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Sulfonylurea Receptor 1 Contributes to the Astrocyte Swelling and Brain Edema in Acute Liver Failure

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Abstract

Astrocyte swelling (cytotoxic brain edema) is the major neurological complication of acute liver failure (ALF), a condition in which ammonia has been strongly implicated in its etiology. Ion channels and transporters are known to be involved in cell volume regulation and a disturbance in these systems may result in cell swelling. One ion channel known to contribute to astrocyte swelling/brain edema in other neurological disorders is the ATP-dependent, non-selective cation channel (NC_{Ca-ATP} channel). We therefore examined its potential role in the astrocyte swelling/brain edema associated with ALF. Cultured astrocytes treated with 5 mM ammonia showed a 3-fold increase in the sulfonylurea receptor type 1 (SUR1) protein expression, a marker of NC_{Ca-ATP} channel activity. Blocking SUR1 with glibenclamide significantly reduced the ammonia-induced cell swelling in cultured astrocytes. Additionally, overexpression of SUR1 in ammonia-treated cultured astrocytes was significantly reduced by co-treatment of cells with BAY 11-7082, an inhibitor of NF-κB, indicating the involvement of an NF-κB-mediated SUR1 upregulation in the mechanism of ammonia-induced astrocyte swelling. Brain SUR1 mRNA level was also found to be increased in the thioacetamide (TAA) rat model of ALF. Additionally, we found a significant increase in SUR1 protein expression in rat brain cortical astrocytes in TAA-treated rats. Treatment with glibenclamide significantly reduced the brain edema in this model of ALF. These findings strongly suggest the involvement of NC_{Ca-ATP} channel in the astrocyte swelling/brain edema in

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Conflict of Interest:

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Human subjects were not involved in this study.

All institutional and national guidelines for the care and use of laboratory animals were followed.

ALF, and that targeting this channel may represent a useful approach for the treatment of the brain edema associated with ALF.

Keywords

Ammonia; acute liver failure; astrocyte swelling; brain edema; glibenclamide; $\text{NC}_{\text{Ca-ATP}}$ channel; sulfonylurea receptor type 1 protein

Introduction

Hepatic encephalopathy (HE) is a clinical condition that occurs in patients with severe liver disease. Acute HE (acute liver failure, ALF) is a life-threatening condition associated with the sudden onset of seizures, delirium and coma, generally occurring after viral hepatitis, acetaminophen toxicity or exposure to other liver toxins. Cytotoxic brain edema (astrocyte swelling) and associated increased intracranial pressure and cerebral herniation represent the most common cause of death in these patients. Elevated blood and brain ammonia have been strongly implicated in its pathogenesis [1].

While the molecular mechanisms by which ammonia contribute to astrocyte swelling/brain edema in ALF are not clear, recent findings suggest the involvement of various ion transporters/ channels/exchangers [2]. One ion channel known to be involved in the brain edema associated with ischemia and trauma is the non-selective cation channel ($\text{NC}_{\text{Ca-ATP}}$ channel) which regulates the transport of most inorganic cations in brain [3].

Up-regulation of the sulfonylurea receptor type 1 protein (SUR1), is coupled to, and regulates the pore-forming region of the $\text{NC}_{\text{Ca-ATP}}$ channel in astrocytes; thus, increased SUR1 gene expression provides a valid measure of the channel activity [4, 5]. SUR1 up-regulation was identified in brain after traumatic brain injury and stroke, and blocking this channel with sulfonylurea compounds such as tolbutamide or glibenclamide, inhibited the astrocyte swelling and reduced the brain edema after stroke [5]. The present study investigated whether activation of $\text{NC}_{\text{Ca-ATP}}$ similarly occurs in a cell culture model of ALF (ammonia-treated cultured astrocytes), as well as in brain of rats with ALF, and whether inhibition of this channel diminishes or prevents the astrocyte swelling and brain edema in ALF.

Materials and Methods

Astrocyte cultures

Primary cultures of cortical astrocytes were prepared as described previously [6]. Briefly, brains of 1–2 day old rat pups were seeded onto 35-mm culture dishes in Dulbecco's modified Eagle's medium containing penicillin, streptomycin and 15% fetal bovine serum, and the culture plates were incubated at 37°C with 5% CO_2 and 95% air. Culture media was changed twice weekly. On day 10 post-seeding, fetal bovine serum was replaced with 10% horse serum. After 14 days, cultures were treated with 0.5 mM dibutyryl cAMP (Sigma, St. Louis, MO, USA) to enhance cellular differentiation [7]. Cultures consisted of at least 95%

astrocytes as determined by glial fibrillary acidic protein (GFAP) immunohistochemistry. Cultures used were 25–28 days old.

The use of ammonia-treated cultured astrocytes as a model of HE is highly appropriate since substantial evidence invokes a crucial role of ammonia in the pathogenesis of HE, and astrocytes are the principal cells affected in this condition [1]. Moreover, many of the findings occurring in HE *in vivo* are also observed in ammonia-treated astrocyte cultures, including characteristic morphological changes, cell swelling, defects in glutamate transport, up-regulation of the 18 kDa translocator protein (TSPO), reduction in levels of glial fibrillary acidic protein and myo-inositol, disturbance in energy metabolism, and evidence of oxidative/nitrative stress [1, 8].

Cell volume measurement

Astrocyte cell volume (intracellular water space) was determined using 3-O-methyl- ^3H -glucose (OMG) equilibration method [9], as modified for astrocyte cultures by Bender and Norenberg (1998) [10]. Briefly, cultures were incubated with ^3H OMG (NEN, Life Sciences, Boston MA) (1 mM containing 1 μCi of radioactive OMG) for 12 h, and at the end of incubation a small aliquot of medium was saved for specific activity determination. Cultures were quickly washed five times with ice-cold buffer containing 290 mM sucrose, 1 mM Tris-nitrate (pH 7.4), 0.5 mM calcium nitrate, and 0.1 mM phloretin. Cells were then lysed in 0.5 ml of 1 N sodium hydroxide. Radioactivity was converted to intracellular water space and expressed as $\mu\text{l}/\text{mg}$ protein. Protein content was determined employing the bicinchoninic acid assay (Pierce, Rockford, IL, USA).

RNA isolation and RT-qPCR

Isolation of mRNA was performed using the RNeasy@-4PCR kit (#AM1914; Ambion, Austin, TX, USA) as described previously [11]. Following RNA extraction from astrocyte cultures or from brain extracts, cDNA was generated with a High Capacity cDNA Reverse Transcription Kit (catalog# 4368814; Applied Biosystems, Foster City, CA, USA) according to manufacturer's instructions, using 2 μg RNA as a template. The cDNA was diluted 1:20 with Nuclease-Free Water (catalog# 10977-015; Invitrogen-Gibco), aliquoted, and stored at -20°C until used. RT-qPCR was performed using 10 μL diluted cDNA on the Mx3005P Multiplex Quantitative PCR System (catalog# 401513; Stratagene/Agilent Technologies, Wilmington, DE, USA) using RT-qPCR SYBR GREEN Reagents (Brilliant® II SYBR® Green QPCR Master Mix; Agilent Technologies) with the ROX reference dye (final reaction volume 25 μL). A 2 μM stock solution containing both forward and reverse primer pairs was reconstituted in Nuclease-Free Water (catalog# 10977-015; Invitrogen-Gibco) and stored at -20°C . RT-qPCR cycling conditions were as follows: an initial 95°C for 10 min, followed by 40 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 15 s. MxPro-Mx3005P v4.10 software (Stratagene/Agilent Technologies, Wilmington, DE) was used to determine the crossing points for each amplification reaction. Results were exported to Microsoft Excel for analysis. SUR1 primer pairs (GenBank accession number L40624), forward primer (base 4774–4792) TGAAGCAACTGCCTCCATC, reverse primer (base 4955–4937) GAAGCTTTTCC GGCTTGTC. All RT-qPCR data were normalized against glyceraldehyde 3-phosphate dehydrogenase.

Immunoblots

SUR1 protein expression (a marker of NC_{Ca}-ATP channel activation) was measured as described previously [12]. Briefly, equal amounts of protein were subjected to gel electrophoresis and the proteins transferred to polyvinylidene difluoride membranes. After blocking with bovine serum albumin, membranes were incubated with anti-SUR1 (mouse monoclonal antibody, cat# 487923, Calbiochem, USA; 1:1000 dilution). Anti-mouse horseradish peroxidase-conjugated secondary antibody (Vector Laboratories) was used at 1:1000. The peroxidase activity was detected by chemiluminescence using the ECL detection system (Amersham, Arlington Heights, IL). Optical density of the bands was determined with the Chemi-Imager (Alpha Innotech Corp, San Leandro, CA) digital imaging system, and the results were quantified with the Sigma Scan Pro program (Jandell Scientific, San Jose, CA) as a proportion of the signal of a housekeeping protein band (α -tubulin).

ALF model

The thioacetamide (TAA)-treated rat model of ALF was used in this study [13, 14]. This model has been well established relative to the clinical status, liver function, blood–brain barrier integrity, and brain edema development [14, 15, 16]. In brief, rats were given TAA (300 mg/kg bwt, intraperitoneally) for 3 consecutive days. Animals were sacrificed 6 h after the last injection. To prevent hypoglycemia and dehydration, rats were given 12.5 ml/kg of fluid therapy (5% dextrose and 0.45% saline with 20 mEq/L of potassium chloride) every 12 hours, s.c. Normal controls received only saline.

In a separate experimental group, TAA-treated rats were given daily doses of glibenclamide (Sigma-Aldrich, St. Louis, MO) (1, 2, 3, 5 and 10 mg/kg bwt, i.p.) 2 h after TAA injection for 3 days. The TAA (alone)-treated, as well as the TAA plus glibenclamide-treated rats were sacrificed 6 h after the third injection of TAA plus glibenclamide-treatment. Glibenclamide was suspended in water using 0.05% (w/v) carboxymethylcellulose; glibenclamide is known to freely cross the blood-brain barrier [17] (Rashidy-Pour, 2001 and references therein). A second group of animals was given only glibenclamide (1, 2, 3, 5 and 10 mg/kg bwt, i.p.) for 3 consecutive days and were sacrificed 6 h after the last injection. Doses of glibenclamide were chosen based on literature data [17] (Rashidy-Pour, 2001 and references therein).

Liver function tests

To assess the extent of liver failure, blood levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined 6 h after the last injection of TAA using a Cobas 0501 automatic analyzer (Roche Diagnostics, IN). For histopathologic correlation, samples of liver from TAA-treated rats were fixed in 10% formalin for 24 h, processed routinely for paraffin sections and stained with hematoxylin and eosin (H&E).

Clinical status

TAA-treated rats were clinically monitored twice a day and stages of encephalopathy were graded according to the criteria of Gammal et al. (1990) [18] : Grade I: lethargy (generalized reduction in spontaneous activity); Grade II: mild ataxia; Grade III: lack of spontaneous

movement but with an intact righting reflex; Grade IV: loss of righting reflex but with an intact pain reflex (measured by reaction to a tail pinch); Grade V: coma.

Brain water measurement

Brain water content was determined by the wet/dry weight method [19]. Approximately 10 mg tissue (three to four pieces from each animal) of cerebral cortex was dissected; wet weights of tissue determined; tissue dried overnight in an oven at 120°C; and dry weights determined. The difference in wet/dry weights was expressed as percent water content.

Immunohistochemistry of SUR1 in rat brain after ALF

At the end of treatment, control and TAA-treated rats (four animals each) were anesthetized and then transcardially perfused with heparinized saline for 1 min, followed by fixation in 4% paraformaldehyde for 15 min. After decapitation, heads were left in the same fixative for an additional 24 h. Brains were cryoprotected (12-24 h) in 30% sucrose, and 20 micron-thick cortical sections obtained with a cryostat were fixed with ice-cold methanol (for 15 min at room temperature). After thorough washing with ice-cold phosphate-buffered saline, sections were blocked with 10% goat serum and incubated overnight at 4°C with anti-SUR1 (mouse monoclonal antibody, cat# 487923, Calbiochem, USA; 1:100 dilution) and glutamine synthetase (GS), an astrocyte-specific enzyme (sc-74430 Santa Cruz Biotechnology, 1:150 dilution). Following incubation with primary antibodies, sections were washed with TBS containing 1% Tween-20 (TBS-T) and incubated with fluorescent HRP-conjugated secondary antibodies (1:500; AlexaFluor-Rhodamine for GS and AlexaFluor-FITC for SUR1) for 2 h. Sections were then mounted with commercial mounting media (Vector Labs., Burlingame, CA) containing DAPI (nuclear stain). Immunofluorescent images were acquired with a Zeiss LSM510/UV Axiovert 200M confocal microscope with a plan apochromat 40× objective lens, and a 2× zoom resulting in images 125×125 µm in area and 1.0 µm optical slice thickness (1.0 Airy units for AlexaFluor 546 or 568 emission channel). Random collection of images from sections of control and TAA-treated rats was achieved by systematically capturing each image in a blinded manner by moving the microscope stage approximately 5 mm in four different directions. At least 15 fluorescent images were captured per rat, and the images merged to localize astrocytic SUR1.

All procedures followed guidelines established by the National Institute of Health *Guide for the Care and Use of Laboratory Animals* and were approved by our Institutional Animal Care and Use Committee (IACUC).

Statistical analysis

All experiments performed in this study were repeated four to seven times using cells derived from five different batches of astrocyte cultures. Data of all experiments were subjected to ANOVA followed by Tukey's *post hoc* comparisons. A value of $p < 0.05$ was considered significant. Error bars represent mean $\pm$ SEM.

Results

1. Sulfonylurea receptor type 1 (SUR1) protein expression in ammonia-treated astrocyte cultures

Cultured astrocytes were exposed to ammonia (5 mM NH₄Cl) for different time periods (1, 3, 6, 12, 24 and 48 h) and at the end of treatment, SUR1 protein expression, a marker of NC_{Ca}-ATP channel activation [4, 5] was measured in the cell extract. Ammonia significantly increased the level of SUR1 protein expression from 3-48 h, with a peak increase at 24 h (3-fold increase as compared to control) (Fig. 1).

2. Sulfonylurea receptor type 1 (SUR1) mRNA in ammonia-treated astrocyte cultures

Astrocytes were treated with ammonia (5 mM NH₄Cl) for different time points (1, 3, 6, 12 and 24 h) as this concentration has been observed in brains of experimental animals with ALF [20, 21, 16]. At the end of treatment, the extent of SUR1 mRNA level was determined in cell extracts. Astrocytes exposed to ammonia had no effect on SUR1 mRNA expression at 1 h (Fig. 2). However, it caused an increase in SUR1 mRNA level in a biphasic manner; an initial increase at 3 h (6.8-fold, as compared to control), followed by a decline at 6 h, and a second rise at 12 and 24 h (3.9- and 7.6-fold) (Fig. 2). Ammonia also caused an increase in SUR1 mRNA level in cultured astrocytes at 48 h which was similar to the level observed at 24 h after ammonia (data not shown).

3. Effect of glibenclamide on the ammonia-induced cell swelling in cultured astrocytes

To examine the potential role of NC_{Ca}-ATP channel in the ammonia-induced astrocyte swelling, cultures were treated with glibenclamide (5-200 nM and 10 and 50 M), alone or with ammonia (5 mM NH₄Cl) for 24 h, and at the end of treatment, the cell volume was measured by the 3-O-methyl-[³H]-glucose method [9, 10]. A 24 h time point was chosen to examine the effect of ammonia and glibenclamide on cell swelling since the peak increase in swelling was previously detected at that time point [22]. A significant increase in cell volume was observed after exposure of cells to ammonia for 24 h (40.2% as compared with control; $p < 0.05$), and such swelling was attenuated by pre-treatment (20 min) of cells with glibenclamide (50 nM) (Fig. 3). Exposure of astrocytes to 5, 10 nM or higher concentrations of glibenclamide (100 and 200 nM; 10 and 50 M) had no significant effect on the astrocyte swelling induced by ammonia (data not shown). Exposure of astrocytes to glibenclamide alone had no effect on cell volume.

We also found a significant reduction in ammonia-induced astrocyte swelling when cells were treated with ammonia plus the ATP-sensitive potassium channel (or KATP channel) blocker repaglinide (50 nM) (39.6%, as compared to the effect of ammonia, 5 mM NH₄Cl, alone on astrocyte swelling; $p < 0.05$) (data not shown), suggesting that KATP channel activation also contribute to the ammonia-induced astrocyte swelling.

4. SUR1 regulation by NF- κ B

We recently found that treatment of cultured astrocytes with ammonia (5 mM NH₄Cl) significantly increased the nuclear translocation (activation) of NF- κ B, and that such activation contributed to the astrocyte swelling/brain edema [23, 11]. To examine whether

the ammonia-induced increase in NF- κ B activation plays a role in the increase in SUR1 protein in cultured astrocytes, cultures were pre-treated for 15 min with BAY 11-7082, an inhibitor of NF- κ B, with and without ammonia, and SUR1 protein expression examined 24 h later. We found a significant increase in SUR1 protein expression when astrocytes were treated with ammonia (Fig. 1), and BAY 11-7082 diminished such effect (Fig. 4). These findings suggest that NF- κ B activation plays a significant role in the up-regulation of SUR1 protein expression following ammonia treatment.

5. SUR1 mRNA level in thioacetamide-treated rat brain cortex

SUR1 mRNA level was determined in brain cortical extracts from TAA-treated rats. Rats treated with TAA for 24 h showed no change in SUR1 mRNA content; however, a significant increase in SUR1 mRNA level was detected at 48 h (1.5-fold increase as compared to sham). SUR1 mRNA level was unaltered in rats treated with TAA for 3 days (i.e., 6 h after the third injection of TAA) (Fig. 5).

6. SUR1 upregulation in cortical astrocytes of rats with acute liver failure

We examined whether SUR1 is also upregulated in cortical astrocytes in an experimental model of ALF (TAA-treated rats). Cortical sections from control and TAA-treated rats (n=4 for each group) were co-immunostained with SUR1 and glutamine synthetase (an astrocyte marker), and images were captured with a confocal microscope. We found faint SUR1 staining of astrocytes in control animals (Fig 6). By contrast, we found intense SUR1 immunofluorescence in GS-positive cells following the administration of TAA, indicating the over-expression of SUR1 in astrocytes in a rat model of ALF (Fig. 6).

7. Effect of glibenclamide on TAA-induced brain edema in rats

We previously established that rats treated with TAA for two consecutive days showed a significant increase in brain edema (0.8% as compared to control) [11] (Jayakumar et al., 2011). However, an approximately 2% increase in brain edema was detected when animals were treated with TAA for 3 consecutive days, at which time the rats were in clinical grade IV-V (pre-coma to coma). We therefore chose the 3-day time point to examine the effect of the NC_{Ca}-ATP channel blocker, glibenclamide, on brain edema. Rats treated with TAA for 3 days showed a 1.96% increase in water content as compared to saline or solvent-treated controls. Treatment with glibenclamide (1, 2, 3, 5 and 10 mg/kg b.wt.), alone, had no significant effect on brain water content. Treatment of rats with 1, 2, 5 and 10 mg/kg b.wt. glibenclamide after TAA administration, had no significant effect on brain edema (data not shown), while treatment with 3 mg/kg b.wt. glibenclamide significantly reduced the brain water content (48.7% reduction in brain edema) (Fig. 7).

In addition to the immunofluorescent detection of SUR1 expression (noted above in the Results, item #6), we also quantified the level of SUR1 in cerebral cortex of TAA-treated rats by Western blots. TAA-treated rats (for 3 consecutive days) showed an increase in SUR1 protein (164.8% increase as compared to control) and such increase in SUR1 protein level was attenuated by treatment of rats with 3 mg/kg b.wt. of glibenclamide (given 2 h post TAA) (Fig. 8).

Clinical grading of encephalopathy in rats (5 per group) treated with TAA and TAA plus glibenclamide was as follows: No changes were observed after 1 day of TAA-treatment. A progressive clinical deterioration was observed on day 2 and 3. Treatment with glibenclamide significantly attenuated the symptoms observed with TAA. Light microscopic examination of liver from TAA-treated rats (3 days) showed microvesicular steatosis with varying degrees of centrilobular necrosis (figure not shown). Identical findings were observed in rats treated with TAA plus glibenclamide.

Discussion

This study demonstrates that exposure of cultured astrocytes to ammonia (5 mM NH_4Cl) showed an increase in SUR1 mRNA and protein expression. Additionally, the SUR1 blocker, glibenclamide, significantly reduced the cell swelling induced by ammonia. Further, in the TAA rat model of ALF, we found a significant increase in SUR1 protein expression in cortical astrocytes, and treatment of rats with glibenclamide, an inhibitor of SUR1, attenuated the brain edema and improved clinical outcome measures.

Sulfonylurea receptors are membrane proteins which are subunits of the inward-rectifier potassium ion channels [24]. There are three forms of the sulfonylurea receptor (SUR1, SUR2A and SUR2B). Sulfonylurea receptor 1 is a regulatory subunit of KATP channel, that also modulates the activity of the nonselective cation channel ($\text{NC}_{\text{Ca-ATP}}$ channel), as well as influence the transport of most inorganic cations in brain. The primary function of the sulfonylurea receptor is to sense intracellular levels of nucleotides (ATP and ADP) and to facilitate the opening (or closing) of its associated potassium channel. Upregulation of SUR1 protein has been identified in brains of rats after traumatic brain injury and stroke, and sulfonylurea compounds, such as tolbutamide and glibenclamide, inhibited the cell swelling in cultured astrocytes and the brain edema, as well as diminished mortality after stroke [12, 4, 5]. Similarly, we also found a significant increase in SUR1 protein expression in cultured astrocytes after ammonia treatment, as well as in cortical astrocytes after ALF, and treatment of cultured astrocytes or rats with glibenclamide partially reversed these effects. These findings suggest that the activation of $\text{NC}_{\text{Ca-ATP}}$ is a common factor in the mediation of the brain edema in various neurological disorders, including ALF.

The change in SUR1 mRNA expression, as well as in protein level, were biphasic, with a rapid and transient increase peaking at 3 h, followed by a decline at 6 h and a second peak at 24 h (Figures 1 and 2). While the reason for the abrupt decrease in both protein and mRNA expression of SUR1 at 6 h after ammonia treatment is unclear, it is possible that free radicals produced in ammonia-treated astrocytes (a rise at 3 min to 1 h followed by a second rise at 4 h) [25] may have altered the level of SUR1 expression. Consistent with this possibility, studies have demonstrated that nitric oxide exerts a similar effect on a number of proinflammatory genes, including COX-2, IL-6 and NF- κB (Connelly et al., 2001 and references therein) [26]. Additionally, in preliminary studies we found a reduction in SUR1 expression when astrocytes were treated with the superoxide scavenger Mn(III) tetrakis (4-benzoic acid) porphyrin (MnTBAP, 10 M), or with the nitric oxide synthase inhibitor L-NAME (250 M) along with ammonia, as compared to the effect of ammonia alone (data not shown).

Brain edema was significantly reduced in animals that received 3 mg/kg glibenclamide after TAA. However, treatment of rats with 2 or 5 mg/kg bwt glibenclamide, had no significant effect on brain water content after TAA treatment. While the reason for such effect is not known, it is possible that lower or higher concentrations of glibenclamide may have exerted non-specific effects. Such an outcome, however, was documented by Delpire et al. 2009 [27] when using ion transporters inhibitors (e.g., KCC2 inhibitors) in other conditions. It is therefore possible that at 2 or 5 mg/kg, glibenclamide may have blocked or activated various ion channels (other than NC Ca-ATP) that might have prevented a therapeutic response.

The means by which ammonia upregulates SUR1 protein expression in ALF is uncertain. Recent studies have suggested that transcriptional mechanisms are responsible for SUR1 upregulation after cerebral ischemia [28]. In particular, the transcription factors hypoxia-inducible factors (HIF1/2), have been shown to play a major role in the upregulation of SUR1 in ischemia [28]. HIF binds to the Sp1 promoter, which in turn stimulates the transcription of the Sp1 gene. The increase in abundance of Sp1 was accompanied by an increase in binding of Sp1 to the Abcc8 promoter, the gene encoding for SUR1 [4]. Thus, it is possible that ammonia might similarly be increasing HIF and Sp1 in astrocytes, resulting in the upregulation of SUR1.

An additional signaling mechanism that may be involved in the regulation of SUR1 is the activation of protein kinases G (PKG). It was shown that treatment of human embryonic kidney cells (HEK293 cells) with caffeine significantly enhanced currents in SUR1 channels in a PKG and intracellular Ca^{2+} -dependent manner [29]. Ammonia is known to activate PKG in cultured astrocytes, and inhibition of such activation was shown to block the ammonia-induced astrocyte swelling [30]. It is therefore possible that activation of PKG in ammonia treated astrocytes may also have contributed to the changes in the SUR1 protein expression and the subsequent $\text{NC}_{\text{Ca-ATP}}$ channel activation observed in this study.

Activation of mitogen-activated protein kinases have also been implicated in the regulation of SUR1 expression [31]. Furthermore, activation (increased nuclear translocation) of the transcription factor NF- κ B was shown to be increased in association with SUR1 upregulation following subarachnoid hemorrhage [32] (Simard et al., 2009). We similarly found a significant increase in SUR1 protein expression, as well as NF- κ B activation, in ammonia-treated astrocytes and BAY 11-7082, an inhibitor of NF- κ B, significantly reduced SUR1 protein expression. Since ammonia is also known to activate PKG and MAPKs, and such activation is well known to stimulate nuclear NF- κ B translocation [33], it is likely that activation of NF- κ B by PKG and MAPKs signaling pathways are involved in the upregulation of SUR1. As SUR1 expression was detected at 6 h, while increased nuclear NF- κ B expression was detected 12 after exposure of astrocytes to ammonia, the early phase of SUR1 protein expression may be regulated by transcription factors other than NF- κ B (e.g., Sp1) [28] (Woo et al., 2012).

We previously demonstrated that exposure of astrocyte cultures to ammonia increases the activation of the ion transporter NKCC1 [34], which requires ATP. By contrast, activation of SUR1 only occurs when ATP levels are significantly decreased. It should therefore be noted that ATP levels have been shown to be decreased in ammonia-treated astrocytes [35].

It is thus possible that this decrease in ATP may contribute to the activation of SUR1 as observed in the present study. Alternatively, NKCC1 activation may be independent of ammonia-induced ATP depletion, since its activation was shown to be enhanced following its oxidation/nitration and subsequent phosphorylation [34].

The administration of glibenclamide to diabetic rats was previously shown to improve decreased hepatic catalase and SOD activities, implicating glibenclamide in its beneficial effect in diabetes [36]. As glibenclamide is reported to have hepato-protective effect, it is possible that the beneficial effect shown by glibenclamide on the brain edema observed in ALF in the present study may have been secondary to improved liver function. However, no histopathological differences, nor changes in serum “liver enzymes” were observed between TAA and TAA plus glibenclamide-treated groups, indicating that the recovery from the brain edema in TAA-treated rats after treatment with glibenclamide was due to a direct effect of glibenclamide on brain edema and not as a consequence of improvement in liver function.

In summary, cultured astrocytes treated with ammonia showed a significant increase in NC_{Ca-ATP} channel activation as measured by SUR1 protein and mRNA levels. Increased SUR1 was associated with cell swelling in cultured astrocytes, and blocking this channel with glibenclamide significantly reduced the cell swelling. SUR1 protein and mRNA levels are increased in rat brain in the thioacetamide model of ALF, and treatment with glibenclamide likewise reduced the brain edema as well as improved their clinical status. Our findings suggest that activation of the ion channel NC_{Ca-ATP}, contributes to the astrocyte swelling/brain edema in ALF, and that targeting NC_{Ca-ATP} may represent a useful therapeutic approach for the brain edema associated with acute liver failure.

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Figure 1.

Sulfonylurea receptor type 1 (SUR1) protein expression in ammonia-treated astrocyte cultures. (A) Western blots show a significant increase in SUR1 protein level when cultures were exposed to 5 mM NH_4Cl . (B) Quantification of NH_4Cl -induced changes in SUR1 protein expression. SUR1 levels were normalized against α -tubulin. Data were subjected to ANOVA ($n = 4$). * $p < 0.05$ vs. control (Cont). Error bars, mean $\pm$ S.E.

Figure 2.

SUR1 mRNA in ammonia-treated astrocyte cultures. Cultures were exposed to ammonia (NH_4Cl , 5 mM) for different time periods (1-24h), and the extent of SUR1 mRNA level was determined by RT-qPCR. Cultures exposed to ammonia showed a significant increase in SUR1 mRNA levels in a biphasic manner, reaching a peak at 3 h, followed by a decline at 6 hr, followed by a second rise at 12-24 hrs. SUR1 mRNA level was normalized against glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Data were subjected to ANOVA ($n = 6$). * $p < 0.05$ vs. control. Error bars, mean $\pm$ S.E.

Figure 3.

Effect of glibenclamide on ammonia-induced astrocyte swelling. Astrocytes were exposed to ammonia (5 mM NH₄Cl) or ammonia plus glibenclamide (a SUR1 inhibitor) and cell swelling was measured 24 h later. A lesser degree of cell swelling was observed following treatment of astrocytes with ammonia and glibenclamide (50 nM), as compared to cells treated only with ammonia. Data were subjected to ANOVA ($n = 6$). * $p < 0.05$ vs. control (Cont). † $p < 0.05$ vs. ammonia (NH₄⁺). Error bars, mean $\pm$ S.E.

Figure 4.

Inhibition of NF-κB activity diminishes SUR1 protein expression. Astrocytes were exposed to ammonia or ammonia plus BAY 11-7082 (BAY), an inhibitor of NF-κB, and SUR1 protein expression was measured 24 h later. A significant reduction in SUR1 protein expression was observed following treatment of astrocytes with ammonia (NH₄⁺) and BAY 11-7082, as compared to cells treated only with ammonia. Data were subjected to ANOVA ($n = 5$). * $p < 0.05$ vs. control (Cont). † $p < 0.05$ vs. ammonia (NH₄Cl). Error bars, mean ± S.E.

Figure 5.

SUR1 mRNA expression in cerebral cortex of thioacetamide (TAA)-induced acute liver failure (ALF) in rats. A significant increase in SUR1 mRNA was identified on Day 2 after TAA treatment. The expression of SUR1 mRNA was normalized against glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Data were subjected to ANOVA ($n = 8$) $*p < 0.05$ vs. control. Error bars, mean $\pm$ S.E.

Figure 6.

Immunohistochemistry of SUR1 in cerebral cortex of rats with acute liver failure following the administration of thioacetamide (TAA, 300 mg/kg bwt. for 3 consecutive days). Control brain: glutamine synthetase (GS, red, astrocytes), and DAPI (blue, nuclei). Co-localization (merged) of SUR1 and GS illustrates a minimal degree of SUR1 protein expression in astrocytes (arrow). TAA-treated rat brain: Note the increase in SUR1 which co-localizes with GS-positive cells (astrocytes) in TAA-treated rat brain cortical tissue (merged image, arrows). Scale bar =10 μ M.

Figure 7.

Effect of glibenclamide on thioacetamide-induced brain edema in rats. Rats treated with TAA (300 mg/kg bwt.) showed an increase in brain water content, and this effect was significantly reduced by treatment of rats (2 h after TAA) with glibenclamide (3 mg/kg b.wt). Data were subjected to ANOVA ($n = 5$ for each group). * $p < 0.05$ vs. control. † $p < 0.05$ vs. TAA. Error bars, mean $\pm$ S.E. Gli, glibenclamide.

Figure 8.

Effect of glibenclamide on thioacetamide (TAA)-induced SUR1 protein level in rats.

Western blots show a significant increase in SUR1 protein level when rats were treated with TAA (300 mg/kg bwt.), and such effect was significantly reduced in TAA-treated rats were given 3 mg/kg b.wt glibenclamide 2 h post TAA (56.2%). Data were subjected to ANOVA ($n = 4$ for each group). * $p < 0.05$ vs. control. † $p < 0.05$ vs. TAA. Error bars, mean $\pm$ S.E. Gli, glibenclamide.