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Brivaracetam does not modulate ionotropic glutamate-, GABA- and glycine-activated channels in hippocampal neurons

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Abstract

Brivaracetam (BRV) is a selective, high affinity ligand for synaptic vesicle protein 2A (SV2A), recently approved as adjunctive treatment for drug refractory partial-onset seizures in adults. BRV binds SV2A with higher affinity than levetiracetam (LEV), and was shown to have a differential interaction with SV2A . Since LEV was reported to interact with multiple excitatory and inhibitory ligand-gated ion channels and that potentially may impact its pharmacological profile, we were interested in determining whether BRV directly modulates inhibitory and excitatory ionotropic receptors in central neurons. Voltage-clamp experiments were performed in primary cultures of mouse hippocampal neurons. At a supratherapeutic concentration of 100µM, BRV was devoid of any direct effect on GABA-A-, glycine-, kainate-, NMDA- and AMPA-gated currents. Similar to LEV, BRV reveals a potent ability to oppose the action of negative modulators on the inhibitory receptors. In conclusion, these results show that BRV contrasts with LEV by not displaying any direct action on inhibitory or excitatory post-synaptic ligand-gated receptors at therapeutic concentrations and thereby supports ~~ that~~ BRV's role as ~~is~~ a selective SV2A ligand. ~~This~~ These findings add~~s~~ further evidence to the validity of SV2A as a relevant antiepileptic drug target and emphasize the potential for exploring further presynaptic mechanisms as a novel approach to antiepileptic drug discovery."

Key words: brivaracetam , GABA, glycine, NMDA, AMPA, kainate

Introduction

Brivaracetam (BRV, Briviact) is a novel antiepileptic drug (AED) identified through rational drug design, following the discovery of levetiracetam (LEV, Keppra)[1]. It was recently approved as adjunctive therapy in adults with refractory partial-onset seizures. LEV and BRV share binding to the synaptic vesicle protein 2A (SV2A)[2] and both modulate the pre-synaptic release of neurotransmitters leading to reduced neuronal excitability[1]. However, BRV displays a 15-30-fold higher affinity than LEV in binding to the native or recombinant SV2A protein[3] and was recently reported to also reveal a differential interaction to SV2A than LEV[2]. These findings appear to translate into superior potency for BRV in enhancing synaptic short term depression during high frequency stimulation and superior efficacy in reducing vesicle release during low frequency stimulation in excitatory hippocampal neuronal networks[4]. This correlates with previous findings showing BRV to possess a different pharmacological profile than LEV in pre-clinical seizure and epilepsy models consisting of both a more potent and complete seizure suppression[1]. BRV also differentiates from LEV by a faster brain entry and free brain concentrations at therapeutic, oral doses for BRV (Cmax of 14µM, 100mg)[2], much lower than for LEV (Cmax of 422µM; 1500mg)[5]. However, several reports suggest additional mechanisms for LEV that involve inhibition of voltage-gated K^+ and Ca^{2+} channels[1; 6] which may influence the generation of action potentials and pre-synaptic neurotransmitter release. BRV further discriminates from LEV by the lack of activity on major voltage-gated ion channels. An initial study with BRV suggested significant inhibition of voltage-gated Na^+ channels in primary cortical neurons but this effect was not confirmed in adult hippocampal neurons[1]. Likewise, repetitive neuronal firings in

primary cortical neurons and adult hippocampal neurons were not impacted by BRV treatment supporting the hypothesis that inhibition of voltage-gated Na^+ currents does not contribute to the antiepileptic mechanism of BRV[1]. Other studies revealed that BRV did not modulate ionic conductances in neurons including high- and low-voltage activated Ca^{2+} currents, voltage-gated delayed rectifier K^+ currents and persistent voltage-gated Na^+ current[7]. The understanding of the molecular mechanisms of LEV has also been extended to study impact on inhibitory post-synaptic receptors and indicated that LEV experimentally modulates GABA-A and glycine receptor activity by reversing the inhibitory effect of negative allosteric modulators such as beta-carbolines and zinc[8]. At therapeutic concentrations, LEV induced a small reduction in the peak amplitude and a prolongation of the decay phase of GABA-gated currents in hippocampal neurons. LEV did not show any direct effect on glycine-gated currents. Additional studies on excitatory post-synaptic receptors indicated that LEV does not modulate kainate or NMDA induced currents (up to 1 mM) but significantly inhibited AMPA-gated currents (IC50=268 µM)[9]. AMPA receptor modulation produced by 200µM LEV was later confirmed in cultured cortical neurons[10]. The modulation of post-synaptic receptors by LEV prompted this study to explore potential modulation by BRV on inhibitory and excitatory post-synaptic receptors. We analyzed the effects of BRV (10-100µM) on native ligand-gated ion channels in hippocampal neurons, including GABA-A, glycine, kainate, NMDA and AMPA receptors.

Materials and Methods

Hippocampal neurons were obtained from 16-days-old mouse embryos using methods fully described previously [11]. Cells dissociation and suspension were prepared as previously described[8]. The medium was renewed once weekly and the cells were used for electrophysiological recordings after 7-14 days-in vitro (DIV), except for studying the inhibition by DMCM of glycine-induced currents, which is only observed in immature neurons. For recording of membrane currents, the cells were transferred to the stage of an inverted Hoffman contrast microscope and maintained at room temperature (20-25°C) in a recording chamber which is continuously perfused permitting application of drugs. The extracellular perfusion solution contained (mM): NaCl, 116.0; D-glucose, 11.1; KCl, 5.4; $CaCl_2$ $2H_20$, 1.8; $MgCl_2$ $6H_20$, 2.0; HEPES, 10.0; pH 7.2. The test substances diluted in the external solution were applied by a rapid microperfusion system (SPS-8, List-Medical, Germany). Recording electrodes were made from borosilicate glass capillaries (Hilgenberg, Germany) using a Flaming-Brown microelectrode puller (Model P97, Sutter Instruments Co). Recording pipettes (10-20 MΩ) were filled with a solution containing (mM): KCl, 130.0; $CaCl_2$ $2H_2O$, 1.0; D-glucose, 11.1; EGTA, 10.0; Na- and Mg-ATP, 5.0; HEPES, 10.0; pH 7.4. Glutamate, γ-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA), kainic acid, N-methyl-D-aspartate (NMDA), dizocilpine (MK801) and 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX), GABA, strychnine, bicuculline, picrotoxin,were purchased from Sigma (USA). Methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM) was from RBI (USA). Glycine was purchased from UCB (Belgium). Voltage-clamp recordings were performed with a Bio-Logic RK-400 patch-clamp amplifier using the tight-seal whole-cell recording configuration. Series resistances were in the range 10-20 MΩ and were electronically

compensated (up to 85-90%). Current traces were digitized and stored on a personal computer. Control of drug application, data acquisition and data analysis were achieved using an ITC-16 acquisition board (Instrutech Corporation, USA) and the TIDA for Windows software (HEKA Elektronik, Pfölz, Germany). Data analysis and graphing were made using GraphPAD Prism software (USA). Except when stated otherwise, the results are expressed as mean ± SEM. Statistical significance of the data was assessed from a Student's t-test or analysis of variance (ANOVA) followed by Dunnett's multiple comparisons post-tests when significance was reached. In all experiments, differences were considered statistically significant when $P < 0.05$.

Results

Brivaracetam does not modify NMDA-, kainate- or AMPA-gated currents

Cultured hippocampal neurons (7-14 DIV) were used to investigate the effect of BRV on glutamate receptors. When recorded in voltage-clamp mode, only cultured neurons displaying fast inactivating inward currents and delayed rectified currents and no run-down of their glutamate responses were further taken into consideration. NMDA, AMPA and kainate were applied at concentrations near their EC_{50} values (determined in separate experiments as 9 µM for NMDA, 2 µM for AMPA and 340 µM for kainate), together with increasing concentrations of BRV (Figure 1A). Figure 1 shows that up to a concentration of 100 µM, BRV does not influence the currents gated by these different ionotropic GluR agonists.

Brivaracetam does not modify inhibitory amino acid-gated currents in cultured hippocampal neurons

In a previous study also involving hippocampal neurons[8], we reported weak, but significant, modulatory effects of LEV on GABA-induced currents (reduction of peak amplitude from 1µM, increase in decay time constant from 100µM). The effect of- BRV on GABA- and glycine-induced currents was therefore assessed by applying GABA and glycine at their EC50 concentrations (12 µM for GABA and 108 µM for glycine) in the presence of increasing BRV concentrations. We observed that both the apparent kinetics of the GABA and glycine currents (activation and decay) (Figure 2A) and their peak amplitudes are not significantly modified in the presence of BRV from 1-100µM (Figure 2B).

Brivaracetam reverses zinc and DMCM inhibition of inhibitory amino acid-gated currents in cultured hippocampal neurons

The main interaction of LEV with inhibitory amino acid receptors was its ability to reverse the negative allosteric modulation of GABA and glycine responses evoked by zinc and β-carbolines[8]. The present study similarly investigated the effect of -BRV (Figure 2C,D,E,F). Under control conditions the addition of zinc produced an inhibition of GABA and glycine evoked currents of 23% ± 4% and 34% ± 6% respectively. Figure 2C,D shows that BRV, at concentrations from 1-100µM, reverses the inhibition induced by zinc of GABA- and glycine-induced currents. This effect is observed for concentrations of BRV as low as 1 µM. Figure 2E,F shows that the inhibitory effect of DMCM on GABA and glycine responses is also reversed by BRV. This effect was visible from a BRV concentration of 1 µM for GABA responses and 10 µM for the reversal of DMCM effects on glycine responses.

Discussion

In this study, we investigated the functional effects of BRV on neuronal ligand-gated excitatory and inhibitory receptors and demonstrated that BRV does not inhibit NMDA-, AMPA-, kainate-, GABA- and glycine-evoked currents at concentrations up to 100 µM. Previous studies have predicted that the exposure levels for BRV in the therapeutic dose range (50mg bid) should reach ~10µM in the brain of patients[3; 12] suggesting that the highest BRV concentrations used in this study (100µM) represent a supra-therapeutic dose. We therefore conclude that BRV does not modulate these ligand-gated ion channels at therapeutic brain concentrations, a finding ~~in patients~~ which is consistent with ~~support~~s its antiepileptic properties ~~are~~ being mediated by its selective action on the presynaptic SV2A protein.

In contrast to the absence of effects of BRV on direct receptor stimulation, BRV significantly reversed the modulation of GABA and glycine receptors by zinc and DMCM. These effects occurred in a concentration range of 1-10µM confirming a similar pattern, as previously described for LEV[8] although BRV produced these effects at 10-30 fold lower concentrations than LEV. Further research will be needed to address ~~the relevance~~how relevant ~~of~~ such a mechanism is in ~~for~~ contributing to the anticonvulsant properties of AEDs. The lack of effects of BRV on excitatory and inhibitory receptors contrasts with the effects of LEV. Indeed, LEV was shown to reduce the peak amplitudes of GABA currents and to inhibit AMPA currents in a concentration range that is relevant for its therapeutic use[10]. The latter mechanism is shared with topiramate and perampanel and coincides with these three AEDs being associated with significant induction of neuropsychiatric adverse effects that limits their clinical use in epilepsy patients[13] . This supports the hypothesis that the behavioral adverse effects, observed in a sub-group of patients exposed to LEV[14] may, at least partly, derive from its- inhibition of AMPA

receptors. The lack of effects of BRV on AMPA receptors may therefore confer it a lower potential for induction of behavioral adverse effects in ~~epileptic patients~~patients with epilepsy. The latter seems supported by a preliminary clinical study[15] that reported a major benefit of switching LEV treated patients with behavioral adverse effects to BRV treatment. It remains to be determined if this mechanistic hypothesis can provide a causal reason for induction of behavioral adverse effects. In conclusion, LEV and BRV show a distinct preclinical profile[1]. From a mechanistic perspective, this may reflect that BRV differs from LEV by higher affinity and a distinct interaction with SV2A contrasted by absence of other significant target interactions. The latter is supported by the findings of this study which also provide a mechanistic rationale for expecting high tolerability and low incidence of behavioral adverse effects with BRV in ~~epileptic patients~~patients with epilepsy. Further elucidation of presynaptic mechanism~~s~~ dysfunctions may provide new insights into the pathophysiological mechanisms of epileptic disorders.

Conflicts of interest

GM and J-MR have served as paid consultants for UCB. All other authors are employed by UCB.

Disclosure

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Legends of figures

Figure 1

Effect of BRV on glutamate-induced currents. **A**: 7-14 days in vitro (DIV) hippocampal neurons were perfused for 10 s with AMPA (3 µM), kainate (100 µM) or NMDA (50 µM) as indicated, alone or in combination with BRV (100µM). **B**: Currents evoked by 3 µM AMPA, 100 µM kainate or 50 µM NMDA (concentrations which elicit a half-maximal response) were recorded in the presence of increasing BRV concentrations. Results are expressed as percentage of excitatory amino acid-induced currents peak amplitudes in the absence of BRV (mean ± SEM)~~, n = 4-5 for AMPA, 6-8 for kainate and 2-9 for NMDA)~~. Numbers of recorded neurons are indicated in graph bars. ANOVA followed by Dunnett's multiple comparison post-tests yielded not significant effect up to 100 µM BRV.

Figure 2

Effect of BRV on GABA- and glycine-induced currents and interaction between BRV and zinc or DMCM inhibition of GABA and glycine responses. **A**: 7-14 days in vitro (DIV) hippocampal neurons were perfused for 10 s with GABA (20 µM) or glycine (100 µM) as indicated, alone or in combination with BRV (100µM). **B**: Currents evoked by 20 µM GABA or 100 µM glycine (concentrations which elicit a half-maximal response) were recorded in the presence of increasing BRV concentrations. Results are expressed as percentage of inhibitory amino acid-induced currents peak amplitudes in the absence of BRV (mean ± SEM)~~., n = 3-5 for GABA and 3-13 for glycine)~~. Numbers of recorded neurons are indicated in graph bars. ANOVA followed by Dunnett's multiple comparison post-testes yielded not significant effect up to 100 µM BRV. **C**: 7-14 DIV hippocampal neurons were perfused for 10 s with 20 µM GABA or 100 µM glycine alone (left traces) or in combination with BRV (right traces) (25 s, 30 µM) and zinc (25 s, half-maximal inhibitory concentrations: 15 µM for GABA-induced currents and 500 µM for glycine-gated currents). **D**: Currents elicited by 20 µM GABA or 100 µM glycine in the presence of 15 µM or 500 µM zinc (for GABA and glycine, respectively) were recorded for increasing BRV concentrations. Results are expressed as percentage of GABA- and glycine-induced current peak amplitudes in the absence of any drug (mean ± SEM). Numbers of recorded neurons are indicated in graph bars.~~, n = 4-36 for GABA and 4-35 for glycine).~~ **E**: 7-14 DIV (for GABA) and 2-4 DIV (for glycine) hippocampal neurons were perfused for 10 s with 20 µM GABA or 100 µM glycine alone (left traces) or in combination with BRV (right traces) (25 s, 30 µM) and DMCM (25 s, half-maximal inhibitory concentrations: 15 µM for GABA-induced currents and 15 µM for glycine-gated currents). **F**: Currents elicited by 20 µM GABA or 100 µM glycine in the presence of 15 µM DMCM were recorded for increasing BRV concentrations. Results are expressed as percentage of GABA- and glycine-induced current peak amplitudes in the absence of any drug (mean ± SEM)~~, n = 6-32 for GABA and 2-18 for glycine)~~. Numbers of recorded neurons are indicated in graph bars. ** $P < 0.01$ and * $P < 0.05$ using ANOVA followed by Dunnett's multiple comparison post-tests.

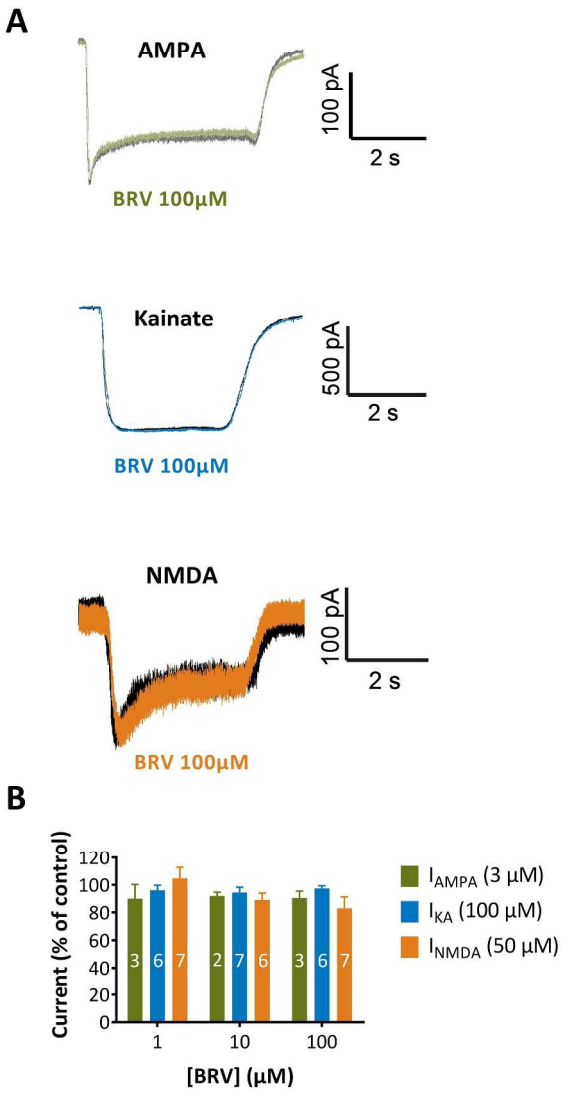

Fig. 1

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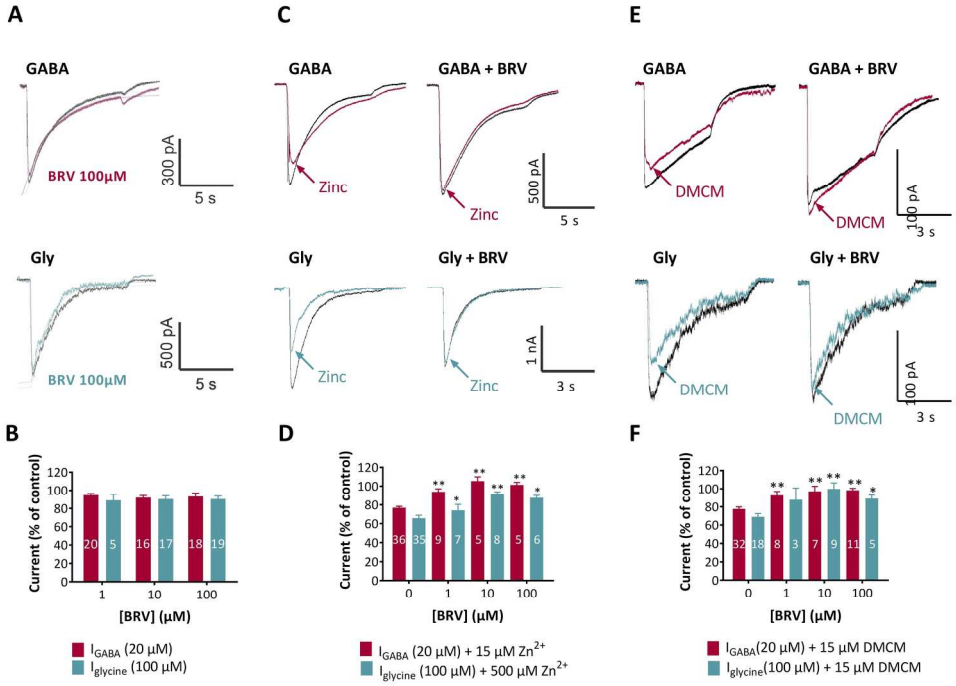

Fig. 2

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