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Riluzole and gabapentinoids activate glutamate transporters to facilitate glutamate-induced glutamate release from cultured astrocytes

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Abstract

We have recently demonstrated that the glutamate transporter activator riluzole paradoxically enhanced glutamate-induced glutamate release from cultured astrocytes. We further showed that both riluzole and the $\alpha_2\delta$ subunit ligand gabapentin activated descending inhibition in rats by increasing glutamate receptor signaling in the locus coeruleus and hypothesized that these drugs share common mechanisms to enhance glutamate release from astrocytes. In the present study, we examined the effects of riluzole and gabapentin on glutamate uptake and release and glutamate-induced Ca^{2+} responses in primary cultures of astrocytes. Riluzole and gabapentin facilitated glutamate-induced glutamate release from astrocytes and significantly increased glutamate uptake, the latter being completely blocked by the non-selective glutamate transporter blocker DL-threo-β-benzyloxyaspartic acid (DL-TBOA). Riluzole and gabapentin also enhanced the glutamate-induced increase in intracellular Ca^{2+} concentrations. Some $\alpha_2\delta$ subunit ligands, pregabalin and L-isoleucine, enhanced the glutamate-induced Ca^{2+} response, whereas another, 3-exo-aminobicyclo[2.2.1]heptane-2-exo-carboxylic acid (ABHCA), did not. The enhancement of glutamate-induced intracellular Ca^{2+} response by riluzole and gabapentin was blocked by the DL-TBOA and an inhibitor of Na^+/Ca^{2+} exchange, 2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiurea (KB-R7943). Gabapentin's enhancement of Ca^{2+} increase was specific to glutamate stimulation, as it was not mimicked with stimulation by ATP. These results suggest that riluzole and gabapentin enhance Na^+-glutamate co-transport through glutamate transporters, induce subsequent Ca^{2+} influx via the reverse mode of Na^+/Ca^{2+} exchange, and thereby facilitate Ca^{2+}-dependent glutamate release by glutamate in astrocytes. The present study also demonstrates a novel target of gabapentinoid action in astrocytes other than $\alpha_2\delta$ subunits in neurons.

Keywords

riluzole; gabapentin; astrocytes; glutamate transporters

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1. Introduction

In the central nervous system, astrocytes regulate extracellular glutamate concentration via two types of glutamate transporters, glutamate transporter-1 (GLT-1) and glutamate-aspartate transporter (GLAST) (Anderson and Swanson, 2000). Under physiological conditions, glutamate transporters take up glutamate from the extracellular space. However, in pathological conditions such as ischemia, astrocytes release large amounts of glutamate via reverse transport, and this increased extracellular glutamate participates in neurotoxicity (Malarkey and Parpura, 2008).

Riluzole, a neuroprotective drug approved for amyotrophic lateral sclerosis (Brooks, 2009), activates GLT-1 and GLAST to enhance glutamate uptake (Frizzo et al., 2004; Fumagalli et al., 2008), and reduces extracellular glutamate concentrations in the spinal cord (Coderre et al., 2007). In contrast to this glutamate lowering effect in the spinal cord, we recently demonstrated that a local injection of riluzole into the locus coeruleus resulted in activation of noradrenergic neurons to induce descending inhibition in rats, possibly via facilitation of glutamate-induced glutamate release from astrocytes (Hayashida et al., 2010). However, the mechanisms by which riluzole might activate glutamate-induced glutamate release from astrocytes is unknown.

Gabapentin inhibits pain transmission via an interaction with $\alpha_2\delta$ subunits of voltage-gated Ca^{2+} channels (Gee et al., 1996). This inhibitory mechanism contrasts with our recent demonstration that gabapentin, like riluzole, activated locus coeruleus neurons via glutamatergic signaling and induced subsequent spinal noradrenaline release in rats and humans (Hayashida et al., 2007; Hayashida et al., 2008). We therefore hypothesized that riluzole and gabapentin may share common mechanisms for glutamate regulation in astrocytes.

Glutamate increases intracellular Ca^{2+} in astrocytes via activation of Ca^{2+} permeable ionotropic glutamate receptors, which respond to AMPA, and metabotropic glutamate receptors, which release Ca^{2+} from internal stores through 1,4,5-inositol-trisphosphate signaling (Hansson et al., 2000; Verkhratsky and Kirchhoff, 2007). In some astrocytes, co-transport of sodium ions and glutamate by glutamate transporters results in Ca^{2+} influx via the reverse mode of Na^+/Ca^{2+} exchange (Kirischuk et al., 1997; Rojas et al., 2007), which in turn leads to glutamate release from astrocytes via Ca^{2+}-dependent mechanisms (Malarkey and Parpura, 2008). By this mechanism, glutamate uptake by astrocytes can paradoxically result in glutamate release. We therefore hypothesized that riluzole and gabapentin enhance Ca^{2+}-dependent glutamate release from astrocytes by activation of glutamate transporters and subsequent Ca^{2+} influx via the reverse mode of Na^+/Ca^{2+} exchange. The present study examined whether riluzole, gabapentin, and other $\alpha_2\delta$ subunit ligands increase glutamate uptake through glutamate transporters, enhance glutamate-induced intracellular Ca^{2+} response by activation of glutamate transporters and subsequent Ca^{2+} influx via the reverse mode of Na^+/Ca^{2+} exchange, and thereby facilitate glutamate-induced glutamate release in primary cultured astrocytes.

2. Materials and Methods

2.1. Astrocyte cultures

Primary astrocyte cultures were prepared from the cerebral cortices of neonatal rats between postnatal days 1 and 2. Cerebral cortices were mechanically dissociated in ice-cold Hank's buffered salt solution (HBSS, pH=7.2) by fire-polished glass pipettes and centrifuged at 300×G for 5 min. Tissues were re-dissociated in ice-cold HBSS and the procedure was repeated two times using smaller pipette tip diameters. Cells were first seeded onto T-50

flasks and incubated in Dulbecco's modified Eagle's medium, containing 10% fetal bovine serum, 100 units/ml penicillin/streptomycin, 2 mM L-glutamine, and 10 ng/ml epidermal growth factor (EGF, BD Biosciences, Bedford, MA, USA) to maintain GLT-1 and GLAST protein expression in astrocytes (Zelenaia et al., 2000), at 37°C and 5% CO_2. After one week, astrocytes were reseeded onto poly-d-lysine-coated glass coverslips (35 mm diameter) at a density of 4×10^4 cells for Ca^{2+} imaging experiments and in 24-well plates at a density of 10^4 cells/well for glutamate uptake/release experiments, then cultured for 6 days. In the present study, cells consisted of >98% of flat polygonal astrocytes with positive immunostaining for glial fibrillary acidic protein.

2.2. Ca^{2+} imaging

Ca^{2+} imaging was performed according to our previous study in neurons (Hayashida et al., 2006) with modifications for cultured astrocytes. Astrocytes were incubated with 5 μM Fura-2 AM (Molecular Probes, Eugene, OR, USA) and 0.01% Pluronic F-127 (Molecular Probes) for 30 min at 37 °C, washed with HEPES buffer containing (in mM): 140 NaCl, 4 KCl, 2 $CaCl_2$, 1.2 $MgSO_4$, 10 glucose, and 10 HEPES, pH=7.4, and then left at room temperature in a dark environment for 20 min. Coverslips were mounted on a chamber equipped with a pressure valve perfusion system (ALA-VM8, ALA Scientific Instruments, New York, NY, USA) and viewed through an inverted microscope. Fura-2 fluorescence was recorded at 510 nm during alternating excitation at 340 and 380 nm at 1 Hz using a monochromater (PTI Deltascan, Photon Technology International, South Brunswick, NJ, USA). Only cells responding to 300 μM ATP at the end of experiment were included in analysis.

2.3. Glutamate uptake and release

For glutamate uptake experiments, astrocytes were incubated with HEPES buffer for 40 min with a change to fresh buffer at 20 min and treated with test drugs for 5 min at 37 °C. Astrocytes were incubated with 1 μM glutamate (combination of both tritiated and unlabeled glutamate) containing test drugs for 1 min at 37 °C, quickly washed twice, and then lysed with 0.4% Triton X-100 for 10 min. Amount of radioactivity in lysates was measured by scintillation spectrometry (LS6500, Beckman Coulter Inc., Fullerton, CA, USA).

For glutamate release experiments, astrocytes were incubated with Dulbecco's modified Eagle's medium containing 1 μM glutamate (combination of both tritiated and unlabeled glutamate) for 1 hr at 37 °C, and washed twice with HEPES buffer. Astrocytes were then incubated with test drugs alone for 5 min, followed by unlabeled 10 μM glutamate in the presence of test drug for 5 min at 37 °C. Supernatants were collected and astrocytes were lysed. The amount of radioactivity in supernatants and lysates was measured as described above.

2.4. Drugs

DL-threo-β-benzyloxyaspartic acid (DL-TBOA, Tocris Bioscience, Ellisville, MO, USA) and 2-[2-[4-(4-nitrobenzyloxy)phenyl]ethyl]isothiurea (KB-R7943, Tocris Bioscience) were dissolved in DMSO and diluted with HEPES buffer. Riluzole (Sigma Chemical CO, St. Louis, MO, USA), gabapentin (Tronto Research Chemicals Inc., North York, ON, USA), pregabalin (gifted from Pfizer Inc., New York, NY, USA), L-isoleucine (Sigma Chemical CO), and 3-exo-aminobicyclo[2.2.1]heptane-2-exo-carboxylic acid (ABHCA, Acros organics, New Jersey, USA) were dissolved in distilled water, and then diluted with HEPES buffer.

2.5. Data analysis

Data from glutamate uptake and release experiments are normally distributed and presented as mean ± S.E.M. Data from Ca^{2+} imaging experiments were not normally distributed and presented as 25^{th}, 50^{th}, and 75^{th} percentiles of Fura-2 fluorescence ratio response. Differences among groups were determined using two-way analysis of variance (ANOVA) or ANOVA on ranks as appropriate. $P<0.05$ was considered significant.

3. Results

3.1. Glutamate uptake and release

For glutamate uptake, we selected relatively low concentration of glutamate (1 μM) to minimize glutamate-induced glutamate release. As previously shown (Frizzo et al., 2004), riluzole (1 μM) significantly enhanced [^{3}H]-glutamate uptake in astrocytes (Fig. 1, $P<0.01$). The concentration of riluzole in the uptake experiment was determined from the previous study (Frizzo et al., 2004). Gabapentin pretreatment also increased [^{3}H]-glutamate uptake in a concentration-dependent manner. However, when acutely administered, gabapentin (100 μM) did not affect [^{3}H]-glutamate uptake, consistent with a previous observation (Su et al., 1995). The non-selective glutamate transporter blocker DL-TBOA (100 μM) strongly reduced [^{3}H]-glutamate uptake ($P<0.001$) and abolished the enhancement of [^{3}H]-glutamate uptake by riluzole and gabapentin.

Riluzole, gabapentin, and the other $\alpha_2\delta$ subunit ligand pregabalin, none of which affected basal [^{3}H]-glutamate release, significantly enhanced glutamate-induced [^{3}H]-glutamate release from astrocytes (Fig. 2, $P<0.01$). Since TBOA (50 μM) alone significantly increased extracellular [^{3}H]-glutamate concentration (16.4 ± 1.0 %, n=6) compared to the buffer control (9.5 ± 0.4 %, n=25, $P<0.01$), the current study could not examine whether blockade of glutamate transporters affects gabapentin and riluzole effects.

3.2. Intracellular Ca^{2+} response

Figure 3A depicts a representative intracellular Ca^{2+} response in an astrocyte evoked by glutamate (10 μM), as reflected in change in ratio of Fura-2 emission with excitation at two different wavelengths. Figure 3B shows the concentration-dependency of glutamate on this increase in Fura-2 ratio. We chose 10 μM glutamate for the subsequent experiments, since this concentration of glutamate induced both glutamate release and Ca^{2+} response in astrocytes. Figure 4A depicts representative intracellular Ca^{2+} responses in astrocytes evoked by glutamate (10 μM) after perfusion with riluzole or gabapentin. Riluzole and gabapentin, neither of which affected basal Fura-2 ratio (Fig. 4B), enhanced the increase evoked by glutamate in a concentration-dependent manner (Fig. 4C). We then tested effects of other $\alpha_2\delta$ subunit ligands glutamate-induced Ca^{2+} response (Fig. 5). Pregabalin and L-isoleucine enhanced the glutamate-induced Ca^{2+} response in a concentration-dependent manner. However, another $\alpha_2\delta$ subunit ligand ABHCA, which has a similar $\alpha_2\delta$ binding affinity to gabapentin (Lynch et al., 2006), failed to alter the glutamate-induced Ca^{2+} response. In contrast to gabapentin's effect on glutamate stimulation, the intracellular Ca^{2+} response evoked by 10 μM ATP (25^{th}, 50^{th}, and 75^{th} percentile; 0.36, 0.41, and 0.46, n=173) was not altered by 100 μM gabapentin (25^{th}, 50^{th}, and 75^{th} percentile; 0.34, 0.40, and 0.46, n=172).

DL-TBOA (50 μM) alone did not affect the basal Fura-2 ratio but significantly reduced the glutamate-induced Fura-2 ratio increase (Fig. 6A and B, $P<0.05$). The facilitatory effect of gabapentin (10 and 100 μM) and riluzole (0.1 and 1 μM) on the glutamate-induced intracellular Ca^{2+} response was significantly reduced by DL-TBOA ($P<0.05$).

The Na^+/Ca^{2+} exchange inhibitor KB-R7943 (30 μM) alone caused a small increase in the basal Fura-2 ratio within 2–3 min that was sustained during the perfusion, but did not affect the glutamate-induced Ca^{2+} response (Fig. 7A and B). The facilitatory effects of gabapentin (100 μM) and riluzole (0.1–1 μM) on glutamate-induced intracellular Ca^{2+} response were significantly reduced by KB-R7943 ($P<0.05$). Since KB-R7943 (30 μM) alone induced $Ca2^+$ response, we did not test higher concentrations of KB-R7943 in the present study.

4. Discussion

4.1. Activation of glutamate transporters by riluzole facilitates glutamate release from cultured astrocytes

Glutamate is the most ubiquitous excitatory neurotransmitter in the central nervous system and its regulation by glutamate transporters in astrocytes has been intensely investigated. The current study confirms previous observations in cultured astrocytes that riluzole activates glutamate transporters to enhance glutamate uptake (Frizzo et al., 2004; Fumagalli et al., 2008), and extends these observations by demonstrating that riluzole enhances glutamate-induced increases in intracellular Ca^{2+} via the reverse mode of Na^+/Ca^{2+} exchange to facilitate glutamate release.

Astrocytes take up glutamate from the extracellular space via glutamate transporters (GLT-1 and GLAST) under physiological conditions, but during pathological states such as ischemia, high extracellular K^+ concentrations can increase extracellular glutamate concentrations by reverse transport through these transporters (Malarkey and Parpura, 2008). However, since riluzole did not alter basal glutamate release in the current study, it is unlikely that riluzole induces glutamate release via reverse transport.

Glutamate primarily acts on AMPA and metabotropic glutamate receptors to increase intracellular Ca^{2+} in astrocytes (Hansson et al., 2000; Verkhratsky and Kirchhoff, 2007). In addition to this glutamate receptor-mediated Ca^{2+} response, co-transport of sodium ions with glutamate or kainite through glutamate transporters results in Ca^{2+} influx via the reverse mode of Na^+/Ca^{2+} exchange in astrocytes *in vitro* and *in situ* (Kirischuk et al., 1997; Rojas et al., 2007), consistent with the current observation that blockade of glutamate transporters by TBOA reduced glutamate-induced Ca^{2+} response in cultured astrocytes. However, we did not observe inhibition of glutamate-induced Ca^{2+} response by KB-R7943 (30 μM), although we did not test higher concentrations of KB-R7943 in the present study. Further studies, such as direct measurements of Na^+/Ca^{2+} exchange current and/or intracellular Na^+ concentration, are required to clarify whether reverse mode of Na^+/Ca^{2+} exchange contributes to glutamate-induced Ca^{2+} response in astrocytes. Nevertheless, the current study demonstrated that both TBOA and KB-R7943 reduced facilitatory effect of riluzole on glutamate-induced Ca^{2+} response, suggesting that glutamate transporters and the reverse mode of Na^+/Ca^{2+} exchange are involved in riluzole's effect. Since riluzole did not affect the basal intracellular Ca^{2+} level in the current study, it is unlikely that riluzole directly reverse Na^+/Ca^{2+} exchange to induce intracellular Ca^{2+} response. These results suggest that activation of glutamate transporters by riluzole can facilitate glutamate-induced glutamate release from cultured astrocytes.

In addition to activation of glutamate transporters (Frizzo et al., 2004; Fumagalli et al., 2008), high concentrations of riluzole (>10 μM) inhibit voltage-dependent sodium and Ca^{2+} channels (Lamanauskas and Nistri, 2008) and glutamate receptors (De Sarro et al., 2000). All of these effects of riluzole would induce inhibition rather than activation in astrocytes and neurons, and would support *in vivo* observations that systemic administered riluzole reduced extracellular glutamate concentration in the spinal cord and some brain regions in rodents (Coderre et al., 2007; Irifune et al., 2007; Takahashi et al., 2011). However, we have

recently demonstrated in rats that riluzole increased glutamate signaling in the locus coeruleus (Hayashida et al., 2010) and we observed facilitation rather than inhibition of glutamate-induced glutamate release by riluzole (1 μM) in the current study. Although the current study added EGF in the culture medium to maintain expression of GLT-1 and GLAST in astrocytes (Zelenaia et al., 2000), we recognize that astrocytes become reactive in the culture condition (Kimelberg et al., 2000) and differ from those *in vivo*. Further study is required to determine whether effects of riluzole on glutamate regulation in astrocytes differ between the locus coeruleus and spinal cord *in vivo*.

4.2. Novel target of gabapentinoid action in astrocytes

Gabapentin has a high affinity for $\alpha_2\delta$ subunits of voltage-gated Ca^{2+} channels, which modulate the release of excitatory neurotransmitters (Gee et al., 1996). Peripheral nerve injury in rats induces up-regulation of $\alpha_2\delta$ subunits in the spinal cord (Luo et al., 2002) and gabapentin shows analgesic effects in transgenic mice with up-regulated $\alpha_2\delta$ subunits but not in normal mice (Li et al., 2006). Although acute inhibition of Ca^{2+} currents by gabapentin is either very minor or absent (Davies et al., 2007), it does inhibit trafficking of voltage-gated Ca^{2+} channels to the cell membrane by binding to $\alpha_2\delta$ subunits (Heblich et al., 2008; Hendrich et al., 2008). These previous results suggest that gabapentin relies on $\alpha_2\delta$ subunits to reduce neuronal excitation. Interestingly, however, some $\alpha_2\delta$ subunit ligands including ABHCA fail to produce behavioral analgesia (Lynch et al., 2006), indicating additional mechanisms, other than $\alpha_2\delta$ interactions, would contribute to the analgesic efficacy of gabapentin.

The current study demonstrates that gabapentin, like riluzole, increases glutamate uptake via TBOA-sensitive glutamate transporters, enhances the glutamate-induced intracellular Ca^{2+} response via the reverse mode of Na^+/Ca^{2+} exchange, and by this mechanism facilitates glutamate release in cultured astrocytes. On the other hand, co-application of gabapentin with glutamate failed to affect glutamate uptake in astrocytes in the current experiment and previous study (Su et al., 1995). These results suggest that gabapentin activates glutamate transporters in astrocytes via direct or indirect interaction, but not via co-transport with glutamate. Since there is no evidence for the presence of $\alpha_2\delta$ subunits in astrocytes, $\alpha_2\delta$ interactions are unlikely to contribute to gabapentin effects in astrocytes. This is further supported by the observation that some $\alpha_2\delta$ ligands, pregabalin and L-isoleucine, enhance glutamate-induced intracellular Ca^{2+} response, while another (ABHCA) did not, despite similar $\alpha_2\delta$ binding affinity of ABHCA as gabapentin (Lynch et al., 2006). In some neurons, pertussis toxin-sensitive G-protein pathways are critical for action of gabapentinoids on ion channels (Bertrand et al., 2003; McClelland et al., 2004). In astrocytes, pertussis toxin itself increases glutamate uptake but reduces noradrenaline-induced facilitation of glutamate uptake in astrocytes (Fahrig, 1993). Further studies are required to examine whether second messenger pathways, such as G-protein pathways, are involved in gabapentin's action in astrocytes.

Systemically administered gabapentin reduces extracellular glutamate concentration in the spinal cord in rats after peripheral nerve injury (Coderre et al., 2007), likely due to the reduction of excitatory neurotransmitters release via $\alpha_2\delta$ interactions (Gee et al., 1996; Li et al., 2006; Luo et al., 2002). However, we previously demonstrated in rats after peripheral nerve injury that systemically administered gabapentin activated locus coeruleus neurons, via glutamate-mediated mechanisms, to induce spinal noradrenalin release and that the anti-hypersensitivity effect of intra- locus coeruleus gabapentin was blocked by intra- locus coeruleus AMPA receptor antagonist, indicating an obligatory role for glutamate signaling in gabapentin's effect in the locus coeruleus (Hayashida et al., 2008), consistent with the current results in astrocytes. The clinical relevance of these laboratory observations was demonstrated in patients with chronic pain that oral administration of gabapentin, in a dose

that produces postoperative analgesia, increased noradrenaline concentrations in cerebrospinal fluid (Hayashida et al., 2007). The current study demonstrates that gabapentinoid directly acts on glutamate regulation in astrocytes and the role of this novel target of gabapentinoid action should be tested *in vivo*.

5. Conclusion

The present study demonstrates that riluzole and gabapentin activate glutamate transporters to induce subsequent Ca^{2+} influx via the reverse mode of Na^{+}/Ca^{2+} exchange, and thereby facilitate glutamate-induced glutamate release in cultured astrocytes. The present study also demonstrates a novel site of gabapentinoid action in astrocytes other than $\alpha_2\delta$ subunits in neurons. Since gabapentin and pregabalin have been recognized as first line drugs for treatment of various chronic pain conditions, this study provides strong rationale for investigating whether astrocytes are important target of gabapentinoid action to reduce chronic pain.

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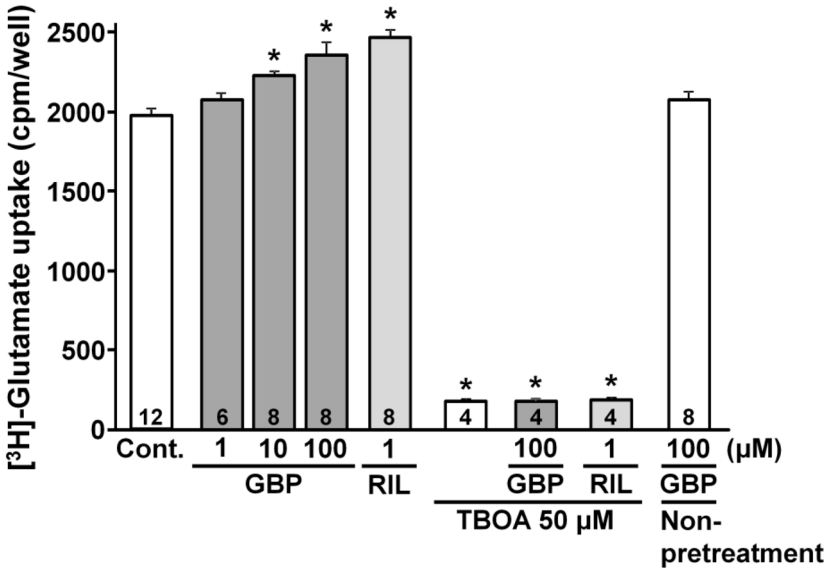

Fig. 1.

[^{3}H]-glutamate uptake in astrocytes is presented as a total radioactivivity (cpm) per well. Cells were pretreated with buffer (control), gabapentin (GBP, 1–100 μM) or riluzole (RIL, 1 μM) in the presence or absence of TBOA (50 μM) for 5 min and glutamate uptake was performed for 1 min in the presence of test drugs. In non-pretreatment group, GBP (100 μM) was co-applied with glutamate without the pretreatment. Number in each column represents sample size. *P<0.01 vs. control.

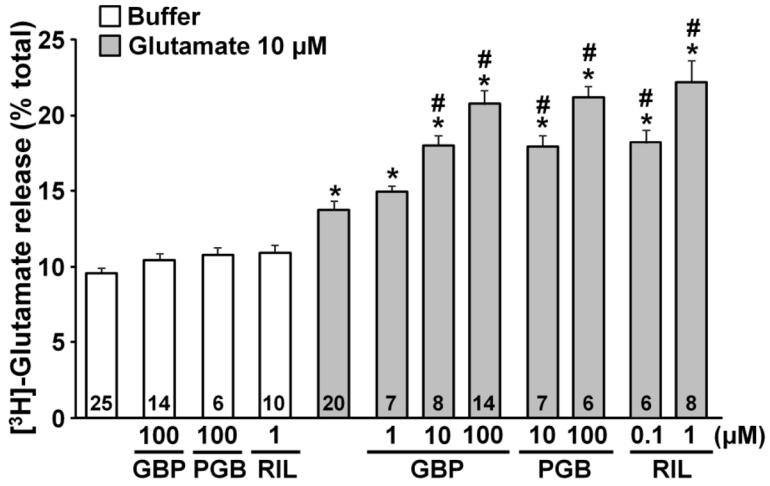

Fig. 2.

Effects of gabapentin, pregabalin and riluzole on glutamate-induced glutamate release from astrocytes. Cells were treated with buffer or glutamate (10 μM) for 5 min in the presence of buffer alone, gabapentin (GBP, 1–100 μM), pregabalin (PGB, 10–100 μM), or riluzole (RIL, 0.1–1 μM). [^{3}H]-glutamate release is presented as a percentage of total radioactivivity. Number in each column represents sample size. *P<0.01 vs. buffer alone. #P<0.01 vs. glutamate alone.

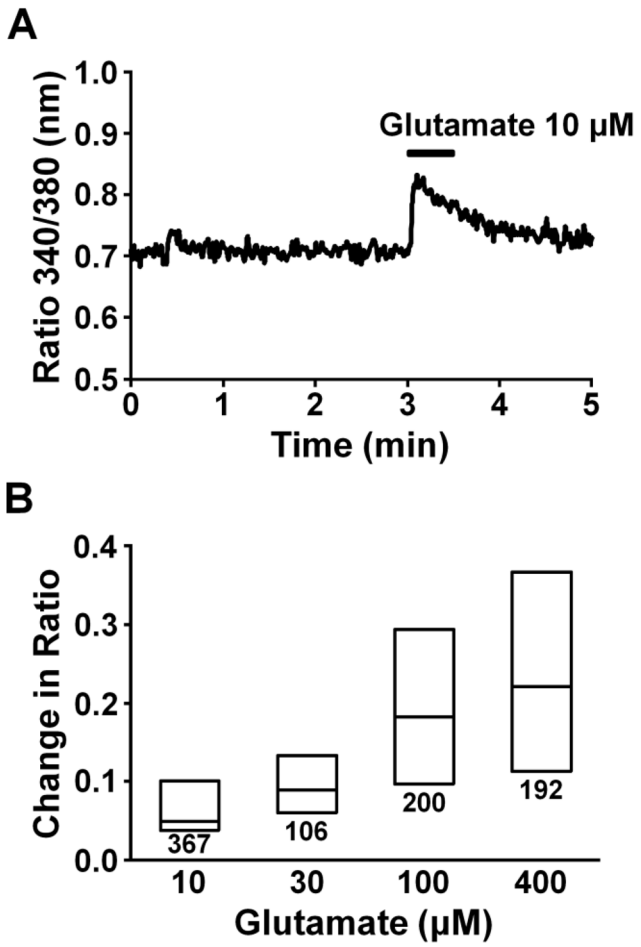

Fig. 3.

(A) A representative intracellular Ca^{2+} response in an astrocyte stimulated with glutamate, presented as change in Fura-2 fluorescence ratio. (B) Concentration response of glutamate (10–400 μM)-induced Fura-2 response. Each box represents the 25^{th}, 50^{th}, and 75^{th} percentiles of Fura-2 fluorescence ratio response. Number under each box represents sample size.

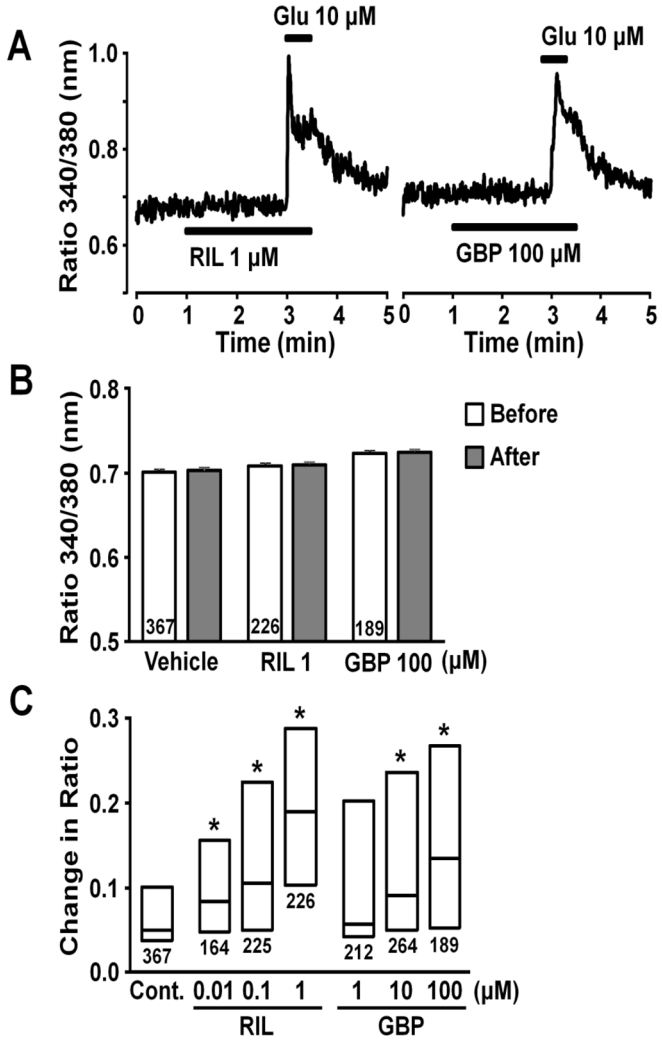

Fig. 4.

(A) Representative intracellular Ca^{2+} responses in astrocytes stimulated with glutamate (Glu) after perfused with gabapentin (GBP) or riluzole (RIL), presented as change in Fura-2 fluorescence ratio. (B) Effects of RIL and GBP on basal Fura-2 fluorescence ratio. Basal values were determined from the average ratio for 1 min before and after starting vehicle, RIL, or GBP perfusion. Number in each column represents sample size. (C) Effects of RIL (0.01–1 μM) and GBP (1–100 μM) on glutamate-induced intracellular Fura-2 response. Each box represents the 25^{th}, 50^{th}, and 75^{th} percentiles of Fura-2 fluorescence ratio response. Number under each box represents sample size. *P<0.05 vs. control (10 μM glutamate alone).

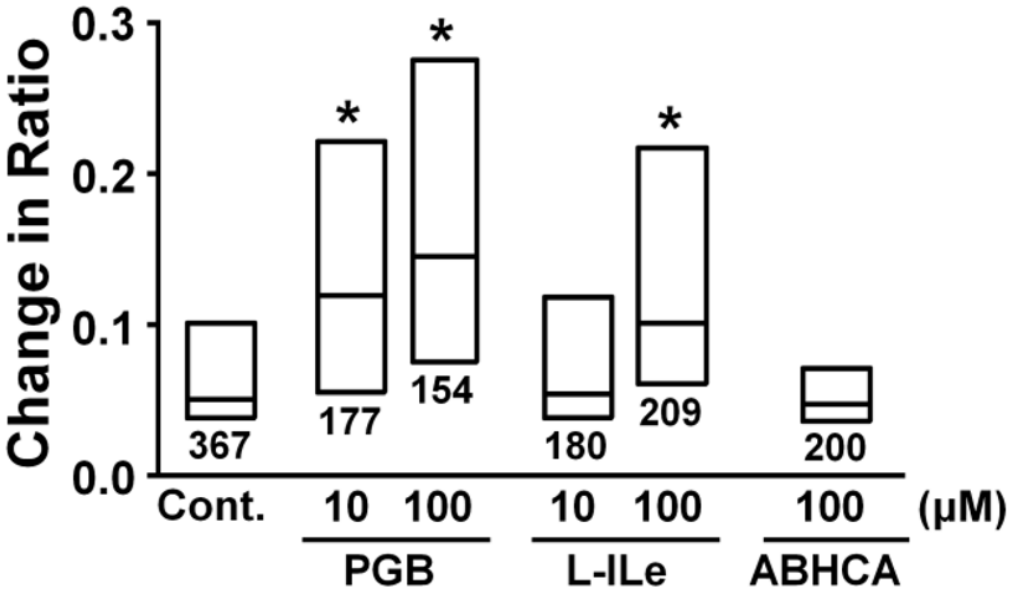

Fig. 5.

Effects of pregabalin (PGB, 10–100 μM), L-isoleucine (L-ILe, 10–100 μM), and ABHCA (100 μM) on glutamate-induced intracellular Fura-2 response. Each box represents the 25th, 50th, and 75th percentiles of Fura-2 fluorescence ratio response. Number under each box represents sample size. *P<0.05 vs. control (10 μM glutamate alone).

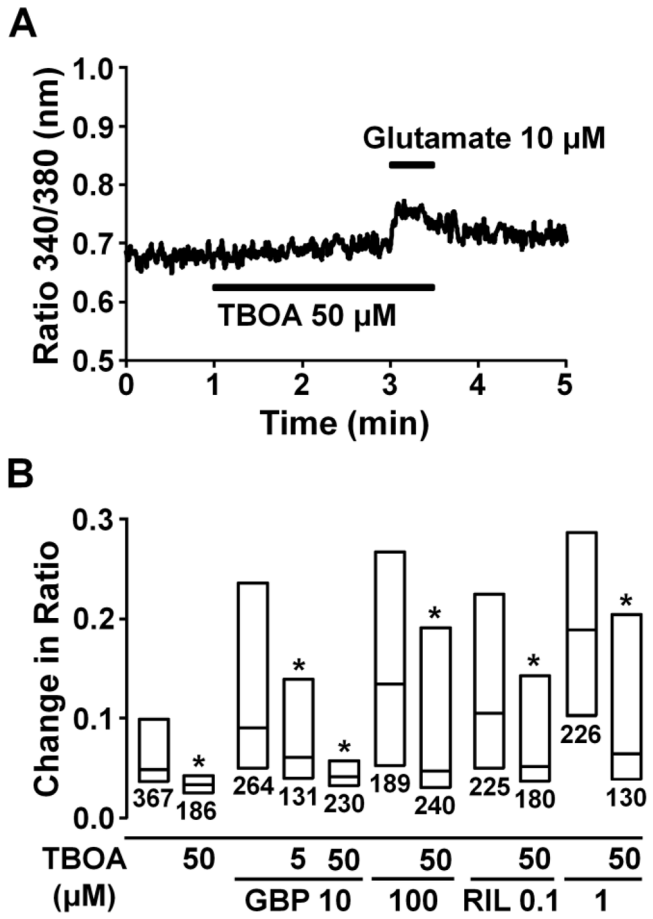

Fig. 6.

(A) A representative intracellular Ca^{2+} concentration response in an astrocyte stimulated with glutamate (10 μM) after perfused with DL-TBOA (50 μM), presented as change in Fura-2 fluorescence ratio. (B) Effects of TBOA (5–50 μM) on gabapentin (GBP, 10 and 100 μM) and riluzole (Ril, 0.1 and 1 μM)-induced enhancement of glutamate-induced intracellular Fura-2 fluorescence ratio response. Each box represents the 25^{th}, 50^{th}, and 75^{th} percentiles of Fura-2 fluorescence ratio response in astrocytes. Number under each box represents sample size. *P<0.05 vs. without TBOA.

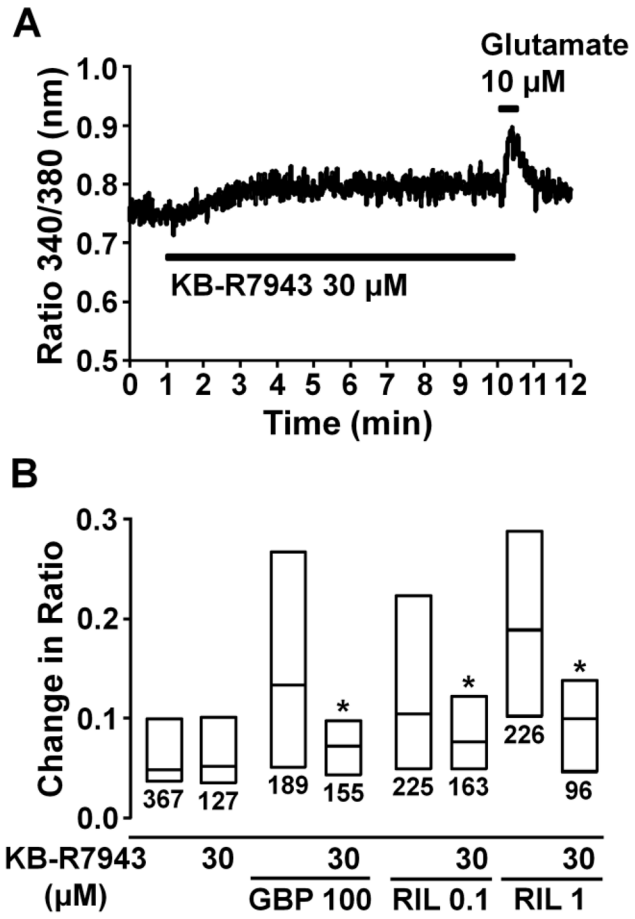

Fig. 7.

(A) A representative intracellular Ca^{2+} concentration response in an astrocyte stimulated with glutamate (10 μM) after perfusion with KB-R7943 (30 μM), presented as change in Fura-2 fluorescence ratio. (B) Effects of KB-R7943 (30 μM) on gabapentin (GBP, 100 μM) and riluzole (Ril, 0.1–1 μM)-induced enhancement of glutamate-induced intracellular Fura-2 fluorescence ratio response. Each box represents the 25^{th}, 50^{th}, and 75^{th} percentiles of Fura-2 fluorescence ratio response. Number under each box represents sample size. *P<0.05 vs. without KB-R7943.