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Gabapentin may inhibit synaptic transmission in the mouse spinal cord dorsal horn through a preferential block of P/Q-type Ca^{2+} channels

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Abstract

Gabapentin is a lipophilic analog of γ-amino butyric acid (GABA) with therapeutic activity against certain forms of epilepsy and neuropathic pain. Despite its structural similarity to GABA, it does not bind $GABA_A$ or $GABA_B$ receptors and the mechanism, especially of its analgesic action, has remained elusive. Here, we have studied its effects on synaptic transmission mediated by the major spinal fast excitatory and inhibitory neurotransmitters, L-glutamate and glycine, in the superficial layers of the spinal cord dorsal horn, a CNS area, which is critically involved in nociception. Gabapentin reversibly reduced evoked excitatory postsynaptic currents mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA-EPSCs) and inhibitory postsynaptic currents mediated by glycine (gly-IPSCs). Inhibition of AMPA-EPSCs and gly-IPSCs occurred with similar potencies (~10–50 nM) and by about the same degree (~40% at 1 μM). Gabapentin did not affect membrane currents elicited by exogenously applied glutamate or glycine arguing against a postsynaptic site of action. Selective blockade of N-type Ca^{2+} channels with ω-conotoxin GVIA dramatically increased and blockade of P/Q-type channels with ω-agatoxin IVA strongly attenuated inhibition of evoked synaptic transmission by gabapentin. These results show that gabapentin affects both excitatory and inhibitory spinal neurotransmission via a presynaptic mechanism which preferentially involves P/Q-type Ca^{2+} channels.

Keywords: Gabapentin; Synaptic transmission; Spinal cord; Dorsal horn; Calcium channel; Pain

1. Introduction

Gabapentin, 1-(aminomethyl) cyclohexane acetic acid (Neurontin®), is widely used against certain forms of epilepsy (Crawford et al., 1987) and neuropathic pain (Rosner et al., 1996). Although several clinical trials have meanwhile confirmed its clinical effectiveness in a variety of pain syndromes (Mao and Chen, 2000), the mechanisms in particular of its analgesic action are still unknown. The only high affinity binding site of gabapentin is the accessory $\alpha_2\delta$ subunit of high voltage-gated Ca^{2+} channels (Gee et al., 1996). Although an interaction with this site might explain inhibitory actions of gabapentin on voltage-gated Ca^{2+} channels (e.g. Stefani et al., 1998; Sutton et al., 2002) and on Ca^{2+} influx into synaptosomes (Meder and Dooley, 2000; Fink et al., 2000, 2002), its significance for Ca^{2+} channel function or therapeutic efficacy is far from being established. Alternative mechanisms suggested in the past, which also may underlie gabapentin's action on synaptic transmission and nociceptive processing, include a, perhaps indirect, activation of $GABA_B$ receptors (Bertrand et al., 2001; Ng et al., 2001) or an interaction with the synthesis (Loscher et al., 1991), degradation (Silverman et al., 1991) or the re-uptake of neurotransmitters (Kocsis and Honmou, 1994).

Despite the lack of a clear mode of action, it is widely accepted that gabapentin exerts its analgesic effect through an interaction with nociceptive processing

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in the spinal cord. When injected into the spinal canal (i.e. intrathecally, i.t.), gabapentin induced antinociception in animal models of inflammatory and neuropathic pain (Shimoyama et al., 1997; Jun and Yaksh, 1998; Kaneko et al., 2000). Several groups have subsequently studied the effects of gabapentin on modulation of synaptic transmission in the spinal cord dorsal horn, which constitutes the first site of synaptic integration in the pain pathway. These experiments yielded a wide variety of results. Shimoyama et al. (2000) reported an inhibition of glutamatergic transmission, while others have found an inhibition only in the hyperalgesic spinal cord (Patel et al., 2000), a selective enhancement of NMDA receptor-mediated synaptic transmission onto GABAergic neurons (Gu and Huang, 2002) or no effect on AMPA receptor-mediated transmission in combination with variably affected NMDA-responses and IPSCs (Moore et al., 2002). Here, we have performed a systematic comparison of the effects of gabapentin on fast excitatory glutamatergic postsynaptic currents mediated by AMPA receptors (AMPA-EPSCs) and on inhibitory synaptic transmission mediated by strychnine-sensitive glycine receptors (gly-IPSCs). We found that gabapentin dose-dependently inhibited both excitatory and inhibitory neurotransmission with similar potencies via a presynaptic mechanism, which involved the preferential inhibition of P/Q-type Ca^{2+} channels.

2. Materials and methods

BALBc mice (7–14 days old) of either sex were killed under deep ether anesthesia by decapitation. Transverse slices (250 μM thick) of the lumbar spinal cord were prepared as described previously (Liebel et al., 1997). Whole-cell patch-clamp recordings were performed from neurons in the superficial layers (substantia gelatinosa) of the dorsal horn under visual control using the infrared gradient contrast technique coupled to a video microscopy system. Slices were completely submerged and continuously superfused with external solution which contained 125 mM NaCl, 26 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM glucose (pH 7.30, 315 mOsm/l) and was bubbled with 95% O_2/5% CO_2. Patch pipettes (4–5 MΩ) were filled with internal solution containing 130 mM K-gluconate, 20 mM KCl, 2 mM $MgCl_2$, 0.05 mM EGTA, 3 mM Na_2-ATP, 0.1 mM Na_3-GTP and 10 mM Na-HEPES (pH 7.30). QX-314 (5 mM) was added to the internal solution to block voltage-gated Na^+ currents. EPSCs and IPSCs were elicited at a frequency of 1/15 s by extracellular electrical stimulation (100 μs, 3–10 V) using a glass electrode filled with standard extracellular solution and placed about 50 μm from the recorded neuron. Combinations of strychnine (300 nM), bicuculline (10 μM), CNQX (10 μM), and D-APV (20 μM) (all from Sigma, Taufkirchen, Germany) were used to isolate AMPA-EPSCs and gly-IPSCs. All recordings were made at −80 mV. Short hyperpolarising voltage steps to −90 mV were applied once per minute to monitor input and access resistance. Drug containing solutions were applied by bath perfusion at a rate of 1–2 ml/min. To study membrane currents elicited by exogenous transmitters, L-glutamate and glycine were applied from glass pipettes to the soma of the recorded neuron by short (1 s) pressure pulses. To investigate the effects of gabapentin on Ca^{2+} channel subtypes slices were incubated with ω-conotoxin GVIA and ω-agatoxin IVA (ω-CgtxGVIA and ω-AgaIVA, both from Sigma, Taufkirchen, Germany) for at least 1 h.

Percent inhibition of current responses was determined from the average amplitude of 10 consecutive currents evoked immediately before application of gabapentin (Sigma, Taufkirchen, Germany) and when a steady state of inhibition was reached, usually 3 min after application. Unless otherwise indicated, all statistical significances were determined using ANOVA followed by Fisher's post hoc test.

3. Results

3.1. Evoked excitatory and inhibitory postsynaptic currents

Excitatory and inhibitory PSCs (EPSCs and IPSCs) mediated by L-glutamate and glycine were evoked by extracellular electrical stimulation and recorded in the whole-cell configuration of patch-clamp technique. Gabapentin (1 μM) reduced the amplitudes of AMPA-EPSCs by 39.9 ± 3.0% (n= 7) (Fig. 1A and C), but had no effects on the decay time course of EPSCs (5.1 ± 1.2 ms versus 5.0 ± 1.0 ms, n= 7, control versus gabapentin). Very similar effects were obtained for gly-IPSCs, which were reduced by 41.5 ± 4.9%, n= 7 (Fig. 1B and C), but showed again no change in their decay time course in response to gabapentin (13.2± 1.5 ms versus 13.7 ± 1.7 ms, n= 7, control versus gabapentin). Recovery from inhibition was slow for both glutamatergic and glycinergic transmission, but partial recovery could be obtained in about 40% of neurons (4/20 and 13/22 for AMPA-EPSCs and gly-IPSCs, respectively). No changes in baseline current or input resistance were observed when gabapentin was applied ($\Delta R_{mem} \leq 1\%$).

Inhibition of AMPA-EPSCs and of gly-IPSCs occurred with comparable potencies. Half maximal effective concentrations (IC_{50}) were 23.0 ± 8.7 nM and 46.1 ± 13.5 nM for AMPA-EPSCs and gly-IPSCs, respectively (Fig. 1C). Significant reduction in PSC amplitudes was obtained at concentrations ≥30 nM. Maximum inhibition (43.1 ± 3.4% and 46.5 ± 3.0% for

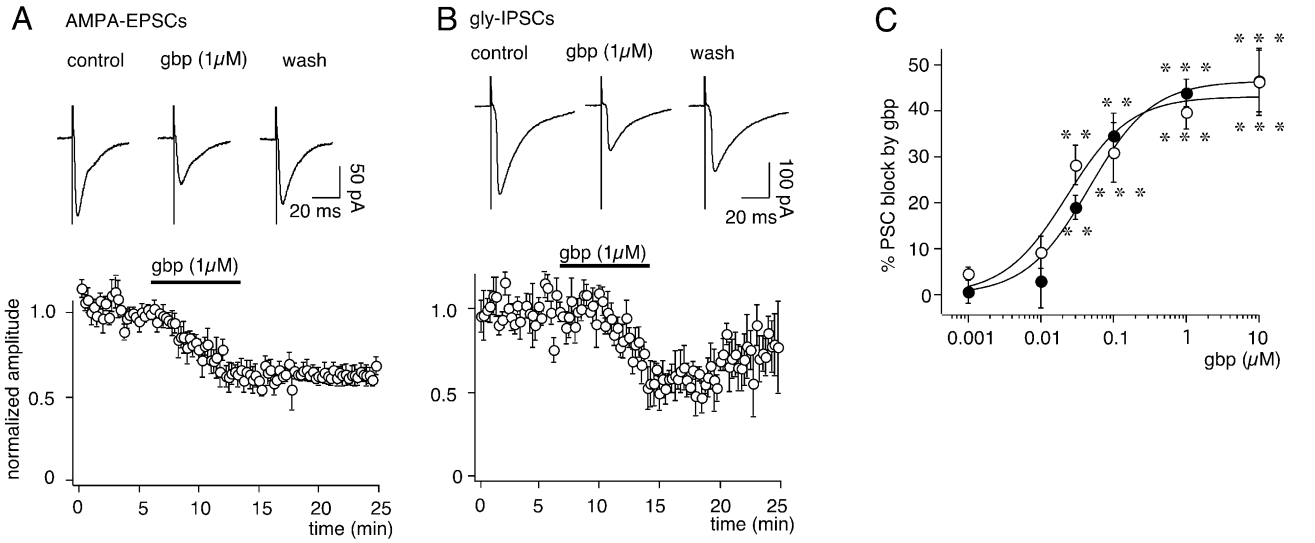

Fig. 1. Effects of gabapentin on evoked synaptic transmission. (A–C) Evoked PSCs mediated by AMPA (A) and strychnine-sensitive glycine (B) receptors. Top traces: Postsynaptic current traces were averaged from 10 consecutive stimulations under control conditions, in the presence of gabapentin (gbp, 1 μM) and after its removal (wash). PSCs were recorded at −80 mV. Bottom diagrams: Time course of gabapentin-mediated inhibition averaged from 7 cells, each. (C) Concentration response curves. AMPA-receptor-mediated EPSCs (white circles) and glycinergic IPSCs (black circles). Percent (mean ± s.e.m.) PSC block in the presence of gabapentin. **, $p \leq 0.01$; ***, $p \leq 0.001$; ANOVA, followed by Fisher's post hoc test, $n = 4$–8 cells per concentration.

AMPA-EPSCs and gly-IPSCs, respectively) occurred at concentrations ≥1 μM (Fig. 1C).

3.2. Membrane currents elicited by exogenously applied neurotransmitters

To distinguish between a pre- and a postsynaptic site of action we tested whether gabapentin would change current responses elicited by exogenous application of Glutamate (1 mM) or glycine (1 mM) (Fig. 2). Gabapentin caused a modest and statistically insignificant decrease in amplitude by $1.63 \pm 0.95\%$, $n = 5$, and $0.82 \pm 1.42\%$, $n = 7$, for glutamate and glycine-evoked currents, respectively, which suggests a pre- rather than a postsynaptic site of action. These results also render an effect on a specific neurotransmitter receptor rather unlikely. They support instead an inhibitory action of gabapentin on a common element involved in presynaptic glutamate and glycine release, e.g. presynaptic N- or P/Q-type Ca^{2+} channels, which mediate most of the presynaptic Ca^{2+} influx coupled to synaptic glutamate and glycine release in the spinal cord (Takahashi and Momiyama, 1993).

3.3. Effects of Ca^{2+} channel toxins on the PSC inhibition by gabapentin

In a further set of experiments we have, therefore, tested whether gabapentin might specifically inhibit synaptic transmission mediated by N- or P/Q-type Ca^{2+} channels. In these experiments, slices were incubated for ≥60 min with ω-CgtxGVIA (Kerr and

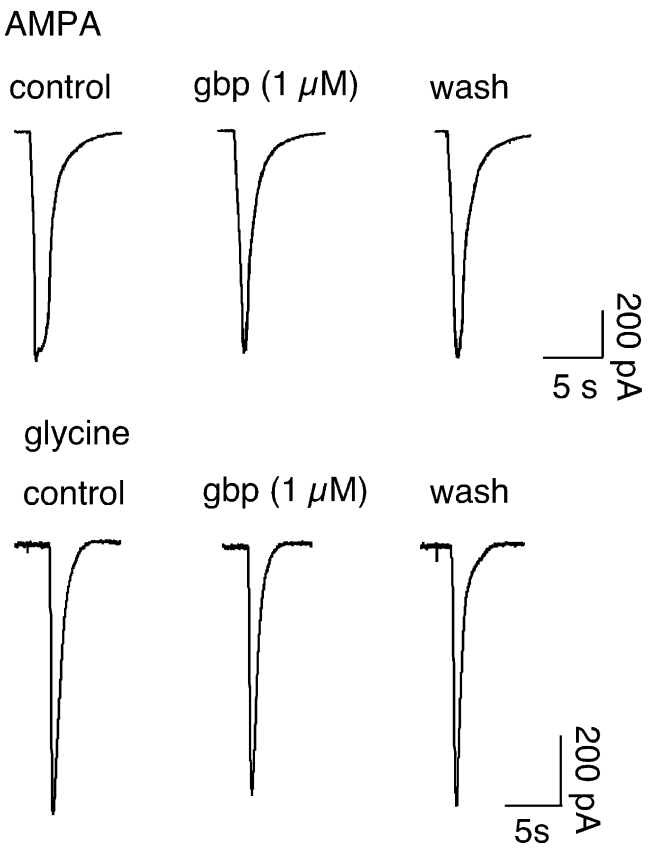

Fig. 2. Whole-cell currents evoked by exogenous application of glutamate (1 mM) or glycine (1 mM) before application of gabapentin (control), during gabapentin application (gbp; 1 μM) and after its removal (wash).

Yoshikami, 1984) or ω-AgaIVA (Mintz et al., 1992), which irreversibly block N- and P/Q-type Ca^{2+} channels, respectively. We first compared synaptic responses in slices preincubated with Ca^{2+} channel toxins (1 μM) and in control slices which had been identically treated except for the Ca^{2+} channel toxin. In slices treated with ω-AgaIVA, inhibition of AMPA-EPSCs and gly-IPSCs was almost complete. Remaining AMPA-EPSCs and gly-IPSCs had average amplitudes of -15.6 ± 6.5 pA ($n= 10$) and -3.6 ± 2.6 pA ($n= 11$), respectively, which corresponds to a block of 95.4% and 98.8%. ω-CgtxGVIA reduced PSC amplitudes to 155.3 ± 30.0 pA (54.2% block, $n= 6$) and to 126.7 ± 22.1 pA (57.8% block, $n= 7$) for AMPA-EPSCs and gly-IPSCs, respectively.

All neurons recorded from ω-CgtxGVIA treated slices exhibited AMPA-EPSCs ($n= 6$) or gly-IPSCs ($n= 7$) sufficiently large to quantify the inhibition by gabapentin. In these cells, the inhibitory effect of gabapentin on synaptic transmission was dramatically increased (Fig. 3A, B, D). Inhibition of gly-IPSCs by gabapentin was nearly complete ($97.3 \pm 1.7\%$, $n= 7$, $p \leq 0.001$) and that of AMPA-EPSCs significantly increased from $39.9 \pm 3.0\%$, $n= 7$, to $65.7 \pm 7.6\%$, $n= 6$ ($p \leq 0.01$).

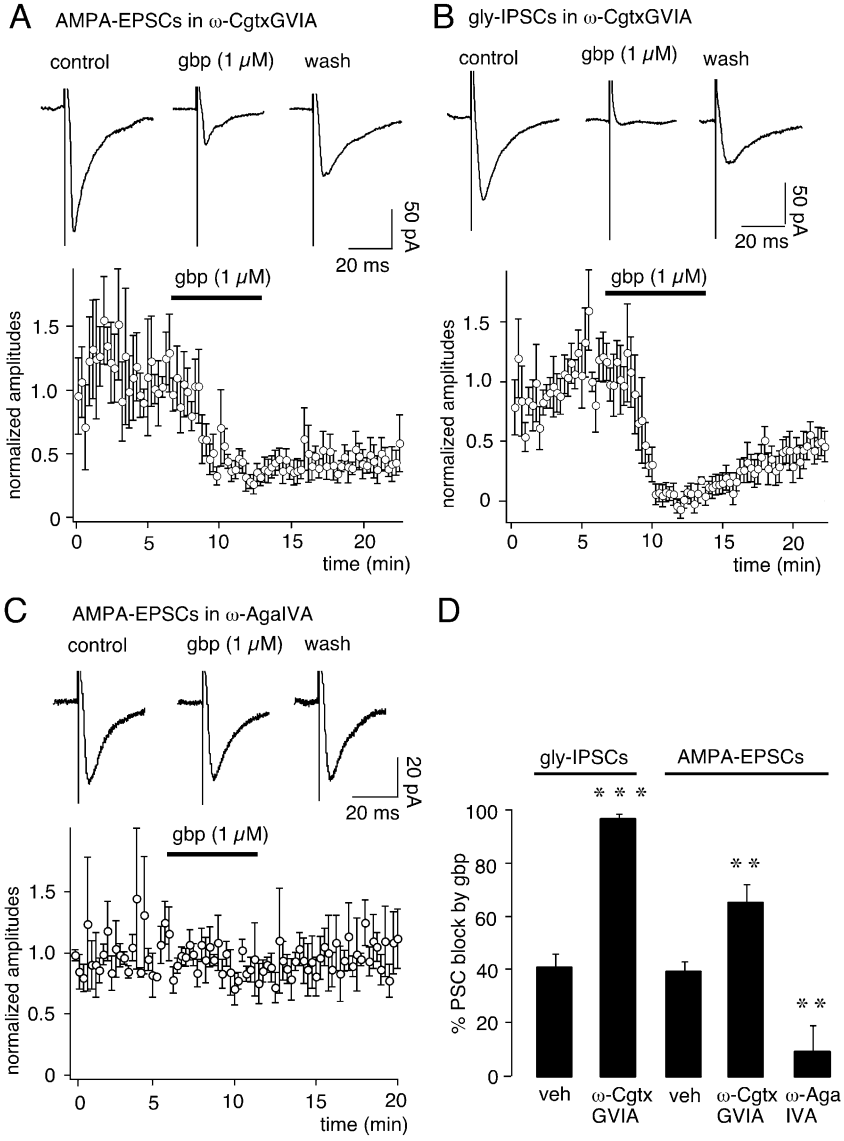

Fig. 3. Effects of Ca^{2+} channel toxins on the inhibitory effect of gabapentin. (A) Average AMPA-EPSCs and (B) gly-IPSCs recorded from slices incubated for ≥60 min with the N-type Ca^{2+} channel blocker ω-CgtxGVIA. Current traces averaged from 10 consecutive stimulations under control conditions, in presence of gabapentin (gbp, 1 μM) and after its removal (wash). Time course of gabapentin-mediated effects on AMPA-EPSCs. (C) Same as (A) but recordings were done from slices incubated ≥60 min with the P/Q-type Ca^{2+} channel blocker ω-AgaIVA. (D) Statistical analysis of PSC inhibition by gabapentin (mean ± s.e.m.) after incubation with different Ca^{2+} channel blockers. **, $p \leq 0.01$; ***, $p \leq 0.001$; ANOVA, followed by Fisher's post hoc test ($n= 6$–7 each, $n= 3$ for AMPA-EPSCs in ω-AgaIVA).

In 3 out of 10 neurons recorded in slices pre-incubated with ω-AgaIVA, AMPA-EPSCs were large enough to evaluate the effect of gabapentin (1 μM) on N-type Ca^{2+} channel-mediated synaptic transmission. Unlike ω-CgtxGVIA, blockade of P/Q-type Ca^{2+} channels with ω-AgaIVA reduced AMPA-EPSC inhibition by gabapentin to $9.6 \pm 3.6\%$ ($n = 3$), which was no longer statistically significant ($p = 0.39$) (Fig. 3C and D). The effect of gabapentin on gly-IPSCs in slices pretreated with ω-AgaIVA was not analyzed, since no detectable gly-IPSCs could be recorded in these slices ($n = 11$). Taken together these results indicate that gabapentin acted primarily on P/Q-type Ca^{2+} channel-mediated synaptic transmission.

4. Discussion

In the present study, we have shown that gabapentin inhibits excitatory glutamatergic and inhibitory glycinergic neurotransmission in the spinal cord dorsal horn in a therapeutically relevant range of concentrations (1–10 μM). The degree of inhibition was nearly identical for glutamatergic transmission mediated by AMPA receptors and inhibitory transmission mediated by strychnine-sensitive glycine receptors and hence apparently independent of the type of neurotransmitter and neurotransmitter receptor involved. In contrast, inhibition of synaptic transmission by gabapentin strongly depended on the Ca^{2+} channel type mediating presynaptic transmitter release. In our preparation, ω-CgtxGVIA and ω-AgaIVA reduced synaptic transmission by about 55% and 95%, respectively. If one assumes a dependence of transmitter release from presynaptic Ca^{2+} influx to the third power, this indicates that N- and P/Q-type channels contribute roughly 25% and ≥60 % to presynaptic Ca^{2+} influx, leaving only little space (≤15%) for a contribution of ω-AgaIVA- and ω-CgTxGVIA-insensitive Ca^{2+} channels. As evident from the experiments in Ca^{2+} channels toxin pretreated slices, P/Q-type-mediated transmission was far more sensitive to inhibition by gabapentin than that mediated by N-type channels. Besides N- and P/Q-type channels, R-type channels are expressed in the superficial spinal cord dorsal horn and probably contribute to transmitter release and nociceptive transmission in this CNS region (Saegusa et al., 2000). An inhibition of these R-type channels by gabapentin cannot be excluded from our data, but due to their apparently small contribution to transmitter release these channels cannot account for the inhibitory action of gabapentin on synaptic transmission. Our results are thus in line with previous reports by other groups who have studied Ca^{2+} influx into rat and human neocortical synaptosomes (Meder and Dooley, 2000; Fink et al., 2000, 2002) and K^+-evoked norepinephrine release from rat cortical slices (Dooley et al., 2002).

4.1. Molecular mechanisms

Inhibition by gabapentin of macroscopic Ca^{2+} channel currents recorded from neuronal somata has also previously been reported in different preparations, but these reports did not provide a coherent picture. Different groups either found a preferential inhibition of L-type (Stefani et al., 1998) or N-type (Sutton et al., 2002) Ca^{2+} channel currents or no acute effect at all (Schumacher et al., 1998). Kang et al. (2002) also found no acute inhibition by gabapentin of P/Q-type (α_{1A}, $\alpha_2\delta$-1 and β_3) Ca^{2+} currents in *Xenopus* oocytes, but a slow-down of inactivation kinetics after prolonged incubation. Despite these discrepancies it appears interesting that inhibition of somatic Ca^{2+} currents occurred with IC_{50} values between 167 nM (rat dorsal root ganglion cells, Sutton et al., 2002) and 3.65 μM (rat cortex, Stefani et al., 1998). If one takes again into account the non-linear dependence to the 3rd–4th power of presynaptic Ca^{2+} influx and transmitter release, these values are in good agreement with those reported here for inhibition of synaptic transmission (23–40 nM).

The fact that gabapentin inhibits different Ca^{2+} channel types in different preparations may indicate that the sensitivity of Ca^{2+} channels to gabapentin is not primarily determined by the pore forming α1 subunit. As outlined above, gabapentin binds with high affinity to two of four known $\alpha_2\delta$ subunits, $\alpha_2\delta$-1 and $\alpha_2\delta$-2 (Gee et al., 1996; Klugbauer et al., 1999; Qin et al., 2002; Marais et al., 2001). The preferential inhibition of P/Q-type-mediated transmission in spinal cord slices might therefore be attributed to a specific association of the gabapentin binding $\alpha_2\delta$-1 and $\alpha_2\delta$-2 subunits with α_{1A}, the pore forming α_1 subunit of P/Q-type channels. α_{1A} is indeed co-expressed with $\alpha_2\delta$-2 in certain tissues (Marais et al., 2001; Hobom et al., 2000). Evidence for a functional co-assembly comes from the mouse mutant *ducky* and from experiments in *Xenopus* oocytes. The mouse mutant *ducky*, which has an epileptic and ataxic phenotype, carries a mutation in the gene coding for the $\alpha_2\delta$-2 subunit (Cacna2d2) and shows strongly decreased ω-AgaIVA sensitive Ca^{2+} channel current densities in cerebellar Purkinje cells (Barclay et al., 2001). In *Xenopus* oocytes, co-expression of α_{1A} with $\alpha_2\delta$-2 increases current density and causes a slight hyperpolarizing shift in the voltage dependence of activation (Barclay et al., 2001), while co-expression of the mutated $\alpha_2\delta$-2 from the *ducky* mouse reduces current density (Brodbeck et al., 2002). The exact expression pattern of gabapentin-sensitive versus -insensitive $\alpha_2\delta$ subunits and their association with α1 subunits is still unknown. It would, therefore, be premature to attribute tissue differences in gabapentin sensitive Ca^{2+} channel types to the distribution of $\alpha_2\delta$ subtypes.

Taken together, these results are at least consistent with a reduction of synaptic transmission via the binding of gabapentin to the $\alpha_2\delta$ subunit of voltage-gated Ca^{2+} channels. Nevertheless, it is still unknown what the exact effect of gabapentin on Ca^{2+} channels is or what conformational change the binding of gabapentin to the $\alpha_2\delta$ subunits might induce.

4.2. Functional consequences

Another important topic is the contribution of the synaptic effects described here to the analgesic effect of gabapentin or, in other words, whether the simultaneous inhibition of excitatory and inhibitory synaptic transmission via an inhibition of presynaptic Ca^{2+} channels can be antinociceptive. N-type Ca^{2+} channel blockade with ω-CtxGVIA and SNX-111 (ziconotide), which also reduce excitatory and inhibitory synaptic transmission, is antinociceptive in the rat formalin test (Malmberg and Yaksh, 1994) and analgesic in clinical trials (Jain, 2000). Less information is available on a possible analgesic effect of P/Q-type Ca^{2+} channel blockade with ω-AgaIVA. Sluka (1998) and Matthews and Dickenson (2001) found a reduction in nociceptive responses after intrathecal injection of ω-AgaIVA in rat models of inflammatory and neuropathic pain, while Yamamoto and Sakashita (1998) reported no antinociceptive effect in a model of neuropathic pain. This discrepancy might be explained by the observation that P/Q-type channels were particularly important for nociceptive transmission under inflammatory (Nebe et al., 1997) and possibly also neuropathic conditions. The prominent role of P/Q type channels under these conditions may also explain why the effects of gabapentin on synaptic transmission were particularly apparent in neuropathic rats (Patel et al., 2000) and is also in good agreement with the observation that gabapentin possesses anti-hyperalgesic rather than general analgesic properties (Bryans and Wustrow, 1999). In this context one might further speculate that the simultaneous inhibition of excitatory and inhibitory transmission observed in naive rats in our study compensates for each other, which might contribute to the low frequency of unwanted side effects seen in patients treated with gabapentin.

4.3. Conclusions and implications

In summary, we have shown that gabapentin reduces fast glutamatergic and glycinergic synaptic transmission in the spinal cord dorsal horn at therapeutically relevant concentrations via a preferential inhibition of P/Q-type Ca^{2+} channels. Our results thus provide further evidence that the binding of gabapentin to certain $\alpha_2\delta$ subunits of voltage gated Ca^{2+} channels inhibits action potential evoked release of fast neurotransmitters, an effect which may account for the spinal antinociceptive properties of gabapentin. They therefore further support the potential of the $\alpha_2\delta$ subunits as targets for a novel class of analgesic drugs.

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