

# Long-term regulation of voltage-gated $\text{Ca}^{2+}$ channels by gabapentin

Myoung-Goo Kang, Ricardo Felix<sup>1</sup>, Kevin P. Campbell\*

Howard Hughes Medical Institute, Department of Physiology and Biophysics, and Department of Neurology,  
The University of Iowa College of Medicine, 400 Eckstein Medical Research Building, Iowa City, IA 52242, USA

Received 22 July 2002; revised 20 August 2002; accepted 20 August 2002

First published online 30 August 2002

Edited by Maurice Montal

**Abstract** Gabapentin (GBP) is a  $\gamma$ -aminobutyric acid analog effective in the treatment of seizures. A high-affinity interaction between GBP and the  $\alpha_2\delta$  subunit of the voltage-gated  $\text{Ca}^{2+}$  channels has been documented. In this report, we examined the effects of the chronic treatment with GBP on neuronal recombinant P/Q-type  $\text{Ca}^{2+}$  channels expressed in *Xenopus* oocytes. GBP did not affect significantly the amplitude or the voltage dependence of the currents. Exposure to the drug did, however, slow down the kinetics of inactivation in a dose-dependent fashion. In addition, biochemical analysis showed that the integrity of  $\text{Ca}^{2+}$  channel complex is not apparently affected by GBP binding, suggesting that chronic treatment with the drug might cause the channel kinetic modification through subtle conformational changes of the protein complex. © 2002 Federation of European Biochemical Societies. Published by Elsevier Science B.V. All rights reserved.

**Key words:**  $\text{Ca}^{2+}$  channel; Gabapentin; Epilepsy;  $\text{Ca}_v2.1$  subunit;  $\alpha_2\delta$  subunit;  $\omega$ -Conotoxin

## 1. Introduction

The influx of  $\text{Ca}^{2+}$  ions through voltage-gated  $\text{Ca}^{2+}$  channels contributes to neuronal excitability and is an important feature of epileptogenesis.  $\text{Ca}^{2+}$  channels contribute significantly to the depolarization shift seen in focal epilepsy and are also implicated in the increase of excitability during the transition to seizures and in the spread of seizure activity [1]. In addition, recent genetic studies have linked  $\text{Ca}^{2+}$  channel subunits to several animal seizure models and human neurological disorders [2,3].  $\text{Ca}^{2+}$  channels are heteromultimers, consisting at least of a main  $\alpha_1$  subunit and auxiliary  $\alpha_2\delta$ ,  $\beta$  and  $\gamma$  subunits. The  $\alpha_1$  subunit is a transmembrane protein that forms the ion-conducting pore and the auxiliary  $\beta$  subunit is an intracellular protein that is thought to play important roles in channel regulation [4]. The auxiliary  $\alpha_2\delta$  and  $\gamma$  subunits are transmembrane proteins also involved in the regulation of the basic biophysical properties and surface expression of  $\text{Ca}^{2+}$  channel [5].

Gabapentin (GBP) is a synthetic drug that has shown anticonvulsant activity in a variety of animal seizure models and is also effective in the treatment of human partial and generalized tonic-clonic seizures [1,6,7]; however, the anticonvul-

sant mechanism of GBP is not known. Despite its structural relationship to the  $\gamma$ -aminobutyric acid (GABA), GBP does not possess a high affinity for GABA receptors [8], and does not affect the uptake or the degradation of this inhibitory neurotransmitter [6,7]. On the other hand, GBP has been shown to be the substrate for a specific binding site in the brain [8], and is capable of modifying the electrophysiological properties of central neurons in culture after prolonged but not brief exposure at concentrations used in clinical trials [9]. A high-affinity GBP-binding protein purified from brain membranes has been characterized, and identified to be the  $\alpha_2\delta$  subunit of the  $\text{Ca}^{2+}$  channel by N-terminal sequencing [10]. Likewise, transient transfection of the  $\alpha_2\delta$  cDNA in COS-7 cells significantly increased GBP binding, consistent with the expression of the  $\alpha_2\delta$  protein as measured by Western blotting [10].

In this report we examined the consequences of the chronic treatment with GBP on the plasma membrane  $\text{Ca}^{2+}$  conductances and on the integrity of the  $\text{Ca}^{2+}$  channel complex. Our results show an alteration in neuronal P/Q-type  $\text{Ca}^{2+}$  channel activity after chronic treatment with GBP presumably caused by a direct effect of the drug on the molecular channel configuration.

## 2. Materials and methods

### 2.1. Materials and preparation of oocytes

$\omega$ -Conotoxin GVIA,  $\omega$ -Conotoxin MVIIC (Peptides International Inc., Louisville, KY, USA) and GBP [1-(aminomethyl) cyclohexane acetic acid, Neurontin®, Parke-Davis Pharmaceuticals Ltd.] were prepared as stock in distilled water and aliquots were stored at  $-20^\circ\text{C}$ . All other chemicals were of reagent grade. *Xenopus laevis* oocytes were prepared as described previously [14].

### 2.2. Heterologous expression of $\text{Ca}^{2+}$ channel subunits

cDNA clones used were as follows: rabbit brain  $\text{Ca}_v2.1$  ( $\alpha_{1.2.1}$ ; formerly  $\alpha_{1A}$ ) [11], rat brain  $\text{Ca}_v\beta_3$  [12], rat brain  $\text{Ca}_v\alpha_2\delta-1$  [13]. As expression vectors, pcDNA3 (Invitrogen, Carlsbad, CA, USA) was used for  $\text{Ca}_v\alpha_2\delta-1$ , pSP72 (Promega, Madison, WI, USA) for  $\text{Ca}_v2.1$ , and pGEM3 (Promega) for  $\text{Ca}_v\beta_3$ . Linearization of those plasmids, in vitro transcription, cRNA purification, and injection of cRNA were performed as described previously [14]. Injected oocytes were incubated at  $18^\circ\text{C}$  for 4 days with or without GBP before electrophysiological recording.

### 2.3. Electrophysiological recordings and data analyses

$\text{Ba}^{2+}$  currents through  $\text{Ca}^{2+}$  channels were recorded by the two-electrode voltage-clamp (TEV) technique [15] as described previously [14]. Briefly, both voltage and current electrodes were filled with 3 M KCl and had initial tip resistances of 0.5–1.0 M $\Omega$ . The recording chamber was filled with recording solution containing 10  $\text{Ba}^{2+}(\text{OH})_2$  mM. Test potentials were applied for 500 ms from a holding potential of  $-90\text{ mV}$  and the results were analyzed by pClamp 6 (Axon Instruments) and SigmaPlot 4.01 (SPSS Inc., Chicago, IL, USA).

\*Corresponding author. Fax: (1)-319-335 6957.

E-mail address: kevin-campbell@uiowa.edu (K.P. Campbell).

<sup>1</sup> Present address: Department of Physiology, Biophysics and Neuroscience, Cinvestav-IPN, Mexico.

#### 2.4. Partial purification of native $\text{Ca}^{2+}$ channels and GBP treatment

$\text{Ca}^{2+}$  channel complex was partially purified from rabbit skeletal muscle and brain as described previously [14]. Briefly, from 50 mg of microsomes, the  $\text{Ca}^{2+}$  channel complexes were extracted from microsomes with solubilization buffer containing 1% digitonin (Biochemical and Synthetica, Switzerland). Out of the solubilized proteins,  $\text{Ca}^{2+}$  channel complexes were then enriched through wheat germ agglutinin-agarose affinity chromatography (Vector Laboratories, Burlingame, CA, USA). The purified channel complexes were then incubated with or without 1.0 mM GBP at 4°C overnight in a solution containing (in mM) 50 Tris-HCl, pH 7.4, 200 NaCl, a mixture of protease inhibitors, and 0.1% Tween-20 (Fisher Scientific). Similar experiments were also performed with different incubation conditions such as various concentration of GBP, 0.1% digitonin instead of 0.1% Tween-20, and room temperature for 1 h instead of 4°C overnight.

#### 2.5. Antibodies and Western blot analysis

Antibodies, IID5E1, Guinea Pig 1, Sheep 6, and Guinea Pig 16, specific for the  $\text{Ca}^{2+}$  channel subunits,  $\alpha_1$ 1.1,  $\alpha_2$ ,  $\beta_{1a}$ , and  $\gamma_1$ , respectively, have been described previously [16–19]. Western blot analysis was performed as described previously [14].

#### 2.6. Statistical analysis

Each experiment was repeated at least three times using different animals. Data are presented as mean  $\pm$  S.E.M. and the number of oocytes is indicated in figures and text. Probability ( $P$ ) of 0.05 or less was considered significant.

### 3. Results

#### 3.1. Acute treatment with GBP does not affect $\text{Ca}^{2+}$ channel activity

GBP exhibits high-affinity binding activity to the  $\alpha_2\delta$  subunit of  $\text{Ca}^{2+}$  channels; however, the link between GBP binding and channel function remains to be clarified. Though some functional studies have shown a modest inhibitory effect of GBP on neuronal  $\text{Ca}^{2+}$  currents in mammalian cultured cells, most of previous data are based on native currents where the expression of different isoforms of  $\alpha_2\delta$  has produced inconsistent results. We therefore decided to further investigate the actions of GBP on neuronal recombinant  $\text{Ca}^{2+}$  channels expressed in *Xenopus* oocytes using the TEV technique. We first analyzed the effects of the acute application of GBP on the recombinant P/Q-type currents expressed in oocytes after cRNA injection of  $\text{Ca}^{2+}$  channel subunits ( $\alpha_1$ 2.1/ $\beta_3$ / $\alpha_2\delta$ -1). Fig. 1A shows normalized peak  $\text{Ba}^{2+}$  current ( $I_{\text{Ba}}$ ) amplitude levels of two oocytes in response to a depolarizing step to 0 mV from a holding potential of  $-90$  mV plotted against time. The effects of GBP were then tested on  $I_{\text{Ba}}$  elicited by the test potential. As can be seen, GBP at a concentration of 30  $\mu\text{M}$  had no effect on the peak amplitude or in the kinetics of the expressed current (Fig. 1B). Peak current amplitude of the  $\alpha_1$ 2.1/ $\beta_3$ / $\alpha_2\delta$ -1 channels was  $-1952 \pm 497$  nA ( $n=8$ ) and  $-1908 \pm 276$  nA ( $n=9$ ) with or without GBP treatment, respectively. To ensure that the negative result with GBP was not due to a lack of access of GBP to its binding site, distinct  $\text{Ca}^{2+}$  channel blockers were added to the recording chamber. Under these circumstances, current amplitude was again unaffected by the N-type  $\text{Ca}^{2+}$  channel antagonist  $\omega$ -Ctx GVIA. In contrast,  $I_{\text{Ba}}$  was significantly reduced by the specific P/Q-type  $\text{Ca}^{2+}$  channel blocker  $\omega$ -Ctx MVIIC and by a non-specific blocker ( $\text{CdCl}_2$ ) confirming that the recombinant P/Q-type expressed in our system were not sensitive to the acute effects of GBP (Fig. 1A). Lastly, the properties of the current-voltage relation of the  $I_{\text{Ba}}$  elicited by voltage steps from  $-40$  to  $+40$  mV with a

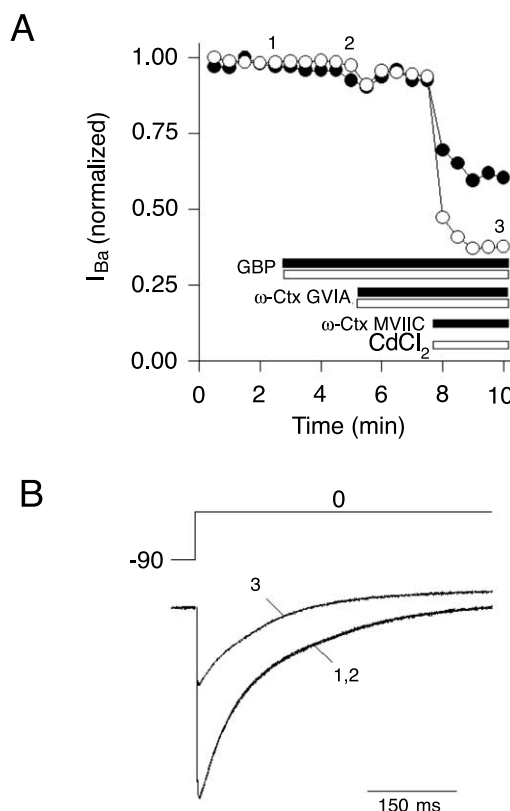


Fig. 1. P/Q-type current in oocytes is insensitive to the acute effects of GBP. A: Representative time courses of block by 200  $\mu\text{M}$   $\text{CdCl}_2$  and 1  $\mu\text{M}$   $\omega$ -Ctx MVIIC of the P/Q-type ( $\alpha_1$ 2.1/ $\beta_3$ / $\alpha_2\delta$ -1) currents expressed in two individual oocytes (open and filled circles). Currents were elicited by stepping from a holding potential of  $-90$  mV to a test potential of 0 mV and normalized to the peak amplitude. Note that in both cases currents were insensitive to the acute application of  $\omega$ -Ctx GVIA (1  $\mu\text{M}$ ) and GBP (30  $\mu\text{M}$ ). B: Representative  $I_{\text{Ba}}$  traces before (1), during application of GBP (2) and after addition of 200  $\mu\text{M}$  of  $\text{CdCl}_2$  (3) to the external recording solution.

holding potential of  $-90$  mV (not shown) in the presence of GBP were very similar to those in the absence of the drug ( $V_{1/2}$  of  $-12.8$  and  $-13.6$  mV, respectively).

#### 3.2. Chronic effect of GBP on $\text{Ca}^{2+}$ channels

Having shown that the acute treatment with GBP does not affect the activity of recombinant  $\text{Ca}^{2+}$  channels, we next examined the potential effects of the chronic exposure to GBP on the fundamental biophysical properties of channels expressed in oocytes. Treatment with 30  $\mu\text{M}$  GBP for 4 days did not seem to have a major effect on peak current amplitude at all voltages tested (not shown); however, it had a significant impact on the waveform. As shown in Fig. 2,  $I_{\text{Ba}}$  recorded from oocytes expressing P/Q-type  $\text{Ca}^{2+}$  channels showed considerable time-dependent inactivation following the peak. To examine a potential effect of GBP treatment on  $\text{Ca}^{2+}$  channel inactivation, the test pulses to  $-10$  and 0 mV eliciting maximum currents were selected to compare the kinetics of inactivation (Fig. 2A). For every trace,  $I_{\text{Ba}}$  was normalized to the peak amplitude and the decay was further analyzed by single exponential functions. The results of this analysis showed that in control cells the current during 500 ms pulses decayed with a time constant ( $\tau$ ) of  $200 \pm 12$  and  $190 \pm 12$  ms at  $-10$  and 0 mV, respectively. As shown in

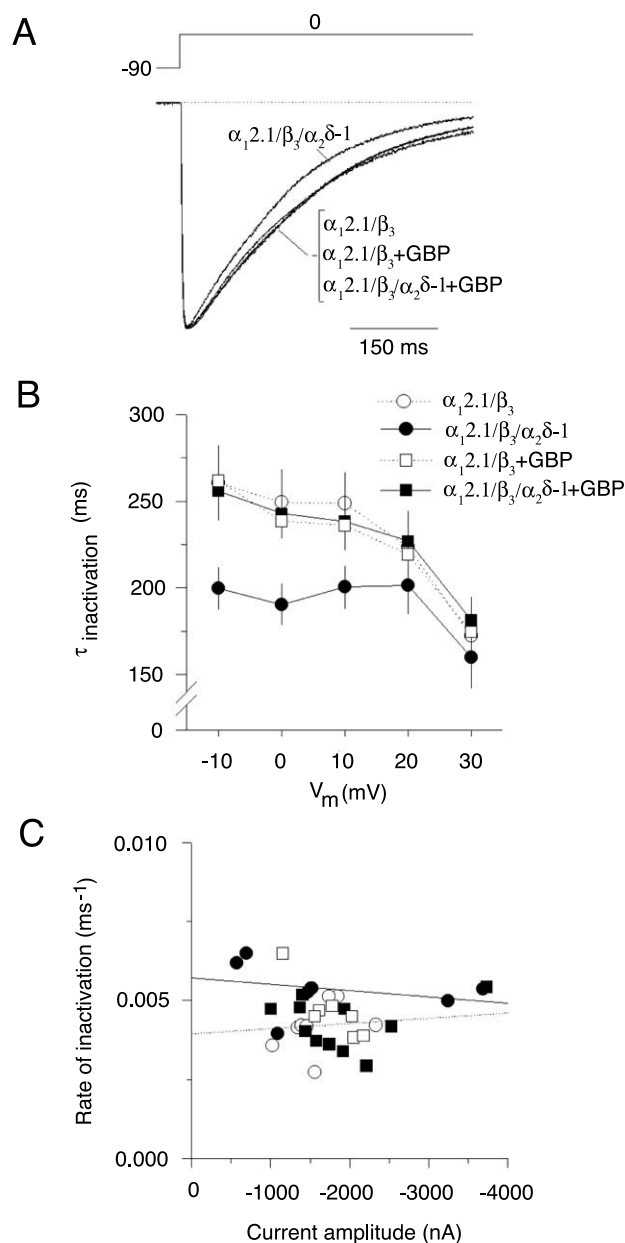


Fig. 2. Evidence for regulation of P/Q-type  $\text{Ca}^{2+}$  channels current in oocytes after chronic treatment with GBP. A: Representative traces of  $I_{\text{Ba}}$  through recombinant P/Q-type channels recorded in the presence and absence of GBP at 0 mV and scaled to the same amplitude. B: A single exponential was used to fit  $I_{\text{Ba}}$  decay to generate the relationship between the time constant,  $\tau$ , of inactivation and the test potential. The curve was obtained by applying 500 ms voltage pulses in the range of from  $-10$  to  $+30$  mV. C: Comparison of the rates of inactivation ( $1/\tau$ ) plotted with respect to peak current at 0 mV in oocytes expressing different  $\text{Ca}_V$  subunit composition:  $\circ$   $\alpha_1 2.1/\beta_3$ ;  $\bullet$   $\alpha_1 2.1/\beta_3/\alpha_2\delta$ ;  $\square$   $\alpha_1 2.1/\beta_3$ +GBP;  $\blacksquare$   $\alpha_1 2.1/\beta_3/\alpha_2\delta$ +GBP.

Fig. 2B, current inactivation in GBP treated cells was significantly slower at all voltages tested (e.g.  $\tau = 256 \pm 13$  and  $249 \pm 14$  ms at  $-10$  and  $0$  mV). Notably, the time course of the currents recorded in cells expressing recombinant channels lacking  $\alpha_2\delta$  was not affected by GBP treatment, suggesting that  $\alpha_2\delta$  is essential for the effect of the drug on channel inactivation (Fig. 2A,B). In addition, one of the concerns when analyzing macroscopic currents in the oocyte system is

that the density of the expressed channels may alter the kinetics of the currents. Fig. 2C summarizes the results obtained in a number of control and GBP treated oocytes from different batches, and expressing a different  $\text{Ca}^{2+}$  channel subunit composition. At  $0$  mV,  $I_{\text{Ba}}$  amplitudes had a six-fold range ( $-571$  to  $-3730$  nA); however, these cells with large differences in channel expression had similar rates of inactivation ( $1/\tau$ ), suggesting that at least over this range of current, the rate of inactivation is independent of the channel density.

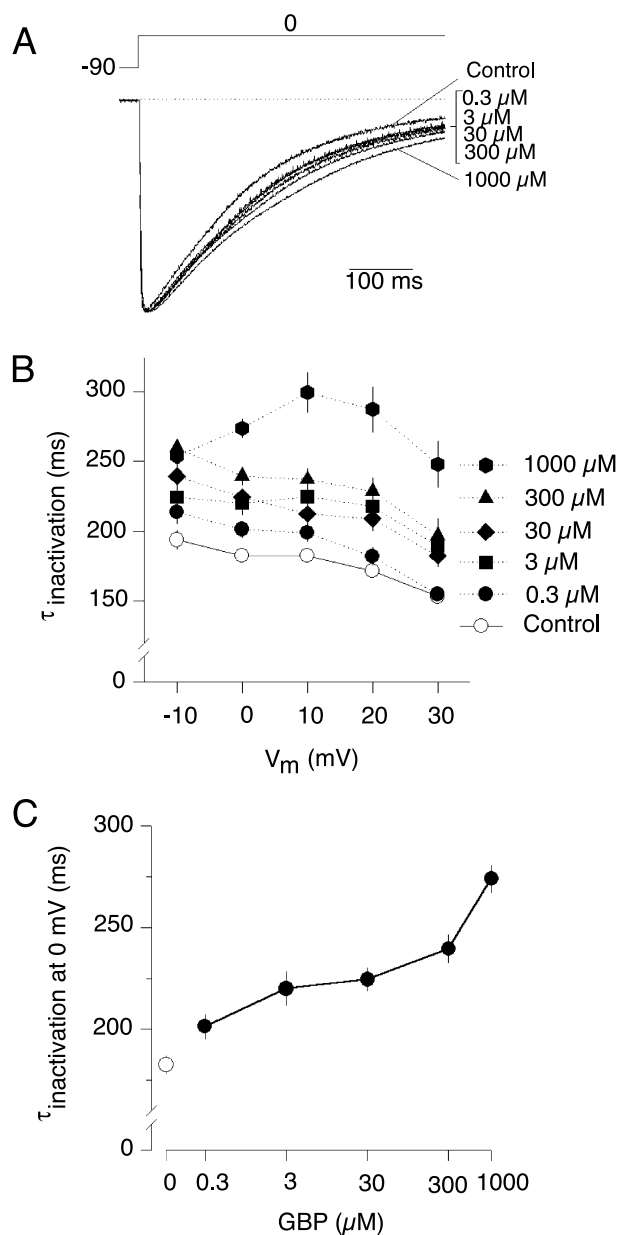


Fig. 3. The decrease in current inactivation by GBP was dose dependent. A: Representative traces of  $I_{\text{Ba}}$  through recombinant P/Q-type  $\text{Ca}^{2+}$  channels ( $\alpha_1 2.1/\beta_3/\alpha_2\delta-1$ ) recorded at  $0$  mV and scaled to the same amplitude. Oocytes were treated for 4 days with GBP as listed. B: Averaged voltage dependence of  $I_{\text{Ba}}$  inactivation kinetics in cells chronically treated with different concentrations of GBP. Time constants were determined by fitting  $I_{\text{Ba}}$  traces sampled in the range of  $-10$  to  $+30$  mV and then plotted as function of test potentials. C: Concentration/response curve for the slowing of  $I_{\text{Ba}}$  inactivation by GBP (mean  $\pm$  S.E.M.,  $n = 6-9$ ). The current was evoked by a voltage pulse from the holding potential of  $-90$  to  $0$  mV.

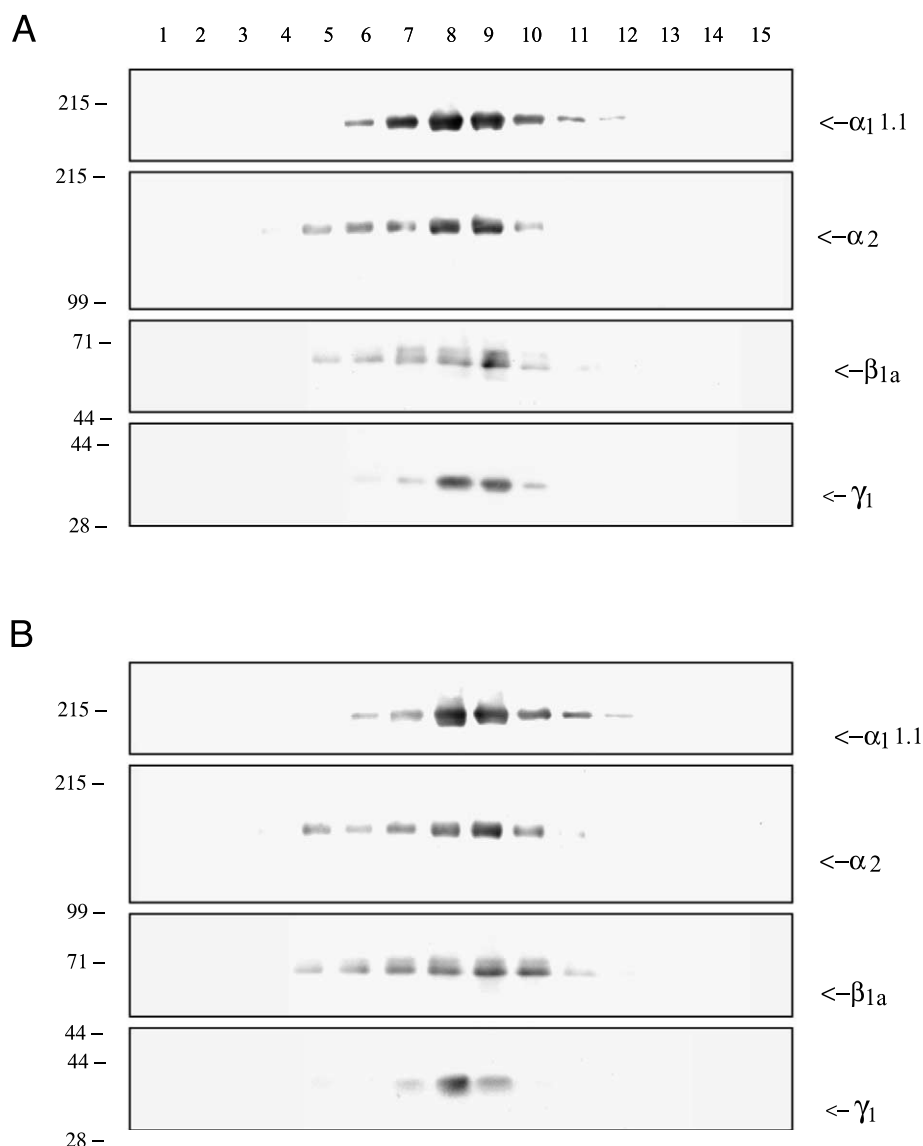


Fig. 4. GBP treatment does not alter the integrity of the Ca<sup>2+</sup> channel complex. A,B: Sucrose gradient fractionation of partially purified skeletal Ca<sup>2+</sup> channels with (B) or without (A) 1 mM GBP treatment. Equal volume of proteins (100  $\mu$ l) were loaded in each lane. After transfer of gels into membranes, the blots were cut and probed separately with affinity-purified antibodies against the subunits of the Ca<sup>2+</sup> channel. The numbers at the top indicate the fraction of the sucrose gradient from top to bottom. The identity of each band is indicated at the right side of panels. Molecular mass standards ( $\times 10^{-3}$ ) are indicated on the left.

The graphs on Fig. 3 show the effect of different concentrations of GBP (300 nM to 1 mM) on the time constant of current inactivation. In Fig. 3A, representative normalized current traces to determine the time course of inactivation are compared at the potential of 0 mV, and the values obtained for the inactivation time constants in different cells are plotted versus membrane voltage in Fig. 3B. The  $\tau$  for  $I_{Ba}$  in control cells was in the range of  $\sim 160$ – $200$  ms and showed no apparent voltage dependence in the  $-10$ – $30$  mV range. Notably, a decrease of the decay kinetics of the current seemed to be an important effect of the drug treatment, as reflected in the concentration/response curve in Fig. 3C, which shows how the time constant for the inactivating component of  $I_{Ba}$  at 0 mV increased as a function of increasing GBP concentrations from a value of  $201 \pm 6$  ms in the cells treated with 300 nM to  $274 \pm 7$  ms in the cells exposed to 1 mM of the drug (Fig. 3C).

### 3.3. GBP seems not to affect the integrity of the Ca<sup>2+</sup> channel complex

Since the inactivation kinetics of GBP treated cells closely resembled that of cells expressing channels lacking  $\alpha_2\delta$ , we hypothesized that GBP binding could cause an alteration in the integrity of the Ca<sup>2+</sup> channel complex. To test this hypothesis, we investigated the potential dissociation of subunits including  $\alpha_2\delta$  from the channel complex through partial purification, GBP treatment (1.0 mM), and sucrose gradient fractionation. Western blot analysis of sucrose gradient fractions with anti- $\alpha_1.1$ ,  $\alpha_2$ ,  $\beta_{1a}$ , and  $\gamma_1$  antibodies showed that the co-sedimentation of all these subunits in fractions 7–10 without GBP treatment (Fig. 4A). Interestingly, as shown in Fig. 4B, the co-sedimentation of Ca<sup>2+</sup> channel subunits was not significantly affected by GBP treatment. None of the Ca<sup>2+</sup> channel subunits including  $\alpha_2\delta$  was apparently dissociated from the complex by the treatment, suggesting that GBP

binding does not disrupt the channel complex. We also tried different incubation conditions of GBP treatment such as various concentrations of the drug, incubation time, and temperature. In none of these experimental conditions, we could observe any appreciable change in the  $\text{Ca}^{2+}$  channel complex integrity by the drug treatment (data not shown). Lastly, similar experiments using partially purified  $\text{Ca}^{2+}$  channel complexes from rabbit brain showed that the treatment with GBP has no effect also on the integrity of native neuronal  $\text{Ca}^{2+}$  channels (data not shown).

#### 4. Discussion

In spite of the demonstrated direct interaction between GBP and the  $\text{Ca}^{2+}$  channel  $\alpha_2\delta$  subunit [10], controversial results regarding the functional effects of the drug on  $\text{Ca}^{2+}$  channels have been reported. No appreciable effects of the acute treatment with GBP on native currents recorded from human hippocampal granule cells [20] and PC12 cells [21] have been found. On the other hand, acute application of GBP has been shown to inhibit L-type  $\text{Ca}^{2+}$  currents in isolated rat neurons [22], mouse skeletal myotubes [23], and neuronal N-type currents in cultured rat dorsal root ganglion neurons [24].

Most of the previous functional assays studying the effects of GBP on  $\text{Ca}^{2+}$  channel activity have been performed after acute exposure to the drug. Nevertheless, previous animal investigations have suggested that GBP should be transported across the plasma membrane of the neurons to exert its effect, and interestingly, the antiepileptic effect of GBP peaked at a time when the brain interstitial level of the drug, as well as its plasma concentration, had declined significantly [25]. Furthermore, previous studies have shown that GBP is actively absorbed by the cells via the system L-amino acid transporter [26]. In this context, the present study focused on testing the potential chronic effects of GBP on neuronal  $\text{Ca}^{2+}$  channel activity.

Our results show that GBP deeply affected the kinetics of channel inactivation after chronic exposure to the drug. Notably, several studies have shown that alteration in the inactivation properties of  $\text{Ca}^{2+}$  channels is an important mechanism for  $\alpha_2\delta$  regulation of ionic current [27,28]. In addition, conversely to what happens with the acute GBP treatment where no alterations in the activity of the channels was observed, the data of the chronic treatment may be better explained by an intracellular interaction between  $\alpha_2\delta$  and GBP during the process of channel assembly, possibly modifying the association of the auxiliary subunit with the channel complex. Therefore, by using partially purified  $\text{Ca}^{2+}$  channels, we tested whether GBP could alter the integrity of the native  $\text{Ca}^{2+}$  channel complex. However, as shown in Fig. 4, none of the  $\text{Ca}^{2+}$  channel subunits including  $\alpha_2\delta$  was dissociated from the complex already formed by the overnight treatment with GBP. Together, the electrophysiological and biochemical analyses in this study suggest that the chronic effects of GBP on the inactivation kinetics of  $\text{Ca}^{2+}$  channel could be explained by an alteration of the  $\alpha_2\delta$  actions on the properties of the  $\text{Ca}^{2+}$  channels possibly by the induction of a conformational change in the channel, although this alteration might not be large enough to cause the dissociation of  $\alpha_2\delta$  from the channel complex.

Our results, however, do not rule out the presence of acute effects of GBP on neuronal  $\text{Ca}^{2+}$  channels. Acute GBP treat-

ment might introduce subtle alterations on the properties of the channels that are not apparent under some experimental conditions or are not easily detected in large currents such as those expressed in the oocytes system. Therefore, a combination of intracellular and extracellular interaction (after chronic and acute treatment, respectively) between  $\alpha_2\delta$  and GBP could be seen as complementary mechanisms of channel regulation. In addition, we could speculate that the change of inactivation kinetics by chronic treatment with GBP could not be observed in cultured mammalian cells [21] due to the fact that they are generally kept in L-glutamine-containing culture media. The concentration of L-glutamine could be high enough to saturate the amino acid transporter system in the cells and GBP may not be efficiently translocated to the interior of the cells. Actually, it was recently reported that GBP could compete with some amino acids for the transportation by the L-type amino acid transporter 1 [29] that has been shown to be a pathway for concentrating GBP intracellularly [26]. In contrast, oocytes are kept in culture in an amino acid-free medium. In this case, the availability of the transporter would be high and the translocation of the drug to the cytoplasm would occur normally. Furthermore, the discrepancy between the data obtained in mammalian cells and oocytes might be also explained by the fact that only  $\alpha_2\delta$ -1 and  $\alpha_2\delta$ -2 isoforms (but not  $\alpha_2\delta$ -3) bind to GBP [30,31] but all three isoforms are expressed differentially in the brain [32]. This may indicate the presence of an exquisite sensitivity to GBP that is dependent on neuronal type and/or localization as well as in  $\text{Ca}^{2+}$  channel  $\alpha_2\delta$  isoform expression.

Lastly, being GBP the first ligand that interacts with the  $\alpha_2\delta$  subunit, demonstration of a direct regulation of neuronal  $\text{Ca}^{2+}$  channels by the drug may be important to the understanding of its antiepileptic properties. Experimental evidence indicates that the epileptic discharges, generated when a physiological tonic mode of neuronal firing shifts to a burst-firing mode [33], are produced by aberrant thalamocortical oscillations [34]. P/Q-type  $\text{Ca}^{2+}$  channels are present and play a role in neurotransmitter release in thalamic neurons [35] providing a potential mechanistic link between  $\text{Ca}^{2+}$  currents and thalamocortical circuits. Interestingly, a reduction of excitatory synaptic transmission in the thalamus of epileptic mice has been documented [36] as a result of reduced  $\text{Ca}^{2+}$  influx, and it was proposed that a net enhanced GABAergic input in thalamocortical neurons may synchronize them into a burst-firing mode. In addition, several studies have revealed that some paroxysmal disorders including epilepsy are linked to  $\text{Ca}^{2+}$  channel mutations leading to reduced  $\text{Ca}^{2+}$  currents in cerebellar neurons of mice and humans [2,3]. Therefore, it is tempting to speculate that one of the consequences of the chronic treatment with GBP may be to revert this increasing  $\text{Ca}^{2+}$  influx by slowing down neuronal  $\text{Ca}^{2+}$  channel inactivation, though further studies will be needed to unveil the precise molecular mechanisms of GBP antiepileptic action.

**Acknowledgements:** M.G.K. is a recipient of pre-doctoral fellowship from The Epilepsy Foundation of America. Work in the laboratory of R.F. is supported by Conacyt (grant 31735-N) and TWAS (grant 263RG/BIO/LA). K.P.C. is an Investigator of the Howard Hughes Medical Institute.

#### References

- [1] Dichter, M.A. (1994) *Epilepsia* 35 (Suppl. 4), S51–S57.



- [2] Felix, R. (2000) *J. Med. Genet.* 37, 729–740.
- [3] Felix, R. (2002) *Cell. Mol. Neurobiol.* 22, 103–120.
- [4] Walker, D. and De Waard, M. (1998) *Trends Neurosci.* 21, 148–154.
- [5] Felix, R. (1999) *Receptor Channel* 6, 351–362.
- [6] MacDonald, R.L. and Kelly, K.M. (1994) *Epilepsia* 35 (Suppl. 4), S41–S50.
- [7] McLean, M.J. (1995) *Gabapentin Epilepsia* 36 (Suppl. 2), S73–S86.
- [8] Suman-Chauhan, N., Webdale, L., Hill, D.R. and Woodruff, G.N. (1993) *Eur. J. Pharmacol.* 244, 293–301.
- [9] Wamil, A.W. and McLean, M.J. (1994) *Epilepsy Res.* 17, 1–11.
- [10] Gee, N.S., Brown, J.P., Dissanayake, V.U.K., Offord, J., Thurlow, R. and Woodruff, G.N. (1996) *J. Biol. Chem.* 271, 5768–5776.
- [11] Mori, Y., Friedrich, T., Kim, M.-S., Mikami, A., Nakai, J., Ruth, P., Bosse, E., Hofmann, F., Flockerzi, V., Furuichi, T., Mikoshiba, K., Imoto, K., Tanabe, T. and Numa, S. (1991) *Nature* 350, 398–402.
- [12] Castellano, A., Wei, X., Birnbaumer, L. and Perez-Reyes, E. (1993) *J. Biol. Chem.* 268, 3450–3455.
- [13] Kim, H.L., Kim, H., Lee, P., King, R.G. and Chin, H. (1992) *Proc. Natl. Acad. Sci. USA* 89, 3251–3255.
- [14] Kang, M.G., Chen, C.C., Felix, R., Letts, V.A., Frankel, W.N., Mori, Y. and Campbell, K.P. (2001) *J. Biol. Chem.* 276, 32917–32924.
- [15] Stuhmer, W. (1992) *Methods Enzymol.* 207, 319–339.
- [16] Leung, A., Imagawa, T. and Campbell, K.P. (1987) *J. Biol. Chem.* 262, 7943–7946.
- [17] Ahern, C.A., Powers, P.A., Biddlecome, G.H., Roethe, L., Vallejo, P., Mortenson, L., Strube, C., Campbell, K.P., Coronado, R. and Gregg, R.G. (2001) *BMC Physiol.* 1, 8.
- [18] Pragnell, M., Sakamoto, J., Jay, S.D. and Campbell, K.P. (1991) *FEBS Lett.* 291, 253–258.
- [19] Sharp, A.H. and Campbell, K.P. (1989) *J. Biol. Chem.* 264, 2816–2825.
- [20] Schumacher, T.B., Beck, H., Steinhauser, C., Schramm, J. and Elger, C.E. (1998) *Epilepsia* 39, 355–363.
- [21] Vega-Hernández, A. and Felix, R. (2002) *Cell. Mol. Neurobiol.* 22, 185–190.
- [22] Stefani, A., Spadoni, F., Giacomini, P., Lavaroni, F. and Bernardi, G. (2001) *Epilepsy Res.* 43, 239–248.
- [23] Alden, K.J. and Garcia, J. (2001) *J. Pharmacol. Exp. Ther.* 297, 727–735.
- [24] Sutton, K.G., Martin, D.J., Pinnock, R.D., Lee, K. and Scott, R.H. (2002) *Br. J. Pharmacol.* 135, 257–265.
- [25] Welty, D.F., Schielke, G.P., Vartanian, M.G. and Taylor, C.P. (1993) *Epilepsy Res.* 16, 175–181.
- [26] Su, T., Lunney, E., Campbell, G. and Oxender, D.L. (1995) *J. Neurochem.* 64, 2125–2131.
- [27] Qin, N., Olcese, R., Stefani, E. and Birnbaumer, L. (1998) *Am. J. Physiol.* 274 (5 Pt 1), C1324–C1331.
- [28] Jones, L.P., Wei, S.K. and Yue, D.T. (1998) *J. Gen. Physiol.* 112, 125–143.
- [29] Uchino, H., Kanai, Y., Kim, K., Wempe, M.F., Chairoungdua, A., Morimoto, E., Anders, M.W. and Endou, H. (2002) *Mol. Pharmacol.* 61, 729–737.
- [30] Marais, E., Klugbauer, N. and Hofmann, F. (2001) *Mol. Pharmacol.* 59, 1243–1248.
- [31] Gong, H.C., Hang, J., Kohler, W., Li, L. and Su, T.Z. (2001) *J. Membr. Biol.* 184, 35–43.
- [32] Klugbauer, N., Lacinova, L., Marais, E., Hobom, M. and Hofmann, F. (1999) *J. Neurosci.* 19, 684–691.
- [33] Steriade, M. and Llinás, R.F. (1988) *Physiol. Rev.* 68, 649–742.
- [34] Snead 3rd, O.C. (1995) *Ann. Neurol.* 37, 146–157.
- [35] Kammermeier, P.J. and Jones, S.W. (1997) *J. Neurophysiol.* 77, 465–475.
- [36] Caddick, S.J., Wang, C., Fletcher, C.F., Jenkins, N.A., Copeland, N.G. and Hosford, D.A. (1999) *J. Neurophysiol.* 81, 20066–20074.