Article

The Integrated Effects of Brivaracetam, a Selective Analog of Levetiracetam, on Ionic Currents and Neuronal Excitability

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Abstract: Brivaracetam (BRV) is recognized as a novel third-generation antiepileptic drug approved for the treatment of epilepsy. Emerging evidence has demonstrated that it has potentially better efficacy and tolerability than its analog, Levetiracetam (LEV). This, however, cannot be explained by their common synaptic vesicle-binding mechanism. Whether BRV can affect different ionic currents and concert these effects to alter neuronal excitability remains unclear. With the aid of patch clamp technology, we found that BRV concentration dependently inhibited the depolarization-induced M-type K+ current (I_K(M)), decreased the delayed-rectifier K+ current (I_K(DR)), and decreased the hyperpolarization-activated cation current in GH3 neurons. However, it had a concentration-dependent inhibition on voltage-gated Na+ current (I_Na). Under an inside-out patch configuration, a bath application of BRV increased the open probability of large-conductance Ca2+-activated K+ channels. Furthermore, in mHippoE-14 hippocampal neurons, the whole-cell I_Na was effectively depressed by BRV. In simulated modeling of hippocampal neurons, BRV was observed to reduce the firing of the action potentials (APs) concurrently with decreases in the AP amplitude. In animal models, BRV ameliorated acute seizures in both OD-1 and lithium-pilocarpine epilepsy models. However, LEV had effects in the latter only. Collectively, our study demonstrated BRV’s multiple ionic mechanism in electrically excitable cells and a potential concerted effect on neuronal excitability and hyperexcitability disorders.

Keywords: brivaracetam; M-type K+ current; voltage-gated Na+ current; large-conductance Ca2+-activated K+ channel; neuron; seizure

1. Introduction

Brivaracetam (BRV; Brivact®, Brivlera®, UCB34714, C11H20N2O2), a chemical analog of levetiracetam (LEV), is an orally or intravenously bioavailable racetam derivative with anticonvulsant (antiepileptic) properties that has appeared in a growing number of research papers [1–33]. Of note, it has also been recognized to be efficacious in the treatment of epilepsy and status epilepticus [9,17,30,34–38].

BRV has also been reported to attenuate pain behavior in a murine model of neuropathic pain [2,39]. BRV was also previously observed to exert anti-neoplastic effects in glioma cells [40]. It has been demonstrated that BRV can interfere with the functional activities of neurons (e.g., hippocampal neurons) or endocrine cells (e.g., pituitary lactotrophs) by binding with high affinity to the synaptic or endocrine vesicle protein 2A (SV2A) [9,15,39,41–45]. SV2A has been recognized as an important broad marker for neuroendocrine cells [41]. It has been reported to be 10–30-fold more potent than LEV, with high efficacy in a wide range of experimental models of focal and generalized seizures. As a potential medication with significantly high SV2A affinity, it is thus important to investigate its mechanism on neuroendocrine cells and hippocampal neurons. Furthermore, BRV has been demonstrated to have higher potency and efficacy than LEV in experimental
models of epilepsy [46]. However, to the best of our knowledge, there is little information available with respect to the effects of BRV on different types of membrane ionic currents in excitatory endocrine cells or neurons or on neuronal excitability, although a previous study showed the ability of BRV to alter the magnitude of voltage-gated Na⁺ current or M-type K⁺ current residing in neurons [42,47]. Furthermore, clinical reports have demonstrated that BRV may be a useful treatment option in patients who have previously failed to respond to or tolerate LEV [48], and it has been reported that BRV has better clinical efficacy and tolerability than LEV [26,28] that cannot be explained simply by the common synaptic vesicle binding property mechanism.

In light of these considerations, we attempted to characterize the effects of BRV on membrane ionic currents (e.g., M-type K⁺ current (I_{K(M)}), a delayed-rectifier K⁺ current (I_{K(DR)}), hyperpolarization-activated cation current (I_h), a voltage-gated Na⁺ current (I_{Na}), and a large-conductance Ca²⁺-activated K⁺ channel (BKCa)) existing in neuroendocrine and hippocampal neurons in order to investigate the integrated effects on neuronal excitability and hyperexcitability using simulation modeling and different animal models of epilepsy.

2. Materials and Methods

2.1. Chemicals, Drugs, and Solutions Used in This Study

Brivaracetam (BRV, Brivact®, Brivlera®, UCB34714, ((2S)-2-[(4R)-2-oxo-4-propylpyrrolidin-1-yl]butanamide, C_{11}H_{20}N_{2}O_{2}, https://pubchem.ncbi.nlm.nih.gov/compound/Brivaracetam, accessed on 25 March 2021) and LEV (Keppra®, (S)-2-(2-oxopyrrolidin-1-yl)butanamide) were kindly provided by UCB (Union Chimique Belge) Pharma (PRA Health Sciences, Taipei, Taiwan). GAL-021 and PF1022A were acquired from MedChemExpress (Everything Biotech, New Taipei City, Taiwan); cilobradine was obtained from Cayman (Excel Biomedical, Taipei, Taiwan); 2-chloro-α,α-diphenylbenzeneacetonitrile (TRAM39) was obtained from Tocris (Union Biomed Inc., Taipei, Taiwan), and tefluthrin, tetraethylammonium chloride and tetrodotoxin were obtained from Sigma-Aldrich (Merck Ltd., Taipei, Taiwan). Unless specified otherwise, culture media, fetal bovine serum, horse serum, L-glutamine, penicillin-streptomycin and trypsin/EDTA were obtained from HyClone™ (Thermo Fisher Scientific; Level Biotech, Tainan, Taiwan), while all other chemicals, including CdCl₂, CsCl, CsOH, EGTA and HEPES, were of laboratory grade and obtained from standard sources. We used twice-distilled water that had been deionized through a Millipore-Q system (Merck, Ltd., Taipei, Taiwan) in all experiments.

The ionic composition of the bath solution (i.e., HEPES-buffered normal Tyrode’s solution) was: NaCl 136.5 mM, KCl 5.4 mM, CaCl₂ 1.8 mM, MgCl₂ 0.53 mM, glucose 5.5 mM, and HEPES 5.5 mM adjusted to pH 7.4 with NaOH. To measure the macroscopic K⁺ currents (e.g., I_{K(M)} or I_{K(DR)}), we filled the recording electrode with a solution: K-aspartate 130 mM, KCl 20 mM, MgCl₂ 1 mM, Na₂ATP 3 mM, Na₂GTP 0.1 mM, EGTA 0.1 mM, and HEPES 5 mM adjusted to pH 7.2 with KOH. To study the BKCa-channel activity measured under an inside-out configuration, the bath solution contained a high K⁺ solution: KCl 130 mM, NaCl 10 mM, MgCl₂ 3 mM, glucose 6 mM, and HEPES 10 mM titrated to 7.4 with KOH, while the pipette solution contained KCl 145 mM, MgCl₂ 2 mM, and HEPES 5 mM titrated to 7.2 with KOH. The value of the free Ca²⁺ concentration was estimated in this study, assuming that there was a dissociation constant of 0.1 µM for EGTA and Ca²⁺ (at pH 7.2). For example, to provide 0.1 µM Ca²⁺ in the bath solution, 1 mM EGTA and 0.5 mM CaCl₂ were added. In this study, we commonly filtered the pipette solutions and culture media with an Acrodisc® syringe filter with a 0.2 µm Super® membrane (Bio-Check Lab., Pall Corp., Taipei, Taiwan).

2.2. Cell Culture

The pituitary adenomatous cell line, GH3, was acquired from the Bioresource Collection and Research Center (BCRC-60015, http://catalog.brcrc.firdi.org.tw/BrcrcContent?bid=60015 (accessed on 26 March 2021), Hsinchu, Taiwan) [49], and the embryonic mouse hippocampal cell line, mHippoE-14, was obtained from Cedarlane CELLutions Biosystems.
Inc. (Hycell International Co., Taipei, Taiwan) [50,51]. The GH3 cells were maintained in Ham’s F-12 medium supplemented with 2.5% fetal bovine serum (v/v), 15% horse serum (v/v), and 2 mM L-glutamine, and the mHippoE-14 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (v/v) and 2 mM L-glutamine. The cells were grown as a monolayer culture in a humidified environment of 5% CO2/95% air at 37 °C. In order to be well differentiated, the GH3 cells were transferred to serum- and Ca2+-free mediums. Electrical recordings were performed 5 or 6 days after the cells had been cultured to 60–80% confluence.

2.3. Electrophysiological Measurements

On the day of the experiments, the cells were dispersed with a 1% trypsin/EDTA solution, and a few drops of cell suspension were quickly transferred to a custom-built recording chamber mounted on the stage of an inverted DM-II microscope (Leica; Major Instruments, Kaohsiung, Taiwan). They were immersed at room temperature (20–25 °C) in normal Tyrode’s solution, the composition of which was provided above. We recorded different types of ionic currents in the whole-cell, cell-attached, or inside-out mode of a standard patch-clamp technique with dynamic adaptive suction (i.e., a decremental change in the suction pressure in response to a progressive increase in the seal resistance), with the aid of either an Axoclamp-2B (Molecular Devices, Sunnyvale, CA, USA) or an RK-400 amplifier (Bio-Logic, Clai, France). Consistent with previous observations [52], the formation of a bleb of membrane lipid in the electrode tip based on microscopic observation of giga-Ω seal formation was observed in our study. The microelectrodes used were prepared from Kimax-51 borosilicate capillaries with a 1.5 mm outer diameter (#34500; Kimble; Dogger, New Taipei City, Taiwan) by using a PP-83 vertical puller (Narishige; Taiwan Instrument, Taipei, Taiwan). Their tip resistance ranged between 3 and 5 MΩ, and they were filled with the various internal solutions described above. During the measurements, the recorded area on the vibration-free table was shielded using a Faraday cage (Scitech, Seoul, Korea). The liquid–liquid junction potential, which commonly appears when the composition of the pipette solution is different from that in the bath, was corrected before the seal formation. The main rationale for the concentrations applied is to use the level which could be clinically achievable and of therapeutic relevance.

2.4. Data Recordings

The signals, comprising potential and current tracings, were monitored on an HM-507 oscilloscope (Hameg, East Meadow, NY, USA) and digitally stored online at 10 kHz in an ASUS VivoBook Flip 14 laptop computer (TP412FA-0131A0210U; ASUS, Tainan, Taiwan) equipped with a 12-bit resolution Digidata 1440A interface (Molecular Devices). During the measurements with either analog-to-digital or digital-to-analog conversion, the latter device was controlled using pCLAMP v.10.7 software (Molecular Devices) run on Microsoft Windows 10 (Redmond, WA, USA). A laptop computer was put on the top of an adjustable Cookskin stand (Ningbo, Zheijiang, China) to allow efficient manipulation during the recordings.

2.5. Data Analyses

To assess the percentage inhibition of BRV on the I\textsubscript{K(M)} amplitude, each cell was voltage-clamped at −50 mV, and a 1-sec depolarizing pulse to −10 mV was applied. The examined cell was briefly depolarized from −80 to −10 mV. The I\textsubscript{K(M)} and I\textsubscript{Na} amplitudes measured at the beginning or end of the depolarizing pulses in the presence of various concentrations of BRV were compared with the control value (i.e., BRV was not present). The concentration-response data for BRV-induced inhibition of I\textsubscript{K(M)} or peak I\textsubscript{Na} were well fitted with the modified Hill equation (i.e., the 3-parameter logistic equation):

\[
\text{Percentage inhibition (\%)} = \frac{E_{\text{max}} \times (BRV)^{nH}}{IC_{50}^{nH} + (BRV)^{nH}}
\]
where (BRV) represents the BRV concentration; IC\textsubscript{50} and n\textsubscript{H} are the concentrations required for a 50% inhibition and Hill coefficient, respectively, and E\textsubscript{max} represents the BRV-mediated maximal block of I\textsubscript{K(M)} or the peak I\textsubscript{Na}.

To assess the steady-state inactivation curve of I\textsubscript{Na} measured with or without the addition of BRV, we employed a two-step voltage profile. The relationship between the normalized amplitude of peak I\textsubscript{Na} and the different conditioning potentials was least-squares fitted to a Boltzmann function in the following form:

\[
\frac{I}{I_{\text{max}}} = \frac{1}{1 + \exp\left\{\frac{(V-V_{1/2})qF}{RT}\right\}}
\]  
(2)

where V is the conditioning potential; V\textsubscript{1/2} the potential at which a half-maximal decrease occurs; I\textsubscript{max} is the maximal amplitude of I\textsubscript{Na}; q is the apparent gating charge in the inactivation curve of the current (i.e., the charge across the membrane’s electrical field between the closed and open conformations); F is the Faraday constant; R is the universal gas constant; T is the absolute temperature, and RT/F = 25.2 mV.

2.6. Single-Channel Analyses

The unitary current events of the digitized BK\textsubscript{Ca} channels were assessed using a pCLAMP v.10.7 (Molecular Devices). Multi-Gaussian adjustments of the amplitude distributions occurring among the channel events were applied to determine the opening channel event. The functional independence among the channels was validated as the observed stationary probabilities were compared. The open-state probabilities were evaluated using an iterative process to minimize the \(X^2\) value calculated from a sufficient number of independent observations.

2.7. Simulation Modeling

To evaluate how BRV could adjust the firing of action potentials (APs), a theoretical model was adapted from a previous work [53]. The model is based largely on the biophysical properties of hippocampal CA1 pyramidal neurons and consists of the delayed-rectifier K\textsuperscript{+} current, the M-type K\textsuperscript{+} current, the transient K\textsuperscript{+} current, the Ca\textsuperscript{2+}-activated K\textsuperscript{+} current, the Na\textsuperscript{+} current, and the Ca\textsuperscript{2+} current. A hyperpolarization-activated cation current adapted from a previous work [54] was also included in the model. The conductance values used to solve the set of differential equations are listed in Table 1.

| Symbol | Description | Value (in Control) | Value (in the Presence of 10 \(\mu\)M BRV) |
|--------|-------------|--------------------|----------------------------------------|
| C\textsubscript{m} | Membrane capacitance (pF) | 1 | 1 |
| g\textsubscript{Na} | Na\textsuperscript{+} current conductance (mS/cm\textsuperscript{2}) | 35 | 17.5 |
| g\textsubscript{Ca} | Ca\textsuperscript{2+} current conductance (mS/cm\textsuperscript{2}) | 0.08 | 0.08 |
| g\textsubscript{KDR} | Delayed-rectifier K\textsuperscript{+} current conductance (mS/cm\textsuperscript{2}) | 6.0 | 5.4 |
| g\textsubscript{A} | A-type K\textsuperscript{+} current conductance (mS/cm\textsuperscript{2}) | 1.4 | 1.4 |
| g\textsubscript{M} | M-type K\textsuperscript{+} current conductance (mS/cm\textsuperscript{2}) | 1 | 0.7 |
| g\textsubscript{KCa} | Ca\textsuperscript{2+}-activated K\textsuperscript{+} current conductance (mS/cm\textsuperscript{2}) | 10 | 20 |
| g\textsubscript{h} | Hyperpolarization-activated cation current conductance (mS/cm\textsuperscript{2}) | 0.4 | 0.36 |

2.8. Animal Experiments

All experiments, including the animal procedures, were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) (Approval No: 110147, Date: 20 February 2021) at National Cheng Kung University, Tainan, Taiwan. Adult Sprague-Dawley male rats weighing 180 to 200 g were purchased from National Cheng Kung University. They were housed in the university’s Animal Center and allowed free access to water and a
pelleted rodent diet (Richmond Standard; PMI Feeds, St Louis, MO, USA). All efforts were made to minimize the number of rats used. The animals were divided into two groups (OD-1 and lithium-pilocarpine). We previously characterized the OD-1 group and its effect as a novel animal model [54].

The OD-1 group was stereotactically injected with 5 ng OD-1 (12 mouse LD50) in the hippocampus (coordinates 4.1 mm caudal, 3.9 mm lateral to the bregma and 3.8 mm below the cortical surface) under general anesthesia (zoletil, 0.1 mL/100 g, intraperitoneal injection (ip)) and analgesia (nalbuphine, 5 mg/kg, ip). After recovery from anesthesia (24 h after injection), the OD-1 group was then divided into three groups given normal saline (NS, 0.1 mL/100g, an intraperitoneal injection (ip)), LEV (500 mg/kg, ip), or BRV (100 mg/kg, ip). The acute seizures were carefully monitored and scored in the following eight hours.

The rats in the pilocarpine group were divided into three groups given NS (0.1 mL/100 g, ip), LEV (500 mg/kg, ip), or BRV (100 mg/kg, ip) 20 min before the pilocarpine injection. The lithium-pilocarpine group was injected with lithium chloride (3 meq/kg; ip) and methylscopolamine (25 mg/kg; subcutaneous (sc)) before they were subjected to pilocarpine (60 mg/kg; subcutaneous injection (sc))-induced seizures.

In both of the OD-1 and lithium-pilocarpine groups, the seizure characteristics of the rats during acute seizures were similar to those reported elsewhere [51,55,56]. The seizures were scored using the Racine scale [56]. The rats were given zoletil (50 mg/kg, ip), xylazine (20 mg, ip), and atropine (0.2 mg/kg, sc) to diminish the seizures if their status epilepticus lasted for 20 min [51,55–58]. Mortality was calculated during the first 24 h after seizure onset. All rats were continuously monitored for the first 24 h after they achieved status epilepticus by two experienced research assistants. The rats were given supportive care: body temperature maintenance with a resistive heating system, food, and adequate hydration with normal saline (0.9% w/v of NaCl, 308 mOsm/L). Any animals showing intense signs of acute respiratory distress were immediately euthanized by overdosing with sodium pentobarbital (150 mg/kg, ip).

In OD-1 model, we evaluated the parameter severe seizures as an indicator of higher neuronal excitotoxicity. In pilocarpine model, the latency to acute stage 3 seizures, number of rats with severe seizures and acute mortality were used to evaluate the neuronal excitotoxicity.

2.9. Statistical Analyses

Linear or nonlinear curve-fitting (e.g., exponential curve or sigmoidal Hill and Boltzmann equations) to any given data sets was undertaken, with the goodness of fit assessed using either Microsoft Excel® v.2016 (Redmond, WA, USA) or OriginPro v.2016 (OriginLab; Schmidt, Taipei, Taiwan). Values are provided as means ± SEM with sample sizes (n), which indicate the number of GH3 cells or mHippoE-14 neurons from which the experimental data were collected. The Student’s t-test and a one-way analysis of variance (ANOVA) followed by post-hoc Fisher’s least-significant difference test for multiple comparisons were performed. However, the data were examined using the nonparametric Kruskal–Wallis test, subject to possible violations in the normality underlying the ANOVA. Differences were considered statistically significant when the p-value was less than 0.05.

3. Results

3.1. Inhibitory Effect of BRV on the Amplitude of M-Type K+ Current (I\textsubscript{K(M)})

The initial stage of the experiments was undertaken to evaluate the effect of BRV on the \textsubscript{I(KM)} inherently existing in the GH3 cells. We bathed the cells in a high-K\textsuperscript{+}, Ca\textsuperscript{2+}-free solution, and, during the recordings, we backfilled the pipette using a K\textsuperscript{+}-containing solution comprising: K-aspartate 130 mM, KCl 20 mM, MgCl\textsubscript{2} 1 mM, Na\textsubscript{2}ATP 3 mM, Na\textsubscript{2}GTP 0.1 mM, EGTA 0.1 mM, and HEPES 5 mM adjusted to pH 7.2 with KOH. When the whole-cell mode was firmly established, we voltage-clamped the cell at −50 mV and thereafter applied a 1 s depolarizing pulse ranging from −50 to −10 mV. As expected, an inward current with a slowly activating time course was evoked. This current has
previously been identified to be \( I_{K(M)} \) \([59–61]\). It was clearly observed that, as the cells were exposed to different concentrations of BRV, the amplitude of \( I_{K(M)} \) progressively decreased (Figure 1A). For example, the addition of 10 \( \mu \)M BRV decreased the \( I_{K(M)} \) amplitude from 78 ± 8 to 42 ± 4 pA \((n = 8, p < 0.05)\). After it was removed, the current amplitude returned to 74 ± 6 pA \((n = 7, p < 0.05)\). The activation time course in the presence of BRV (10 \( \mu \)M) slowed, as evidenced by a significant prolongation in the activation time constant \( (\tau_{act}) \) of the current from 92 ± 11 to 122 ± 14 msec \((n = 8, p < 0.05)\). Moreover, when the differences in the current traces between the absence and presence of 10 or 30 \( \mu \)M BRV were taken, the net change in the membrane currents, i.e., the BRV-sensitive component, was obtained (Figure 1B). These BRV-sensitive inward currents exhibited time-dependent activation and deactivation. Figure 1C shows that cell exposure to BRV can result in a concentration-dependent decrease in the amplitude of \( I_{K(M)} \) elicited in response to 1 s step depolarization. The \( IC_{50} \) value needed for BRV-perturbed inhibition of \( I_{K(M)} \) was estimated to be 6.5 \( \mu \)M. Therefore, BRV is capable of producing a depressant action on depolarization-induced \( I_{K(M)} \).

![Figure 1](image-url)

**Figure 1.** Inhibitory effect of BRV on M-type \( K^+ \) current \( (I_{K(M)}) \) in GH3 cells. In these experiments, cells were immersed in high-\( K^+ \), \( Ca^{2+} \)-free solution, and the recording electrode was filled with the \( K^+ \)-containing solution comprising: K-aspartate 130 mM, KCl 20 mM, MgCl2 1 mM, Na2ATP 3 mM, Na2GTP 0.1 mM, EGTA 0.1 mM, and HEPES 5 mM adjusted to pH 7.2 with KOH; (A) Representative \( I_{K(M)} \) traces evoked by a 1 s depolarizing pulse from −50 to −10 mV (indicated in the upper part of the figure). (a): control (i.e., BRV was not present); (b): 10 \( \mu \)M BRV; (c): 30 \( \mu \)M BRV; (d): washout of BRV. It should be noted that the trajectories of the deactivating currents were partly cut off for the purpose of illustration; (B) BRV-sensitive inward current (a–b (green trace) or a–c (red trace) in panel (A)) (a): BRV-sensitive current at 10 \( \mu \)M BRV; (b): BRV-sensitive current at 30 \( \mu \)M BRV; (C) Concentration-dependent inhibition of BRV on \( I_{K(M)} \) amplitude measured from GH3 cells (mean ± SEM; \( n = 8)\). Current amplitude obtained in the absence and presence of different BRV concentrations (0.3–100 \( \mu \)M) was taken at the endpoint of depolarization pulses ranging from −50 to −10 mV. A smooth, continuous line was obtained by fitting the experimental results to the modified Hill equation (as elaborated in the Materials and Methods section). The vertical broken line points out the \( IC_{50} \) value (i.e., 6.5 \( \mu \)M) needed for BRV-mediated inhibition of \( I_{K(M)} \) in these cells.

### 3.2. Mild Inhibition of BRV on Delayed-Rectifier \( K^+ \) Current \( (I_{K(DR)}) \)

The question as to whether BRV affects other types of \( K^+ \) currents (e.g., \( I_{K(DR)} \)) was raised. Previous reports have shown the ability of LEV to modify the amplitude and gating of \( I_{K(DR)} \) \([62]\). The subsequent experiments were therefore performed to evaluate whether \( I_{K(DR)} \) could be modified in the presence of BRV. To elicit a family of \( I_{K(DR)} \) (Figure 2A), cells
were immersed in Ca\textsuperscript{2+}-free Tyrode’s solution containing 1 \textmu M tetrodotoxin and 0.5 mM CdCl\textsubscript{2}, and the recording electrode was filled with K\textsuperscript{+}-containing solution. During the measurements, the examined cell was maintained at \(-50\) mV, and various voltage steps ranging between \(-60\) and \(+50\) mV at intervals of 10 mV were applied to evoke \(I_{K(DR)}\) \[61,62\]. Under these conditions, BRV at a concentration of 3 \textmu M was not found to have any effect on \(I_{K(DR)}\), as measured throughout the entire voltage-clamp step. For example, as the cells were depolarized from \(-50\) to \(+50\) mV, the \(I_{K(DR)}\) amplitude in the absence and presence of 3 \textmu M BRV did not differ significantly (822 \pm 42 pA (control) vs. 821 \pm 22 pA (the presence of BRV); \(n = 8, p > 0.05\)). However, when the cells were exposed to 10 \textmu M BRV, the amplitude of \(I_{K(DR)}\) slightly, but significantly, decreased (Figure 2A,B). For example, at +50 mV, the current amplitude significantly declined from 834 \pm 49 to 711 \pm 38 pA (\(n = 8, p < 0.05\)) during cell exposure to 10 \textmu M BRV. The current-voltage (I-V) relationship to \(I_{K(DR)}\) collected with or without the addition of 10 \textmu M BRV is illustrated in Figure 2B. It could be observed that, unlike \(I_{K(M)}\), \(I_{K(DR)}\) tends to be less subject to being altered by BRV.

3.3. Mild Inhibitory Effect on Hyperpolarization-Activated Cation Current (\(I_h\)) Caused by BRV

We further examined whether BRV could produce any modifications on hyperpolarization-induced \(I_h\). The experiments were conducted in cells bathed in Ca\textsuperscript{2+}-free Tyrode’s solution containing 1 \textmu M tetrodotoxin, and the recording electrode was filled with a K\textsuperscript{+}-containing solution comprising: K-aspartate 130 mM, KCl 20 mM, MgCl\textsubscript{2} 1 mM, Na\textsubscript{2}ATP 3 mM, Na\textsubscript{2}GTP 0.1 mM, EGTA 0.1 mM, and HEPES 5 mM adjusted to pH 7.2 with KOH. (A) Representative \(I_{K(DR)}\) traces obtained in the absence (upper) and presence of 10 \textmu M BRV. The inset in the upper part of the figure indicates the voltage profile used; (B) Comparison of the mean current-voltage (IV) of \(I_{K(DR)}\) without (○) or with the addition (■) of 10 \textmu M BRV (mean \pm SEM; \(n = 8\)). The current amplitude was measured at the end of each voltage command pulse. It should be noted that BRV (30 \textmu M) mildly inhibited the \(I_{K(DR)}\) amplitude at +50 mV.

![Figure 2. Mild inhibition of BRV on delayed-rectifier K\textsuperscript{+} current (\(I_{K(DR)}\)) in GH\textsubscript{3} cells. In these experiments, the cells were bathed in Ca\textsuperscript{2+}-free Tyrode’s solution containing 1 \textmu M tetrodotoxin and 0.5 mM CdCl\textsubscript{2}, and the recording electrode was filled with a K\textsuperscript{+}-containing solution comprising: K-aspartate 130 mM, KCl 20 mM, MgCl\textsubscript{2} 1 mM, Na\textsubscript{2}ATP 3 mM, Na\textsubscript{2}GTP 0.1 mM, EGTA 0.1 mM, and HEPES 5 mM adjusted to pH 7.2 with KOH. (A) Representative \(I_{K(DR)}\) traces obtained in the absence (upper) and presence of 10 \textmu M BRV. The inset in the upper part of the figure indicates the voltage profile used; (B) Comparison of the mean current-voltage (IV) of \(I_{K(DR)}\) without (○) or with the addition (■) of 10 \textmu M BRV (mean \pm SEM; \(n = 8\)). The current amplitude was measured at the end of each voltage command pulse. It should be noted that BRV (30 \textmu M) mildly inhibited the \(I_{K(DR)}\) amplitude at +50 mV.

3.3. Mild Inhibitory Effect on Hyperpolarization-Activated Cation Current (\(I_h\)) Caused by BRV

We further examined whether BRV could produce any modifications on hyperpolarization-induced \(I_h\). The experiments were conducted in cells bathed in Ca\textsuperscript{2+}-free Tyrode’s solution containing 1 \textmu M tetrodotoxin, and the recording electrode was filled with a K\textsuperscript{+}-containing solution. As depicted in Figure 3A, the 2 s long hyperpolarizing command voltages ranging from \(-40\) to \(-120\) mV could readily evoke an inward current with slowly activating and deactivating time courses in response to such sustained hyperpolarization. This type of ionic current has been previously identified to be \(I_h\) \[49,63,64\]. When the cells were exposed to BRV (3 \textmu M), the \(I_h\) amplitude was unaffected. However, BRV at a concentration of 10 \textmu M resulted in a lessening in the \(I_h\) amplitude from 347 \pm 28 to 313 \pm 24 pA (\(n = 7, p < 0.05\)). Additionally, in the continued presence of 10 \textmu M BRV, subsequent addition of 3 \textmu M cilobradine was observed to lessen the current amplitude further, as demonstrated.
This type of ionic current has been previously identified to be idly depolarized from adjusted to pH 7.2 with CsOH. As shown in Figure 4A, when the examined cell was rapidly depolarized from −80 to −10 mV, INa with both a rapid activation and inactivation time course was robustly evoked. As the cells were exposed to BRV, the peak amplitude of INa in response to a brief depolarizing command voltage progressively declined. For example, BRV (10 µM) resulted in an evident reduction in peak INa from 298 ± 19 to 153 ± 11 pA (n = 8, p < 0.05). After the washout of BRV, the current amplitude returned to 291 ± 17 pA (n = 8, p < 0.05). However, neither activation, inactivation, nor deactivation of the time course of INa in response to brief step depolarization was measurably perturbed in the presence of BRV (3 or 10 µM). µ illustrates the mean I-V relationships of peak INa, in the absence and presence of 3 or 10 µM BRV. It was observed that the overall I-V relationship to peak INa in these cells was not altered during exposure to BRV, in spite of an obvious lessening in peak INa. The concentration-dependent inhibitory effect of BRV on peak INa amplitude was then determined, as illustrated in Figure 4C. According to the modified Hill equation elaborated in the Materials and Methods Section, the IC₅₀ values for BRV-induced inhibition of peak INa measured at the start of the depolarizing command voltage was by a significant reduction in current amplitude to 129 ± 18 pA (n = 7, p < 0.05) (Figure 3B). Cilobradine was previously used to effectively suppress INa [65].

Figure 3. Mild inhibition of BRV on hyperpolarization-activated cationic current (Ih) in GH3 cells. The experiments were conducted in cells immersed in Ca²⁺-free Tyrode’s solution containing 1 µM tetrodotoxin, where the pipette was filled with a K⁺-containing solution comprising K-aspartate 130 mM, KCl 20 mM, MgCl₂ 1 mM, Na₂ATP 3 mM, Na₂GTP 0.1 mM, EGTA 0.1 mM, and HEPES 5 mM adjusted to pH 7.2 with KOH. (A) Representative Ih traces activated by a 2 s hyperpolarizing voltage pulse ranging from −40 to −120 mV (indicated in the upper part of the figure). (1): control, (2): 10 µM BRV, and (3): 10 µM BRV plus 3 µM cilobradine (Cil); (B) Summary bar graph showing the effects of BRV and BRV plus cilobradine (Cil) in hyperpolarization-activated Ih (mean ± SEM; n = 8). The current amplitude was measured at the endpoint of a 2 s hyperpolarizing pulse ranging from −40 to −120 mV. * indicates significantly different from control (p < 0.05) and † indicates significantly different from BRV (10 mM) alone group (p < 0.05).

3.4. Effect of BRV on Voltage-Gated Na⁺ Current (INa)

In the next set of experiments, the INa was examined in response to a short depolarizing command voltage to determine whether BRV could modify it. Cells were bathed in Ca²⁺-free Tyrode’s solution containing 10 mM tetraethylammonium chloride, and the electrode was filled with a Cs⁺-containing solution comprising Cs-aspartate 130 mM, CsCl 20 mM, MgCl₂ 1 mM, Na₂ATP 3 mM, Na₂GTP 0.1 mM, EGTA 0.1 mM, and HEPES 5 mM adjusted to pH 7.2 with CsOH. As shown in Figure 4A, when the examined cell was rapidly depolarized from −80 to −10 mV, INa with both a rapid activation and inactivation time course was robustly evoked. As the cells were exposed to BRV, the peak amplitude of INa in response to a brief depolarizing command voltage progressively declined. For example, BRV (10 µM) resulted in an evident reduction in peak INa from 298 ± 19 to 153 ± 11 pA (n = 8, p < 0.05). After the washout of BRV, the current amplitude returned to 291 ± 17 pA (n = 8, p < 0.05). However, neither activation, inactivation, nor deactivation of the time course of INa in response to brief step depolarization was measurably perturbed in the presence of BRV (3 or 10 µM). µ illustrates the mean I-V relationships of peak INa, in the absence and presence of 3 or 10 µM BRV. It was observed that the overall I-V relationship to peak INa in these cells was not altered during exposure to BRV, in spite of an obvious lessening in peak INa. The concentration-dependent inhibitory effect of BRV on peak INa amplitude was then determined, as illustrated in Figure 4C. According to the modified Hill equation elaborated in the Materials and Methods Section, the IC₅₀ values for BRV-induced inhibition of peak INa measured at the start of the depolarizing command voltage was
found to be 12.2 μM, and BRV at a concentration of 300 μM almost completely eliminate the current amplitude.

![Figure 4](image_url)

**Figure 4.** Inhibitory effects of BRV on voltage-gated Na⁺ current (I_{Na}) in GH₃ cells. The cells were bathed in Ca²⁺-free Tyrode’s solution containing 10 mM tetraethylammonium chloride, where the recording electrode was filled with a Cs⁺-containing solution. (A) Representative I_{Na} traces activated by rapid membrane depolarization (indicated in the upper part of the figure). Inset shows an expanded record from the dashed box. (a): control (i.e., BRV not present), (b): 3 μM BRV, (c): 10 μM BRV, and (d): washout of BRV. (B) Mean I-V relationships of peak I_{Na} obtained in the absence (●) and in the presence of 3 μM BRV (○) and 10 μM BRV (△) (mean ± SEM; n = 7–8). The current amplitude was measured at the beginning of each brief depolarization. It should be noted that cell exposure to BRV depressed the amplitude of the peak I_{Na}; however, the overall I-V relationship with the current was not altered. Furthermore, the activation and inactivation time courses of the peak I_{Na} were clearly modified in the presence of BRV. (C) Concentration-dependent inhibition of peak I_{Na} caused by different BRV concentrations (mean ± SEM; n = 7). Peak I_{Na} was activated by rapid depolarization from −80 to −10 mV. The vertical broken line was placed on the IC₅₀ value of BRV used to show the inhibition of peak I_{Na} amplitude in response to the depolarizing command voltage.

3.5. Steady-State Inactivation Curve of Peak I_{Na} Taken with or without Addition of BRV

The following experiments were undertaken to investigate the effects of BRV on the steady-state inactivation of I_{Na}. In this set of whole-cell recordings, a two-pulse protocol was used (Figure 5A), and the inactivation parameters of peak I_{Na} were then estimated in the absence or presence of 10 μM BRV. The normalized amplitude of peak I_{Na} (i.e., I/\text{I}_{\text{max}}) versus the conditioning potential was then derived, as presented in Figure 5B. Thereafter, the experimental data were least-squares fitted (indicated in the continuous smooth line) to a Boltzmann function elaborated in the Materials and Methods Section (Figure 5B). Based on the experimental observations, in the control (i.e., BRV was not present), \( V_{1/2} = -35.5 \pm 2.9 \text{ mV}, q = 3.8 \pm 0.2 e \) \((n = 7)\), whereas in the presence of 10 μM BRV, \( V_{1/2} = -44.3 \pm 3.1 \text{ mV}, q = 3.7 \pm 0.2 e \) \((n = 7)\). Noticeably, as cells were exposed to 10 μM BRV, the midpoint of the steady-state inactivation curve was shifted in a hyperpolarizing direction by approximately 9 mV, regardless of its inability to alter the estimated gating charge (i.e., q value) of the curve. The results from these experiments made it possible to indicate that exposure to BRV is capable of altering the inactivation curve of I_{Na}.
Figure 5. BRV-induced modifications to the steady-state inactivation curve of peak $I_{Na}$ recorded from GH3 cells. In this set of experiments, the cells were immersed in a $Ca^{2+}$-free Tyrode’s solution containing 10 mM tetraethylammonium chloride, where the pipette was filled with $Cs^+$-containing solution. (A) Representative $I_{Na}$ traces obtained without (upper view) and with addition of 10 µM BRV (lower view). The uppermost part indicates the voltage profile used, where the different colors correspond to the different command voltages; (B) Steady-state inactivation curve of peak $I_{Na}$ in the absence (■) and presence (○) of 10 µM BRV (mean ± SEM; $n = 7$). It should be noted that a significant leftward (i.e., hyperpolarizing) shift along the voltage axis in the inactivation curve of the current was detected in the presence of 10 µM BRV, while the slope (i.e., gating charge [$q_{1/2}$]) of the curve remained unaffected.

3.6. Stimulatory Effect of BRV on the Activity of Large-Conductance $Ca^{2+}$-Activated K$^+$ (BK$_{Ca}$) Channels

Next, an effort was made to explore whether the presence of BRV could produce any perturbations on the probability of which BK$_{Ca}$ channels would be actively open. Cells were bathed in high-K$^+$ solution (i.e., 130 mM K$^+$) that contained 1 µM $Ca^{2+}$, and the recording pipette was filled with the K$^+$-containing solution. When the inside-out configuration was firmly established, the excised membrane was voltage-clamped at +60 mV. As depicted in Figure 6A, the activity of the BK$_{Ca}$ channels was drastically increased as the intracellular leaflet of the detached patch was exposed to 10 µM of BRV. For example, the presence of resulted in a clear increase in the channel open-state probability from 0.083 ± 0.007 to 0.138 ± 0.011 ($n = 8, p < 0.05$); however, it was observed that 10 µM of BRV was unable to alter the single-channel amplitude. When the BRV was washed out, the channel activity returned to 0.094 ± 0.008 ($n = 6, p < 0.05$). Alternatively, in the continued presence of BRV, the subsequent addition of GAL-021 or PF1022A reversed the BRV-mediated decrease in BK$_{Ca}$-channel activity; however, further application of TRAM39 failed to exert any effects (Figure 6B). TRAM39 was previously reported to be an inhibitor of intermediate-conductance $Ca^{2+}$-activated K$^+$ channels [66], whereas GAL-021 or PF1022A alone was shown to effectively suppress BK$_{Ca}$-channel activity [64,67].
should be noted that the upward deflection shows the channel opening event. The lower part (red color) of the figure BRV was not present) (left side of the figure) and after the bath addition of 10 µM of BRV (right side of the figure). It

**Figure 6.** Stimulatory effect of BRV on the activity of the large-conductance Ca\(^{2+}\)-activated K\(^{+}\) (BK\(_{Ca}\)) channels in GH\(_3\) cells. In this set of inside-out current recordings, the cells were immersed in a high-K\(^{+}\) solution containing 1 µM Ca\(^{2+}\), where the holding potential was clamped at +60 mV. (A) Representative BK\(_{Ca}\)-channel traces (blue color) obtained in the control (i.e., BRV was not present) (left side of the figure) and after the bath addition of 10 µM of BRV (right side of the figure). It should be noted that the upward deflection shows the channel opening event. The lower part (red color) of the figure shows the expanded records taken from the uppermost part of the figure; (B) Summary bar graph showing the open-state likelihood of the presence of BK\(_{Ca}\) channels in the presence of BRV (10 µM), BRV (10 µM) plus TRAM39 (3 µM), BRV (10 µM) plus GAL-021 (3 µM), and BRV (10 µM) plus PF1022A (3 µM) (mean ± SEM; \(n = 7–8\)). The channel activity was measured when the membrane patch was maintained at +60 mV. * Significantly different from the control \((p < 0.05)\) and † significantly different from the 10 µM BRV-alone group \((p < 0.05)\).

### 3.7. Inhibitory Effect of BRV on \(I_{Na}\) in Hippocampal Neurons

BRV has been reported to produce significant changes in the functional activities of neurons or neural networks that include the hippocampus [12,29,39,43,47]. To investigate this result, experiments were undertaken to determine whether the \(I_{Na}\) residing in mHippoE-14 cells [68] could be subject to any modifications by BRV. Whole-cell current recordings were conducted in cells that were bathed in Ca\(^{2+}\)-free Tyrode’s solution, and the electrode was filled with a Cs\(^{+}\)-containing solution. As the cells were exposed to different concentrations of BRV, the amplitude of the peak \(I_{Na}\) was activated in response to a brief depolarizing pulse (Figure 7A,B). For example, BRV, at a concentration of 3 µM lessened the amplitude of peak \(I_{Na}\) from 1.97 ± 0.14 to 0.95 ± 0.08 nA \((n = 8, p < 0.05)\). Furthermore, when 3 µM BRV was continually present, further addition of tefluthrin (10 µM) was found to reverse BRV-mediated inhibition of the \(I_{Na}\) amplitude, as evidenced by a decrease in the peak \(I_{Na}\) amplitude to 1.32 ± 0.11 nA \((n = 8, p < 0.05)\) (Figure 7B). Tefluthrin, a type-I pyrethroid insecticide, has been previously demonstrated to activate \(I_{Na}\) [69]. Therefore, in keeping with the observations in the GH\(_3\) cells discussed above, the presence of BRV was shown to produce a depressant action on the \(I_{Na}\) observed in mHippoE-14 cells; however, the activation and inactivation time courses of the current were not modified in its presence.
3.7. Inhibitory Effect of BRV on INa in Hippocampal Neurons

As demonstrated in Figure 8A, the probability that BKCa channels would be open was elevated when the excised patch was exposed to 10 or 30 µM of BRV. Figure 8B provides a summary bar graph showing the stimulatory effects of BRV on the channel opening probability in these cells. Therefore, the presence of BRV had a stimulatory effect on the BKCa-channel activity in these cells.

3.8. Effect of BRV on BKCa-Channel Activity Recorded from mHippoE-14 Hippocampal Neurons

A further investigation of the effects of BRV on the activity of BKCa channels in mHippoE-14 cells was carried out. Under inside-out current recordings, cells were bathed in a high-K+ solution containing 1 µM Ca2+, and the potential was voltage-clamped at +60 mV. As demonstrated in Figure 8A, the probability that BKCa channels would be open was elevated when the excised patch was exposed to 10 or 30 µM of BRV. Figure 8B provides a summary bar graph showing the stimulatory effects of BRV on the channel opening probability in these cells. Therefore, the presence of BRV had a stimulatory effect on the BKCa-channel activity in these cells.

Figure 7. Inhibitory effect of BRV on INa in mHippoE-14 hippocampal neurons. (A) Representative INa traces obtained in the absence (a, blue color) and presence (b, red color) of 3 µM BRV. The upper part is the voltage protocol delivered; (B) Summary bar graph showing the effects of BRV and BRV plus tefluthrin (10 µM) on the peak amplitude of INa in these cells (mean ± SEM; n = 8). * Significantly different from control (p < 0.05) and † significantly different from 3 µM BRV-alone group (p < 0.05).

Figure 8. Stimulatory effects of BRV on the activity of BKCa channels identified in mouse mHippoE-14 hippocampal neurons. In these experiments, cells were bathed in high K+ solution containing 1 µM of Ca2++; inside-out current recordings were performed, and channel activity was measured at a holding potential of +60 mV. (A) Representative channel current traces obtained in the control period (left, blue color) and after bath addition of 10 mM BRV (red color). Channel-opening event is denoted as an upward deflection (i.e., outward current); (B) Summary bar graph showing effects of BRV on the probability of channel opening during exposure to 10 or 30 mM of BRV (mean ± SEM; n = 8). * Significantly different from control (p < 0.05).
3.9. Simulated Firing of Action Potentials (APs) in Modeled Neurons Used to Mimic the Effect of BRV

For further evaluation of the effect of BRV on neuronal excitability, we explored how the firing of APs in a modeled neuron can be adjusted by adding BRV. The descriptions of this modeled neuron were detailed previously [53], and the formula for a hyperpolarization-activated cation current was incorporated into the model [54]. The parameters used in this work are illustrated in Table 1. In attempts to study AP firing, a long depolarizing current with 1, 1.5, or 2 mA/cm² was applied to this modeled neuron. It is clear from these simulations that, with different current stimulus strengths, the presence of 10 μM of BRV (i.e., with arbitrary changes in the different types of ionic currents demonstrated above) led to decreases in the AP firing frequency as well as the AP amplitude of the modeled neuron (Figure 9).

![Figure 9](simulated_firing.png)

Figure 9. Simulated firing of action potentials (APs) generated from the modeled neuron to mimic the effects of BRV. AP traces in the upper and lower part of the figure indicate those theoretically simulated to mimic the control condition in which BRV was not present and the experimental condition in the presence of BRV (10 μM), respectively. The AP firing in each panel was evoked by a 500 ms current stimulus at a strength of 1, 1.5 and 2 μA/cm². The values used for this numerical simulation are illustrated in Table 1.

3.10. Effects of BRV versus LEV on Acute Seizures in Different Animal Models

In a final series of studies, we evaluated the effects of BRV versus LEV on acute seizures in different animal models, including a sodium channel agonism-based OD-1 model [55] and the well-established lithium-pilocarpine-induced epilepsy model. We have recent characterized OD-1, a scorpion toxin, which could produce a concentration-, time-, and state-dependent rise in the peak amplitude of I_{Na}, shifting the I_{Na} inactivation curve to a less negative potential and increasing the frequency of spontaneous action currents. It could generate a significantly higher frequency of spontaneous seizures and epileptiform discharges compared with lithium-pilocarpine- or kainic acid-induced epilepsy, with comparable pathological changes [55]. We found in the OD-1 model that BRV had significant effects on the sustained time of severe seizures (stage 4 and above, seconds) (NS: 450 ± 25, LEV: 390 ± 22, BRV: 220 ± 20) (p < 0.05) compared to the control group (Figure 10A). The BRV group also had a significantly lower number of animals with severe seizures (NS: 60%, LEV: 45%, BRV: 35%) (p < 0.05) and severe seizure counts within the 8 h study duration (NS: 35, LEV: 24, BRV: 19) (p < 0.05) as compared to the control group (Figure 10B,C). In comparison, in the lithium-pilocarpine-induced epilepsy model, compared to the control
group, both LEV and BRV had significant effects on the latency of stage 3 seizures and above, NS: 25.8 ± 1.5, LEV: 35 ± 1.8, BRV: 37 ± 2) \((p < 0.05)\) (Figure 10D), the number of animals with severe seizures (NS: 58%, LEV: 38%, BRV: 36%) \((p < 0.05)\) (Figure 10E), and mortality (NS: 27%, LEV: 17%, BRV: 15%) \((p < 0.05)\) (Figure 10F). The observations in these animal models demonstrated the differential effects of BRV and LEV on acute seizures and neuronal hyperexcitability.

![Figure 10](image)

Figure 10. Effects of BRV versus LEV on acute seizures in OD-1 and lithium-pilocarpine animal models. (A–C) In the OD-1 model, BRV had a significant effect on the sustained time of severe seizures (stage 4 and above) compared to the control group. The BRV group also had a low number of rats with severe seizures and a lower severe seizure count, as compared to the control group (* \(p < 0.05, n = 7\) in each group); (D–F) In the lithium-pilocarpine-induced epilepsy model, compared to the control group, both LEV and BRV had significant effects on the latency of stage 3 seizures, the number of rats with severe seizures, and mortality (* \(p < 0.05, n = 7\) in each group). The data were analyzed using an ANOVA followed by Fisher’s least significant difference tests.

4. Discussion

The principal findings presented in this study are as follows: First, cell exposure to brivaracetam (BRV), a bioavailable LEV derivative, resulted in a concentration-dependent inhibition of \(I_{K(M)}\); however, it mildly depressed the amplitude of \(I_{K(DR)}\) and \(I_{h}\). Second, the presence of this agent inhibited the peak amplitude of \(I_{Na}\) in a concentration-dependent manner together with a leftward shift in the steady-state inactivation curve of the current. Third, in the case of the inside-out current recordings, addition of BRV to the intracellular side of the excised patch enhanced the probability \(BK_{Ca}\) channels that would be open. Fourth, in the mHippoE-14 hippocampal neurons, BRV was effective in suppressing the amplitude of peak \(I_{Na}\) with minimal changes in the activation and inactivation time courses of the current. These observations thus suggest evidence that, besides being a high-affinity ligand for SV2A [9,15,39,41–45], BRV can perturb the ionic currents specified herein, hence disclosing a potential additional impact on the functional activities of different excitable cells.

The effective IC\(_{50}\) values needed for BRV-induced inhibition of the \(I_{K(M)}\) or peak \(I_{Na}\) observed in the GH3 cells were estimated to be 6.5 or 12.2 \(\mu M\), respectively. In addition to a measurable reduction in the \(I_{K(M)}\) amplitude in response to long-lasting maintained depolarization, the activation time course of the current became slower in the presence of BRV. The BRV molecule can therefore reach the binding site once the \(I_{K(M)}\) channels are overly activated and reside in either the open state or in the open conformation. Additionally, despite the decrease in peak \(I_{Na}\) combined with a lack of the overall IV relationship to the current, the steady-state inactivation curve of the current was found to shift along the voltage axis toward a hyperpolarized potential (about 9 mV), with no measurable per-
turbations on the estimated gating charge related to the conditioning potential versus the relative current amplitude. Consequently, the window \( I_{Na} \) [70] was expected to decrease in the presence of BRV. By extension, in the inside-out current recordings, the addition of BRV to the internal leaflet of the excised patch conceivably elevated the probability of \( B_{K_{Ca}} \) channel openings, notwithstanding its inability to modify the single-channel amplitude. In the continued presence of BRV, subsequent application of either GAL-021 or PF1022A were detected to effectively attenuate increases in channel activity.

In previous recent pharmacokinetic studies on BRV, following intravenous administration of this agent, its plasma concentration was reported to range between 1 and 3 mg/L (i.e., 4.7 and 14.1 \( \mu \)M) [34,71,72]. Therefore, the ionic channels (i.e., M-type (\( KCNQx \)) \( K^+ \), \( Na_V \) (\( SCNx \)), and \( B_{K_{Ca}} \) (\( KCNMA1 \)) channels) are a relevant target for the pharmacological actions of this drug and may virtually occur within the clinically therapeutic range although the detailed mechanism by which BRV interferes with actions on these types of ion channels still requires further detailed investigation.

It should be mentioned that glioma cells can functionally express the magnitude of \( I_{Na} \), which may be linked to the malignant transformation of neoplastic cells [73]. BRV was also previously reported to exert anti-neoplastic actions identified in glioma cells [40]. In keeping with previous observations [47], it was possible to determine that the presence of BRV can lead to a possible reduction in the amplitude of peak \( I_{Na} \) identified in \( GH_3 \) or \( mHippoE-14 \) cells although the activation and inactivation time course of the current in response to the depolarizing command voltage remained unperturbed when the cells were exposed to BRV. As such, to what extent BRV-mediated changes in the magnitude of \( I_{Na} \) residing in glioma cells will participate in its anti-neoplastic actions remains to be established. Alternatively, BRV-induced relief of pain sensation as reported previously [1,2,39] could be partly explained by its inhibition of peak \( I_{Na} \) in sensory neurons.

It is worth noting that Levetiracetam was previously shown to decrease the amplitude of \( I_{K(DR)} \) when accompanied by an enhanced inactivation current time course [62]. However, in this study, the amplitude of \( I_{K(DR)} \) or \( I_h \) was mildly inhibited by adding BRV, and minimal changes in \( I_{K(DR)} \) inactivation in response to different levels of sustained depolarization were observed in its presence. It is conceivable, therefore, that in contrast to LEV, the \( I_{K(DR)} \) may not be an obligate target with which the BRV molecule can interfere. Moreover, the effects of BRV and LEV on the various types of ionic currents demonstrated herein could not be solely explained by their binding to the synaptic vesicle protein 2A (SV2A) in hippocampal neurons and pituitary cells [41–43,45,74], which was thought to be the case in both synaptic or endocrine vesicle exocytosis and neurotransmitter release, although they have been observed to be high-affinity SV2A ligands [9,15,39,43,44].

In the theoretical study, it was possible to mimic the BRV action on central neurons. It was noted that when cells were exposed to BRV, the simulated frequency of neuronal AP firing elicited in response to different depolarizing stimuli was obviously decreased owing to the changes in the conductance values of the different types of ionic currents referenced above, in combination with the reduced AP amplitude. It is conceivable, therefore, that its effects on neuronal APs occurring in vivo will be affected.

There were fundamental differences between the two animal models. In OD-1 model, it was possible to mimic the BRV action on central neurons. It was noted that when cells were exposed to BRV, the simulated frequency of neuronal AP firing elicited in response to different depolarizing stimuli was obviously decreased owing to the changes in the conductance values of the different types of ionic currents referenced above, in combination with the reduced AP amplitude. It is conceivable, therefore, that its effects on neuronal APs occurring in vivo will be affected.

There were fundamental differences between the two animal models. In OD-1 model, we needed to anesthetize these animals first to stereotactically inject the OD-1 toxin and observed the seizure parameter 24 h later. There was thus no acute mortality in this group because of the initial anesthetic effect, which reduced the initial acute neuronal excitotoxicity. We evaluated the parameter severe seizures as an indicator of high neuronal excitotoxicity. For pilocarpine model, the acute excitotoxicity was prominent after initial intraperitoneal injection, thus it is not uncommon to lead to acute mortality following stage 4–5 seizure and status epilepticus. The latency to acute stage 3 seizures, number of rats with severe seizures and acute mortality were used to evaluate the high neuronal excitotoxicity. Therefore, the seizure parameters evaluated were different in both models.
The present evaluation of the effects of BRV versus LEV on acute seizure animal models further demonstrated the different ionic effects of BRV and LEV. Compared to LEV, sodium channel modulation of BRV, as demonstrated in the present study, explained its significant effect on OD-1, a unique sodium channel-agonism-based animal model of epilepsy and seizure. However, our previous study on LEV’s ionic mechanism did not reveal the underlying LEV mechanism for sodium channel modulation [62]. Nevertheless, both LEV and BRV had significant effects on the lithium-pilocarpine-induced seizure model, which was in line with previous observations regarding LEV’s effect on this model and the common SV2A mechanism of both medications in this model [75,76]. Furthermore, the various actions associated with the mechanism accounted for the finding that the response in the presence of BRV has been found to be several minutes faster than that with LEV in patients with photosensitive epilepsy [77], and BRV has been shown to be a useful treatment option in patients with epilepsy who have previously failed to respond to or tolerate LEV [48,78]. The unique ionic mechanism, in addition to the common SV2A modulation, justifies the role of BRV in rationale polytherapy for epileptic disorders, in terms of both efficacy and adverse events.

5. Conclusions

BRV’s multiple ionic mechanism in electrically excitable cells and a concerted effect on neuronal excitability underlies its therapeutic potential in clinical neuronal hyperexcitability disorders.

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