Interleukin-1 Stimulates Glutamate Uptake in Glial Cells by Accelerating Membrane Trafficking of Na⁺/K⁺-ATPase via Actin Depolymerization[▽]

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Interleukin-1 (IL-1) is a mediator of brain injury induced by ischemia, trauma, and chronic neurodegenerative disease. IL-1 also has a protective role by preventing neuronal cell death from glutamate neurotoxicity. However, the cellular mechanisms of IL-1 action remain unresolved. In the mammalian retina, glutamate/aspartate transporter (GLAST) is a Na⁺-dependent, major glutamate transporter localized to Müller glial cells, and loss of GLAST leads to glaucomatous retinal degeneration (T. Harada, C. Harada, K. Nakamura, H. A. Quah, A. Okumura, K. Namekata, T. Saeki, M. Aihara, H. Yoshida, A. Mitani, and K. Tanaka, *J. Clin. Invest.* 117:1763–1770, 2007). We show here that IL-1 increases glutamate uptake in Müller cells by a mechanism that involves increased membrane Na⁺/K⁺-ATPase localization, required for counteracting the Na⁺-glutamate cotransport. IL-1 activated the p38 mitogen-activated protein kinase (MAPK)/capase 11 pathway, which destabilizes the actin cytoskeleton allowing Na⁺/K⁺-ATPase membrane redistribution. Furthermore, pretreatment with IL-1 protected retinal neurons from glutamate neurotoxicity through p38 MAPK signaling. Our observations suggested that IL-1 acts as a potential neuroprotective agent by modulating the functions of the glia-neuron network.

It is well known that the release of excitatory amino acids, such as glutamate, can cause neuronal cell death. Excessive extracellular concentrations of glutamate induce an uncontrolled elevation of intracellular calcium that enters through chronically activated glutamate receptors. Glutamate uptake by glial cells is a well-known mechanism for maintaining low extracellular level of glutamate and promoting efficient inter-neuronal signaling in the central nervous system. In addition, the same process is considered a neuroprotective mechanism during neurodegeneration. Clearance of glutamate from the extracellular space is accomplished primarily by the action of glutamate transporters (9). In the central nervous system, glutamate/aspartate transporter (GLAST) and glutamate transporter 1 (GLT-1) are Na⁺-dependent glutamate transporters found in astrocytes (49, 53). Genetic deletion of GLAST and/or GLT-1 causes abnormal brain development and neurological symptoms such as motor deficits, increased susceptibility to seizures, and exacerbation of noise-induced hearing loss (15, 35, 52, 53). We previously identified GLAST as the only glial-type glutamate transporter in the retina, whereas GLT-1 is expressed in neurons including bipolar cells and photoreceptors (20, 23). Not surprisingly then, GLAST is more active in preventing glutamate neurotoxicity after ischemia than GLT-1 (18). In addition, glaucomatous retinal and optic nerve degeneration were observed in GLAST-deficient mice (20). Since glutamate transport is coupled with the cotransport of 3Na⁺, the efficiency of glutamate uptake is influenced by intracellular

and extracellular Na⁺ concentrations (34, 48). Elevated intracellular Na⁺ is decreased by Na⁺/K⁺-ATPase, which is in turn dependent on ATP levels (2, 11, 16). However, severe ischemia and other states that cause ATP depletion in glial cells lead to elevated intracellular Na⁺ and resultant failure of glutamate uptake (34).

Interleukin-1 (IL-1) is an important mediator of brain injury induced by ischemia or trauma and has been implicated in chronic brain diseases including Alzheimer's disease, Parkinson's disease, and multiple sclerosis (1, 43). Deletion of IL-1 in mice conferred approximately 80% neuroprotection against neuronal damage due to ischemia (4). Now, conflicting evidence has proposed a neuroprotective role for IL-1. For example, pretreatment of IL-1 protects glutamate-induced neuronal cell death in cortical and retinal neurons (6, 30, 47) by increasing the synthesis of neurotrophic factors (8). This neuroprotective effect of IL-1 was reduced by administration of nerve growth factor, nerve growth factor neutralizing antibody, or IL-1 receptor antagonist. These observations suggested that IL-1 might mediate beneficial effects on neurons through its receptor; however, the detailed mechanism and intracellular signaling underlying such a role remain unknown. This study examined the putative role of IL-1 in glutamate uptake by using cultured retinal glial cells as well as possible mechanisms of IL-1-induced neuroprotection. We showed that IL-1 stimulation enhances glutamate uptake without affecting GLAST expression and protects retinal neurons from glutamate neurotoxicity.

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MATERIALS AND METHODS

Animals. C57BL/6J mice were obtained from CLEA Japan (Tokyo, Japan), and all animal procedures were performed in accordance with the Tokyo Metropolitan Institute for Neuroscience Guidelines for the Care and Use of Ani-

mals. Intraocular injection of IL-1 (100 ng/eye; ProSpec-TecnoGene, Rehovot, Israel) and glutamate (8.8 μ g/eye; Wako, Osaka, Japan) and induction of ischemia were achieved essentially as previously described (18). Briefly, we introduced sterile saline into the anterior chamber of the left eye at 120 cm of H₂O pressure for 20 min, while the right eye served as a nonischemic control. Animals were sacrificed 6 h after reperfusion, and the posterior parts of the eyes were sectioned sagittally.

Immunohistochemistry. Retinal ganglion cells (RGCs) were retrogradely labeled from the superior colliculus with Fluoro-Gold (Fluorochrome, Englewood, CO) as previously reported (18). The 7- μ m-thick retinal sections were double labeled with mouse anti-glutamine synthetase (1.0 μ g/ml; Chemicon, CA) and rabbit anti-IL-1 receptor (0.5 μ g/ml; IBL, Gunma, Japan), rabbit anti-GLAST (0.5 μ g/ml) (23), or rabbit anti-caspase 11 (0.5 μ g/ml; Santa Cruz, CA) as primary antibodies. Cy-3-conjugated goat anti-rabbit immunoglobulin G (IgG; Jackson ImmunoResearch, PA) and Cy-2-conjugated donkey anti-mouse IgG (Jackson ImmunoResearch) were used as secondary antibodies. For terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling (TUNEL) staining, paraffin sections were treated with 10 μ g/ml proteinase K and then incubated in 0.26 U/ μ l terminal deoxynucleotidyltransferase in the supplied buffer (Invitrogen, CA) and 20 μ mol/liter biotinylated 16-dUTP (Roche, Mannheim, Germany) for 1 h at 37°C. Sections were viewed by epifluorescence on a light microscope (BX51; Olympus, Tokyo, Japan) equipped with Plan Fluor objectives and connected to a DP70 camera (Olympus).

Retinal explant culture. Retinal explant cultures were made as described previously (25) with some modification. Briefly, the neural retina without pigment epithelium was placed on a Millicell chamber filter (30-mm diameter, 0.4-mm pore size; Millipore, MA) with the ganglion cell layer (GCL) upwards. The chambers were transferred to a six-well culture plate, with each well containing 1 ml of Dulbecco's modified Eagle's medium-F12 medium (Invitrogen) containing 20% heat-inactivated horse serum (Invitrogen), changed every other day. The cells were cultured at 34°C in 5% CO₂. In some experiments, retinas were preincubated with or without 50 ng/ml IL-1 for 24 h and then stimulated with 5 mM glutamate for 1 h. After 72 h, retinal explants were immunostained with antibody against NeuN (1.0 μ g/ml; Chemicon).

Glutamate uptake assay. Primary cultured Müller cells were prepared as described previously (19). Müller cells were cultured in 5.5 mM glucose-containing Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The culture media were replaced with a modified Hanks balanced salt solution for a 20-min preincubation, before the addition of 0.025 mCi/ml L-[³H]glutamate (Amersham, Uppsala, Sweden) and 100 μ M unlabeled glutamate to the medium. Uptake was terminated after 7 min by three washes in ice-cold Hanks balanced salt solution, immediately followed by cell lysis in 0.1 M NaOH. Aliquots were taken for scintillation counting, and protein concentration was determined using bovine serum albumin standards. In some experiments, Müller cells were stimulated with IL-1 alone or with both IL-1 and cytochalasin D (0.3 μ M; Biomol Research Laboratories, PA) for 12 or 24 h before assay. Inhibitors of p38 mitogen-activated protein kinase (MAPK) (10 μ M; Calbiochem, CA) or Jun N-terminal kinase (JNK) (10 μ M; Calbiochem) were applied 10 min before IL-1 treatment. MK-801 (100 μ M; Tocris Cookson, MO), DNQX (100 μ M; Tocris Cookson), QX-314 (3 mM; Calbiochem), or ouabain (10 mM; Calbiochem) was applied to Müller cells 20 min before the assay.

Immunoblotting. Retinas and cultured cells were homogenized in ice-cold 50 mM Tris-HCl (pH 7.4) containing 150 mM NaCl and a protease inhibitor cocktail (Roche). Surface proteins were purified using a cell surface protein isolation kit (Pierce, IL) according to the manufacturer's instructions. Briefly, cell surface proteins were labeled with EZ-Link sulfo-NHS-SS-biotin, which binds to the amino group on the extracellular protein domain, and purified on an avidin column. The bound (cell surface) and unbound (intracellular) proteins were subjected to immunoblot analysis. Protein concentrations were determined using a Bio-Rad protein assay kit (Bio-Rad, CA). Samples were separated on sodium dodecyl sulfate (SDS)-polyacrylamide gels and subsequently electrotransferred to an Immobilon-P filter (Millipore). Membranes were incubated with antibodies against GLAST (1:1,000), p38 MAPK (1:1,000; BD Biosciences, Ontario, Canada), phospho-p38 MAPK (1:1,000; BD Biosciences), JNK (1:1,000; BD Biosciences), phospho-JNK (1:1,000; BD Biosciences), Na⁺/K⁺-ATPase (1:1,000; Santa Cruz), cofilin (1:1,000; BD Biosciences), phosphocofilin (1:1,000; BD Biosciences), or IL-1 (1:1,000; Rockland, PA). Primary antibody binding was detected using horseradish peroxidase-labeled anti-mouse IgG secondary antibody (Amersham, NJ) and visualized using the ECL Plus Western blotting system (Amersham).

Intracellular Na⁺ measurement. Cultured Müller cells grown on glass-bottomed dishes were imaged live to record the dynamic intracellular ion state using the fluorescent dye CoroNa Green AM, as described previously (36). Müller cells were loaded with 10 μ M CoroNa Green AM in Hanks balanced salt solution at

37°C for 45 min and then placed in an open-bath imaging chamber. Cells were excited every 10 s at 345 nm, and the emission fluorescence at 510 nm was recorded. In some experiments, 1 mM ouabain or 3 mM QX-314 was applied together with the CoroNa Green AM. Inhibitors of p38 MAPK or JNK were applied to cells 10 min before IL-1 treatment. Image acquisition was computer controlled using Metaview software (Universal Imaging, PA).

RT-PCR. Total RNA was isolated from cultured Müller cells with Isogen reagent (Nippon Gene, Tokyo, Japan) and then reverse transcribed with a Revertra Ace instrument (Toyobo, Osaka, Japan) to obtain cDNA. Reverse transcription-PCR (RT-PCR) analysis was performed as previously described (19). The primer sequences used in PCR were as follows: caspase 11, 5'-ATG GCTGAAAACAAACACCC-3' and 5'-TAGCCTAAGTCTTCAAGAAG-3'; glyceraldehyde 3-phosphate dehydrogenase, 5'-ACCACAGTCCATGCCATCA C-3' and 5'-TCCACCACCCTGTTGCTGTA-3'. Reactions were conducted under the following conditions: preheating at 94°C for 2 min and then 35 cycles consisting of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and polymerization at 72°C for 30 min. The expected sizes of the amplified cDNA fragments of caspase 11 and actin were 500 and 452 bp, respectively.

RNA interference. RNA oligomers containing 21 nucleotides were synthesized in the sense and antisense directions corresponding to mouse caspase 11 at nucleotides 270 to 288 (5'-GGAAAUGGAGGAACCAGAA-3') with dTdT overhangs at each 3' terminus (JBioS, Saitama, Japan). A scrambled sequence, 5'-UUCUGGUUCCUCCUCC-3', was used as a negative control. Annealing was performed as described previously (10). Transfection into Müller cells was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Immunocytochemistry. Cells grown on glass coverslips were fixed in 4% paraformaldehyde for 20 min and permeabilized with 0.5% Triton X-100 for 15 min. The coverslips were incubated in 5% horse serum in phosphate-buffered saline for at least 1 h at room temperature for blocking and then incubated overnight with rabbit anti-caspase 11 (1.0 μ g/ml; Santa Cruz) at 4°C. They were then incubated with Cy-3-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch). F-actin was also visualized in the cells by incubation with phalloidin conjugated with rhodamine for 30 min at room temperature.

Statistics. Data are presented as means $\pm$ standard errors except as noted. When statistical analyses are performed, Student's *t* test was used to estimate the significance of the results. Statistical significance was accepted at *P* < 0.05.

RESULTS

Glutamate uptake by Müller cells is enhanced by IL-1-MAPK signaling. We first examined the expression of IL-1 receptor in mouse retina by immunohistochemical analysis. IL-1 receptor was mainly localized to the inner retina (Fig. 1A), where it colabeled (Fig. 1C) with glutamine synthetase, a marker of Müller glial cells (Fig. 1B). Double-positive cells were also observed in the GCL (Fig. 1D to F), but IL-1 receptor expression was not detected in retrogradely labeled RGCs (Fig. 1G to I). IL-1 receptor is thus found in Müller glial cells but not RGCs.

Glutamate uptake by GLAST in Müller cells (Fig. 1J to L) is important for retinal neuroprotection *in vivo* (20, 23). Since IL-1 receptor was expressed in Müller cells, we examined how IL-1 affects the capacity of glutamate uptake in cultured Müller cells. Glutamate transport activity was measured as uptake of L-[³H]glutamate. Treatment with 0.1 to 200 ng/ml IL-1 for 24 h significantly increased the glutamate uptake activity in Müller cells (Fig. 2A), and IL-1 at 50 ng/ml increased the activity to significant levels after 6 h (Fig. 2B).

IL-1 activates MAPKs such as p38 MAPK and JNK (14, 40, 50). Based on these previous findings, we carried out immunoblot analysis and found that IL-1 strongly induced p38 MAPK and JNK phosphorylation within 15 min (Fig. 2C). We further examined whether p38 MAPK and JNK are involved in IL-1-dependent glutamate uptake by using SB203580 and JNK inhibitor II, which are pharmacological blockers for p38

FIG. 1. Expression of IL-1 receptor in the mouse retina. (A to C) Immunohistochemical analysis of mouse retina double stained (C) with antibodies against IL-1 receptor (A) and glutamine synthetase (B), a specific marker for Müller glial cells. (D to F) Enlarged images of the GCL in panels A to C, respectively. (G to I) Expression of IL-1 receptor (G) and retrogradely labeled RGCs (H) in the GCL. (J to L) Immunohistochemical analysis of mouse retina double stained (L) with antibodies against GLAST (J) and glutamine synthetase (K). INL, inner nuclear layer; ONL, outer nuclear layer. Bars, 50 μm (A to C and J to L) and 25 μm (D to I).

MAPK and JNK, respectively. SB203580 suppressed IL-1-induced enhancement of glutamate uptake, while JNK inhibitor II had no effect (Fig. 2D), indicating that activation of the IL-1-p38 MAPK, but not the IL-1-JNK, pathway is necessary for IL-1-dependent glutamate transport. On the other hand, IL-1 had no effect on GLAST expression levels in cultured Müller cells (Fig. 2E).

Our present data suggested that IL-1 prevents retinal degeneration caused by glutamate neurotoxicity. Retinal explants stimulated with 5 mM glutamate for 1 h (Fig. 3B) showed a clear decrease in the number of NeuN-positive neurons in the GCL compared with nontreated controls (Fig. 3A) 72 h after treatment. However, pretreatment of the cells with IL-1 significantly increased the number of surviving neurons (Fig. 3C and D). Addition of a MAPK inhibitor, SB203580, to the retinal explants at the same time as glutamate and IL-1 abolished the neuroprotective effect of IL-1 (Fig. 3D). Consistent with our *in vitro* study (Fig. 2D), IL-1 thus seemed to protect retinal neurons from glutamate neurotoxicity via the p38 MAPK pathway. We also examined the effect of IL-1 on RGC apoptosis *in vivo*. Intraocular injection of glutamate (8.8 $\mu\text{g}/\text{eye}$) increased the number of TUNEL-positive cells in the GCL (Fig. 3F) compared with nontreated controls (Fig. 3E). IL-1 pretreatment (100 ng/eye) significantly suppressed apoptotic cell death due to glutamate neurotoxicity *in vivo* (Fig. 3G and H).

FIG. 2. IL-1 increases glutamate uptake by Müller glial cells. (A) Concentration dependency of glutamate transport activity in Müller cells treated with IL-1 for 24 h. *, $P < 0.05$. (B) Time dependency of glutamate transport activity in Müller cells treated with 50 ng/ml of IL-1. *, $P < 0.05$. (C) Activation of p38 MAPK and JNK in Müller cells treated with 50 ng/ml of IL-1 for indicated times. Two micrograms of proteins was separated on an SDS-polyacrylamide gel followed by immunoblot analysis using anti-p38 MAPK, anti-phospho-p38 MAPK, anti-JNK, and anti-phospho-JNK antibodies. (D) Effect of p38 MAPK or JNK inhibition on IL-1-induced glutamate uptake activity in Müller cells, showing suppression by the p38 MAPK inhibitor but not by the JNK inhibitor. (E) GLAST expression levels in Müller cells treated with 50 ng/ml of IL-1 for 24 h. The data are means $\pm$ standard errors of three samples for each group.

IL-1 suppresses intracellular Na^+ accumulation by altering intracellular Na^+/K^+ -ATPase expression pattern. Glutamate transport is coupled to the cotransport of 3Na^+ ; thus, glutamate uptake by Müller cells may be influenced by intracellular Na^+ concentrations (34, 48). To address this possibility, we used a sodium channel inhibitor, QX-314, or an Na^+/K^+ -ATPase inhibitor, ouabain. The intracellular Na^+

FIG. 3. IL-1 protects retinal neurons from glutamate neurotoxicity. (A to C) Immunohistochemical analysis of mouse retinal explants stained with anti-NeuN antibody. Explants were nontreated (A), treated with glutamate alone (B), or treated with both IL-1 and glutamate (C). (D) Quantification of NeuN-positive cells in the GCL. (E to G) TUNEL staining of mouse retinal sections. Retinas were nontreated (E), treated with glutamate alone (F), or treated with both IL-1 and glutamate (G). (H) Quantification of TUNEL-positive cells in the GCL. The data are means $\pm$ standard errors of three samples for each group. INL, inner nuclear layer; ONL, outer nuclear layer. Bar, 50 μ m.

concentration was decreased in cultured Müller cells by QX-314 and increased by ouabain (Fig. 4A). In contrast, glutamate uptake was increased by QX-314 and decreased by ouabain (Fig. 4B). In addition, ouabain suppressed IL-1-induced glutamate uptake. Two glutamate receptor inhibitors, MK-801 and DNQX, had no effect on glutamate uptake activity. We next evaluated the effect of IL-1 on Na^+ concentration in cultured Müller cells. Intracellular Na^+ concentration was increased after stimulation with 2 mM glutamate, but IL-1 pretreatment suppressed the upregulation (Fig. 4C). KCl induces cell depolarization and Na^+ influx through voltage-gated Na^+ channels. As for gluta-

FIG. 4. IL-1 suppresses intracellular Na^+ accumulation in Müller cells. (A) Na^+ imaging of Müller cells after treatment with QX-314 or ouabain. Müller cells were loaded with Na^+ indicator CoroNa Green and stimulated with bath application of 3 mM QX-314 or 1 mM ouabain. Bar, 40 μ m. (B) Glutamate uptake activity by Müller cells after treatment with MK-801, DNQX, QX-314, ouabain, IL-1, or both IL-1 and ouabain. *, $P < 0.01$ versus control. **, $P < 0.05$ versus IL-1. (C) Quantification of Na^+ accumulation in Müller cells stimulated with bath application of 2 mM glutamate or 50 mM KCl. Pretreatment of IL-1 suppressed glutamate- and KCl-induced Na^+ accumulation. The data are means $\pm$ standard errors of 9 to 15 cells for each group from three independent cultures. (D) Na^+ imaging of Müller cells treated with KCl. Fluorescence images are shown in pseudocolor, with blue and red representing the lowest and highest intensities, respectively. The indicated times are seconds after initial application. Pretreatment of IL-1 (lower panels) suppressed KCl-induced Na^+ accumulation (upper panels). Bar, 20 μ m. (E) Effect of p38 MAPK or JNK inhibition on KCl-mediated Na^+ accumulation in Müller cells, showing suppression by the p38 MAPK inhibitor but not by the JNK inhibitor. The data are means $\pm$ standard errors of 10 to 13 cells for each group from three independent cultures. (F) IL-1 altered the intracellular localization of Na^+/K^+ -ATPase in Müller cells. After 24 h of IL-1 treatment, cell surface proteins were labeled with biotin and purified using avidin column. The bound (cell surface) and unbound (intracellular) proteins were separated on SDS-polyacrylamide gels followed by immunoblot analysis using anti- Na^+/K^+ -ATPase, anti-GLAST, and antitubulin antibodies. ND, not detectable.

mate, KCl increased the intracellular Na⁺ concentration in cultured Müller cells, and IL-1 significantly suppressed the accumulation (Fig. 4C and D). In addition, SB203580 masked the inhibitory effect of IL-1 on KCl-mediated Na⁺ accumulation, while JNK inhibitor II had no effect (Fig. 4E). Since Na⁺ extrusion is mediated mainly by Na⁺/K⁺-ATPase, we examined its intracellular localization by immunoblot analysis after biotinylation of cell surface proteins. Interestingly, IL-1 increased Na⁺/K⁺-ATPase expression on the cell surface, whereas less was found in the intracellular fraction (Fig. 4F). Total expression levels of Na⁺/K⁺-ATPase were not influenced by IL-1 treatment. As shown in Fig. 2E, IL-1 had no effect on intracellular localization of GLAST.

IL-1 stimulates actin disassembly and membrane trafficking of Na⁺/K⁺-ATPase. Actin filament assembly is essential for numerous cellular events, including membrane trafficking (29, 39, 42, 54), and thus may participate in IL-1-induced relocation of Na⁺/K⁺-ATPase. Cytochalasin D, which disrupts actin filaments by inhibiting polymerization, further enhanced Na⁺/K⁺-ATPase accumulation on the cell surface of Müller cells when used in combination with IL-1 (Fig. 5A). Cytochalasin D also synergistically suppressed Na⁺ accumulation (Fig. 5B) and stimulated glutamate uptake (Fig. 5C). Cofilin promotes depolymerization of actin filaments (3), and IL-1 may affect cofilin expression levels in cultured Müller cells. Immunoblot analysis revealed that both total and phosphorylated cofilin expression levels were upregulated by IL-1 (Fig. 5D and E). These results suggested that IL-1 stimulates membrane accumulation of Na⁺/K⁺-ATPase through actin depolymerization.

A recent study showed that caspase 11 promotes cofilin-mediated actin depolymerization (33). Since actin depolymerization enhanced IL-1-dependent glutamate uptake (Fig. 5C), we examined the effect of IL-1 on caspase 11 expression in cultured Müller cells. Caspase 11 mRNA and protein were undetectable in nontreated Müller cells but clearly upregulated after IL-1 treatment (Fig. 6A and B). Intracocular injection of IL-1 (Fig. 6C) and ischemic retinal injury (Fig. 6D) also increased caspase 11 expression in Müller cells *in vivo*. This upregulation occurred concomitantly with IL-1 upregulation at 3 and 6 h after ischemic injury (Fig. 6E).

We next examined whether caspase 11 is involved in actin depolymerization in cultured Müller cells. Stimulation with IL-1 induced strong caspase 11 expression in some cells. F-actin in such cells was clearly reduced compared that in with other cells expressing low caspase 11 (Fig. 7A). Caspase 11 induction was p38 MAPK, but not JNK, pathway dependent (Fig. 7B). We further investigated whether caspase 11 is involved in IL-1-dependent glutamate uptake. Cultured Müller cells transfected with caspase 11 small interfering RNA (siRNA) showed a decrease in IL-1-induced caspase 11 expression to about 50% (Fig. 7C and D). In this situation, caspase 11 siRNA significantly decreased IL-1-induced glutamate uptake (Fig. 7E). Scrambled siRNA had no effect on caspase 11 expression and glutamate uptake. These results suggested that IL-1 induces caspase 11 expression and promotes actin depolymerization, leading to membrane accumulation of Na⁺/K⁺-ATPase in Müller cells.

FIG. 5. Effects of actin assembly on IL-1-induced glutamate uptake. (A) Effect of IL-1 and cytochalasin D (CyD) on membrane trafficking of Na⁺/K⁺-ATPase. After treatment with IL-1 alone or with both IL-1 and CyD, cell surface proteins were biotinylated and purified on avidin affinity columns. Samples were separated on SDS-polyacrylamide gels followed by immunoblot analysis using anti-Na⁺/K⁺-ATPase and antitubulin antibodies. (B) Effect of IL-1 and CyD on KCl-induced Na⁺ accumulation. The data are means $\pm$ standard errors of 12 to 18 cells for each group from three independent cultures. (C) Effect of IL-1 and CyD on glutamate uptake activity. The data are means $\pm$ standard errors of three samples for each group. (D) Increased cofilin expression after IL-1 treatment in Müller cells treated with IL-1 for 24 h, lysed, and subjected to immunoblot analysis using anticofilin and anti-phosphorylated cofilin (anti-P-cofilin) antibodies. (E) Quantification of total and P-cofilin expression levels. The data are means $\pm$ standard errors of three samples for each group.

DISCUSSION

Here we showed that IL-1 increases glutamate uptake by Müller glial cells and pretreatment of IL-1 partially protects retinal neurons from glutamate neurotoxicity. Together with

FIG. 6. IL-1 induces caspase 11 expression in Müller cells. (A) RT-PCR analysis of caspase 11 expression in Müller cells treated with IL-1 for the indicated times. Total RNA was isolated, reverse transcribed, and subjected to PCR analysis. GAPDH, glyceraldehyde-3-phosphate dehydrogenase. (B) Immunocytochemical analysis of caspase 11 in Müller cells following 24 h of IL-1 treatment. The cells were fixed and immunostained with anti-caspase 11 antibody. Bar, 20 μ m. (C) Effect of IL-1 on caspase 11 expression in the mouse retina, which was double stained with antibodies against caspase 11 (green) and glutamine synthetase (red) 6 h after intraocular injection of IL-1. Bar, 50 μ m. (D) Effect of ischemia on caspase 11 expression in the mouse retina. After 3 h of ischemia, mouse retinas were double stained with antibodies against caspase 11 (green) and glutamine synthetase (red). Bar, 50 μ m. (E) Immunoblot analysis of ischemic retina. Two micrograms of proteins from ischemic retinas was separated on SDS-polyacrylamide gels followed by immunoblot analysis using anti-caspase 11 and anti-IL-1 antibodies.

IL-1 receptor expression pattern, IL-1 seems to mediate neuroprotection through Müller cells. Because GLAST is a major, glial-type glutamate transporter, we investigated the mechanisms by which IL-1 stimulates the function of GLAST. A Na^+/K^+ -ATPase inhibitor, ouabain, stimulated Na^+ accumulation and suppressed glutamate uptake. IL-1 had no effect on the expression levels of GLAST, total Na^+/K^+ -ATPase, and ATP (data not shown). However, IL-1 stimulated the mem-

FIG. 7. Effect of caspase 11 on IL-1-induced glutamate uptake by Müller cells. (A) Effect of caspase 11 expression on actin filaments. After IL-1 treatment, cells were stained with anti-caspase 11 antibody (green) and rhodamine-labeled phalloidin (red). F-actin was reduced in the cell expressing caspase 11 (arrowhead) compared with other cells (arrows). Bar, 20 μ m. (B) Effect of p38 MAPK or JNK inhibition on caspase 11 expression. IL-1-induced caspase 11 expression was suppressed by the p38 MAPK inhibitor but not by the JNK inhibitor. The data are means $\pm$ standard errors of three samples for each group. (C) RT-PCR analysis of caspase 11 expression levels in Müller cells. (D) Quantification of caspase 11 expression levels in Müller cells. The data are means $\pm$ standard errors of three samples for each group. (E) Quantification of glutamate uptake by Müller cells. The data are means $\pm$ standard errors of three samples for each group.

brane trafficking of Na^+/K^+ -ATPase, suppressed Na^+ accumulation, and increased glutamate uptake.

In this study, IL-1 increased total cofilin expression levels in Müller cells, implying a role for IL-1 in actin network dynamics. In addition, cytochalasin D enhanced the membrane trafficking of Na^+/K^+ -ATPase and suppressed Na^+ accumulation after IL-1 treatment. Furthermore, phosphorylated cofilin binds to and activates Na^+/K^+ -ATPase (28, 31, 32). Our find-

ings therefore suggested that IL-1 suppresses intracellular Na⁺ concentrations by accumulating Na⁺/K⁺-ATPase at the cell surface via cofilin-mediated actin depolymerization and directly stimulating Na⁺/K⁺-ATPase activity in Müller cells. Since IL-1 stimulates glutamate uptake in cultured astroglia from mouse brain (data not shown), this mechanism may be a more general phenomenon in glial cells. Recent study showed that GLAST also directs cell surface expression of Na⁺/K⁺-ATPase in human astrocytes (13).

Caspase 11 expression is induced by stimulation with lipopolysaccharide and mediates the activation of caspase 1 by physical interaction, inducing IL-1 secretion (51). In the present study, we detected strong caspase 11 expression in Müller cells after intraocular injection of IL-1. Thus, IL-1 might stimulate IL-1 production and secretion in an autocrine manner. A recent study showed caspase 11 forming a complex with cofilin and promoting actin depolymerization that resulted in enhanced cell migration (33). We showed a reduction of actin staining in cultured Müller cells that strongly expressed caspase 11 after IL-1 treatment, suggesting that caspase 11 expression promotes cofilin-mediated actin depolymerization and the membrane trafficking of Na⁺/K⁺-ATPase. Consistent with this, caspase 11 knockdown suppressed IL-1-dependent glutamate uptake. On the other hand, cytochalasin D or caspase 11 siRNA alone had no effect on intracellular Na⁺ concentration and glutamate uptake. These results suggest that IL-1 activates multiple signal transduction pathways regulating intracellular localization of Na⁺/K⁺-ATPase. For example, IL-1 is known to activate Rho family GTPases, which regulate actin assembly (26, 27, 46).

MAPKs such as p38 MAPK and JNK regulate a spectrum of processes including inflammation, cell proliferation, differentiation, and cell death (18, 37). IL-1 activates p38 MAPK and JNK in several cell types (14, 40, 50). In epithelial cells, IL-1 reduced Na⁺ accumulation by downregulating Na⁺ channels via p38 MAPK signaling (44). In addition, stimulation of p38 MAPK in hepatocytes suppressed Na⁺ accumulation during hypoxia, whereas p38 MAPK inhibition increased membrane Na⁺ permeability (7, 12). In cultured Müller cells, we demonstrated that inhibition of p38 MAPK, but not JNK, suppressed caspase 11 expression, Na⁺ accumulation, and glutamate uptake. Taken together, our observations suggested that the IL-1/p38 MAPK signaling pathway is essential for maintaining intracellular Na⁺ concentration and modulating glutamate uptake activity in Müller cells (Fig. 8).

Glutamate excitotoxicity is involved in various eye diseases including retinal artery occlusion, diabetic retinopathy, and glaucoma. We recently reported that GLAST is essential not only to keep the extracellular glutamate concentration below the neurotoxic level but also to maintain glutathione levels in Müller cells (20). Glutathione is a tripeptide of glutamate, cysteine, and glycine and is central in protecting RGCs against oxidative stress. Oxidative stress is also involved in glaucoma and other forms of retinal degeneration (1). Since glutamate uptake is a rate-limiting step in glial glutathione synthesis (24, 45), GLAST activation by IL-1 may be an effective strategy by which to manage these pathological conditions. On the other hand, a major part of the available intracellular glutamate is used for glutamine synthesis catalyzed by glutamine synthetase. In the retina, this enzyme is exclusively located in

FIG. 8. Proposed model for IL-1-induced glutamate uptake in Müller cells. Activation of IL-1/p38 MAPK signaling increases caspase 11 and subsequently induces cofilin activation, in turn disrupting the F-actin network. This actin depolymerization stimulates membrane trafficking of Na⁺/K⁺-ATPase, suppresses Na⁺ accumulation in Müller cells, and enhances glutamate uptake.

Müller cells, which in turn have rapid glutamate turnover (41). Taken together, overexpression of glutamine synthetase/GLAST, in combination with IL-1 stimulation, may have synergistic effects on retinal neuroprotection. Future studies will thus examine the therapeutic effect of IL-1 in various animal models of retinal degeneration, including normal tension glaucoma (20).

In conclusion, this study demonstrated that IL-1, a mediator of brain injury, might also protect retinal neurons via stimulating the major glutamate transporter in Müller glial cells. We suggest that such a glia-neuron network is functional in various forms of neurodegenerative diseases (19, 21). Furthermore, recent studies showed that Müller cells could proliferate after neurotoxic damage and produce some neural cell types in the adult mammalian retina (22, 38). Therefore, Müller cells may be a new therapeutic target for both neuroprotection and regeneration in retinal degenerative diseases (5, 17).

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