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Gabapentin inhibits presynaptic Ca^{2+} influx and synaptic transmission in rat hippocampus and neocortex

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Abstract

Gabapentin is a widely used drug with anticonvulsant, antinociceptive and anxiolytic properties. Although it has been previously shown that Gabapentin binds with high affinity to the $\alpha_2\delta$ subunit of voltage-operated Ca^{2+} channels (VOCC), little is known about the functional consequences of this interaction. Here, we investigated the effect of Gabapentin on VOCCs and synaptic transmission in rat hippocampus and neocortex using whole-cell patch clamp and confocal imaging techniques. Gabapentin (100–300 μM) did not affect the peak amplitude or voltage-dependency of VOCC currents recorded from either dissociated or in situ neocortical and hippocampal pyramidal cells. In contrast, Gabapentin inhibited K^+-evoked increases in $[Ca^{2+}]$ in a subset of synaptosomes isolated from rat hippocampus and neocortex in a dose-dependent manner, with an apparent half-maximal inhibitory effect at ~ 100 nM. In hippocampal slices, Gabapentin (300 μM) inhibited the amplitude of evoked excitatory- and inhibitory postsynaptic currents recorded from CA1 pyramidal cells by 30–40%. Taken together, the results suggest that Gabapentin selectively inhibits Ca^{2+} influx by inhibiting VOCCs in a subset of excitatory and inhibitory presynaptic terminals, thereby attenuating synaptic transmission.

Keywords: Gabapentin; Ca^{2+} channel, voltage-operated; Patch clamp; Ca^{2+} imaging

1. Introduction

Gabapentin (1-(aminomethyl)cyclohexane acetic acid, Neurontin®) is an anticonvulsant used as add-on therapy for the treatment of epileptic seizures. In addition, Gabapentin exhibits antinociceptive and anxiolytic effects (Macdonald and Kelly, 1993; Morris, 1999). Despite the plethora of literature on the therapeutic efficacy of Gabapentin, there is no consensus on its molecular mechanism of action (Taylor et al., 1998). A single high affinity (K_D ~ 35 nM) binding site for [^{3}H]Gabapentin has been described in the outer layers of the cortex, and the dendritic regions of the hippocampal CA1 area (Suman-Chauhan et al., 1993; Hill et al., 1993), and it has been suggested that this binding site corresponds to the auxiliary $\alpha_2\delta$ subunit of voltage-operated Ca^{2+} channels (VOCCs; Gee et al., 1996). However, there are conflicting reports on the functional effects of Gabapentin on VOCCs.

It has been reported that Gabapentin inhibits high voltage-activated Ca^{2+} channels in dissociated rat cortical pyramidal cells, medium spiny striatal neurons and large globus pallidus cells (Stefani et al., 1998, 2001) and in isolated dorsal root ganglion neurones (Alden and Garcia, 2001; Sutton et al., 2002). In contrast, Rock et al. (1993) reported no modulation of VOCCs by Gabapentin (up to 1 mM) in rat nodose and dorsal root ganglion neurons. Moreover, Gabapentin does not inhibit VOCCs in cardiac myocytes (Alden and Garcia, 2001) and in hippocampal granule cells from patients with temporal lobe epilepsy (Schumacher et al., 1998).

In contrast to the unequivocal effects of Gabapentin on postsynaptic VOCCs, there is now substantial evidence that Gabapentin inhibits presynaptic VOCCs and attenuates neurotransmitter release from central nerve terminals (Dooley et al., 2000; Fink et al., 2000; Meder and Dooley, 2000; Fink et al., 2002). In addition, it has been reported that

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Gabapentin inhibits the frequency, but not the amplitude, of miniature excitatory postsynaptic currents (EPSCs) in the superficial lamina of the spinal cord dorsal horn (Shimoyama et al., 2000). These results suggest a presynaptic site of action for Gabapentin.

In this study, we compared the effect of Gabapentin on pre- and postsynaptic Ca^{2+} influx in rat hippocampus and neocortex. In addition, we investigated the effect of Gabapentin on synaptic transmission in hippocampus. Parts of this study have appeared as abstract (van Hooft et al., 2000).

2. Materials and methods

2.1. Dissociated cells and slices

Dissociated cells and slices were prepared as described before (Vreugdenhil and Wadman, 1992). Transverse or saggital hippocampal and neocortical slices (250 μm) from young adult male Wistar rats were cut on either a tissue chopper or a vibroslicer. For the preparation of dissociated cells, subslices of areas CA1, CA3 and neocortex were incubated (1 h for CA1 and CA3, 30 min for neocortex) in dissociation solution containing (in mM): NaCl (120), KCl (5), $CaCl_2$ (1), $MgCl_2$ (1), $NaHCO_3$ (10), glucose (25), trypsin (bovine pancreas, type XI, 1 mg/ml), continuously bubbled with 95% O_2/5% CO_2 at 31 °C. Before use, 2–3 tissue pieces were mechanically triturated using fire-polished Pasteur pipettes. For in situ patch clamp experiments, slices were allowed to recover for 1 h at 31 °C in artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl (124), KCl (3.5), $CaCl_2$ (2), $MgSO_4$ (1.5), $NaHCO_3$ (25), NaH_2PO_4 (1.25), D-glucose (10), continuously bubbled with 95% O_2/5% CO_2. Slices were subsequently kept at room temperature until use.

2.2. Electrophysiology

VOCCs in dissociated cells were recorded as described before (Vreugdenhil and Wadman, 1992). Cells were continuously superfused with external solution containing (in mM): NaCl (110), KCl (5), $CaCl_2$ (5), $MgCl_2$ (1), HEPES (10), D-glucose (25), CsCl (5), tetraethylammonium-Cl (10), 4-aminopyridine (5), tetrodotoxin (0.0005), pH = 7.4 (adjusted with NaOH). For recording of VOCCs in slices, pyramidal cells in area CA1 of hippocampal and layer 5 of neocortical slices were visualized using video microscopy with Hoffman modulation contrast (Faas et al., 1996). Slices were continuously superfused with ACSF, supplemented with the following blockers to isolate VOCCs (in mM): CsCl (5), tetraethylammonium-Cl (10), 4-aminopyridine (5), tetrodotoxin (0.0005). Patch pipettes were pulled from borosilicate glass and had resistances of 2.5–4 MΩ when filled with internal solution containing (in mM): CsF (100), $CaCl_2$ (0.5), $MgCl_2$ (2), EGTA (10), HEPES (10), tetraethylammonium-Cl (20), phosphocreatine (20), phosphocreatine kinase (50 u/ml), MgATP (2), NaGTP (0.1), leupeptin (0.1), pH = 7.3 (adjusted with CsOH). Cells were whole-cell voltage clamped (holding potential −70 mV) using an Axopatch 200B amplifier controlled by an Atari computer and custom-made interface and software. Series resistance ranged from 8 to 15 MΩ, and was compensated for 70–80%. VOCC currents were evoked by 500-ms step depolarizations from a 3-s hyperpolarizing prepulse. Currents were low-pass filtered at 1 kHz and sampled at 2 kHz. Drugs were delivered via the bath solution.

Evoked excitatory- and inhibitory postsynaptic currents (EPSCs and IPSCs) were recorded in hippocampal CA1 pyramidal cells. Slices were continuously superfused with ACSF. Patch pipettes were pulled from borosilicate glass and had resistances of 2.5–4 MΩ when filled with internal solution containing (in mM): K-gluconate (140), $MgCl_2$ (2), EGTA (5), HEPES (10), pH = 7.3 (adjusted with KOH). Cells were whole-cell voltage clamped using an EPC-9 amplifier (HEKA Electronic, Lambrecht, Germany) at a holding potential of −90 mV or 0 mV, resulting in inward EPSCs and outward IPSCs, respectively. EPSCs and IPSCs were evoked by stimulation of the Schaffer collaterals using a bipolar stainless-steel electrode (stimulation 100 μs at 50–200 μA). To test the postsynaptic responsiveness of the cells, 100 μM glutamate or 100 μM GABA was applied via a picospritzer (100 ms, 5–15 psi) to the dendritic region of hippocampal CA1 pyramidal cells. Correct application of the ligands was visually monitored by including Fast Green (1 mg/ml) in the picospritzer pipette. Signals were low-pass filtered at 2 kHz and sampled at 4 kHz. Drugs were delivered via the bath solution.

2.3. Confocal imaging of synaptosomes

Synaptosomes were isolated from hippocampi and cerebral cortices of adult male Sprague–Dawley rats (Taconic Farms) according to the method described by Dunkley et al. (1986). In brief, rats were decapitated and their brains removed into ice-cold 0.32 M sucrose following a protocol approved by the MCP Hahnemann Institutional Animal Care and Use Committee. Using standard landmarks, hippocampi and cerebral cortices were dissected and then homogenized in ice-cold 0.32 M sucrose using a glass-Teflon tissue grinder. The homogenized tissue was differentially centrifuged to obtain a P_2 fraction. The resulting pellet was resuspended and applied onto step-gradients of Percoll in sucrose/EDTA (pH 7.4). The highly enriched synaptosomal interface was collected and washed with oxygenated HEPES-buffered saline (HBS) with the composition (in mM): NaCl (142), KCl (2.4), K_2HPO_4 (1.2), $MgCl_2$ (1), D-glucose (5), HEPES (10), pH = 7.4 (adjusted with NaOH).

Purified hippocampal or cerebral cortical synaptosomes were loaded with the fluorescent Ca^{2+} indicator fluo-3 by incubating with the acetoxymethyl ester derivative of the dye in HBS at 5 μM for 30 min at 37 °C and were then imaged essentially as previously described (Nichols and

Mollard, 1996). In brief, the fluo-3-loaded synaptosomes were washed with HBS containing 1 mM Ca^{2+}, plated onto Cell-Tak-coated 15-mm circular coverslips, and then mounted into a Warner RC-20 closed perfusion chamber (36 μl exchange volume). Preparations were maintained under constant perfusion at 3–5 ml/min with HBS containing 1 mM Ca^{2+}. Imaging was performed using a Bio-Rad MRC-600 argon/krypton laser-scanning confocal system attached to a Zeiss Axiovert 135M inverted microscope or a Nikon PCM 2000 laser-scanning confocal imaging system attached to a Nikon Diaphot inverted microscope. Changes in fluorescence were monitored in response to excitation at 488 nm, collecting images at 4-s intervals. After collecting five images as baseline, stimulation was initiated by rapid solution switching via an attached manifold (~ 0.7 s for solution exchange). Resultant digitized (eight-bit) images were analyzed using OPTIMAS image analysis software (Optimas), quantifying the fluorescence intensities associated with individual synaptosomes as ratios of intensities over the collected time sequence (F) to the fluorescence intensity observed at t_0 (F_0). The intensity ratios (F/F_0) over time correspond to the relative (uncalibrated) changes in $[Ca^{2+}]_i$ in the individual synaptosomes. All time-series were corrected for photobleaching, which was typically <3%, based on the baseline.

To examine the effects of Gabapentin, a sequential stimulation protocol was performed as follows. Initial responses to stimulation with 50 mM K^+ were acquired. The preparation was washed without (control) or with various concentrations of Gabapentin. Finally, the preparation was restimulated with 50 mM K^+ without (control) or coapplied with the same concentration of Gabapentin as used during the wash to acquire a second response. Typically, the second control Ca^{2+} response ranges from 85 to 115% (S.D.) of the initial control response. Thus, the threshold for sensitivity for Gabapentin was set at 85% of the initial response. Though this criterion may lead to a small underestimate of the proportion of synaptosomes sensitive to Gabapentin, it increases the confidence level.

2.4. Data analysis

VOCCs were analyzed as previously described (Vreugdenhil and Wadman, 1992; Faas et al., 1996; Kortekaas and Wadman, 1997). Activation curves were fitted to a Goldman–Hodgkin–Katz current–voltage equation, with a voltage-dependent permeability according to a Boltzmann function, and inactivation curves were fitted to a Boltzmann function (Kortekaas and Wadman, 1997). All results are expressed as means ± S.E.M. of n independent experiments and compared using Student's t-test or one-way analysis-of-variance (ANOVA), where appropriate. All experiments were performed at room temperature. Gabapentin was generously provided by Parke-Davis Pharmaceuticals (Hoofddorp, The Netherlands). All other chemicals were obtained from either Merck or Sigma.

3. Results

3.1. Gabapentin does not inhibit postsynaptic Ca^{2+} currents

Dissociated pyramidal neurons from hippocampal CA1 and CA3 areas and neocortex were whole-cell voltage clamped, and VOCCs were evoked by depolarization. Activation- and inactivation curves were similar to those described previously (Fig. 1A; Vreugdenhil and Wadman, 1992), and were not affected in the presence of 100 μM Gabapentin (Table 1). Fig. 1C shows that the application of 100 μM Gabapentin did not affect the peak amplitude of the VOCC recorded from dissociated hippocampal CA1 pyramidal neurons. In contrast, application of 100 μM Cd^{2+} rapidly and completely blocked the VOCC (Fig. 1C). The peak amplitude of VOCCs recorded from dissociated pyramidal neurons from hippocampal CA1 and CA3 areas and neocortex was not affected by 100 μM Gabapentin (Fig. 1D, solid bars). Even at a concentration of 300 μM, Gabapentin did not affect the peak amplitude of VOCCs in dissociated hippocampal CA1 pyramidal neurons (mean normalized peak amplitude: 101.2 ± 2.1% of control, $n=5$).

It was previously demonstrated that [^{3}H]Gabapentin binding sites are located in the dendritic regions of both hippocampus and neocortex (Suman-Chauhan et al., 1993; Hill et al., 1993). Because dissociated neurons are likely to have lost most of their dendrites during the dissociation procedure, we tested the effect of Gabapentin on VOCCs recorded from intact neurons in slices. In addition to high voltage-activated VOCCs as recorded from dissociated neurons, VOCCs recorded from neurons in slices contain a low voltage-activated component, which mainly arises from channels located at dendrites (Fig. 1B; Karst et al., 1993). Application of 100 μM Gabapentin did not affect the peak amplitude of high voltage-activated VOCCs recorded from hippocampal CA1 and neocortical pyramidal neurons in slices (Fig. 1D). The mean normalized peak amplitude in the presence of 100 μM Gabapentin of low voltage-activated VOCCs recorded from hippocampal and neocortical slices amounted to 100.2 ± 3.4% ($n=4$) and 99.3 ± 2.5% ($n=6$) of control, respectively. The combined results suggest that postsynaptic VOCCs are not likely to be the target of Gabapentin.

3.2. Gabapentin inhibits presynaptic Ca^{2+} influx

As an alternative hypothesis, the reported [^{3}H]Gabapentin binding sites in the dendritic regions of hippocampus and neocortex (Suman-Chauhan et al., 1993; Hill et al., 1993) could be located on presynaptic nerve terminals. Therefore, we tested the effect of Gabapentin on K^+-evoked Ca^{2+} influx in individual isolated synaptosomes using Ca^{2+} imaging. Rapid application of elevated extracellular K^+ concentration induces the immediate depolarization of synaptosomes, which, in turn, evokes Ca^{2+} entry via voltage-sensitive Ca^{2+} channels (see, for example, Suszkiw et al.,

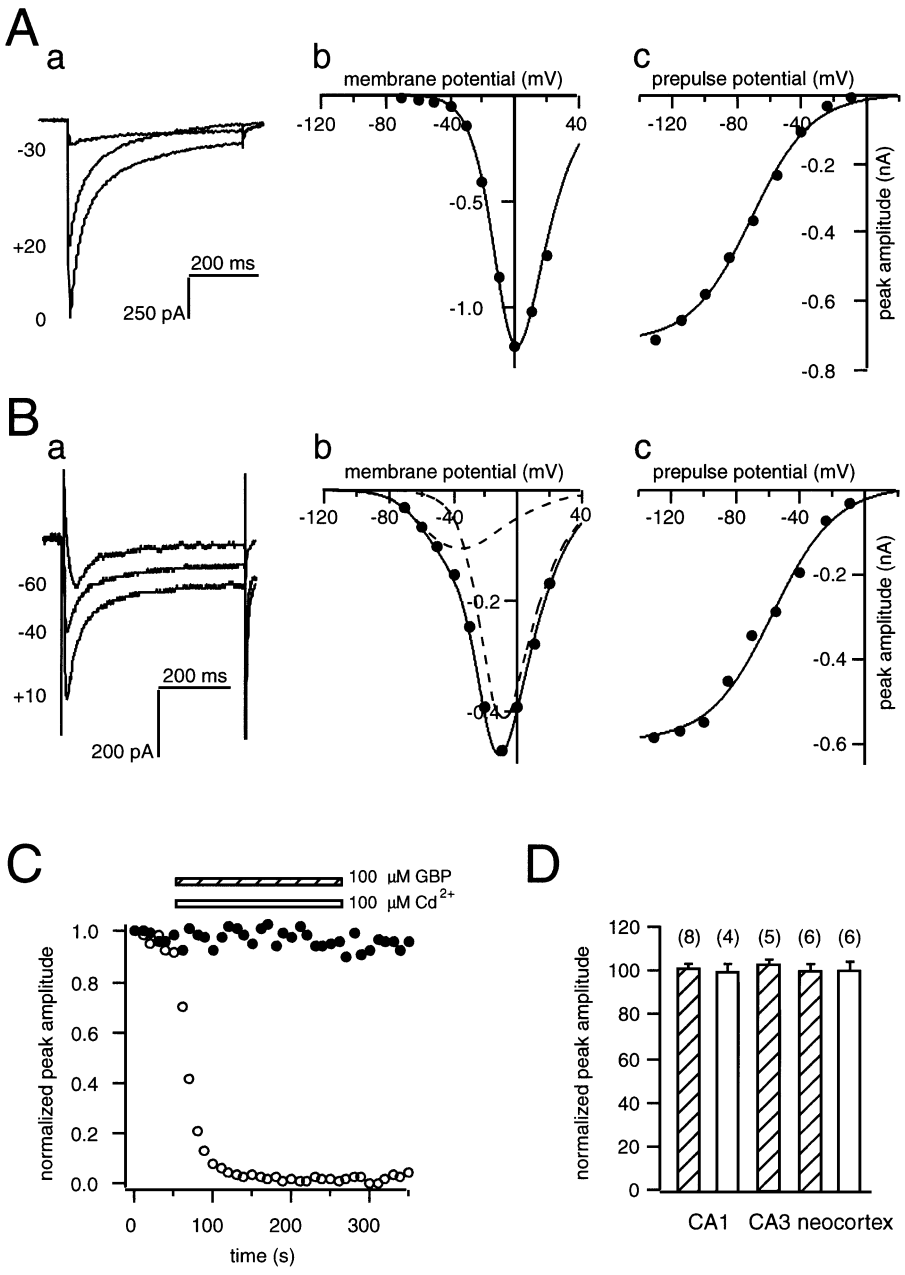

Fig. 1. Gabapentin does not affect VOCCs in hippocampal and neocortical pyramidal neurons. VOCCs recorded from dissociated hippocampal CA1 pyramidal neurons (A) and hippocampal CA1 pyramidal neurons in slices (B). (Aa,Ba) Example of VOCCs evoked by depolarization from a 3-s prepulse at − 120 mV to the indicated membrane potential. (Ab,Bb) *I*–*V* curve of activation, evoked by depolarization from − 70 to 20 mV with 10-mV increments. The *I*–*V* curve was fitted to a Goldman–Hodgkin–Katz current–voltage equation, with a voltage-dependent permeability according to a Boltzmann function (solid line). The *I*–*V* curve recorded from neurons in slices was fitted with a double current–voltage equation. The two separate components, indicating the low- and high voltage-activated VOCCs, are shown (dashed lines). (Ac,Bc) *I*–*V* curves of inactivation evoked by a depolarization to − 10 mV from a 3-s prepulse at the indicated membrane potentials. (C) Normalized peak amplitudes of VOCCs in dissociated hippocampal CA1 pyramidal neurons during application of 100 µM Gabapentin (GBP, hatched bar, solid circles) or 100 µM Cd^{2+} (open bar, open circles). Currents were evoked by a depolarization to − 10 mV from a 3-s prepulse at − 120 mV every 10 s. (D) Summary of the effect of 100 µM Gabapentin on high voltage-activated VOCCs recorded from the cell types indicated. Hatched bars refer to measurements in dissociated cells, and open bars refer to measurements in slices. Numbers above the bars indicate the number of cells. Values are not significantly different from 100%.

1989). Typically, depolarization causes the intrasynaptosomal levels of Ca^{2+} to rise to an initial peak, followed by a maintained plateau phase, as shown in Fig. 2. It has been shown previously that the peak of the Ca^{2+} response is sensitive to N-type Ca^{2+} channel blockers and the plateau is sensitive to P/Q-type Ca^{2+} channel blockers (Suszkiw et al., 1989; Ronde and Nichols, 1998; Dougherty and Nichols, unpublished observations). Gabapentin was found to inhibit substantially both the peak and the plateau of the depolarization-evoked changes in $[Ca^{2+}]_i$ in hippocampal synapto-

Table 1
Gabapentin does not affect voltage-dependent activation and inactivation of VOCCs in dissociated hippocampal CA1 pyramidal neurons

		Control	+100 μM Gabapentin
Activation	V_h	−4.8 ± 1.8	−4.5 ± 1.4
	V_c	−8.6 ± 0.9	−10.2 ± 0.7
Inactivation	V_h	−80.1 ± 7.0	−81.8 ± 5.8
	V_c	13.3 ± 5.1	15.4 ± 3.5

Activation curves were fitted to a Goldman–Hodgkin–Katz current–voltage equation, with a voltage-dependent permeability according to a Boltzmann function, and inactivation curves were fitted to a Boltzmann function. Estimates (in mV) of V_h (potential of half-maximal activation) and V_c (proportional to the slope of the curve) in the presence and absence of 100 μM Gabapentin do not differ significantly ($n=4$).

somes, with apparent half-maximum effect at ~100 nM and maximum inhibition occurring at 3 μM (Fig. 2). At 100 μM Gabapentin, the K^+-evoked responses were inhibited to 22–40% of controls. Similar results were observed for synaptosomes obtained from rat cerebral cortices. At 100 μM Gabapentin, Ca^{2+} responses in 12 out of 33 cortical synaptosomes were inhibited to 32 ± 9% of controls. At 300 μM Gabapentin, Ca^{2+} responses in 13 out of 18 cortical synaptosomes were inhibited to 38 ± 5% of controls. Because Gabapentin inhibited both the peak and the plateau phase of the Ca^{2+} response, this suggests that both N- and P/Q-type VOCCs were involved.

3.3. Synaptic transmission is attenuated by Gabapentin

The combined data suggest that Gabapentin preferentially inhibits presynaptic Ca^{2+} influx, but not postsynaptic VOCCs in hippocampus and neocortex. Given the pivotal role of presynaptic Ca^{2+} influx in the release of neurotransmitter, we tested whether Gabapentin affects synaptic

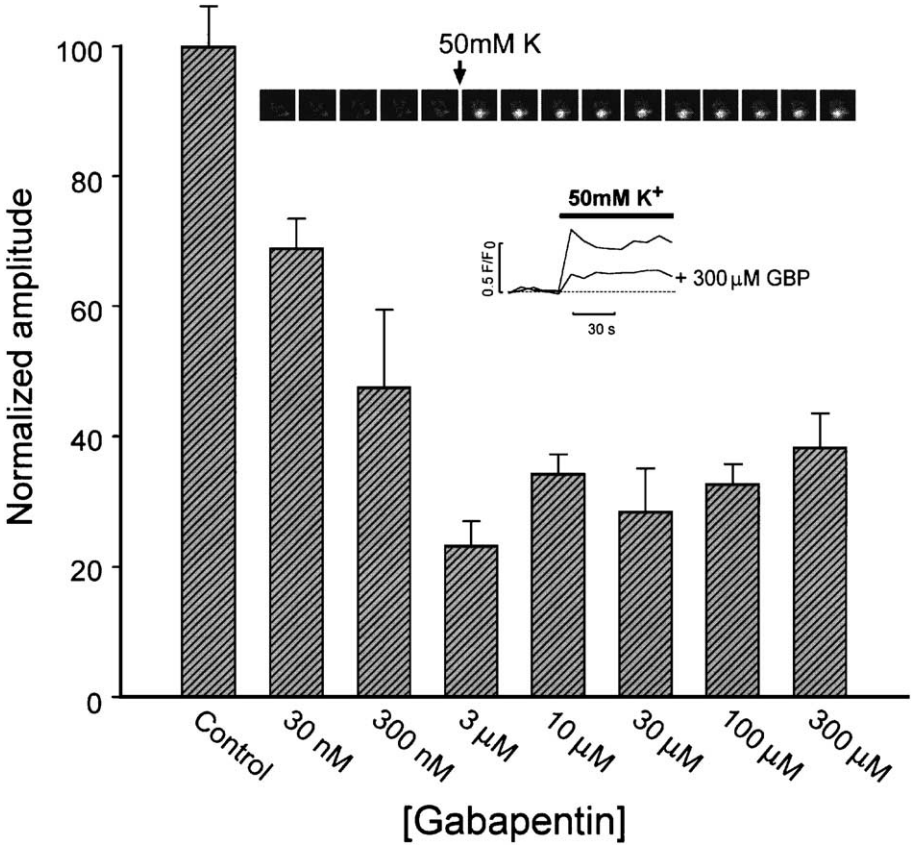

Fig. 2. Inhibition of K^+-evoked changes in $[Ca^{2+}]_i$ in hippocampal synaptosomes by various concentrations of Gabapentin. Synaptosomes isolated from hippocampi and loaded with the Ca^{2+} indicator fluo-3 were stimulated with 50 mM K^+ to obtain an initial response. The preparation was then washed for 10 min with HBS without (control) or with various concentrations of Gabapentin. The preparation was re-stimulated with 50 mM K^+ in the absence (control) or presence of Gabapentin, imaging the same synaptosomal field. Relative fluorescence intensities (F/F_0) associated with individual synaptosomes were quantified from the digitized images. The peak amplitudes of each synaptosome response in the presence of various concentrations of Gabapentin were normalized, as percents, to the respective peak amplitudes of the response to the first stimulation in the absence of Gabapentin. Normalized values are means ± s.e.m. (five experiments). As described in Methods, the threshold for sensitivity for Gabapentin evident in the second response was set at 85% of the initial response, based on 15% S.D. for the sequential control responses. Fractions of synaptosomes displaying sensitivity to Gabapentin (out of the total number examined) were: Gabapentin 30 nM—13/14; 300 nM—6/8; 3 μM—20/40; 10 μM—32/49; 30 μM—15/31; 100 μM—39/60; 300 μM—19/23. All values for Gabapentin-treated preparations are significantly different from control ($p<0.01$; one-way ANOVA). Apparent half-maximal inhibitory effect of Gabapentin was estimated to be ~100 nM. Inset, Image sequence of typical individual synaptosome response to application of elevated K^+; representative traces of responses in individual synaptosomes to 300 μM Gabapentin (GBP). Vertical scales denote units of fluorescence ratios (F/F_0).

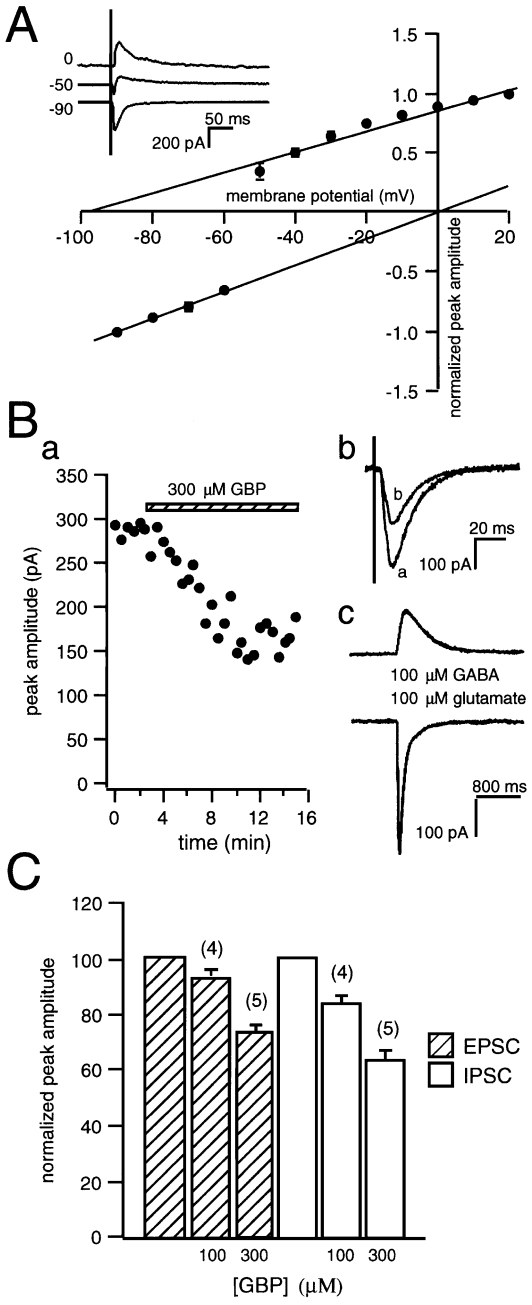

Fig. 3. Gabapentin reduced the amplitude of evoked EPSCs and IPSCs. (A) Plots of the amplitude of evoked synaptic responses, recorded in hippocampal CA1 neurons by stimulation of Schaffer collaterals, against the holding potential. Solid lines are obtained by linear regression. Inset shows currents recorded at the indicated holding potentials. At −50 mV, a combined EPSC-IPSC is recorded. (Ba) Peak amplitude of evoked EPSCs recorded in a hippocampal CA1 neuron during application of 300 μM Gabapentin (hatched bar). (Bb) Example of evoked postsynaptic currents before (a) and during (b) application of Gabapentin. (Bc) Superimposed current traces after picospritzer application of 100 μM GABA (top) or 100 μM glutamate (bottom) in the absence and presence of 300 μM Gabapentin (GBP). Note that the traces overlap completely. (C) Summary of the effects of Gabapentin (GBP) on evoked EPSCs and IPSCs in hippocampal CA1 neurons. Numbers above the bars indicate the number of cells. Values are significantly different from control ($P<0.01$).

transmission. Hippocampal CA1 pyramidal neurons in slices were whole-cell voltage clamped, and synaptic currents were evoked by stimulation of the Schaffer collaterals. As the pipette solution contained low [Cl^-], the reversal potentials for the EPSCs and the IPSCs were calculated to be around 0 and −90 mV, respectively (Fig. 3A). By shifting the holding potential back and forth from 0 to −90 mV, both evoked EPSCs and IPSCs were recorded from the same neuron. Fig. 3B shows that upon bath application of 300 μM Gabapentin, the peak amplitude of the evoked EPSC was reduced. Both evoked EPSCs and IPSCs were sensitive to Gabapentin in a dose-dependent manner (Fig. 3C). At 300 μM, Gabapentin inhibited the evoked EPSCs and IPSCs to 72.6 ± 3.5% ($n=5$) and 63.2 ± 3.7% ($n=5$) of control, respectively. In order to exclude a direct effect of Gabapentin on the postsynaptic responsiveness of the cell, the effect of Gabapentin on ion current evoked by application of 100 μM glutamate or 100 μM γ-aminobutyric acid (GABA) was examined. Fig. 3Bc shows that Gabapentin did not affect the glutamate- and GABA-evoked ion currents.

4. Discussion

Our results demonstrate that Gabapentin inhibits presynaptic Ca^{2+} influx, and that Gabapentin inhibits excitatory as well as inhibitory synaptic transmission in hippocampus and neocortex. We did not detect an inhibitory effect of Gabapentin on postsynaptic VOCCs recorded in either dissociated cells or slices from hippocampus and neocortex. However, VOCCs were rapidly and completely blocked by Cd^{2+} in these preparations (Fig. 1C), indicating that a putative blocking effect of Gabapentin could have been resolved. Our results contrast with those reported by Stefani et al. (1998, 2001), who showed that postsynaptic VOCCs in dissociated rat cortical neurons are blocked by Gabapentin. The reason for this discrepancy remains unclear. Although we cannot rule out an effect of Gabapentin on postsynaptic VOCCs at certain sites in the CNS, it is unlikely that postsynaptic VOCCs are the main target of Gabapentin.

The results of confocal Ca^{2+} imaging on single hippocampal synaptosomes show that Gabapentin dose-dependently inhibits presynaptic Ca^{2+} influx, with apparent half-maximum effect at ~100 nM (Fig. 2). Recently, it was shown that Gabapentin inhibits Ca^{2+} influx in cortical synaptosomal suspensions with an IC_{50} of ~10 μM (Fink et al., 2000; Meder and Dooley, 2000). In our study, we also observed the inhibition of Ca^{2+} influx by Gabapentin in single synaptosomes isolated from neocortex. The amount of inhibition at 100 and 300 μM Gabapentin was similar to that observed in hippocampal synaptosomes. Although the different estimates of the sensitivity of hippocampal and neocortical synaptosomes to Gabapentin may suggest that Gabapentin acts at different targets in those brain areas, it is more likely that this apparent discrepancy is due to methodological differences (single synaptosomes vs. synapto-

some suspensions). Fink et al. (2000) reported that the inhibition of K^+-evoked Ca^{2+} influx in synaptosomes isolated from neocortex could be blocked by P/Q-type VOCC blockers, but not by N-type VOCC blockers. In our study, we observed the inhibition of both the peak phase (mediated by N-type VOCCs) and the plateau phase (mediated by P/Q-type VOCCs) by Gabapentin, suggesting that Gabapentin acts on several types of presynaptic VOCCs. It should be noted that we used confocal imaging on single synaptosomes (in contrast to synaptosomal suspensions), which allows for a kinetic distinction between Ca^{2+} responses mediated by different VOCC subtypes (peak- vs. plateau phase). Moreover, only a fraction of synaptosomes is sensitive to inhibition by Gabapentin (approximately 50%) and therefore a putative effect of N-type VOCC blockers may be obscured when using synaptosome suspensions.

Previous studies have shown that Gabapentin inhibits K^+-evoked excitatory amino acid release from hippocampal and neocortical slices (Fink et al., 2000; Dooley et al., 2000). In concordance with these findings, we show that Gabapentin inhibits evoked EPSCs in hippocampus (Fig. 3). In addition, we show that evoked IPSCs are also inhibited by Gabapentin, without affecting postsynaptic glutamate- and GABA-activated ion currents (Fig. 3B). From the confocal imaging experiments, it appeared that not all synaptosomes were sensitive to Gabapentin: Ca^{2+} influx in approximately 10–50% of the synaptosomes was not inhibited by Gabapentin (Fig. 2). It is unlikely that this difference can be attributed to a differential sensitivity of excitatory and inhibitory terminals, as evoked EPSCs and IPSCs were inhibited by Gabapentin to the same extent (Fig. 3C). This raises the possibility that the molecular target of Gabapentin is selectively expressed in a subset of presynaptic terminals. Initial studies suggested that the molecular target of Gabapentin is an $\alpha_2\delta$ subunit of VOCCs (Gee et al., 1996). Recently, it has been shown that Gabapentin can also act as an agonist on $GABA_B$ receptors with a specific heterodimeric composition, which are negatively coupled to VOCCs (Ng et al., 2001; Bertrand et al., 2001; but see Lanneau et al., 2001). It remains to be determined whether these targets are differentially expressed in presynaptic terminals and provide the molecular basis of inhibition by Gabapentin of presynaptic Ca^{2+} influx in the CNS.

An overall attenuation of both inhibitory and excitatory synaptic transmission by Gabapentin could be one of the mechanisms that contribute to the anticonvulsant properties of Gabapentin. However, many studies have shown additional effects of Gabapentin (for review see Taylor et al., 1998), such as an increase in the levels of GABA (Petroff et al., 1996), an increase in the levels of whole blood serotonin (Rao et al., 1988), and a reduction of glutamate synthesis by inhibition of the branched-chain amino acid aminotransferase (Goldlust et al., 1995). Therefore, it still remains to be determined whether the acute effects of Gabapentin reported thus far contribute to any of the therapeutic effects in patients receiving long-term exposure to Gabapentin.

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