Blocked Gap Junctional Coupling Increases Glutamate-Induced Neurotoxicity in Neuron-Astrocyte Co-Cultures

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Abstract. Gap junctional communication is likely one means by which neurons can endure glutamate cytotoxicity associated with CNS insults (i.e. ischemia). To examine this neuroprotective role of gap junctions, we employed gap junctional blockers to neuronal and astrocytic co-cultures during exposure to a high concentration of extracellular glutamate. Co-cultures were treated with the blocking agents carbenoxolone (CBX; 25 μM), 18α-glycyrrhetinic acid (AGA; 10 μM), or the inactive blocking analogue glycyrrhizinic acid (GZA; 25 μM). Twenty-four hours following the insult, cell mortality was analyzed and quantified by the release of lactate dehydrogenase (LDH) into the media, the cells’ inability to exclude propidium iodide, and terminal dUTP nick end labeling (TUNEL). Measurement of LDH release revealed that the glutamate insult was detrimental to the co-cultures when gap junctions were blocked with CBX and AGA. Based on propidium iodide and TUNEL labeling, the glutamate insult caused significant cell death compared to sham vehicle and mortality was amplified in the presence of CBX and AGA. Since blockers were not themselves toxic and did not affect astrocytic uptake of glutamate, it is likely that blocked gap junctions lead to the increased glutamate cytotoxicity. These findings support the hypothesis that gap junctions play a neuroprotective role against glutamate cytotoxicity.

Key Words: Astrocyte; Cytotoxicity; Gap junctions; Glutamate; Neuron.

INTRODUCTION

Glutamate is a negatively charged amino acid used for excitatory synaptic transmission in the mammalian nervous system. Although the concentration of glutamate can reach the millimolar range in nerve terminals (1), its extracellular concentration is maintained at a low level to prevent neurotoxicity. As early as 1957, Lucas and Newhouse (2) noted that glutamate can be toxic to neurons if presented at a high concentration. Later, Olney and Sharpe (3) implemented the term “excitotoxicity” to describe the cytotoxic effect that glutamate (and other such excitatory amino acids) has on neurons when applied at high dosages. Physiologically, high levels of glutamate can be achieved by excessive release, inhibition of uptake, or both. Normally, a low concentration of extracellular glutamate is maintained by both neurons and astrocytes. Neurons store glutamate in intracellular stores and regulate its release (4). Astrocytes take up extracellular glutamate by specific transporters and convert the glutamate into glutamine, which is then released for neuronal uptake (5–8). However, excitotoxic levels of extracellular glutamate can occur and are associated with acute and chronic neurodegenerative disorders such as trauma (9), stroke/ischemia (10–13), epilepsy (14, 15), Huntington disease (16, 17), amyotrophic lateral sclerosis (18), and hypoglycemic encephalopathy (19).

Various interventions are currently being examined to reduce neuronal death associated with central nervous system injuries and diseases (20). Such therapies include glutamate release inhibitors, glutamate receptor antagonists, Ca2+ channel blockers, GABA receptor agonists, gangliosides, neurotrophic factors, calpain inhibitors, caspase inhibitors, free radical scavengers, as well as immune and cell metabolism modulators. A novel therapeutic area being examined for brain injury and disease targets the regulation of gap junctional communication.

Gap junctions are specialized channels linking the cytosol of 2 adjacent cells. The limited size of the gap junction pore permits the passage of ions, intracellular messengers, and other molecules that are less than 1 kDa in size (21–23). These channels are formed by each cell donating a hemi-channel (the connexon), which itself consists of proteins called connexins (Cxs) (24).

In regards to brain injury and disease, it is not yet clear whether gap junctions play a neuroprotective or neurodestructive role. On the neuroprotective stance, gap junctions could allow the astrocytic syncytium to buffer extracellular space from cytotoxic levels of metabolites and ions (such as glutamate and K+) (25–29). In addition, gap junctions could promote the viability of energy-exhausted cells by allowing essential molecules (e.g. ATP and glucose) to move into areas of high demand. Alternatively, in a neurodestructive role, gap junctions could permit the movement of potentially toxic metabolites into cells that are already compromised by the insult.

The role of gap junctions in neuroprotection or neurodestruction may be inferred by inhibiting gap junctional transmission while insulting the cells. Gap junctional coupling can be impairedblocked by administering various agents including carbenoxolone (3-hydroxy-11-oxoolean-12-en-30-oic acid) 3 hemisuccinate; CBX) and
18α-glycyrrhetinic acid (3-hydroxy-11-oxo-18,20-olean-12-en-29-oic acid; AGA). CBX is a water-soluble synthetic gap junction blocker and has an inactive analog, glycyrrhizic acid (GZA), which is a derivative of Glycyrrhiza glabra (30). AGA is also derived from G. glabra and also blocks gap junctions; the exact mechanism by which AGA and CBX block the junctions has not yet been deciphered. Previous studies have reported that these blocking agents have no effect on cell viability if presented at concentrations less than 100 μM (30–35).

In this study, we examined the role of gap junctions in primary co-cultures of cortical neurons and astrocytes when exposed to a high level of glutamate. By blocking gap junctions, each cell (neuron and astrocyte) would no longer be part of a collective and, therefore, be forced to endure the insult on its own. We hypothesized that blocking gap junctions with either CBX or AGA would increase neurotoxicity to glutamate. It was determined that when neural cells are uncoupled, their resilience to glutamate cytotoxicity is compromised.

MATERIALS AND METHODS

Dulbecco’s Modified Eagle Medium (DMEM), fetal bovine serum (FBS), DMEM/F12, Neurobasal Medium, penicillin-streptomycin, Hank’s balanced salt solution (HBSS) without CaCl₂, phosphate buffered saline (PBS), and Earle’s Balanced Salt Solution were obtained from GIBCO Laboratories (Burlington, ON, Canada). Cytosine arabinoside, poly-D-lysine, D-glucose, insulin, transferrin, putrescine-HCl, progesterone, sodium selenite, CBX, GZA, AGA, propidium iodide, Hoechst 33342 dye, glutamate, anti-microtubule associated protein 2 (MAP2) antibody, anti-glial fibrillary acidic protein (GFAP) antibody, and lactate dehydrogenase (LDH) Detection kit were products of Sigma (Oakville, ON, Canada). L-[³H]Glutamate ([³H]Glutamate (38–46 Ci/mmol) was purchased from Amersham Canada (Oakville, ON, Canada). Apoptosis Detection System (terminal dUTP nick end labeling, TUNEL, Fluorescein) was from Promega (Madison, WI). Cell strainers (pore size 70 μm) and 6-well plates were acquired from VWR (Mississauga, ON, Canada). DiI (1,1’-dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate) and calcein acetoxymethyl ester (calcein-AM) were purchased from Molecular Probes (Eugene, OR). Solution I consisted of HBSS supplemented with 10% FBS. Media I was comprised of Neurobasal Medium and DMEM/F12 (2:3) supplemented with 10% FBS. Media II was prepared with 54 ml Neurobasal Medium, 36 ml DMEM/F12, 50 μl penicillin-streptomycin, and 2 ml of N2 Supplements. N2 Supplements (36) consisted of the following (given in final concentrations in Media II): glucose (0.6%), insulin (10 μg/ml), transferrin (20 μg/ml), putrescine-HCl (62 μM), progesterone (20 nM), and sodium selenite (30 nM). Sodium Hepes Buffer contained (final concentrations) 134 mM NaCl, 5.2 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄, 10 mM glucose, and 20 mM HEPES, pH 7.3.

Cell Culture

Primary cultures of type 1 astrocytes were prepared from murine cortices using a method modified from Fedoroff and Richardson (37). In brief, cortices were dissected from 1- to 2-day-old mouse pups, placed into PBS, and subsequently freed of meninges. Cortices were then placed into DMEM supplemented with 10% FBS and mechanically dissociated using a serological pipette. This cell suspension was then passed through a cell strainer and diluted using DMEM supplemented with 10% FBS and penicillin-streptomycin at a ratio of 7 ml/cortex. Cells were plated onto 6-well plates (previously coated with poly-D-lysine, 100 μg/ml) in 2 ml aliquots. Cultures were maintained within a humidified incubator at 37°C in 95% air/5% CO₂. Culture media was continuously replaced every 3 days in addition to shaking the cultures. Astrocytic cultures were maintained for 15–17 days from initial plating prior to experiments or co-culturing with neurons. Immunocytochemical characterization of cell types present in the final astrocytic cultures revealed that 95%–97% of the population were GFAP-immunopositive.

Co-cultures consisted of a monolayer of astrocytes (prepared using the method described above) to which cortical neurons were seeded on top. Neurons were prepared from mouse cortices using a modified method by Mazzoni and Kenigsberg (36). In brief, cortices were dissected from embryonic mice of gestation age 16 days and placed into HBSS supplemented with 10% FBS. Cortices were then freed of meninges, minced with scalpels, and mechanically disrupted by gently triturating through a fire-polished Pasteur pipette (bore size of approximately 0.6-mm internal diameter). Cell suspension was passed through a cell strainer, centrifuged, and subsequently resuspended in HBSS and Media I (1:1) supplemented with 10% FBS. Cells (1 × 10⁶ cells/well) were seeded on top of astrocyte cultures and allowed to settle for 2 h. Subsequently, media was replaced with 2 ml Media II. On day 4, growth media was replaced with fresh Media II containing cytosine arabinoside (2 μM). Thereafter, two-thirds of the conditioned media was replaced every third day with fresh Media II and cultures were maintained for 14 days prior to experiments. Immunocytochemical analysis indicated that cells cultured on top of the astrocytes expressed the neuronal marker MAP2.

Pre-Loading

Blocking of astrocytic gap junctions was determined by dye coupling using the pre-loading method as described by Goldberg et al (38). In brief, astrocyte cultures prepared by the method outlined above were maintained in Media II for 7 days. Various concentrations of the blocking agents CBX and AGA and the inactive analogue GZA were tested. To minimize indirect effects of the blocker CBX, we performed similar experiments in the presence of GZA; GZA has a similar chemical structure to CBX yet does not block gap junctions (30–32).

All agents were added to media 1 h prior to pre-loading and were present in all solutions during the experiment. Cultures were bathed in dye solution (5 μM calcein-AM and 10 μM DiI in an isotonic (0.3 M) glucose solution) for 20 min in a humidified incubator (37°C, 5% CO₂/95% air), rinsed twice with isotonic glucose solution, trypsinized, suspended in DMEM with 1% FBS, and plated onto unlabeled cells at a 1:500 ratio of labeled to unlabeled cells. Cells were maintained for 3 h in
the incubator and were subsequently examined with a photomicroscope (Zeiss Axiopt; Carl Zeiss, NY). Gap junctional communication was assessed by the passage of calcein from the donor cells (labeled with both calcein and DiI) to the underlying recipient cells.

Glutamate Exposure

Blocking agents were added to co-cultures 1 h prior to experiments and were present in all solutions throughout the experiment. Co-cultures were rinsed with PBS and then bathed in EBSS with glutamate (1 mM) or sham (vehicle) for 3 h in a humidified incubator (37°C, 95% air/5% CO2). Cells were subsequently maintained for 24 h after glutamate/sham exposure in fresh Media II.

Mortality Assessment

Samples of the 24-h-conditioned media were collected and analyzed for LDH activity. Cell cultures were exposed to propidium iodide (30 μM in PBS) for 5 min and subsequently fixed in 4% formaldehyde solution. Cells were then stained with TUNEL and Hoechst and examined by fluorescence microscopy.

[3H]Glutamate Uptake

Glutamate uptake experiments were performed in a manner similar to that described by Sitar et al (39). In brief, confluent cultures of astrocytes were switched to Media II for 1 wk prior to experiment. Blocking agents were added to cultures 1 h prior to glutamate uptake and were present in all solutions during transport experiments. Cells were rinsed with Sodium Hepes Buffer and exposed to glutamate solution for 120 s. Uptake was terminated by washing cultures with ice-cold Tris-sucrose (pH 7.3) solution. Cells were harvested by osmotic lysis (1 ml water/dish) and mechanical scraping. The radioactive contents of the buffer and cells were measured by liquid scintillation counting. Glutamate uptake was based on the specific activity of radiolabeled glutamate in the buffer and that taken-up by the cells.

Data Analysis

All experiments were performed on 3 or more culture preparations from individual litters of mice. Data are presented as means ± standard error of the mean (SEM). Comparisons between mean values were evaluated using one-way analysis of variance with Student-Newman-Keuls Multiple Comparisons test. A p value of <0.05 was considered significant.

RESULTS

Blockage of Gap Junctions by CBX and AGA

A minimal concentration of CBX and AGA required to block astrocytic gap junctions was determined by assaying unlabeled cells receiving calcein (green) from a labeled cell (DiI and calcein; red and green, respectively). Final concentrations of 1, 10, 25, and 50 μM of blockers were tested. Figure 1 demonstrates the blocking effect of CBX on astrocytic gap junctions after the cells have been maintained in serum-free media. While vehicle (water) and GZA had no effect on the passage of calcein from donor cells to, and among, recipient cells, concentrations including and exceeding 25 μM CBX effectively blocked the passage of this dye. A lower concentration of AGA (10 μM) was required to similarly block astrocytic gap junctions while its vehicle dimethylsulfoxide (DMSO) had no effect. From the results obtained in the pre-loading experiment concentrations of 25 μM CBX and 10 μM AGA were chosen for the remainder of the experiments to characterize the effect of glutamate cytotoxicity when gap junctions are blocked. There was no toxicity observed to astrocytes or neurons with the concentrations of CBX, AGA, or GZA used in these experiments.

Blockage of Gap Junctions Increases Cytotoxicity to Glutamate

Glutamate cytotoxicity experiments were performed on primary co-cultures of murine cortical astrocytes and neurons. Immunocytochemical analysis revealed MAP-2-positive neurons situated on top of a confluent layer of GFAP-positive astrocytes (Fig. 2). The 2 cell types occupied different focal planes, which hindered phase-contrast images but, in conjunction with Hoechst dye staining, allowed for cell-type identification based on nuclear size and its plane of focus.

Glutamate cytotoxicity was analyzed by 3 mechanisms: LDH release, the inability to exclude propidium iodide, and TUNEL labeling. When presented with a sham insult, neither vehicle, CBX nor GZA caused the cells to release a significant amount of LDH (Fig. 3). When glutamate was administered in the presence of vehicle or GZA, no significant LDH release occurred. However, when gap junctions were blocked with CBX, a similar glutamate insult caused the cells to release a significant amount of LDH.

Analysis of propidium iodide-labeled cells revealed a similar pattern between agents compared to LDH released (Fig. 4). While no difference was ascertained between vehicle, CBX, and GZA under sham conditions, glutamate caused a significant increase in propidium iodide-labeled cells in the presence of vehicle and CBX. This glutamate insult, in conjunction with blocked gap junctions (by CBX), caused a substantial number of cells to
be labeled with propidium iodide (compared to all other treatments; Fig. 5).

A third method of cytotoxicity analysis was performed on the co-cultures following glutamate exposure with/without blocking gap junctions using TUNEL. Similar to the other 2 analyses, neither vehicle, CBX nor GZA alone caused a large proportion of labeling (Fig. 4). However, while glutamate exposure induced cytotoxicity (comparing vehicle sham with vehicle glutamate insult), the magnitude of this insult was enhanced when the gap junctions were blocked (glutamate insult with CBX; Fig. 6).

To confirm that increased glutamate cytotoxicity in the presence of CBX was caused by blocked gap junctions, another blocker, AGA, was employed in similar experiments. Neither vehicle (DMSO) nor AGA caused a significant LDH release (data not shown). However, AGA did cause a significant amount of LDH release when present during the glutamate exposure.

**Gap Junction Blocker Does Not Affect Astrocytic Glutamate Transporters**

As glutamate cytotoxicity was amplified in the presence of a gap junction blocker, it was necessary to determine if the blocker was affecting astrocytic glutamate transporters. Figure 7 demonstrates that there was no difference between the uptake of [H]glutamate by cortical astrocytes in the presence of vehicle, CBX, or GZA.
DISCUSSION

Increasing evidence suggests that gap junctions play a role above and beyond everyday normal functioning. Supporting other studies, the results of this paper show the importance of functional gap junctions in preventing cell death associated with CNS injury and disease. By blocking functional gap junctions in co-cultures of neurons and astrocytes, we have demonstrated the important role of intercellular communication in allowing cells to endure high levels of extracellular glutamate.

Glutamate can induce a cytotoxic effect to sensitive cells (i.e. neurons) by 1 of 2 mechanisms. In the first mechanism, glutamate activates ionotropic receptors with subsequent depolarization of the cell, resulting in cell swelling and, potentially, necrosis. In the second mechanism, glutamate metabotropic receptor overstimulation may cause the cell to undergo transcriptionally active suicide (apoptosis). A recent study suggests that, depending on the cellular environment and energy supply, a single cell can switch between necrosis and apoptosis (40). Therefore, to quantify cell death in our study, we examined markers for both necrosis (LDH release and the inability to exclude PI) and apoptosis (TUNEL labeling).

While glutamate exposure did not significantly affect LDH release in our cultures, PI and TUNEL staining indicated that the dosage of glutamate employed significantly insulted the cells. More specifically, the PI and TUNEL labeling was highly associated with the glutamate-sensitive neurons and labeled very few underlying astrocytes (similar results to Choi [41]). Astrocytic tolerance to the glutamate insult is likely due to astrocytes lacking NMDA receptors (42) and housing large glycogen stores (a crucial metabolite for energy production) (43, 44).

Although glutamate alone induced cell death, this detrimental effect was amplified in the presence of gap junction blockers, as demonstrated by released LDH, PI- and TUNEL-labeling. Again, mortality markers (PI and TUNEL) were specific to the glutamate-sensitive neurons. Interestingly, most of the PI-stained cells were also labeled by TUNEL, demonstrating either the ability of TUNEL to label necrotic cells (45) or the overlapping properties of necrosis and apoptosis.

The increased glutamate cytotoxicity in the presence of the gap junction blockers was likely directly related to the blockers impeding intercellular gap junctional communication. To address the specificity of the blockers, we employed an inactive form of one of the blockers in addition to administering the blockers in the absence of glutamate. The blockers alone caused no significant cell death and no significant difference in mortality was determined between vehicle and the inactive blocker in the presence of glutamate. Furthermore, the blockers did not affect astrocytic uptake of radiolabeled glutamate; thus, the increased mortality seen with the blockers in combination with glutamate could not be attributed to the blockers inhibiting normal astrocytic glutamate transport and sequestering. Taken together, increased glutamate cytotoxicity (in particular neurotoxicity) in the presence of the blockers suggests that one major parameter affecting neuronal survival following an excitotoxic insult is coupling to its neighboring cells.

The increased cytotoxicity to glutamate in the presence of gap junction blockers may have occurred by way of the blockers hindering neuro-neuronal and/or neuro-astroglial coupling. It is likely that neuro-neuronal coupling does not directly provide neuroprotection from a glutamate insult based on the following reasons. First, although neurons are known to be highly coupled electrically during development, gap junctional coupling becomes limited as they mature (46). Second, neighboring glutamate-sensitive neurons would be dealing with the same insult under similar conditions and, therefore, likely cannot afford to offer support. However, if neuro-astrocytic coupling occurs, then neuro-neuronal coupling may indirectly play a key role in neuroprotection. Even if neuronal coupling is limited, every neuron could be supported by the glial syncytium either through direct contact to an astrocyte or to another neuron that is itself directly coupled to the astrocytes. Coupling to the more resilient astrocytes could permit the import of necessary...
metabolites and the export (buffering) of ions and second messengers that may otherwise reach detrimental levels.

Although there has been much debate in regards to functional neuro-astrocytic coupling, increasing evidence supports such heterocellular coupling. It was originally thought that neurons do not couple to astrocytes based on their different connexin constituents. To date, neurons have been reported to express Cx26, Cx32, Cx33, Cx36, Cx37, Cx40, Cx47, and possibly Cx43 (47–49). On the other hand, astrocytic gap junctions consist of Cx30, Cx40, Cx43, and Cx45 (22, 50, 51). Whether the gap junctions consist of similar Cxs (homotypic gap junctions consisting of Cx43) or a combination of different Cxs (heterotypic gap junctions), neuro-astroglial coupling has repeatedly been demonstrated (6, 52–59).

The protective role of gap junctions has been demonstrated in this report as well as in previous studies. By blocking the gap junctions, we have demonstrated that intercellular communication plays a neuroprotective role when neurons and astrocytes are exposed to a high level of extracellular glutamate. A similar finding was previously reported by Blanc et al, who demonstrated that hippocampal astrocytic gap junctional communication decreased neuronal vulnerability to oxidative stress (FeSO\textsubscript{4} exposure) (60). However, unlike the results reported by Blanc et al, the blockers used in our experiments (i.e. CBX and AGA) had no toxic effects on cortical co-cultures when presented in the absence of glutamate exposure. This finding leads us to conclude that blocking gap junctions inhibits the passage of certain substances (donation of certain essential metabolites or buffering noxious substances) between cells during the insult. Another notable difference between these 2 studies is the insult employed: FeSO\textsubscript{4} indiscriminately impairs both neurons and astrocytes, while glutamate can selectively damage the more sensitive neurons. Injuring the neurons in conjunction with blocking the gap junctions of relatively unstressed astrocytes allows the importance of gap junctional coupling to be examined.

Fig. 4. An example of necrosis (propidium iodide) and apoptosis (TUNEL) markers in mixed cultures following glutamate cytotoxicity in the presence of either vehicle (water, A–C) or CBX (25 μM, D–F). Number of cells stained with propidium iodide (red, A, D) and TUNEL (green, B, E) was increased when cells received a glutamate insult with CBX (compared with glutamate insult with vehicle). Hoechst staining (blue, C, F) was used to calculate total number of cells in the field. Scale bar = 50 μm.
Blocking gap junctions increased the co-culture's inability to exclude propidium iodide (PI) following glutamate exposure. Three-hour exposure to sham insult or the gap junction blocker CBX (25 μM) or its inactive analogue GZA (25 μM) resulted in no change in PI labeling in mixed cultures, while glutamate (1 mM), and glutamate in combination with CBX, significantly increased the number of PI-labeled cells. Glutamate exposure with CBX resulted in an increased number of PI-labeled cells compared to sham with CBX, glutamate with vehicle (H2O), and glutamate with GZA. The number of PI-labeled cells is expressed as a percent of total cells within the field examined. Data are mean ± SEM. * p < 0.001 compared to sham with CBX, compared to vehicle with glutamate, and compared to glutamate with GZA. # p < 0.05 compared to sham with vehicle.

Blocking gap junctions increased apoptosis in co-cultures following glutamate exposure. Three-hour exposure to glutamate (1 mM) or the gap junction blocker CBX (25 μM) or its inactive analogue GZA (25 μM) produced no change of TUNEL labeling in mixed cultures, while glutamate in combination with CBX significantly increased the number of TUNEL-labeled cells. Glutamate exposure with CBX resulted in an increased number of TUNEL-labeled cells compared to sham with CBX, glutamate with vehicle (H2O), and glutamate with GZA. The number of TUNEL-labeled cells is expressed as a percent of total cells within the field examined. Data are mean ± SEM. * p < 0.001 compared to sham with CBX, compared to vehicle with glutamate, and compared to glutamate with GZA. # p < 0.05 compared to sham with vehicle.

Blocking of astrocytic gap junctions does not affect ability to transport glutamate. The presence of CBX (25 μM) did not cause a significant difference in [3H]glutamate uptake by astrocytes compared to either vehicle (H2O) or its inactive analogue, GZA (25 μM).

High levels of glutamate and K+ have been shown to increase astrocytic coupling (61) while mild or moderate ischemia increases Cx43 immunostaining (62); an increase in coupling suggests that the cells are attempting to manage the insult. Complementing these findings, Cotrina et al have demonstrated that astrocytic gap junctions remain open during ischemia (63). Finally, using the rat mucosa model, Iwata et al found that inhibition of gap junctional coupling in combination with ischemia-reperfusion increases tissue damage (64). In contrast, it has been suggested that gap junctions have a neurodestructive role. Maintaining gap junctional intercellular communication to severely injured/diseased areas may allow the spread of potentially harmful levels of certain ions (i.e. K+ and Ca2+) and second messengers (i.e. IP3) to tissue already compromised. In accordance with this theory, supporting evidence suggests that the surrounding insulted cells do attempt to sever their contact and isolate the heavily damaged area (62, 65). Furthermore, it is believed that the gap junction network may itself propagate spreading depression (66).

While in vitro approaches similar to the one used in the present study have provided valuable insight into the mechanisms of neuronal injury (67), the complex structural and chemical interactions that occur in the CNS cannot be maintained in typical dissociated tissue culture approaches. It is possible to maintain a significant degree of integrity through the use of culturing organotypic brain slices, and these have been used for studies on ischemia and glutamate cytotoxicity (68). However, in vivo studies have also been pursued to address the role of gap junctions in neuronal injury. In support of the current findings, we have recently shown that heterozygote Cx43-null mice that have a deficiency in astrocytic gap
junctons exhibit increased infarct volume 4 days after unilateral middle cerebral artery occlusion (69).

The present study concludes that the blockade of gap junctions in cortical neuron-astrocyte co-cultures decreases cytotoxicity to glutamate. Since blocking of gap junctions under sham insults had no effect, we suggest that functional gap junctions are neuroprotective and neuronal survival is increased through coupling of the astrocytic syncytium during a glutamate insult. Thus, it is possible that some neurological diseases may have diminished or compromised gap junctions which would increase neuronal vulnerability. Accordingly, a potential therapy for some neurological injuries or diseases may lie in upregulating functional gap junctions.

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