performed with optimal conditions specific for individual primer pairs. The products were subcloned into the pGEM-T vector and sequenced to confirm their molecular identity.
diffusion. Therefore, in subsequent experiments, we measured specifically system N-mediated glutamine uptake by performing uptake measurements in an Na\(^{+}\)/H\(^{+}\)-containing buffer in the presence of 5 mM MeAIB and adjusting the measured uptake for the Na\(^{+}\)/H\(^{+}\)-independent component.

System N is known to be influenced by pH, being stimulated by an outwardly directed H\(^{+}\)/H\(^{+}\) gradient.\(^{16-19}\) To confirm that the Na\(^{+}\)/H\(^{+}\)-dependent, MeAIB-insensitive glutamine uptake observed in rMC1 cells does indeed occur via system N, we investigated the influence of extracellular pH (Fig. 2A). We found the uptake to be influenced significantly by pH of uptake buffer. The uptake decreased when pH was made acidic (less than pH 7) and increased when pH was made alkaline (greater than pH 7). The uptake was optimal at pH 8.

**Substrate Selectivity of System N in rMC1 Cells**

The substrate selectivity of system N in rMC1 cells was investigated by competition experiments in which the ability of various amino acids (5 mM) to compete with \[^{3}H\]glutamine for uptake via system N. Among the amino acids tested, glutamine, cysteine, alanine, asparagine, serine, threonine, methionine, and leucine were the most potent inhibitors of system N-mediated \[^{3}H\]glutamine uptake (>70% inhibition; Fig. 2B). Tryptophan, valine, phenylalanine, isoleucine, glycine, tyrosine, and histidine showed moderate inhibition (30%–50% inhibition). Thus, all the neutral amino acids tested exhibited moderate-to-robust inhibition. In contrast, the anionic amino acids glutamate and aspartate and the cationic amino acids arginine and lysine showed very little inhibition (<20%).

**Relative Affinities of Selected Amino Acids for System N in rMC1 Cells**

To determine the affinity of glutamine to system N, we performed saturation kinetic analysis by measuring system N-specific uptake with increasing concentrations of glutamine (Fig. 3A). The uptake was saturable with a \(K_m\) of 128 ± 10 \(\mu\)M and a \(V_{max}\) of 7.8 ± 0.4 nanomoles/mg protein per 15 minutes. We then examined the affinities of other amino acids selected for competition with glutamine.
dependent and MeAIB-insensitive uptake of [3H]glutamine (5 μM) to the Hill equation gave a value of 0.99. Uptake was hyperbolic (Fig. 4). Analysis of the data according to the Hill equation described the relationship between Na+/H+ concentrations of Na+/H+ ions and glutamine uptake, by analyzing the dose–response relationship of glutamine uptake in these cells. The substrate specificity of system N was determined by measuring system N-mediated glutamine uptake in these cells in the presence (NaCl) and absence (NMDG) of Na+. Activation kinetics for glutamine (Gln) uptake via an Na+-dependent and MeAIB-insensitive transport process. Na+-dependent and MeAIB-insensitive glutamine uptake was measured for 15 minutes in the absence of competing amino acids.

**Na+ Activation Kinetics for Glutamine Uptake via System N in rMC1 Cells**

The kinetics of activation of glutamine uptake via system N in rMC1 cells by Na+ was analyzed by measuring system N-specific glutamine uptake in the presence of increasing concentrations of Na+. The curve describing the relationship between Na+ concentration and system N-specific glutamine uptake was hyperbolic (Fig. 4). Analysis of the data according to the Hill equation gave a value of 0.99 ± 0.02 for b, suggesting an Na+-to-glutamine stoichiometry of 1:1.

**Characterization of Primary Müller Cells**

We used the transformed Müller cell line rMC1 in our initial studies because of the ease with which these cells can be maintained in culture for uptake measurements. There is, however, no guarantee that the findings in a transformed cell line can be extrapolated to the corresponding normal cells. Therefore, we wanted to see whether system N could be demonstrated at the functional level in primary cultures of Müller cells. Before using the primary Müller cells for uptake measurements, we evaluated the purity of the cells by analyzing the expression of various Müller cell markers. As shown in Figure 5A, the phase-contrast analysis indicated that the cells had flattened morphology with thin processes characteristic of Müller cells. Using immunofluorescence methods, we examined the expression of several proteins that are considered markers for Müller cells.7,8,22,23 The cells were highly positive for CRALBP (Fig. 5B) and EAAT1 (Fig. 5C). They were also positive for glutamine synthetase (Fig. 5D) and weakly positive for GFAP (Fig. 5E). Immunofluorescence for the neuronal marker NF-L was not positive (Fig. 5F). In addition, immunofluorescence analysis of reduced-folate transporter 1 (RFT-1), known to be expressed in RPE cells,24,25 did not detect this protein in the primary Müller cells (data not shown). Taken together, these data suggest that the method used to isolate these cells yielded a nearly pure population of Müller cells.

**Analysis of Glutamine Uptake in Primary Müller Cells**

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Glutamine plays an important metabolic role in glutamatergic neurotransmission. Glutamatergic neurons synthesize the neurotransmitter glutamate using glutamine as the immediate precursor. These neurons express a highly efficient glutamine transport system for the active uptake of glutamine from extracellular space. In the brain, astrocytes serve as the source of extracellular glutamine because these cells have the ability to synthesize glutamine via glutamine synthetase.

**Molecular Evidence for Expression of Glutamine Transporters in rMC1 Cells and in Primary Müller Cells**

We investigated the expression of different isoforms of systems A, N, and L in rMC1 cells and in primary Müller cells by RT-PCR. The data in Figure 7 show that rMC1 cells, as well as primary Müller cells expressed all these isoforms: LAT1 and LAT2 (system L), ATA1 and ATA2 (system A), and SN1 and SN2 (system N).

**DISCUSSION**

Glutamine plays an important metabolic role in glutamatergic neurotransmission. Glutamatergic neurons synthesize the neurotransmitter glutamate using glutamine as the immediate precursor. These neurons express a highly efficient glutamine transport system for the active uptake of glutamine from extracellular space. In the brain, astrocytes serve as the source of extracellular glutamine because these cells have the ability to synthesize glutamine via glutamine synthetase. Because astrocytes are the principal contributors to the clearance of glutamate from synapse by uptake via EAAT1 or EAAT2, glutamate entering the cells by this mechanism is used as the substrate for glutamine synthetase. Thus, glutamate released by glutamatergic neurons is recycled through the participation of metabolic pathways in astrocytes. An essential feature of this glutamate-glutamine cycle is the ability of astrocytes to release glutamate. This process does not occur by simple diffusion; a transport system mediates the release. The molecular identity of this transport system remained unknown until recently. Several amino acid transporters that are capable of glutamine transport have been cloned in recent years. Among them, the two subtypes of system N, namely SN1 and SN2, possess functional characteristics suitable to participate in the release of glutamine. Available evidence strongly suggests that SN1 and/or SN2 are responsible for glutamine release from astrocytes.

The glutamate-glutamine cycle also occurs between Müller cells and glutamatergic neurons in the retina. Müller cells actively take up extracellular glutamate via EAAT1 and convert it to glutamine via glutamine synthetase. Even though it is logical to expect that these cells must possess a transport mechanism for the release of glutamine for subsequent utilization by glutamatergic neurons, no information was available on the identity of the transport mechanism. Therefore, we initiated studies to characterize glutamine transport systems in these cells to understand better the role of Müller cells in glutamatergic neurotransmission in the retina. These studies were performed in the rat Müller cell line rMC1 and also in primary cultures of mouse Müller cells. Our results were that system N was the predominant contributor to glutamine uptake in rMC1 cells, even though other glutamine transporters such as systems A and L were also expressed. We monitored in this cell line the activity of system N with its transport mechanism operating in the influx mode by maintaining an inwardly directed Na$^+$ gradient and an outwardly directed H$^+$ gradient.

Our conclusion that system N is the principal glutamine transporter in rMC1 cells is based on the following observations. A majority (~90%) of glutamine uptake in these cells was Na$^+$-dependent, indicating that diffusion and system L contribute very little to glutamine uptake. Within the Na$^+$-dependent glutamine uptake component, only a minor fraction was sensitive to inhibition by MeAIB. This rules out system A as a significant contributor to the glutamine uptake observed in these cells. The Na$^+$-dependent and MeAIB-insensitive glutamine uptake exhibits functional characteristics expected of system N, including stimulation by an outwardly directed H$^+$ gradient and high affinity for glutamine. ATB$^{60}$ is an amino acid transporter that can mediate glutamine uptake by an
Na\(^+\)-coupled mechanism, but glutamine uptake via this system is expected to be sensitive to inhibition by cationic amino acids such as arginine and lysine.\(^{30-32}\) Our results show that cationic amino acids have little or no effect on Na\(^+\)-dependent and MeAIB-insensitive glutamine uptake in rMC1 cells. As expected of system N, the Na\(^+\)-dependent and MeAIB-insensitive glutamine uptake is inhibitable by several neutral amino acids. Similar results have been obtained with primary mouse Müller cells. RT-PCR studies show that mRNA transcripts for both isoforms of system N (SN1 and SN2) are expressed in rMC1 cells as well as in primary Müller cells. Based on these data, we conclude that Müller cells in intact retina express both isoforms of system N (SN1 and SN2) and that this system is likely to be responsible for the release of glutamine into the extracellular space. Our functional and molecular evidence for the expression of system N in Müller cells is supported by the recent studies by Boulland et al.,\(^{33}\) which have described the presence of SN1 in Müller cells in intact retina by immunohistochemical techniques. The biological importance of these findings is readily apparent because the glutamine release mechanism is a major component of the glutamate-glutamine cycle that occurs between the Müller cells and the glutamatergic neurons within the retina.

**References**


