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ORIGINAL ARTICLE

Differential modulation of K^+-evoked ^{3}H-neurotransmitter release from human neocortex by gabapentin and pregabalin

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Abstract Anticonvulsant, analgesic, and anxiolytic effects have been observed both in preclinical and clinical studies with gabapentin (GBP) and pregabalin (PGB). These drugs appear to act by binding to the $\alpha_2\delta$ subunit of voltage-sensitive Ca^{2+} channels (VSCC), resulting in the inhibition of neurotransmitter release. In this study, we examined the effects of GBP and PGB (mostly 100 μM, corresponding to relatively high preclinical/clinical plasma levels) on the release of neurotransmitters in human neocortical slices. These slices were prelabeled with ^{3}H-dopamine (^{3}H-DA), ^{3}H-choline (to release ^{3}H-acetylcholine (^{3}H-ACh)), ^{3}H-noradrenaline (^{3}H-NA), and ^{3}H-serotonin (^{3}H-5-HT), and stimulated twice in superfusion experiments by elevation of extracellular K^+ in the presence and absence of GBP and PGB. The $\alpha_2\delta$ ligands produced significant inhibitions of K^+-evoked ^{3}H-ACh, ^{3}H-NA, and ^{3}H-5-HT release between 22% and 56% without affecting ^{3}H-DA release. Neither drug reduced ^{3}H-NA release in the presence of L-isoleucine, a putative $\alpha_2\delta$ antagonist. Interestingly, this antagonism did not occur using the enantiomer, D-isoleucine. These results suggest that GBP and PGB are not general inhibitors of VSCC and neurotransmitter release. Such $\alpha_2\delta$ ligands appear to be selective modulators of the release of certain, but not all, neurotransmitters. This differential modulation of neurotransmission presumably contributes to their clinical profile.

Keywords Gabapentin · Pregabalin · Neurotransmitter release

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Introduction

Gabapentin (GBP) and pregabalin (PGB) have clinical efficacy corresponding to their anticonvulsant, analgesic, and anxiolytic effects observed in several preclinical models (Taylor et al. 1998, 2007). Both drugs are derivatives of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), and GBP was originally designed as a GABA-mimetic agent that can cross the blood–brain barrier (Satzinger 1994). The precise mechanism by which GBP and PGB exert their clinical efficacy is still not completely understood. There is, however, a high-affinity binding site for these drugs: the $\alpha_2\delta$ subunit of voltage-sensitive Ca^{2+} channels (VSCC; Belliotti et al. 2005; Bian et al. 2006). The VSCC mediate Ca^{2+} entry into cells in response to membrane depolarization. Presynaptically, Ca^{2+} entry then initiates exocytotic release of neurotransmitters with vesicular storage, as investigated in the present study, i.e., dopamine (DA), acetylcholine (ACh), noradrenaline (NA),

and serotonin (5-HT). High voltage-activated Ca^{2+} channels are complexes of a pore-forming α_1 subunit; a transmembrane, disulfide-linked complex of α_2 and δ subunits; an intracellular β subunit; and, in some cases, a transmembrane γ subunit (Catterall 2000). GBP was the first ligand described to interact with the $\alpha_2\delta$ subunit of VSCC (Gee et al. 1996).

An interaction with the $\alpha_2\delta$ subunit of VSCC is considered to underlie the pharmacological effects of GBP, PGB, and related drugs; this interaction appears to diminish the amount of Ca^{2+} entering the presynaptic terminal following depolarization, leading to a decrease in neurotransmitter release (Dooley et al. 2007). There are four different subtypes of $\alpha_2\delta$ protein ($\alpha_2\delta$-1, -2, -3, and -4), yet only $\alpha_2\delta$-1 and -2 bind PGB and GBP with high affinity (Taylor et al. 2007; Dooley et al. 2007). Moreover, a mutation in the gene of mice encoding the $\alpha_2\delta$-1 protein substantially reduced specific ^{3}H-PGB (and ^{3}H-GBP) binding in central nervous system (CNS) regions known to preferentially express the $\alpha_2\delta$-1 subtype (e.g., neocortex, hippocampus, basolateral amygdala, dorsal horn of spinal cord), suggesting that $\alpha_2\delta$-1 is the major binding site for PGB (Bian et al. 2006). Additionally, this reduction of ^{3}H-PGB binding in R217A mice led to a loss of the analgesic activity of PGB in different preclinical pain models (Field et al. 2006).

Several previous studies have examined the modulation of neurotransmitter release by GBP and PGB. GBP inhibited electrically evoked ^{3}H-NA and ^{3}H-5-HT release from rat neocortical slices in a concentration-dependent manner (Schlicker et al. 1985). It also reduced electrically evoked ^{3}H-DA release from rabbit caudate nucleus slices (Reimann 1983), but it did not alter ^{3}H-ACh release from these slices (Schlicker et al. 1985). Both GBP and PGB (1–100 μM) inhibited electrically and K^+-evoked ^{3}H-NA release, but not that induced by veratridine, from rat neocortical slices (Dooley et al. 2000). The electrically evoked ^{3}H-NA release was inhibited to a smaller extent, leading the authors to conclude that GBP and PGB are more effective to modulate neurotransmitter release requiring only partial or intermittent Na^+ channel activation. GBP also decreased K^+-evoked ^{3}H-NA release from human neocortical and rat hippocampal slices in a concentration-dependent manner (Freiman et al. 2001). This inhibitory effect was antagonized by the K_{ATP}-channel antagonist glibenclamide, implicating that opening of K_{ATP} channels may be involved in the inhibitory action of GBP.

Thurlow et al. (1993) showed that some large neutral L-amino acids (e.g., L-leucine, L-isoleucine, L-methionine) are potent, stereospecific displacers of ^{3}H-GBP binding to mouse and pig brain membranes, whereas the corresponding D-enantiomers were all much less active. This observation proved also the case for rat brain membranes (Thurlow et al. 1996a). These findings point to a modulatory role of endogenous amino acids in neurotransmission (Dooley et al. 2007).

In the present investigation, we tested a relatively high concentration of GBP and PGB (100 μM) on the release of several neurotransmitters from human neocortical tissue under similar experimental conditions (with the exception of ^{3}H-5-HT release experiments, where PGB was tested in a concentration of 1, 10, and 100 μM). This concentration is pharmacologically relevant, as it is still within the therapeutic range in plasma and/or brain tissue of 1 to 100 μM (Dooley et al. 2000). We chose this concentration because it was a saturating inhibitory concentration in previous experiments addressing the effects of both drugs on ^{3}H-NA release in rat brain slices (Dooley et al. 2000). The results of these in vitro experiments provide further information on the pharmacological effects of $\alpha_2\delta$ ligands in the target species, potentially relevant to their clinical profile.

Materials and methods

Tissue source

Fresh human neocortical tissue was obtained during surgical removal of deep-seated brain tumors or epileptic foci. The tissue, derived from 19 patients of either sex (age range 11–73 years), was removed in a gentle, atraumatic manner and immediately placed in ice-cold saline to ensure viability. Each patient was informed of the experimental protocol and signed a declaration of consent in accordance with the Declaration of Helsinki, as requested by the local ethics committee. After premedication with midazolam, patients were anesthetized with propofol plus fentanyl. Cisatracurium was given for muscle relaxation. Patients received cefuroxim as intraoperative one-time antibiotic prophylaxis. Tissue macroscopically infiltrated with tumor was excluded, and only that tissue appearing unaffected by the underlying disease process was used in subsequent in vitro experiments. The human neocortical tissue included frontal, parietal, temporal, and occipital areas. The white matter was separated (and discarded) from the gray matter, containing all six neocortical layers (i.e., I–VI).

Superfusion model of neurotransmitter release

Tissue slices (350 μm) were prepared using a McIllwain Tissue Chopper (Bachofer; Reutlingen, Germany) and immersed in ice-cold buffer. The buffer used for tissue preparation, incubation and superfusion contained was (in mM) NaCl 121, KCl 1.8, $CaCl_2$ 1.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 10, and ascorbic acid 0.06. Before use, the buffer was saturated with 95% O_2/5% CO_2 and the pH adjusted to 7.4.

The neocortical slices were incubated at 37°C in buffer gassed with 95% O_2/5% CO_2 and containing 0.1 μM ^{3}H-NA, ^{3}H-choline (as precursor to neuronally releasable ^{3}H-ACh), or ^{3}H-DA for 45 min or ^{3}H-HT for 60 min. For ^{3}H-DA, the 5-HT uptake inhibitor fluvoxamine (1 μM) and the NA uptake inhibitor (+)-oxaprotiline (1 μM) were present during incubation (and during superfusion; see below) to prevent false labeling of noradrenergic and serotonergic terminals (Löffler et al. 2006).

After incubation, the slices were washed, transferred into superfusion chambers, and superfused with prewarmed buffer at a rate of 0.4 ml/min. The buffer routinely contained uptake inhibitors and autoreceptor blockers corresponding to the neurotransmitter under investigation. These substances prevent neuronal uptake of released neurotransmitters and autoinhibition, respectively: ^{3}H-NA, (+)-oxaprotiline (1 μM) and idazoxan (1 μM); ^{3}H-ACh, hemicholinium-3 (10 μM) and atropine (1 μM); ^{3}H-DA, nomifensine (1 μM) and sulpiride (10 μM) (in addition to fluvoxamine (1 μM) and (+)-oxaprotiline (1 μM)); and ^{3}H-5-HT, fluvoxamine (1 μM) and methiotepin (1 μM). Collection of 5-min fractional samples began after 60 min of superfusion. The slices were stimulated twice (S_1, S_2) by elevating the K^+ concentration from 3 mM to 15, 25, or 30 mM and maintaining osmolarity by reducing buffer Na^+ accordingly. The S_1 was applied after 75 min of superfusion for 2 min and followed by S_2 after 115 min of superfusion for 2 min. GBP and PGB (100 μM) were present in the superfusion buffer 20 min before S_2. In the case of experiments addressing the effect of PGB on ^{3}H-5-HT release, lower concentrations were also tested (10 and 1 μM). In other experiments, addressing an interaction of GBP and PGB (100 μM) with putative endogenous $\alpha_2\delta$ ligands, D- and L-isoleucine (D-/L-ILe; 100 μM) were present throughout superfusion to determine if these amino acids share a common site of action with GBP and PGB. Additional experiments evaluated L-ILe and D-ILe (100 μM) per se for direct effects on ^{3}H-NA release by addition to the buffer 20 min before S_2. Solvent-treated slices were routinely run in parallel to drug-treated slices. At the end of experiments, slices were solubilized with 0.5 ml Soluene (Packard Instruments; Frankfurt, Germany), and radioactivity of fractional samples and slices were determined by scintillation spectrometry.

Calculations and statistics

The K^+-evoked tritium overflow, expressed as the fractional rate of the total radioactivity in the slice, was calculated as the difference between total tritium outflow (from the start of stimulation and the following 20 min onwards) and basal tritium outflow (during this 20-min period). Basal ^{3}H-efflux was defined as ^{3}H-outflow as percentage of the total radioactivity in the slice during the 5-min period before the second stimulation. The evoked tritium overflow was assumed to represent the release of the ^{3}H-neurotransmitter under investigation. The effects of GBP and PGB on ^{3}H-DA, ^{3}H-NA, ^{3}H-5-HT, and ^{3}H-ACh release were evaluated by calculating the S_2/S_1 ratio of the evoked tritium overflow during the first and second stimulations and normalizing to control values. The effects of L-ILe and D-ILe on ^{3}H-NA release were assessed in a similar manner.

Results are given as arithmetic means with 95% confidence intervals (CI_{95}) to indicate statistical probability. The number of experiments, *n*, is indicated in brackets in the figures or given in the text. Significant differences between two means were tested using the Student's *t* test after analysis of variance. The minimal level of significance was $p<0.05$ (two-tail criterion).

Materials

Substances from commercial sources included idazoxan hydrochloride, hemicholinium-3, atropine sulfate, sulpiride, nomifensine maleate, fluvoxamine maleate, D-isoleucine, L-isoleucine (Sigma, Taufkirchen, Germany), methiothepin maleate, and tetrodotoxin (TTX; Tocris, Cologne, Germany). GBP and PGB were kindly donated by Pfizer (Ann Arbor, MI, USA); (+)-oxaprotiline hydrochloride was a gift from Novartis (Basel, Switzerland). Tritiated neurotransmitters were purchased from Perkin Elmer (Boston, MA, USA). ^{3}H-choline was purchased from Amersham (Buckinghamshire, UK).

Results

GBP and PGB (100 μM) did not change the basal ^{3}H-efflux in all the experiments on ^{3}H-ACh, ^{3}H-DA, ^{3}H-5-HT, and ^{3}H-NA release (data not shown).

K^+-evoked ^{3}H-ACh release

The mean S_1 value of 25 mM K^+ as stimulus was 1.08% (CI_{95}=[0.77, 1.38]). Basal ^{3}H-efflux was 0.18% (CI_{95}=[0.15, 0.21]). GBP (100 μM) decreased K^+-evoked ^{3}H-ACh release by 22% (CI_{95}=[12, 32]), while that of PGB (100 μM) was by 22% (CI_{95}=[6, 38]). The mean S_2/S_1 ratios of GBP- and PGB-treated slices were significantly different from control-treated slices (Fig. 1).

The mean S_1 value using 30 mM K^+ was 1.88% (CI_{95}=[1.32, 2.44]). Basal ^{3}H-efflux was 0.17% (CI_{95}=0.15, 0.20]). GBP and PGB (100 μM) reduced ^{3}H-ACh release by 24% (CI_{95}=[8, 39]) and 18% (CI_{95}=[−29, 47]), respectively. This reduction was significant for GBP but not for PGB (Fig. 1).

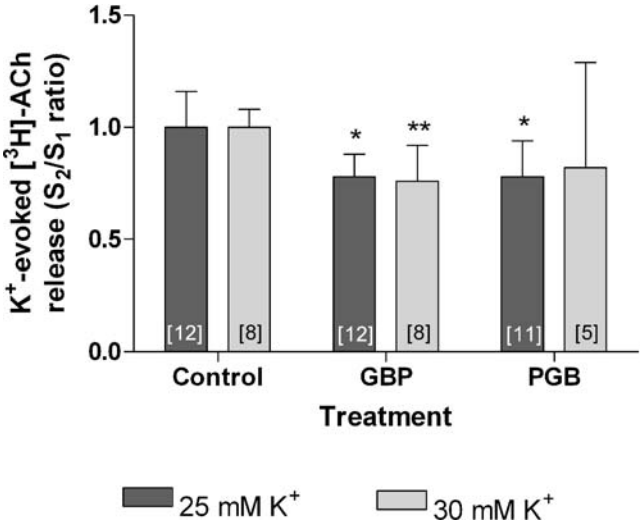

Fig. 1 Effects of GBP (100 μM) and PGB (100 μM) on K^+ (25 mM or 30 mM)-evoked ^{3}H-ACh release from human neocortical slices. Both drugs were present in the buffer 20 min before S_2. Values in *brackets* represent the number of observations. The S_2/S_1 ratios are given as mean values normalized to control values $\pm CI_{95}$. A significant difference from the respective control value is indicated by *asterisks* (*single asterisks*, $p<0.05$; *double asterisks*, $p<0.01$)

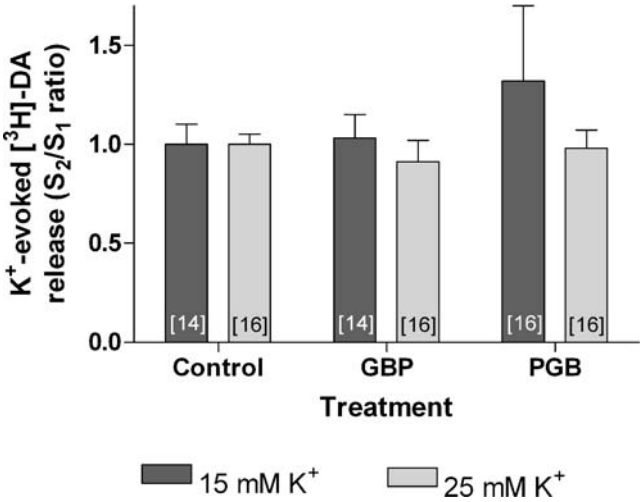

Fig. 2 Effects of GBP (100 μM) and PGB (100 μM) on K^+ (15 mM or 25 mM)-evoked ^{3}H-DA release from human neocortical slices. Both drugs were present in the buffer 20 min before S_2. Values in *brackets* represent the number of observations. The S_2/S_1 ratios are given as mean values normalized to controls $\pm CI_{95}$. There were no significant differences from the respective control value (i.e., $p>0.05$)

K^+-evoked ^{3}H-DA release

GBP and PGB (100 μM) did not alter K^+ (25 mM)-evoked ^{3}H-DA release compared to control-treated slices. As the mean S_1 value was relatively high using 25 mM K^+ (10.21%, CI_{95}=[8.66, 11.92]), the stimulus was decreased to 15 mM K^+. The mean S_1 value for this latter condition was 2.81% (CI_{95}=[2.17, 3.44]), and, again, GBP and PGB (100 μM) did not alter ^{3}H-DA release (Fig. 2). Basal ^{3}H-efflux was 0.32% (CI_{95}=[0.30, 0.33]) in the experiments with 15 mM K^+ and 0.33% (CI_{95}=[0.32, 0.34]) in the experiments with 25 mM K^+.

K^+-evoked ^{3}H-5-HT release

The mean S_1 value of K^+ (25 mM)-evoked ^{3}H-5-HT release was 2.24% (CI_{95}=[2.02, 2.45]). Basal ^{3}H-efflux was 0.40% (CI_{95}=[0.37, 0.43]). GBP (100 μM) significantly decreased ^{3}H-5-HT release by 36% (CI_{95}=[23, 48]), and PGB (100 μM) caused a more pronounced inhibition of 56% (CI_{95}=[46, 66]) (Fig. 3). Also, lower PGB concentrations still inhibited the evoked ^{3}H-5-HT release: At 10 μM, release was reduced by 34% (CI_{95}=[24, 43], n=8; $p<0.0001$) and at 1 μM by 16% (CI_{95}=[−4, 36], n=10; $p<0.05$).

The inhibition of ^{3}H-5-HT release by 100 μM PGB was also tested in the presence of TTX (0.32 μM) throughout superfusion: The inhibition of 53% (CI_{95}=[39; 66], n=9) was similar to that in the absence of TTX (see discussion). By itself, TTX reduced the mean S_1 value by about 31%, just at the level of the p=0.05 significance (data not shown).

K^+-evoked ^{3}H-NA release

The mean S_1 value of K^+ (15 mM)-evoked ^{3}H-NA release was 6.95% (CI_{95}=[5.76, 8.15]). Basal ^{3}H-efflux was 0.41% (CI_{95}=[0.38, 0.44]). GBP and PGB (100 μM) decreased ^{3}H-NA release by 24% (CI_{95}=[20, 32]) and 19% (CI_{95}=[3, 26]), respectively. In contrast, neither L-ILe nor D-ILe (100 μM) affected ^{3}H-NA release. When L-ILe (100 μM) was present throughout superfusion, however, the inhibitory effects of GBP and PGB (100 μM) were abolished. The presence of the stereoisomer D-ILe (100 μM) throughout superfusion did not attenuate the GBP- and PGB-mediated reductions of ^{3}H-NA release (i.e., 21% (CI_{95}=[17, 41]) and 17% (CI_{95}=[6, 27]), respectively; Fig. 4).

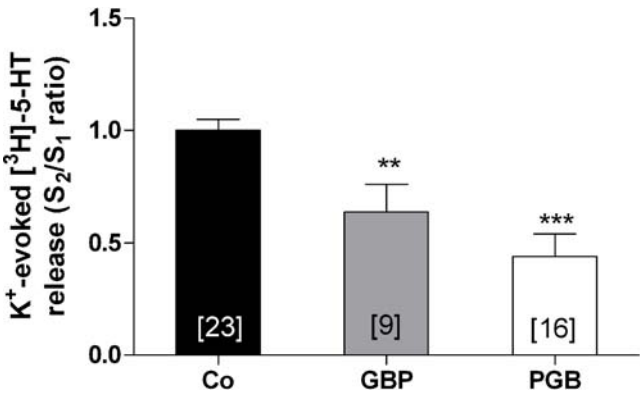

Fig. 3 Effects of GBP (100 μM) and PGB (100 μM) on K^+ (25 mM)-evoked ^{3}H-5-HT release from human neocortical slices. Both drugs were present in the buffer 20 min before S_2. Values in *brackets* represent the number of observations. The S_2/S_1 ratios are given as mean values normalized to control values $\pm CI_{95}$. A significant difference from the control value is indicated by *asterisks* (*double asterisks*, $p<0.01$; *triple asterisks*, $p<0.001$)

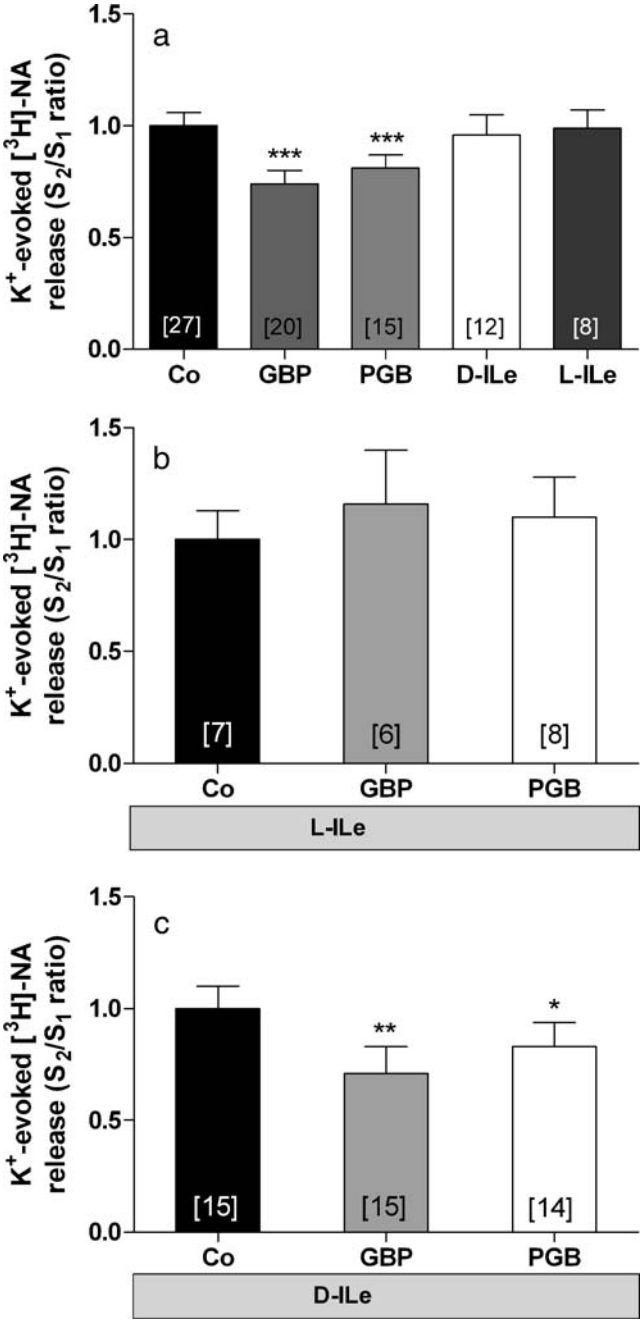

Fig. 4 Comparative effects of GBP (100 μM), PGB (100 μM), D-lLe (100 μM), and L-lLe (100 μM) on K^+ (15 mM)-evoked ^{3}H-NA release from human neocortical slices. The slices in these experiments were exposed to **a** normal conditions (drugs present in the buffer 20 min before S_2, **b** L-lLe (100 μM) present throughout superfusion, and **c** D-lLe (100 μM) present throughout superfusion. Values in *brackets* represent the number of observations. The S_2/S_1 ratios are given as mean values normalized to control $\pm CI_{95}$. A significant difference from the respective control value is indicated by *asterisks* (*single asterisks*, $p<0.05$; *double asterisks*, $p<0.01$; *triple asterisks*, $p<0.001$)

Discussion

An increase of buffer K^+ leads to the depolarization of neurons, followed by exocytosis of vesicles preloaded with ^{3}H-neurotransmitters. The uptake of ^{3}H-neurotransmitters occurs by transporters generally corresponding to the transmitters under investigation. Previous studies have demonstrated that an elevated K^+ concentration evokes a Ca^{2+}-dependent, quasiphysiological release of ^{3}H-DA, ^{3}H-NA, ^{3}H-5-HT, and ^{3}H-ACh in mammalian brain slices (Taube et al. 1977; Verbeuren et al. 1984; Johnson et al. 1993). In our experiments, we used autoreceptor antagonists and uptake inhibitors throughout the experiments to bias the system to be free of both uptake and negative feedback and to enhance the magnitude of release to a given K^+ stimulus; thus, we tried to meet the concern that the magnitude of release of a particular transmitter in some brain regions may be relatively small. To be consistent with our procedures across transmitters and brain regions, autoreceptor antagonists and uptake inhibitors were applied in all experimental approaches.

Except for some experiments on ^{3}H-5-HT release to evaluate a concentration dependence of the inhibition by PGB, we decided to use GBP and PGB at the quite high concentration of 100 μM, because 100 μM has been shown to be a saturating inhibitory concentration in previous experiments addressing the effects of both drugs on ^{3}H-NA release in rat brain slices (Dooley et al. 2000). Because it is known that the clinically effective plasma concentration of GBP in epilepsy can be up to 100 μM, this rather high concentration is still clinically relevant, especially because GBP (and PGB) readily crosses the blood–brain barrier and concentrates in brain tissue via the system-L active transport process (see, for instance, Luer et al. 1999).

Although the VSCC $\alpha_2\delta$ subunit is generally thought to be the primary target of GBP and PGB action, there remains a poor understanding of the mechanistic aspects by which these drugs modulate synaptic transmission, especially considering the different inhibitory effects across various neurotransmitter systems. Both drugs caused modest yet significant decreases of ^{3}H-ACh, ^{3}H-NA, and ^{3}H-5-HT release evoked by various K^+ concentrations. These drugs did not, however, alter K^+-evoked ^{3}H-DA release. It has been shown before by our group that a modulation of K^+ (30 mM)-evoked ^{3}H-DA release in human neocortical slices is possible, e.g., by the D_2 receptor agonist (−)-quinpirole (Löffler et al. 2006). So, most probably, we did not overlook an effect of GBP and PGB (100 μM) in our experimental setup, which was similar to the conditions of our own above-mentioned study. In the case of ^{3}H-NA, the reduction of release was antagonized by L-lLe, an α-amino acid that binds with high affinity to the VSCC $\alpha_2\delta$ subunit. This antagomism was not

observed with the stereoisomer D-ILe, confirming the highly stereoselective nature of the binding site demonstrated previously in radioligand binding assays (Thurlow et al. 1996a, b; Dooley et al. 2002). These findings provide indirect support for the hypothesis that GBP and PGB reduce presynaptic Ca^{2+} influx by binding to the VSCC $\alpha_2\delta$ subunit, consequently resulting in decreased neurotransmitter release. A presynaptic location of $\alpha_2\delta$ subunits is supported by our finding of an unchanged amount of inhibition by PGB of ^{3}H-5-HT release in the presence of TTX, which functionally isolates the nerve endings from action potentials. The inhibition via $\alpha_2\delta$ subunits is consistent with previous investigations into the molecular actions of GBP and PGB, namely, an arginine-to-alanine mutation of the $\alpha_2\delta$-1 protein (transgenic R217A mice) dramatically reduced in vitro ^{3}H-PGB binding to this protein in several CNS regions, as measured audiographically (Bian et al. 2006). These data provide strong evidence that the $\alpha_2\delta$-1 subunit of VSCC is the major binding protein for PGB (and GBP). At the in vivo level, this view is corroborated by the attenuated analgesic efficacy of PGB in R217A mice (Field et al. 2006).

As GBP and PGB bind only to the $\alpha_2\delta$-1 and -2 subtypes of the three homologous proteins found in the CNS ($\alpha_2\delta$-1, -2, and -3), one could speculate that varying ratios of these three subtypes exist on presynaptic terminals of different neurotransmitter systems. This hypothesis may underlie the current observation that GBP and PGB lack effect on ^{3}H-DA release.

In situ hybridization studies have shown that the three $\alpha_2\delta$ subtypes exhibit differential patterns of expression in rat brain; $\alpha_2\delta$-1 and -2 mRNA patterns are largely complementary, whereas $\alpha_2\delta$-3 mRNA is expressed in regions that also expressed $\alpha_2\delta$-1 or -2 mRNA (Cole et al. 2005). Although GBP and PGB also bind to the $\alpha_2\delta$-2 subtype, their affinity for $\alpha_2\delta$-1 is higher (Cole et al. 2005). If the density of $\alpha_2\delta$-1-containing VSCC in dopaminergic neurons (presynaptic terminals) of human neocortex is low relative to that of $\alpha_2\delta$-2 and $\alpha_2\delta$-3, then this could conceivably explain the inactivity of GBP and PGB on ^{3}H-DA release. Our findings could be further clarified by assessing the densities of $\alpha_2\delta$-subtypes on presynaptic terminals of different neurotransmitter systems using immunohistochemical methods for colocalizing transmitter and $\alpha_2\delta$ subtype(s). Such colocalization experiments would determine the existence of distinct ratios of $\alpha_2\delta$-subtypes in different neurotransmitter systems, potentially substantiating our functional results.

The effects of GBP and PGB could also reflect the activity state of VSCC. GBP modulated Ca^{2+} currents in a concentration- and voltage-dependent manner, but its effect was dependent on the culture conditions of F-11 cells and cultured dorsal ganglion neurons (Martin et al. 2002).

GBP failed to modulate P/Q-type VSCC-mediated glutamatergic neurotransmission in the rat hippocampus (Brown and Randall 2005), yet depressed glutamatergic transmission in the mouse spinal cord (Bayer et al. 2004). Brown and Randall concluded that GBP is not a general inhibitor of presynaptic P/Q-type VSCC and that the hippocampal and spinal cord VSCC may have different features that affect sensitivity to GBP (and other $\alpha_2\delta$ ligands).

Our results show, in accordance with the study of Brown and Randall (2005), that the $\alpha_2\delta$ ligand drug class cannot simply be categorized as general inhibitors of VSCC and neurotransmitter release. The $\alpha_2\delta$ ligands, like GBP and PGB, differ in their effects across distinct neurotransmitter systems. The reason for these differences remains elusive, but they may correlate with subunit composition or activity state of presynaptic VSCC. These differences may also explain why, in earlier studies, the electrically evoked ^{3}H-DA release from rabbit caudate nucleus slices was reduced (Reimann 1983) but ^{3}H-ACh release from these slices was not (Schlicker et al. 1985), in contrast to the effects seen with these neurotransmitters in human neocortex slices (present paper).

This is the first study that investigated the effects of PGB on superfused brain slices of humans. The results of a marked inhibition by this drug of ^{3}H-5-HT release seems clinically interesting: An increase in serotonergic transmission in the CNS induces anxiety (see, for instance, Feuerstein 2008). Hence, the therapeutic effect of PGB as a highly effective anxiolytic drug (Bandelow et al. 2007) may be explained, at least in part, by reduction of 5-HT release in cortical areas.

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