Research Square

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Depressive Effectiveness of Vigabatrin (γ-Vinyl-GABA), an Antiepileptic Drug, in Intermediate-conductance Calcium-Activated Potassium Channels in Human Glioma Cells

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Abstract

Background: Vigabatrin (VGB) is an approved non-traditional antiepileptic drug that has been revealed to have potential for treating brain tumors; however, its effect on ionic channels in glioma cells remains largely unclear.

Methods: With the aid of patch-clamp technology, we investigated the effects of VGB on various ionic currents in the glioblastoma multiforme cell line 13-06-MG.

Results: In cell-attached configuration, VGB concentration-dependently reduced the activity of intermediate-conductance Ca^{2+}-activated K^+ (IK_{Ca}) channels, while DCEBIO (5,6-dichloro-1-ethyl-1,3-dihydro-2H-benzimidazol-2-one) counteracted the VGB-induced inhibition of IK_{Ca} channels. However, the activity of neither large-conductance Ca^{2+}-activated (BK_{Ca}) nor inwardly rectifying K^+ (K_{IR}) channels were affected by the presence of VGB in human 13-06-MG cells. However, in the continued presence of VGB, the addition of GAL-021 or $BaCl_2$ effectively suppressed BK_{Ca} and K_{IR} channels.

Conclusions: The inhibitory effect of VGB on IK_{Ca} channels demonstrated in the current study could be an important underlying mechanism of VGB-induced antineoplastic (*e.g.*, anti-glioma) actions.

Background

Vigabatrin (VGB; y-vinyl-gamma-aminobutyric acid [y-vinyl-GABA]) is an approved antiepileptic drug, which is tailored as an adjuvant therapy for adults with refractory partial epilepsy; it is also used for the treatment of infantile spasms (Lux et al., 2004; Hancock et al., 2008; Hemming et al., 2008). VGB is a structural analog of GABA, which irreversibly inhibits GABA-transaminase (Grant & Heel, 1991) and thus consequently increases levels of the inhibitory neurotransmitter GABA (Schechter, 1989) in the brain. It has been shown to attenuate astroglial TWIK-related acid-sensitive K^+ channel-1 in the hippocampus of seizure-sensitive gerbils (Kim et al., 2007). Although most of VGB's effects are thought to be attributed to its GABA-ergic actions, its perturbations on the amplitude or gating of ionic effects are not clear.

The degree of functional expression in the intermediate-conductance Ca^{2+}-activated K^+ (IK_{Ca}) channels identified in glioma cells has recently been reported to interfere with the progression of malignant tumors (Wulff et al., 2007). IK_{Ca} channels (also known as $K_{Ca}3.1$, SK4, $IK_{Ca}1$, and KCNN4) are encoded by the *KCNN4* gene. These channels have been cloned from human, mouse, or rat tissues and they are linked to many cellular functions, such as hormonal secretion, cell motility, cell proliferation, and Ca^{2+} influx and K^+ efflux regulation. All of these underlying mechanisms have been extensively studied in many non-excitable and neoplastic cells (Lallet-Daher et al., 2009; Liang et al., 2011; Ohya et al., 2011). These channels exhibit to have single-channel conductance of 20-60 pS and their biophysical and pharmacological profiles are distinct from those of large- or small-conductance Ca^{2+}-activated K^+ channels (Schwab et al., 2012; Leanza et al., 2014). Perhaps more importantly, the modulators of IK_{Ca}

channels represent a potential therapeutic approach for a variety of diseases, particularly at malignant gliomas (Jensen et al., 2001; Wulff et al., 2007).

VGB has been reported to decrease oligodendrocyte precursor cell proliferation and to increase the number of mature oligodendrocytes (Zonouzi et al., 2015). Interestingly, it has been also shown to have promising therapeutic efficacy for treating brain metastases *in vivo* (Schnepp et al., 2017). However, VGB's ion mechanism on anti-neoplastic actions has yet to be determined. In this study, we sought to investigate VGB's ion mechanism on anti-neoplastic actions in the glioblastoma multiforme cell line (*i.e.*, human 13-06-MG glioma cells).

Methods

Chemicals, drugs and solutions

VGB ((±)-y-vinyl-GABA, $C_6H_{11}NO_2$) was acquired from Sigma-Aldrich (Merck Ltd., Taipei, Taiwan), GAL-021 was obtained from MedChemExpress (Everything Biotech Ltd., New Taipei City, Taiwan), and DCEBIO (5,6-dichloro-1-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one) and TRAM-34 (1-((2-chlorophenyl)-(diphenyl)methyl)-1*H*-pyrazole) were obtained from Tocris (Union Biomed, Taipei, Taiwan). Unless specified otherwise, the culture media, fetal bovine serum, L-glutamine, and trypsin/EDTA were acquired from HyClone^{TM} (Thermo Fisher Scientific, Taipei, Taiwan); all other chemicals or reagents were of analytical grade.

The composition of the bathing solution (*i.e.*, HEPES-buffered normal Tyrode's solution) was 136.5 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.53 mM $MgCl_2$, 5.5 mM glucose, and 5.5 mM HEPES titrated with NaOH to pH 7.4. To measure K^+ currents, patch pipettes were backfilled with an internal solution consisting of 130 mM K-aspartate, 20 mM KCl, 1 mM KH_2PO_4, 1 mM $MgCl_2$, 3 mM Na_2ATP, 100 μM Na_2GTP, 0.1 mM EGTA, and 5 mM HEPES titrated with KOH to pH 7.2 (Hung et al., 2019; Lai et al., 2020). To preclude the contamination of whole-cell Cl^- currents, we replaced Cl^- ions inside the pipette solution with aspartate.

For recording large-conductance Ca^{2+}-activated (BK_{Ca}) channels, a high K^+-bathing solution was used, and its composition was 145 mM KCl, 0.53 mM $MgCl_2$, and 5 mM HEPES titrated with KOH to 7.2, and the pipette solution contained 145 mM KCl, 2 mM $MgCl_2$, and 5 mM HEPES titrated with KOH to 7.2. All solutions were prepared using demineralized water from a Milli-Q water purification system (Merck, Ltd., Taipei, Taiwan). The pipette solution and culture medium were filtered on the day of use with an Acrodisc® syringe filter with a Supor® membrane (Bio-Check; New Taipei City, Taiwan).

Cell preparations

The glioblastoma multiforme cell line (13-06-MG) used was kindly provided by Professor Dr. Carol A. Kruse (Department of Neurosurgery, Ronald Reagan UCLA Medical Center, LA, U.S.A). The 13-06-MG cells

were routinely grown at a density of 10^6/ml in high glucose (4 g/l) Dulbecco's modified Eagle media (Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin and 10 µg/ml streptomycin. Cells were maintained at 37˚C in a 5% CO_2 incubator as monolayer cultures and then sub-cultured weekly; fresh media was added every 2-3 days to maintain a healthy cell population. Glial cells were verified by identifying glial fibrillary acidic protein, which is a cytoskeletal protein.

To evaluate concentration-dependent inhibition of VGB on the probability of IK_{Ca}-channel openings, 13-06-MG cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$, and each cell examined was held at -80 mV relative to the bath. The probability of channel opening was measured in the control and during cell exposure to different concentrations (0.3-100 µM) of VGB; and these values were then compared with those measured after the addition of TRAM-34 (3 µM). TRAM-34 is a known selective blocker of IK_{Ca} channels. The concentration required to suppress 50% of channel activity was determined by means of a Hill function: **see formula 1 in the supplementary files section.**

where [C] represents the VGB concentration; IC_{50} or n_H is the concentration required for a 50% inhibition or the Hill coefficient, respectively; and E_{max} indicates the maximal reduction in channel opening probability (*i.e.*, TRAM-34-sensitive channel activity) caused by VGB.

Statistical analyses

Linear or nonlinear curve-fitting (*e.g.*, sigmoidal or exponential curve) was performed on the data sets collected using either Microsoft Excel® (Redmond, WA) or OriginPro 2016 (Microcal). Values are presented as the mean ± standard error of the mean (SEM) with sample sizes (n) indicating the number of 13-06-MG cells from which the experimental data was collected. A paired or unpaired Student's *t*-test or one-way analysis of variance (ANOVA) followed by a *post-hoc* Fisher's least-significant difference test, were performed to analyze multiple groups. The data were examined using a nonparametric Kruskal-Wallis test, subject to possible violation in the normality underlying ANOVA. Differences were considered statistically significant when the *P*-value was below 0.05.

Results

VGB and the activity of IK_{Ca} channels in in 13-06-MG cells

Experiments to evaluate the effect of VGB on IK_{Ca}-channel activity were performed. In this set of experiments, 13-06-MG cells were bathed in normal Tyrode's solution containing 1.8 mM $CaCl_2$ and single-channel current recordings were made. The probability of IK_{Ca} channel opening was measured at -80 mV relative to the bath. In the presence of VGB, the IK_{Ca} channels were significantly less likely to be open, compared with the control (**Figure 1A**). Similar effects were observed after TRAM-34 was added to the control group **(Figure 1B)**. IK_{Ca} channels that were closed in VGB-treated cells were reopened after the cells were treated with DCEBIO, an activator of IK_{Ca} channels. This data is summarized in **Figure 1C**,

which shows the effects of VGB, TRAM-34 (3 μM), and VGB (10 μM) plus DCEBIO (10 μM) on IK_{Ca}-channel activity. Each bar indicates the mean ± SEM (n=9-11). Additionally, as cells were exposed to Tyrode's solution containing 3.6 mM $CaCl_2$, the presence of VGB (10 mM) effectively decreased IK_{Ca}-channel activity, while it had minimal effect on it in cells bathed in Ca^{2+}-free Tyrode solution.

VGB effect on single-channel conductance of IK_{Ca} channels

How VGB treatment affected IK_{Ca} channels at different membrane potentials was further evaluated. Plots of current amplitude as a function of holding potential were then constructed. Single-channel amplitudes at the potentials ranging between -80 and -40 mV were measured. The single-channel conductance of IK_{Ca} channels calculated from a linear *I-V* relationship in the control was calculated to be 32.4±4 pS (n=9) over the voltage ranging between -80 and -40 mV, and 9.5±1.1 pS (n=9) over that between +130 and +150 mV, respectively (**Figure. 2**). Of notice, the conductance measured at negative potentials was greater than that at positive voltages. However, the single-channel slope conductance (32.1±4 pS; n=9, $P>0.05$) of IK_{Ca} channels was not significantly changed after VGB (10 mM) treatment, despite the observed reduction in the probability of channel openings. Similarly, no change in single-channel conductance of the channel measured over the potentials ranging between +130 and +150 mV was demonstrated in the presence of VGB.

Concentration-dependent inhibitory effect of VGB on the activity of IK_{Ca} channels

The relationship of the percentage suppression of IK_{Ca}-channel activity versus VGB concentration was further analyzed. In this set of experiments, each cell was maintained at -80 mV relative to the bath, and the channel open probability in the absence and presence of different VGB concentrations was measured. As depicted in **Figure 3**, the addition of VGB (0.3-100 μM) suppressed the activity of IK_{Ca} channels in a concentration-dependent manner. The IC_{50} value required for its inhibitory effect on channel activity in 13-06-MG cells was calculated to be 4.21 μM, and it at a concentration of 100 μM nearly abolished the probability of channel openings. These findings led us to indicate that VGB is able to exert a depressive action on the activity of IK_{Ca} channels expressed in 13-06-MG cells.

Effect of VGB and VGB plus GAL-021 on the probability of BK_{Ca}-channel opening

We further examined whether the presence of VGB could affect the activity of BK_{Ca} channels in 13-06-MG cells. In these experiments, cells were immersed in a high-K^+ solution that contained 1.8 mM $CaCl_2$, and the examined cells were held at +80 mV. As the cells were exposed to 10 μM VGB, the probability of BK_{Ca} channels opening was not altered **(Figure 4)**. However, following the addition of GAL-021 (10 μM) channel activity was significantly decreased. GAL-021 has been previously reported to be a blocker of BK_{Ca} channels (Roozekrans et al., 2014). Unlike IK_{Ca} channels, which were suppressed by VGB, the BK_{Ca} channels were resistant to being blocked by this agent.

Effect of VGB and VGB plus $BaCl_2$ on K_{IR}-channel activity

In another set of single-channel current recordings, we tested whether other K^+ channels (*i.e.*, K_{IR} channels) could be affected by the presence of VGB. Cells were bathed in Ca^{2+}-free Tyrode's solution and the holding potential was set at -80 mV relative to the bath. However, the presence of 10 μM VGB was unable to produce any modifications in K_{IR} channel activity in these cells (**Figure 5**). However, the subsequent addition of 1 mM $BaCl_2$ in the continued presence of 10 μM VGB, effectively suppressed the probability of channel opening. $BaCl_2$ is regarded as an inhibitor of K_{IR} channels (Wang et al., 2012).

Discussion

VGB is an anti-epileptic agent that is reported to be an inhibitor of gamma-aminobutyric acid breakdown. It has been approved for use as an adjunctive treatment for resistant epilepsy, and as a monotherapy for infantile spasms or West syndrome (Hancook et al., 2008; Hemming et al., 2008). In this study, we found that VGB dose-dependently reduced the probability of IK_{Ca} channel openings, and that this reduction in channel activity is voltage-dependent and associated with an increase in mean channel time closed. The reduction of the channel open probability accounts primarily for its suppression in IK_{Ca}-channel activity, despite the inability to modify single-channel conductance in IK_{Ca} channels. However, the activity of neither BK_{ca} nor K_{ir} channels was perturbed by the presence of VGB. Therefore, in addition to the inhibition of GABA breakdown, this study revealed that VGB suppressed the activity of IK_{Ca} channels. This effect could be partly responsible for its suppression of neoplastic cells (Lee et al., 2016). Therefore, caution needs to be appropriately exercised when the effect of this compound is explained solely by its action on GABA-ergic dysregulation (Zonouzi et al, 2015). However, whether there is functional coupling between GABA-receptor(s) signaling and IK_{Ca}-channel activity remains to be further studied.

The single-channel conductance of IK_{Ca} channels in human glioma cells (13-06-MG) was calculated to be 32 pS, a value similar to the prototypical IK_{Ca} channels present in other cell types (Turner et al., 2015; Jensen et al., 2001; Wulff et al., 2007), but apparently less than that of BK_{Ca} channels (Wu et al., 2008; Koekkoek et al., 2015). VGB-mediated inhibition of IK_{Ca}-channel activity depends on membrane voltage and it is thought to occur via a direct interaction with the KCa3.1 channel protein in glioma cells.

In this study, the IC_{50} value required for VGB-induced inhibition of IK_{Ca} channels was 4.21 μM. There is a wide range of serum/plasma concentrations (0.8–36 mg/L) associated with successful epilepsy treatment (McMillin et al., 2016). The concentration in cerebrospinal fluid was noted to be approximately 30-40% of plasma concentration, supporting that the IC_{50} value of VGB in the current study could be of clinical relevance. The presence of VGB inhibits IK_{Ca} channels in humans at these relatively low concentrations, and in contrast to other GABA compounds, it is lipophilic and able to cross the blood-brain barrier (Silverman, 2004). Therefore, findings from the present observations could be important in determining VGB's *in vivo* anti-neoplastic mechanism.

Different types of kinetic behaviors perturbed by VGB might facilitate its inhibition of IK_{Ca}-channel activity. VGB has no discernible effect on IK_{Ca} single-channel conductance; therefore, the VGB molecule unlikely acts within the channel's central pore. However, the mean closed time of the channel was conceivably lengthened in its presence. Based on minimal kinetic analyses, we were able to characterize VGB-mediated inhibition of IK_{Ca} channels by a greater affinity for the IK_{Ca} channel in the closed (or resting) state. The activity of IK_{Ca} channels has been previously reported to regulate the proliferation of prostate cancer cells by controlling Ca^{2+} entry into these cells (Lallet-Daher et al., 2009). However, significant changes in neither BK_{Ca}- nor Kir-channel activity were observed in these cells. The effectiveness of VGB in inhibiting IK_{Ca} channels demonstrated here in glioma cells does not arise secondary to the reduction of intracellular Ca^{2+} (McFerrin et al., 2012). In the present study, VGB inhibited IK_{Ca}-channel activity within a few minutes in the 13-06-MG cells. As the onset of inhibition was rapid, its action on channel activity was most unlikely to ascribe from the binding to nuclear DNAs. The mechanism through which the VGB molecule binds to and then interact with IK_{Ca} channels tends to be direct and not genomic, despite the detailed mechanism of VGB action remains to be further resolved.

An earlier study in which immunolabelling of KCa3.1 channels was performed, disclosed that IK_{Ca} channels tended to be differentially expressed in excitatory and inhibitory neurons of the central nervous system (Turner et al., 2015). Different isoforms of KCa3.1 might also be present in various types of body tissue, including gliomas; however, whether VGB is capable of modifying different types of IK_{Ca} channels remains unknown. Further studies investigating the extent to which VGB-induced effects on glioma cells may be attributed to direct inhibitory perturbations on IK_{Ca} channels, are thus imperatively warranted.

Of notice, the expression and function of glial Kir channels have been previously studied in retinal Müller glial cells, Schwann cells, astrocytes, and oligodendrocytes. Expression of Kir4.1 was identified in brain and retinal glial cells, while those of Kir2.1 and Kir2.3 were reported to be present in Schwann cells (Horie and Kurachi, 1999; Hibino et al., 2010). Whether VGA can perturb the activity of different types of Kir channels in glial cells still remains to be further resolved.

Interestingly, one *in vitro* study suggested that VGB should not be used for prophylaxis or the short-term treatment of epilepsy in glioblastoma (Lee et al., 2016). However, another study suggested that blocking GABA flux into the TCA cycle, either through genetic depletion of GAD1 or pharmacological treatment with VGB, significantly suppressed aggressive metastatic outgrowth in the brain. Furthermore, it suggests that VGB might bring an additional benefit of stabilizing tumor-induced seizures (Schnepp et al., 2017).

Our previous study on temozolomide, which demonstrated its inhibitory effect on IK_{Ca} accompanied by membrane depolarization, could describe an important underlying mechanism of temozolomide-induced antineoplastic actions (Yeh et al., 2016). Supportively, it has been reported ionizing radiation could stimulate BK_{Ca} channel activity, resulting in Ca^{2+}/calmodulin-dependent kinaces II, leading to glioblastoma cell migration (Steinle et al., 2011). As KCa3.1 has been reported to confer radioresistance

to breast cancer cells (Mohr et al., 2019a), strategies targeting KCa3.1 in anti-cancer treatment may have good potential in modulating anti-tumor immune activity (Mohr et al., 2019b).

The inhibitory effect of VGB on IK_{Ca} channels demonstrated herein sheds light on and supports the potential of VGB on antineoplastic actions. The possible link between vigabatrin/IK_{Ca} channel activity and neoplastic cell behavior, including migration, spread, survival and proliferation is worth further investigation.

Declarations

Ethics approval and consent to participate: Not applicable. This study did not involve human participants and animals.

Consent for publication: Not applicable.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' Contributions: TYH, SNW, and CWH conceived the study. TYH, HYIH, SNW, and CWH performed the experiments. SNW and CWH participated in the statistical analysis. All authors approved the final manuscript. Each author contributed substantially during manuscript drafting or revision.

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Figures

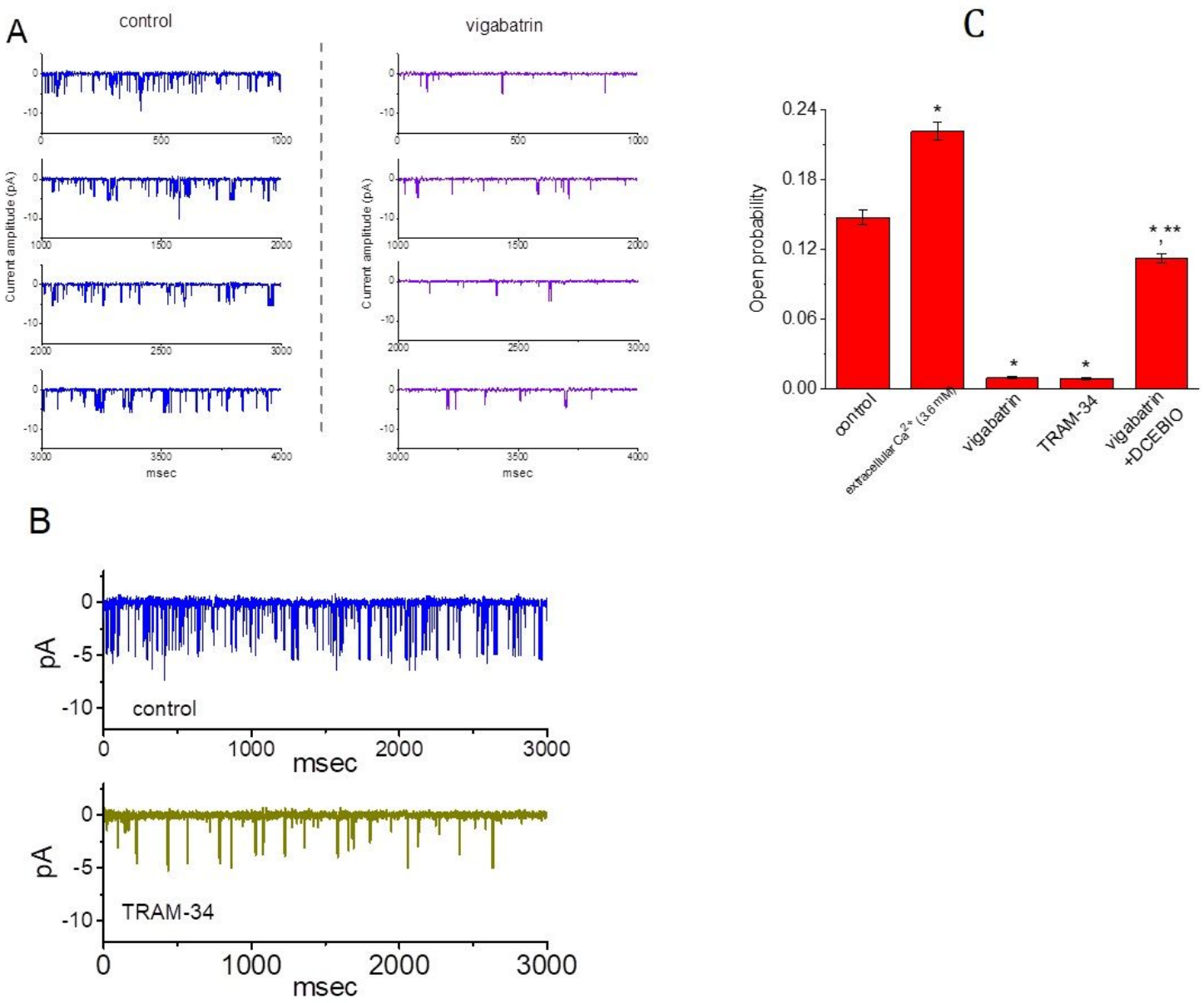

Figure 1

Effect of VGB on the activity of intermediate-conductance Ca2+-activated K+ (IKCa) channels expressed in human 13-06-MG glioma cells. In this set of experiments, 13-06-MG cells were bathed in normal Tyrode's solution containing 1.8 mM CaCl2 and single-channel current recordings were made. The probability of IKCa-channels opening was measured at -80 mV relative to the bath. (A) Original current traces for IKCa channels obtained in the absence (left) and presence (right) of VGB (10 μM). Note that channel opening gives a downward deflection in current. (B) Original IKCa-channel traces taken in the absence (upper) and presence (lower) of TRAM-34 (3 □M). (C) Summary of the data showing the effects of VGB, TRAM-34 (3 μM), and VGB (10 μM) plus DCEBIO (10 μM) on IKCa-channel activity. The probability of IKCa channel opening was measured at -80 mV relative to the bath. Each bar indicates the mean ± SEM (n=9-11). *Significantly different from control (i.e., in the presence of 1.8 mM Ca2+) (P<0.05) and **significantly different from the VGB alone group (P<0.05).

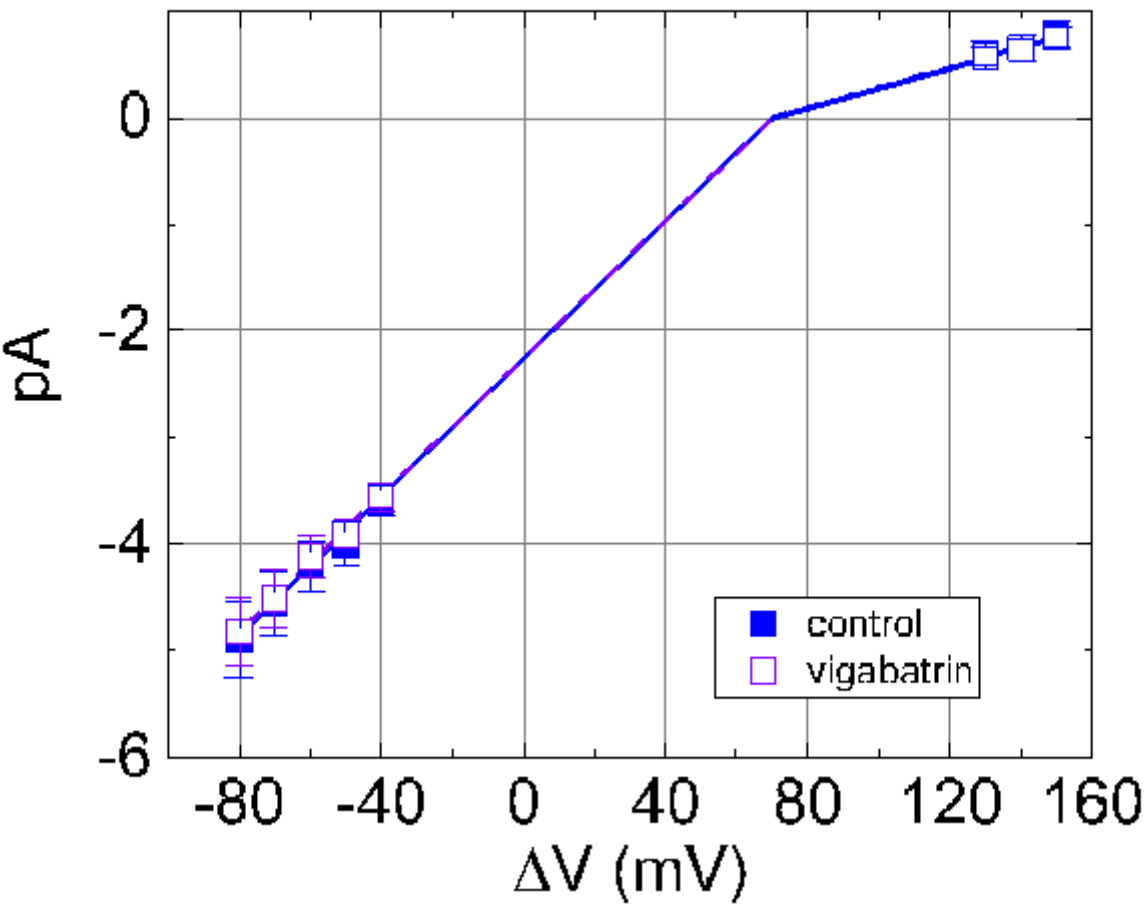

Figure 2

The association between single IKCa-channel amplitude and membrane potential (i.e., voltage) in the absence (■) and presence (□) of 10 μM VGB (mean ± SEM; n=8-13 for each point). Single-channel amplitudes over the potentials ranging from -80 to -40 mV or from +130 to +150 mV were measured. Note that the single-channel conductance of IKCa channels over the voltage range between -80 and -40 mV obtained in the absence (32.4 pS) and presence (32.1 pS) of VGB did not differ significantly in human 13-06-MG cells.

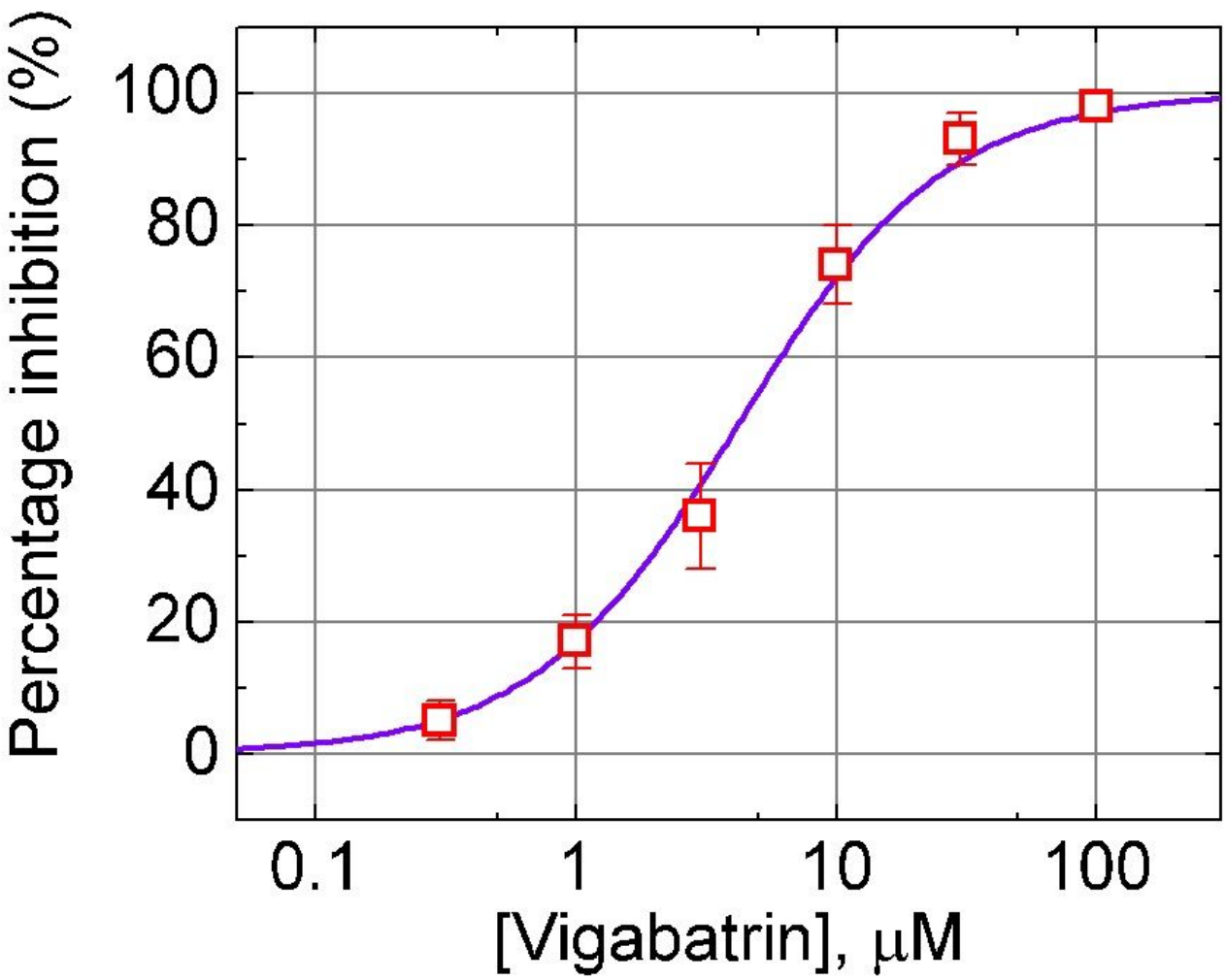

Figure 3

Concentration-response curve for VGB-induced suppression of IKCa channels recorded in human 13-06-MG cells (mean ± SEM; n=11-14 for each point). VGB was added at various concentrations (0.3-100 μM) to the bath, and the activity of IKCa channels was detected at -80 mV relative to the bath. The smooth curve was well fitted with a least-squares procedure to a modified Hill function.

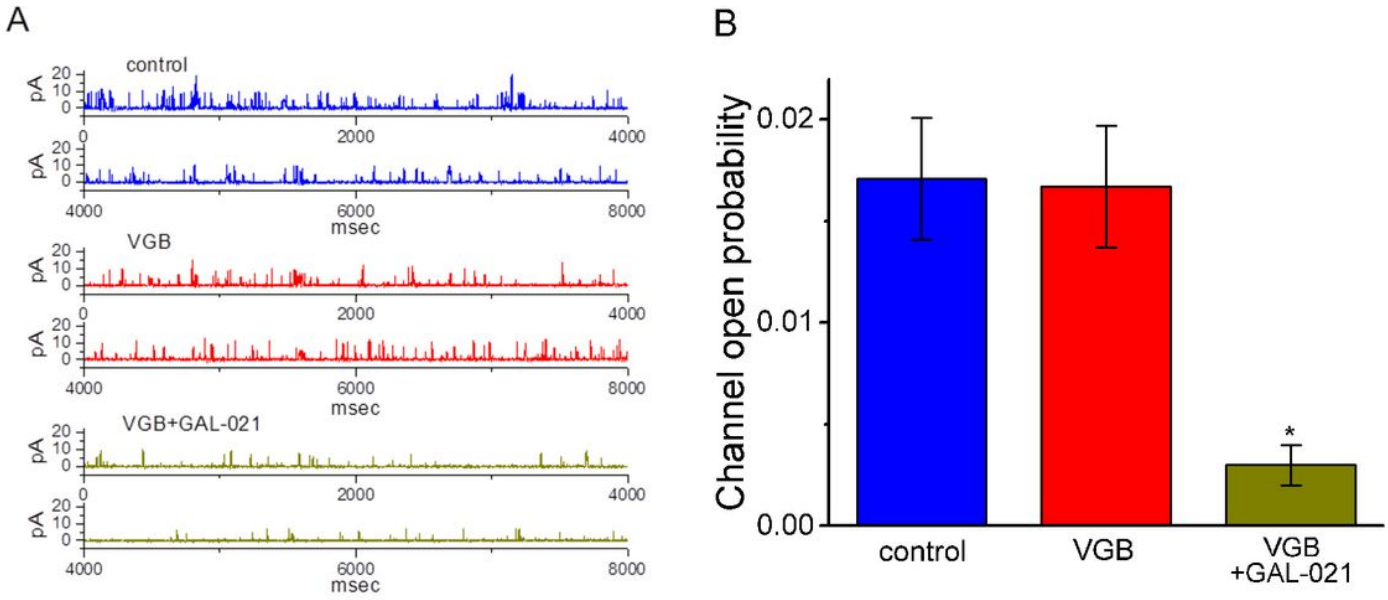

Figure 4

The inability of VGB to alter the activity of BKCa channels was recorded in human 13-06-MG cells. The experiments were conducted under cell-attached configuration. The cells were bathed in a high-K+ solution containing 1.8 mM CaCl2, and the examined cells were clamped at a level of +80 mV. (A) Original trace of single BKCa channels obtained in the control (upper) and after the addition of 10 μM VGB (middle) or 10 μM VGB plus 10 μM GAL-021 (lower). The upward deflection indicates the opening event of the channel. (B) Summary bar graph of the effects of VGB or VGB plus GAL-021 on the probability of BKCa channel opening (mean ± SEM; n=7 for each bar). *Significantly different from the control or 10 μM VGB alone ($P<0.05$).

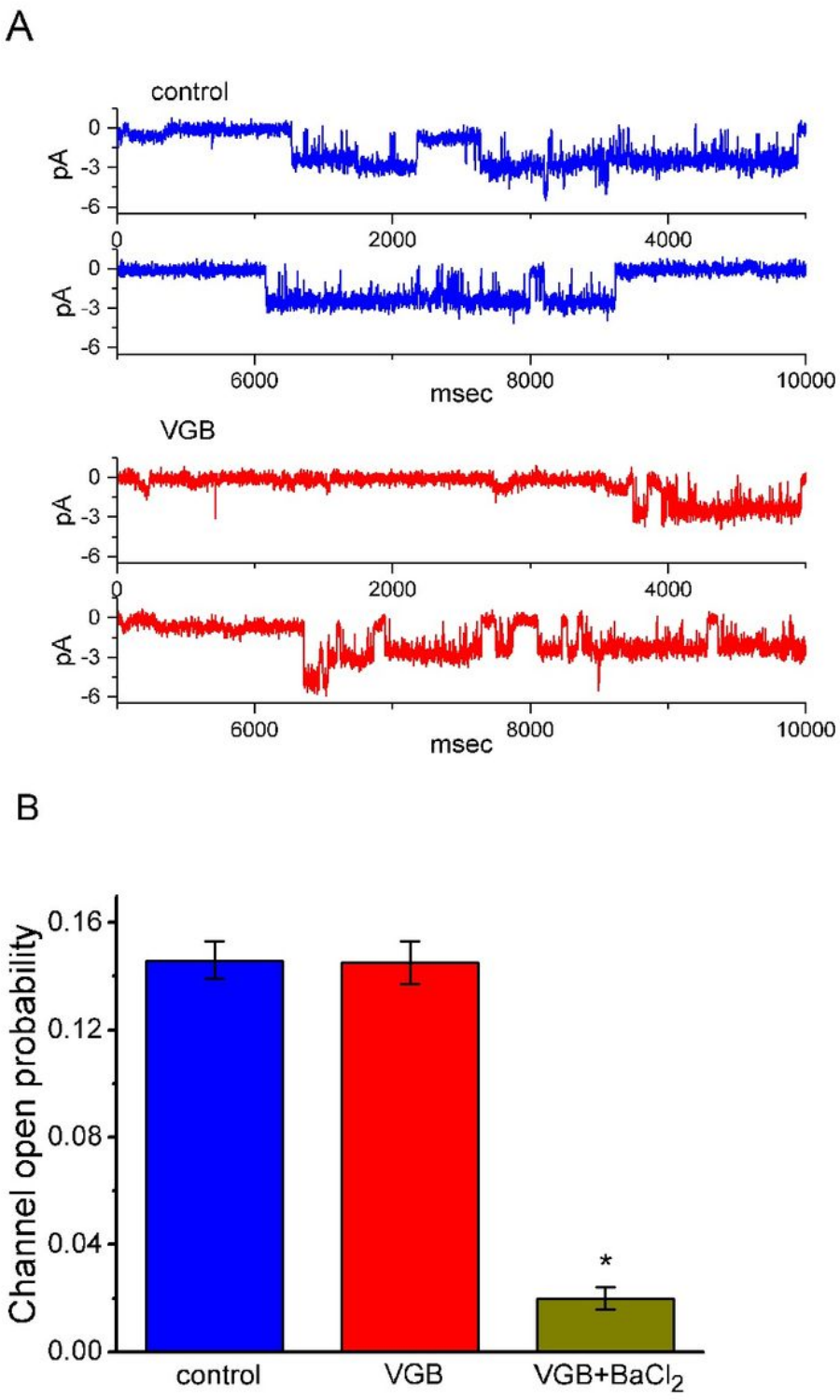

Figure 5

Failure of VGB to modify the activity of KIR channels in human 13-06-MG cells. In this set of experiments, we bathed cells in Ca2+-free Tyrode's solution and, during the recording, we backfilled the pipette by using K+-containing solution. The activity of KIR channels was detected at -80 mV relative to the bath. (A) Single KIR channels obtained in the absence (upper) and presence (lower) of 10 μM VGB. The downward deflection denotes the channel opening event. (B) Summary bar graph depicting the effect of VGB and

VGB plus $BaCl_2$ on the activity of KIR channels in human 13-06-MG cells (mean ± SEM; n=7 for each bar). *Significantly different from the control or 10 μM VGB alone ($P<0.05$). VGB: 10 μM VGB; $BaCl_2$: 1 mM $BaCl_2$.