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Research report

Characterization of an L-type calcium channel expressed by human retinal Müller (glial) cells

Donald G. Puro [a,*], J.-J. Hwang [b], Oh-Joo Kwon [b], Hemin Chin [b]

[a] *Departments of Ophthalmology and Physiology, W.K. Kellogg Eye Center, University of Michigan, 1000 Wall Street, Ann Arbor, MI 48105, USA*
[b] *Laboratory of Neurochemistry, NINDS, National Institutes of Health, Bethesda, MD 20892, USA*

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Abstract

The traditional notion that glial cells are permeable only to potassium has been revised. For example, glia from various parts of the nervous system have calcium-permeable ion channels. Since characterization of the calcium channels in glia is limited, the purpose of this study was to determine the molecular identity and examine the functional properties of a voltage-gated calcium channel expressed by Müller cells, the predominant glia of the retina. Whole-cell and perforated-patch recordings of human Müller cells in culture revealed a high threshold voltage-activated calcium current that is blocked by dihydropyridines, but not by ω-conotoxin GVIA or ω-conotoxin MVIIC. RT-PCR of cultured human Müller cells using primers specific for the calcium channel subunits demonstrated the expression of an L-type channel composed of the α_1D, α_2 and β_3 subunits. The α_2 subunit of the Müller cell calcium channel is a splice variant which is distinct from either the skeletal muscle α_2s or the brain α_2b. Our electrophysiological experiments indicate that the α_1D/α_2/β_3 calcium channel is functionally linked with the activation of a potassium channel that may serve as one of the pathways for the redistribution by Müller cells of excess retinal potassium.

Keywords: Calcium channel subunits; Retina; Glia; Potassium homeostasis; Conotoxin; Potassium currents

1. Introduction

Glial cells are more complex than previously thought. For example, they express a diversity of ion channels [1] and are no longer thought to simply have a passive function in the nervous system. However, at present, the molecular basis for glial cell function is poorly understood. Since the ion channels expressed by these cells are likely to have a functional role, characterization of the structure and physiology of glial channels should help elucidate the basis of glial activity. In this study, we examined the molecular identity and functional properties of a voltage-dependent calcium channel expressed by Müller glial cells from the adult human retina.

Voltage-dependent calcium channels are found in the plasma membranes of many cells, including some glia such as Müller cells [20,25]. By regulating calcium entry, these channels can modulate a variety of physiological functions although their specific roles in glial cells remain unclear. Voltage-dependent calcium channels are classified into multiple types based on their electrophysiological and pharmacological properties: e.g. L-, T-, N-, P- and Q- and R-types [2,3,29]. The dihydropyridine (DHP)-sensitive L-type calcium channel purified from skeletal muscle is a multimeric complex that is composed of the pore-forming α_1 subunit and the regulatory/ancillary α_2-δ, β and γ subunits [4,7]. Molecular cloning has determined the primary structures of the calcium channel subunits expressed by skeletal muscle [6]. The α_1, β and γ subunits are encoded by separate genes, whereas the α_2 and δ subunits are produced by proteolytic cleavage of a larger precursor produced by a single gene. Subsequently, two additional L-type α_1 subunit genes have been identified; the first gene (α_1C) encoding the channels expressed in cardiac and smooth muscles as well as in the brain, and the second (α_1D) gene encoding channels found in the brain, endocrine system and a number of other cell types [8,14,30,32]. At present, four mammalian β subunit cDNAs have been reported [13,16].

The subunit compositions of calcium channels expressed by glial cells have not been reported. Here we

* Corresponding author. Fax: (1) (313) 936-2340.

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show that human Müller cells express mRNAs corresponding to the α_1D, α_2 and β_3 subunits, which could form DHP-sensitive class D L-type calcium channels. This channel is functionally linked with activation of a potassium channel that may play a role in the adaptive response of Müller cells to changes in the retinal microenvironment.

2. Materials and methods

2.1. Cell cultures

Cultures of retinal glial cells were prepared as detailed elsewhere [24]. Postmortem eyes from adult donors were supplied within 24 h of death by the Michigan Eye Bank and Transplantation Center. Retinas were removed, exposed to a calcium- and magnesium-free phosphate buffer (CMF) supplemented with 0.1% trypsin (3 × crystallized; Worthington), 0.2% hyaluronidase and 4% chicken serum for 45 min at 37°C and then dissociated mechanically in medium A (40% Dulbecco's modified Eagle's medium, 40% Ham's F12 medium and 20% fetal bovine serum). Dissociated cells from one retina were added to three 35-mm Petri dishes, kept in a humidified environment of 96.5% air/3.5% CO_2 at 37°C and fed medium A twice per week. Cells that had been in culture for up to 5 passages were used. As reported elsewhere, since virtually all of the cells prepared by this method are immunoreactive to antibodies against glutamine synthetase [31], which is localized in the retina to only the Müller glial cells [19], it appears that the cells in these cultures are Müller cells.

2.2. Electrophysiology

One day prior to recording, cultures were washed twice and kept in medium A without serum. During experiments, cultures were examined at 400 × magnification with an inverted microscope equipped with phase-contrast optics. Ionic currents were recorded at room temperature with the patch clamp technique using the whole-cell or perforated-patch configurations as detailed elsewhere [25,26]. Patch pipets of Corning no. 7052 glass were coated with Sylgard no. 184 (Dow Corning, Midland, MI) to within 100 μm of their tips and heat polished. Pipets for whole-cell recording contained 140 mM CsCl, 1 mM $MgCl_2$, 10 mM Cs-EGTA and 10 mM Cs-HEPES (pH 7.4) and had resistances of 4–8 MΩ. The initial ~400 μm of the perforated-patch pipets contained a solution of 135 mM CsCl, 6 mM $MgCl_2$, 10 mM Cs-HEPES (pH 7.4); the remainder of the pipet contained this solution supplemented with 120 μg/ml amphotericin B. The resistance of perforated-patch pipets was 2–5 MΩ. The pipets were mounted in the holder of a Dagan (Minneapolis, MN) 3900 patch-clamp amplifier and sealed to Müller cells. Seals generally formed over a period of 1–30 s and reached resistances of greater than 1 Ω. As amphotericin perforated the patch, the access resistance to the studied cells decreased to < 20 MΩ within approximately 15 min. Recordings were used after the ratio of cell membrane to series resistance was greater than 10. The delivery of voltage step protocols and the sampling of currents were performed using pClamp software (Axon Instruments, Foster City, CA), which was also used for data analysis. Currents were filtered at 1 kHz. Recordings are shown after subtraction of capacitive currents. Unless stated otherwise, the bathing solution contained 125 mM CsCl, 10 mM $BaCl_2$, 20 mM glucose, 5 μM tetrodotoxin (TTX) and 10 mM Ba-HEPES (pH 7.4). Solutions could be miniperfused in the area of the glial cell by applying < 0.5 psi from a pressure ejection system (Medical Systems, Greenvale, NY) to the back end of a pipet which had a tip size of ~3 μm and was positioned ~50 μm from the cell being recorded. A stock solution (1000 ×) of nimodipine was dissolved in DMSO, which at 0.1% had no effect on calcium currents. Stock solutions of conotoxins were dissolved in distilled water containing 0.1% bovine serum albumin.

2.3. RT-PCR analysis

Total RNAs were prepared from monolayers of Müller cells by a guanidinium thiocyanate-phenol-chloroform extraction method [10]. First strand cDNA was synthesized using 1–2 μg of total RNA, which had been treated with RNase-free DNase, using SuperScript Preamplification System (Life Technologies, Gaithersburg, MD). Following the first strand cDNA synthesis, the polymerase chain reaction (PCR) (50 μl) was carried out in 10 mM Tris, pH 8.3, containing 4 μl cDNA, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.01% (w/v) gelatin, 1.25 U Taq polymerase (Perkin-Elmer Cetus, Norwalk, CT) and with 50 pmol each of one of the following primer pairs for voltage-sensitive calcium channel subunits: na3fc/wolf4 (α_1B), a1cs/a1cas (α_1C), m1f/m2r(α_1D), m5f/m6r (α_1D), hskmf/hskmr (α_1S), ha2f/ha2r (α_2b), bt11f11/bt11r12 (β_1), Beta2-5P/Beta2-3P (β_2), NB830S/NB1225A (β_3), and *Xba*I5/*Xba*I3 (β_4). Human hippocampus and human fetal brain cDNAs used in the PCR were purchased from Clontech Laboratories (Palo Alto, CA).

The primers used were as follows (the nucleotide residue number and accession number are in parentheses): na3fc, 5′-AGC GGA GGC AGC CCT CAT CTT CCT-3′ (6483–6506, M94172); wolf4, 5′-CTA GCA CCA GTG GTC TTG GTC AGG-3′ (7142–7165, M94172); a1cs, 5′-TCG TGG GCT TCG TCA TCG TCA-3′ (3408–3428, L29534); a1cas, 5′-CCT CTG CGT TCA TAG AGG GAG AG-3′ (3859–3881, L29534); m1f, 5′-TCC CTG TGC AAC TTA GTG GCA TCC TTA-3′ (2413–2439, M76558); m2r, 5′-CTG AGC AGT GTT CAG ACT TTC AGC ATC-3′ (2779–2805, M76558); m5f 5′-GCC ATT GTC TAT TTC ATC AGT TTT-3′ (4828–4851, M76558); m6r, 5′-CGA TAT AGC ACG CCG GAT TTC TGG CCC-3′ (5461–5487, M76558); hskmf, 5′-AGG AGC ACC AGG GAG

AAT ACT TCC-3′ (5533–5556, L33798); hskmr, 5′-GCA CTT TTT GAG GTG GTT CCT GAC-3′ (5985–6008, L33798); ha2f, 5′-TGG TGT GAT GGG AGT AGA TGT GTC T-3′ (1489–1513, M76559); ha2r, 5′-CTT GGT GCT ATG AAT GTA TAG CCA G-3′ (1965–1989, M76559); bt11f11, 5′-ACT CCA GTT CCA GTC TGG G-3′ (566–584, M76560); bt11r12, 5′-TGC CAT CAA ACC GAT GCT TCA-3′ (767–787, M76560); Beta2-5P, 5′-ACG TTT GAC TCT GAA ACC CAA GAG-3′ (1866–1887, M80546); Beta2-3P, 5′-TGT AAG GCA AGA CTG AAT CTG TCC C-3′ (2537–2557, M80545); NB830S, 5′-GTG CAG AGT GAG ATT GAG CGC-3′ (782–803, U07139); NB1225A, 5′-CTG AAG TCC AGG GAT GGC ACT G-3′ (1153–1174, U07139). *Xba*I5 5′-GCT CTA GAG CGG AGG CAT ATT GGC GTG CCA CC-3′ (1384–1405, L02315); *Xba*I3 5′-GCT CTA GAG CTC AAA GCC TAT GTC GGG AGT C-3′ (1743–1763, L02315).

The PCR was performed for 35 cycles (1 min at 94°C, 2 min at 55°C, and 2 min at 72°C). The final extension was carried out at 72°C for 5 min at the end of cycling. The amplified products were separated on polyacrylamide gels and stained with ethidium bromide, or run on 1.5% agarose gels, eluted and cloned into pCR11 vector (Invitrogen, San Diego, CA), and sequenced using dideoxy chain termination reaction [27].

Unless stated otherwise, chemicals were from Sigma (St. Louis, MO); growth media and serum were from Gibco-BRL (Grand Island, NY).

3. Results

Whole-cell recordings of membrane currents in human Müller cells were performed with bathing and pipet solu-

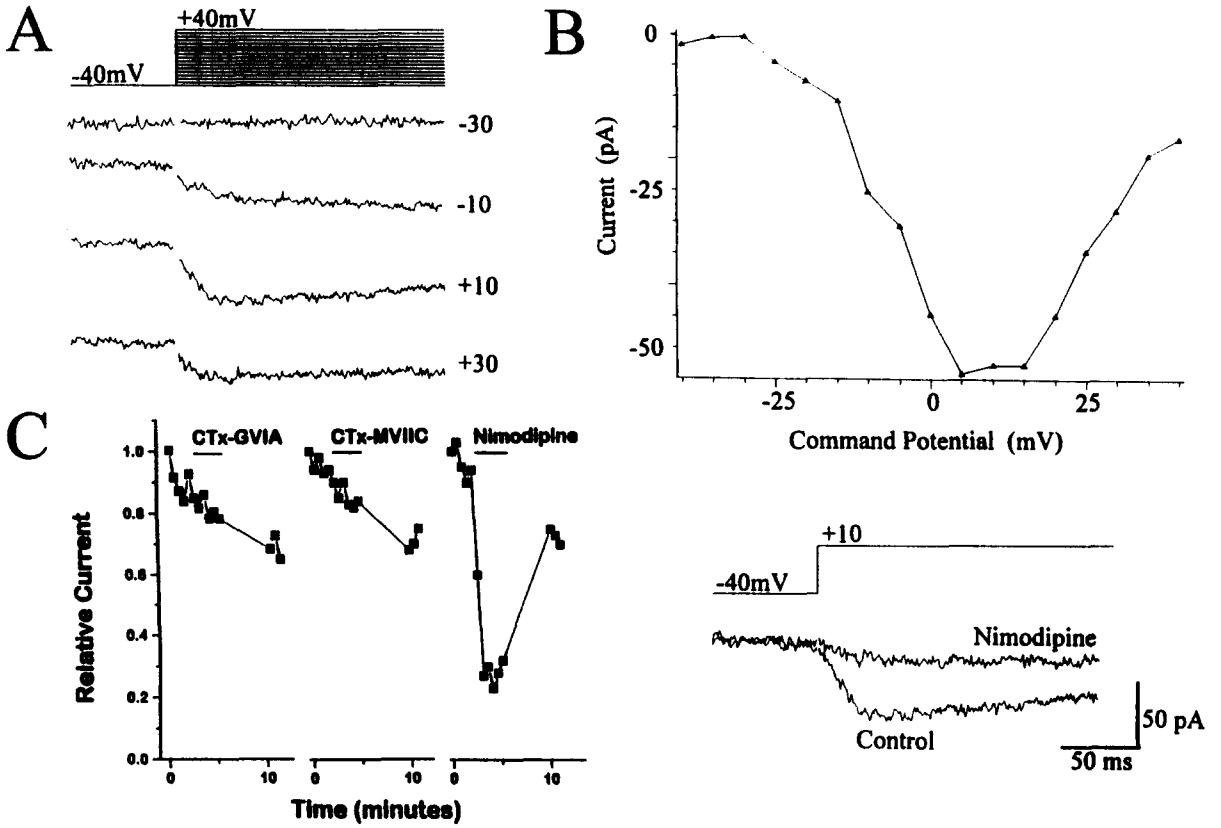

Fig. 1. Inward currents observed in human Müller cells under whole-cell recording conditions in which potassium and sodium channels were blocked. A: The top panel shows the stimulus protocol used to study high threshold voltage-activated calcium currents. The lower panels present examples of currents evoked with pulses from a holding potential of −40 mV to the depolarizing command potentials indicated. B: Peak inward currents plotted against command potentials using the protocol shown in A. C: The plot in the left panel illustrates the effects of ω-conotoxin GVIA, ω-conotoxin MVIIC and nimodipine on peak inward currents evoked by a pulse from a holding potential of −40 mV to +10 mV. Currents were normalized to the peak amplitude current evoked ~ 3 min prior to exposure to toxins or drug. The horizontal lines indicate the periods of miniperfusion of the bathing solution supplemented with 5 μM of the putative channel blocker. The mean values from three experiments are shown. Standard deviations were < 0.2. During exposure to the toxins the changes in currents were not significant ($P > 0.3$, Student's t-test) although some 'run down' of currents was evident. In control experiments in which no drugs or toxins were tested, the relative current after 5 min of recording decreased to a mean of 78 ± 4 (SD), i.e. to an amplitude similar to that observed when the toxins were present. In contrast, perfusion with nimodipine significantly ($P < 0.01$, Student's t-test) reduced the inward current. The panel on the right shows currents in a Müller cell before and after exposure to a perfusate containing 5 μM nimodipine. The time and current scales shown in this panel also apply to the records in A. Müller cells from the human retina express high threshold voltage-activated currents which are nimodipine-sensitive.

tions that effectively isolated inward currents carried by barium and calcium. Potassium channel currents were abolished by having cesium in the bathing and pipet solutions and having barium externally. Possible sodium channel current was blocked with 5 μM TTX and replacement of external sodium with cesium. Under these recording conditions, sampled Müller cells typically had inward currents evoked by depolarization (Fig. 1A). When evoked from a holding potential of −40 mV, the inward currents had a high threshold of activation, i.e. ~ −25 mV (Fig. 1B). The pharmacological profile of these high threshold calcium currents in Müller cells is consistent with an L-type channel. Specifically, these currents are sensitive to the dihydropyridine, nimodipine, but minimally inhibited by ω-conotoxin GVIA or ω-conotoxin MVIIC (Fig. 1C). These findings are in contrast to the T-type calcium currents of Müller cells, which are near totally inactivated at a holding potential of −40 mV, have a threshold of activation of ~ −55 mV and are insensitive to dihydropyridines [25].

Molecular biological techniques were used to identify the subunit composition of the L-type calcium channel expressed in human Müller glial cells. Four sets of primers specific for the three known L-type calcium channel α_1 subunits (α_1C, α_1D and α_1S) as well as those for the α_1 subunit of ω-conotoxin-sensitive N-type calcium (α_1B) were used. Only the two α_1D primer pairs amplified detectable products from Müller cell cDNA of the predicted diagnositic size (Fig. 2). In contrast, other α_1 subunit primer sets did not yield PCR products indicating that α_1C, α_1S, and α_1B subunit transcripts were either not expressed or were present at very low levels. These results are consistent with electrophysiological data (Fig. 1 and [25]) and strongly indicate that the α_1D isoform of the L-type calcium channels are expressed in Müller glia cells.

The two Müller cell α_1D PCR products (360 bp and 660 bp in size) were subcloned and completely sequenced in order to confirm their identity. As expected, the deduced amino acid sequence of the 360-bp product was 100% identical to the region of the human brain α_1D subunit containing IIS5 and IIS6 (residue number 640–760) [32] while that of the 660-bp amplified product corresponded to the C-terminal cytoplasmic region (residue number 1441–1660) (sequence not shown). The expression of α_1D isoform has previously been demonstrated in the brain, endocrine system and kidney [8,28,29,32].

It has been found that both human and rat skeletal muscle and brain tissue express distict alternatively spliced

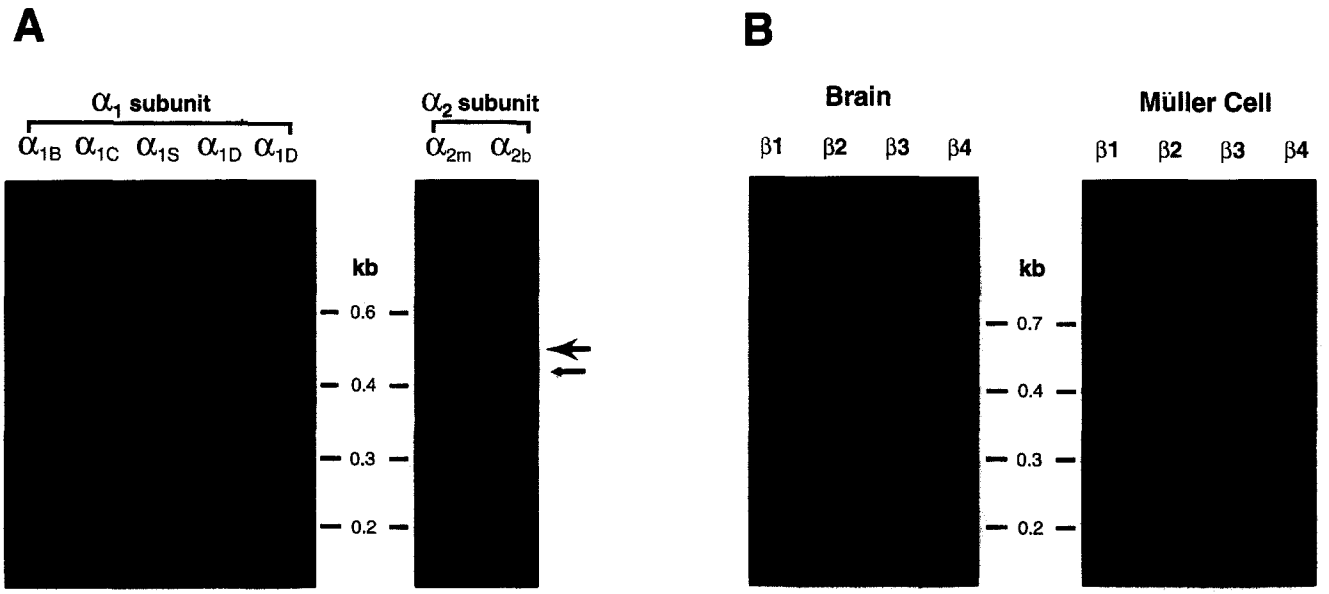

Fig. 2. RT-PCR analaysis of mRNA encoding voltage-dependent calcium channel subunits. cDNAs from human Müller glia and other tissues were generated as described in Materials and methods. A: cDNAs were PCR amplified with primers specific for the α_1B, α_1S, α_1C, α_1D, and α_2 subunits of calcium channels. PCR products were separated in a 4–20% polyacrylamide gradient gel in TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA) and visualized using ethidium bromide staining. The migration of a 100-bp ladder (Life Technologies/BRL, Gaithersburg, MD) is indicated at the center. The two primer pairs specific for human brain α_1D subunit transcript, m1f/m2r and m5f/m6r, yielded the predicted 360- and 660-bp PCR products. The primer pairs specific for α_2 subunit transcripts amplified a 498-bp product from brain (α_2b, as indicated by a thick arrow), and a 462-bp product from Müller cells (α_2m, indicated by a thin arrow). B: Human brain and Müller cell cDNAs were amplified with the primer pair specific for four calcium channel β subunit transcripts (β_1, β_2, β_3 and β_4). Each of the primer pairs amplified a product of expected size when human brain cDNA was used as template, bt11f1/bt11r1, which was designed to amplify a unique region of the β_1 subunit mRNA, amplified a product of 256-bp representing a spliced variant expressed in the brain. The β_2 primer pair (Beta2-5P/Beta2-3P), β_3 primer pair (NB830S/NB1225A) and β_4 pair (*Xba*I5/*Xba*I3) amplified 755-, 407- and 376-bp products corresponding to the respective β subunit transcripts present in the brain. In contrast, only the β_3 primer pair (NB830S/NB1225A) amplified a product of expected size 407 bp from Müller cell cDNA.

variants of the α_2 subunit [17,32]. Williams and coworkers [32] have termed the skeletal muscle splice variant α_2s and the brain variant α_2b. To determine which splice variant is present in Müller glial cells, primers flanking the differentially spliced region for the α_2 subunit were used to amplify cDNA. As shown in Fig. 2A, a 500-bp fragment was amplified from human hippocampus cDNA as expected, but a smaller product of 460 bp was amplified from Müller cDNA, indicating that Müller cells express an α_2 variant distinct from either skeletal muscle α_2s of the brain variant α_2b. The comparison of the deduced amino acid sequences shown in Fig. 3 suggests that the Müller cell α_2 variant would encode an α_2 polypeptide that is at least 12 amino acids shorter than the brain isoform and 25 amino acids smaller than the skeletal muscle isoform. The presence of the Müller cell splice variant for the α_2 subunit has not been demonstrated previously.

A

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GGTGTGATGGGAGTAGATGTGTCTTTGGAAGATATTAAAAGACTGACACCACGTTTTACA
G V M G V D V S L E D I K R L T P R F T  (20)

CTGTGCCCCAATGGGTATTACTTTGCAATCGATCCTAATGGTTATGTTTTATTACATCCA
L C P N G Y Y F A I D P N G Y V L L H P  (40)

AATCTTCAGCCAAAGGAGCCAGTAACATTGGATTTCCTTGATGCAGAGTTAGAGAATGAT
N L Q P K E P V T L D F L D A E L E N D  (60)

ATTAAAGTGGAGATTCGAAATAAGATGATTGATGGGGAAAGTGGAGAAAAAACATTCAGA
I K V E I R N K M I D G E S G E K T F R  (80)

ACTCTGGTTAAATCTCAAGATGAGAGATATATTGACAAAGGAAACAGGACATACACATGG
T L V K S Q D E R Y I D K G N R T Y T W  (100)

ACACCTGTCAATGGCACAGATTACAGTTTGGCCTTGGTATTACCAACCTACAGTTTTTAC
T P V N G T D Y S L A L V L P T Y S F Y  (120)

TATATAAAAGCCAAACTAGAAGAGACAATAACTCAGGCCAGATATTCGGAAACCCTGAAG
Y I K A K L E E T I T Q A R Y S E T L K  (140)

CCAGATAATTTTGAAGAATCTGGCTATACATTCATAGCACCAAG
P D N F E E S G Y T F I A P              (154)
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B

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M02   (1)   GVMGVDVSLE DIKRLTPRFT LCPNGYYFAI DPNGYVLLHP NLQPK..... (45)
h02b  (486) GVMGVDVSLE DIKRLTPRFT LCPNGYYFAI DPNGYVLLHP NLQPK..... (530)
r02m  (488) GVMGVDVSLE DIKRLTPRFT LCPNGYYFAI DPNGYVLLHP NLQPKPIGVG (537)

M02   (46)  .......... .........E PVTLDFLDAE LENDIKVEIR NKMIDGESGE (76)
h02b  (531) GVMGVDVSLE ....NPKSQE PVTLDFLDAE LENDIKVEIR NKMIDGESGE (566)
r02m  (538) GVMGVDVSLE DIKRLTPRFT PVTLDFLDAE LENDIKVEIR NKMIDGESGE (587)

M02   (77)  KTFRTLVKSQ DERYIDKGNR TYTWTPVNGT DY.SLALVLP TYSFYYIKAK (125)
h02b  (567) KTFRTLVKSQ DERYIDKGNR TYTWTPVNGT DY.SLALVLP TYSFYYIKAK (615)
r02m  (488) KTFRTLVKSQ DERYIDKGNR TYTWTPVNGT DYSSLALVLP TYSFYYIKAK (637)

M02   (126) LEETITQARY .......SET LKPDNFEESG YTFIAP (154)
h02b  (616) LEETITQARS KKGKMKDSET LKPDNFEESG YTFIAP (651)
r02m  (638) LEETITQARY .......SET LKPDNFEESG YTFIAP (666)
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Fig. 3. A: Nucleotide and deduced amino acid sequences of Müller α_2 subunit PCR clone. The nucleotide sequence of Müller α_2 subunit PCR clone was determined by sequencing both strands of a PCR II plasmid (Invitrogen, San Diego, CA) containing the amplified α_2 subunit PCR product. The nucleotide sequences corresponding to the 5′ sense and 3′ antisense PCR primers are underlined. The deduced amino acid sequence is given in single-letter code below the corresponding nucleotide sequence. B: Alignment of deduced amino acid sequences of three splice variant of $\alpha 2$ subunit. Shown is the alignment of the deduced amino acid sequences of human Müller cell (Mα_2), human brain (hα_2b), and rabbit skeletal muscle (rα_2m) α_2 subunits. The hα_2b sequence is from Williams et al. [32] (GenBank accession number M76559), and rα_2m sequence is from Ellis et al. [12] (GenBank accession number M21948). Dots represent gaps in the sequence.

Based on PCR amplification of the human Müller glial cells with the primers specific for the four known β subunit isoforms, we found that Müller cells express only β_3 subunit mRNA (Fig. 2B, Müller cell). All four β subunits were detected in human fetal brain when using these primers (Fig. 2B, Brain). The Müller β subunit PCR clone that we isolated contained a 407-bp amplified product corresponding to the amino acid residues from 250 to 385 of the β_3 subunit (sequence not shown), and its deduced amino acid sequence differed by 2 amino acids from that of rat β_3 subunit [5], but was 100% identical to that of human brain β_3 subunit previously reported in the GenBank (accession number U01739 and X76556).

To further analyze the physiology of the high threshold calcium currents expressed by human Müller cells, recordings of ionic currents were made using the perforated-patch technique. This configuration tends to maintain the intracellular environment in a more physiological state than standard whole-cell recordings which often are associated with a 'run down' of calcium channel activity (e.g. Fig. 1C) as the cytoplasm is 'washed out'. When monitored with the perforated-patch technique, the high threshold voltage-activated calcium currents showed markedly more inactivation (Fig. 4A) than when recorded under the whole-cell recording conditions used in this study (Fig. 1). The sensitivity to nimodipine (Fig. 4B) provides pharmacological evidence that these inactivating calcium currents observed with the perforated-patch configuration are of an L-type. No other high threshold voltage-activated calcium channels were detected.

The role of calcium channels in Müller cell function is not well understood. Since potassium homeostasis is one important putative function of glial cells [21], we examined the possibility that calcium channel activity can regulate potassium currents in Müller cells. Using the perforated-patch configuration with bathing and pipet solutions designed not to block potassium currents, we found that the depolarization-evoked outward current in human Müller cells is sensitive to tetraethylammonium (Fig. 5A), an inhibitor of many types of potassium channels. In the presence of nimodipine, which inhibits the high threshold voltage-activated calcium currents of these cells (Figs. 1 and 4), the outward potassium current was substantially reduced (Fig. 5B,C). Specifically, in a series of five cells the outward current evoked with a depolarization from -40 mV to 0 mV was reduced by 51% (SD = 8) with exposure to 1 μM nimodipine. A role for an influx of calcium is further suggested by Fig. 5D which demonstrates that a substantial portion of the depolarization-evoked current is dependent on extracellular calcium. These observations are consistent with our earlier documentation of the presence of calcium-activated (K_{Ca}) channels in human Müller cells [23]. Taken together, these experiments indicate that an influx of calcium via L-type channels activates K_{Ca} channels. Thus, in human Müller cells there appears to be a functional dependence of some

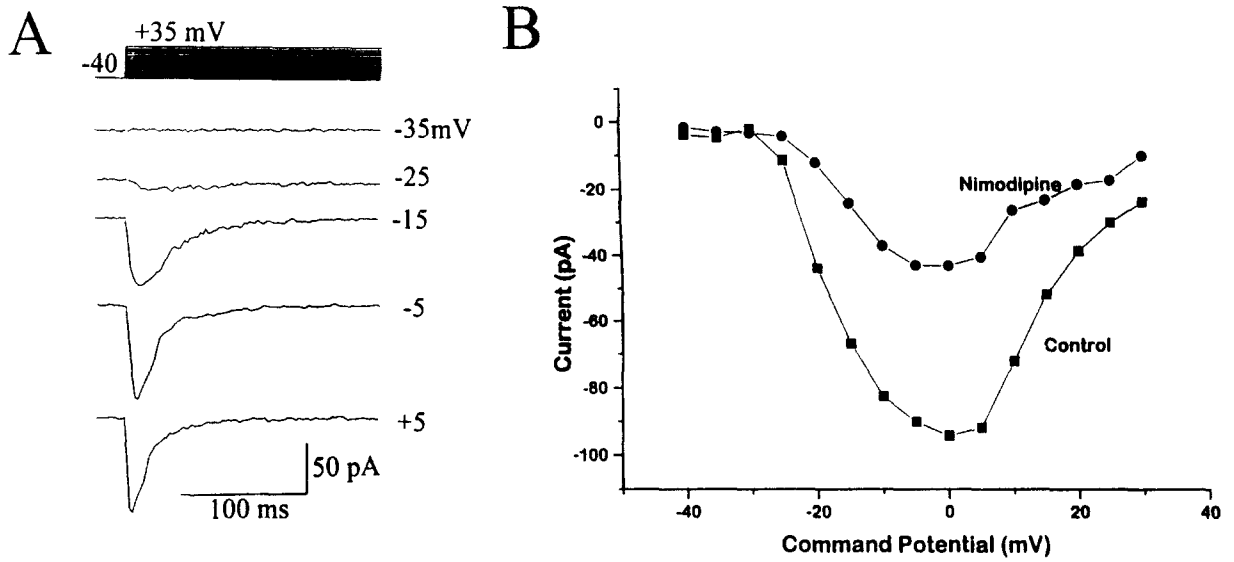

Fig. 4. Inward currents observed in human Müller cells using the perforated-patch configuration under conditions to block potassium and sodium currents. A: The top panel shows the stimulus protocol used. The lower panels present examples of currents evoked from a holding potential of −40 mV to various depolarizing potentials as indicated. B: Plot of peak inward currents versus command potentials using the protocol shown in A. The squares show values before exposure to 1 μM nimodipine. The voltage-activated, nimodipine-sensitive calcium current recorded in Müller cells with the perforated-patch technique shows significant inactivation.

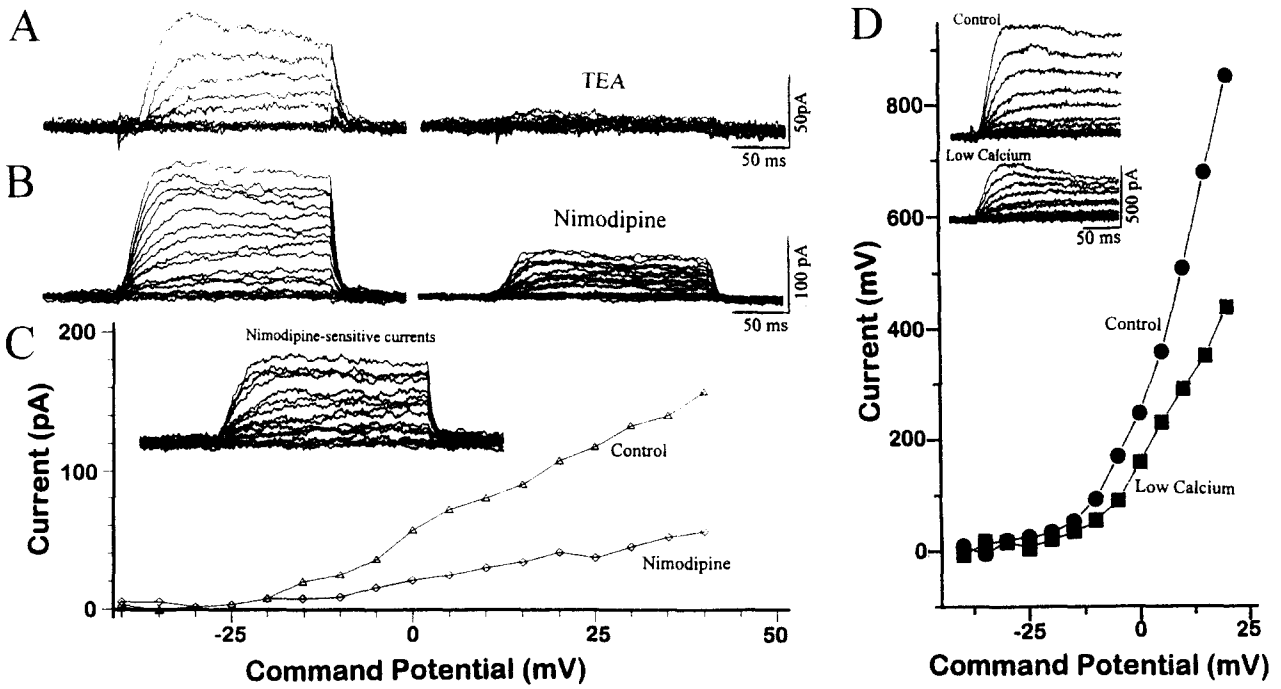

Fig. 5. Outward currents of human Müller cells recorded using the perforated-patch configuration. In contrast to experiments illustrated in Fig. 1 and Fig. 4, the bathing and pipet solutions used here did not block potassium channels and were not designed to enhance calcium currents. A: Currents evoked with pulses from a holding potential of −40 mV to 13 successive test potentials increased at 5-mV steps. The left hand panel shows evoked currents under control conditions; on the right are currents during miniperfusion of the bathing solution supplemented with 20 mM tetraethylamonium, a potassium channel blocker. B: Currents evoked before and during miniperfusion of the bathing solution supplemented with 1 μM nimodipine. The holding potential was −40 mV; command potentials increased at 5-mV steps from −40 to +40 mV. C: Current-voltage plots for the traces shown in B. Values are the mean currents at 140–190 ms after the onset of a 200-ms depolarizing pulse. The inset shows the nimodipine-sensitive currents obtained by digital subtraction of the currents observed in the presence (B, right hand panel) and absence (B, left hand panel) of nimodipine. For A–C, the bathing solution contained (mM) 135 NaCl, 10 KCl, 1.8 $CaCl_2$, 0.8 $MgCl_2$ 10 Na-HEPES (pH 7.4), 20 glucose and 0.005 TTX; the pipet contained a solution of (mM) 40 KCl, 65 K_2SO_4, 6 $MgCl_2$ and 10 K-HEPES (pH 7.4) with 120 μg/ml amphotericin B as described in Materials and methods. D: Current-voltage plots for a Müller cell in the bathing solution used in A–C (circles) and in the bathing solution lacking added calcium and having 1 mM EGTA. The holding potential was −40 mV. The families of current traces recorded under control and low calcium conditions are shown in the upper part of the panel. Exposure of Müller cells to the L-type of calcium channel blocker nimodipine markedly reduces the depolarization-evoked potassium current.

potassium currents on the L-type calcium channel composed mainly of $\alpha_1D/\alpha_2/\beta_3$ subunits.

4. Discussion

Our molecular analysis revealed that human Müller glial cells in culture express L-type calcium channels consisting of the α_1D and α_2 subunits, in combination mainly with β_3 subunits. A physiological correlate of this expression is the presence in Müller cells of a high threshold voltage-activated calcium current that is sensitive to dihydropyridines. This is the first molecular characterization of a glial calcium channel. Whether glial cells from other parts of the nervous system express channels with the same subunit composition remains to be determined.

The $\alpha_1D/\alpha_2/\beta_3$ combination is unlikely to be the only calcium channel expressed by human Müller cells. We also detected expression of β_4 mRNA albeit at a much lower level. It may be reasonable to conclude that the β_4 subunit does not contribute significantly to the L-type calcium channels in Müller cells since this subunit has been shown to be preferentially associated with the ω-conotoxin MIIC-sensitive α_1A subunit in vitro [11] and during cerebellar development [18]. In addition to the L-type of calcium channels, human Müller cells also have a low threshold voltage-activated T-type of calcium current [25]. However, molecular characterization of the T-type channel in human Müller cells awaits discovery of the genes encoding subunits for this family of channels.

The knowledge that human Müller cells express the $\alpha_1D/\alpha_2/\beta_3$ calcium channel subunits contributes to an understanding of the molecular basis for the physiology and pathophysiology of these cells. From studies of α_1D expression in oocytes, it is evident that this subunit is capable of forming a channel that is responsive to dihydropyridines [32] and modulated by a β subunit [7]. Of the subunits studied electrophysiologically, β_3 most potently enhances the rate of inactivation of calcium currents [15]. Our finding of rapid inactivation of the high threshold voltage-activated, nimodipine-sensitive calcium current is consistent with the presence of the β_3 isoform in Müller cells. However, the observation that the calcium currents monitored in the perforated-patch configuration had more inactivation than those detected with whole-cell recordings in which the intracellular calcium concentration was low ($\sim 10^{-8}$ M) and there was no GTP/ATP regeneration system suggests that molecules in the cytoplasm of Müller cells may modulate L-type calcium channel inactivation perhaps by affecting the function of the β_3 subunits. Clearly, future studies are needed to better understand the functional regulation of the L-type calcium channels in human Müller cells.

The role of the α_2 subunit in calcium channel function has yet to be clarified. Interestingly, a single α_2 subunit gene encodes at least three different splice variants [9]. These include the skeletal muscle α_2s [12], the brain α_2b [17,32], and the newly identified Müller cell α_2 subunit. DNA sequence analysis presented in this study indicates that the α_2 subunit polypeptide of the Müller cell L-type Ca^{2+} channel would be at least 12 amino acids smaller than the brain $\alpha 2b$ and 25 amino acids shorter than the skeletal muscle α_2s. Although further structural analysis of the α_2 subunit gene is required, the three forms of the α_2 subunit are likely to have arisen by alternative use of exons in a manner analogous to that for the β_1 subunit [22]. An unanswered question is whether the α_2 subunit expressed by human Müller cells is unique to this type of glial cell.

While evidence is good that human Müller cells in culture have functional $\alpha_1D/\alpha_2/\beta_3$ calcium channels it remains to be demonstrated that this calcium channel is expressed by Müller cells in vivo. This will not be readily accomplished in the human retina. However, preliminary findings by one of us (D.G.P.) indicate that freshly dissociated human Müller cells have high threshold voltage-activated calcium channels. Also, the finding of high threshold voltage-activated calcium currents in Müller cells in a slice preparation of the salamander retina [20] strongly suggests that calcium channels of this class are present in these cells in vivo at least in some vertebrates.

The role of the $\alpha_1D/\alpha_2/\beta_3$ calcium channels in the functioning of Müller cells is uncertain. Since the threshold of activation (~ -25 mV) is far from the resting membrane potential, this calcium channel may be active under pathophysiological conditions, such as ischemia and cell injury, which are associated with profound depolarization. In agreement with this, it appears that dihydropyridine-sensitive channels play a role in Müller cell proliferation [32], which commonly occurs in response to retinal injury.

In the present study, we asked whether the $\alpha_1D/\alpha_2/\beta_3$ calcium channel of Müller cells may influence the activity of potassium channels, which may be involved in the glial regulation of extracellular potassium levels under conditions of severe depolarization. Our finding that exposure of Müller cells to nimodipine blocks approximately 50% of the depolarization-evoked potassium current indicates that there is a significant functional dependence of this current on the activity of L-type calcium channels. An enhancement of K_{Ca} channel activity induced by an influx of calcium through L-type channels may facilitate the ability of Müller cells to redistribute excess extracellular potassium. Thus, evidence is accumulating that the $\alpha_1D/\alpha_2/\beta_3$ calcium channel of Müller cells may play a role in the responses of the retinal glia to pathophysiological conditions.

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