Stimulus-Dependent Modulation of \(^{3}\text{H}\)Norepinephrine Release from Rat Neocortical Slices by Gabapentin and Pregabalin\(^1\)

DAVID J. DOOLEY, CINDY M. DONOVAN, and THOMAS A. PUGSLEY

Department of Neuroscience Therapeutics, Pfizer Global Research & Development, Ann Arbor, Michigan

Accepted for publication August 29, 2000 This paper is available online at http://www.jpet.org

ABSTRACT

Gabapentin (GBP; Neurontin) has proven efficacy in several neurological and psychiatric disorders yet its mechanism of action remains elusive. This drug, and the related compounds pregabalin [PGB; CI-1008, S(\(+\))-3-isobutylgaba] and its enantiomer R(\(–\))-3-isobutylgaba, were tested in an in vitro superfusion model of stimulation-evoked neurotransmitter release using rat neocortical slices prelabeled with \(^{3}\text{H}\)norepinephrine (\(^{3}\text{H}\)NE). The variables addressed were stimulus type (i.e., electrical, K\(^+\), veratridine) and intensity, concentration dependence, onset and reversibility of action, and commonality of mechanism. Both GBP and PGB inhibited electrically and K\(^+\)-evoked \(^{3}\text{H}\)NE release, but not that induced by veratridine. Inhibition by these drugs was most pronounced with the K\(^+\) stimulus, allowing determination of concentration-effect relationships (viz., 25 mM K\(^+\) stimulus: GBP IC\(_{50}\) = 8.9 \(\mu\)M, PGB IC\(_{50}\) = 11.8 \(\mu\)M). 

GBP demonstrated the dependence of \(^{3}\text{H}\)NE release inhibition on optimal stimulus intensity. The inhibitory effect of GBP increased with longer slice exposure time before stimulation, and reversed upon washout. Combination experiments with GBP and PGB indicated a similar mechanism of action to inhibit K\(^+\)-evoked \(^{3}\text{H}\)NE release. GBP and PGB are concluded to act in a comparable, if not identical, manner to preferentially attenuate \(^{3}\text{H}\)NE release evoked by stimuli effecting mild and prolonged depolarizations. This type of modulation of neurotransmitter release may be integral to the clinical pharmacology of these drugs.

Gabapentin (GBP; Neurontin, 1-(aminomethyl)cyclohexaneacetic acid) (Fig. 1) is efficacious in several neurological and psychiatric disorders, including epilepsy (Chadwick et al., 1998), neuropathic pain (Rosenberg et al., 1997), migraine (Mathew et al., 1999), tremor (Gironell et al., 1999), social phobia (Pande et al., 1999), bipolar depression (Young et al., 1999), and drug abuse/withdrawal (Myrick et al., 1998). The mechanism(s) of action that explains this broad spectrum of therapeutic utility is controversial (Taylor et al., 1998). One interesting hypothesis originates from the observation that \(^{3}\text{H}\)GBP binds to the auxiliary \(\alpha_2\delta\) subunit of voltage-sensitive calcium channels (VSCC) (Gee et al., 1996). Because the \(\alpha_2\delta\) subunit can modulate Ca\(^{2+}\) conductance of the \(\alpha_1\) subunit (Walker and De Waard, 1998), there exists the possibility that the GBP-\(\alpha_2\delta\) subunit complex negatively modulates neuronal \(\alpha_1\) VSCC subunits (viz., L-, N-, and P/Q-type VSCC) to decrease depolarization-induced Ca\(^{2+}\) influx in various neuronal cells (Stefani et al., 1998), and to attenuate K\(^+\)-induced Ca\(^{2+}\) influx in synaptosomes (or presynaptic axon terminals) (Meder and Dooley, 2000).

Decreases of depolarization-evoked Ca\(^{2+}\) entry into neurons by GBP presumably translate into reductions of neuronal excitability and neurotransmitter release. Such changes offer a logical explanation for the normalization or attenuation of neuronal dysfunction seen in GBP-responsive central nervous system disorders. Previous investigations have, in fact, demonstrated that GBP can cause relatively small yet consistent inhibitions (i.e., \leq 20\% of control values at relevant physiological concentrations) of electrically evoked, calcium-dependent \(^{3}\text{H}\)neurotransmitter release from mammalian brain slices (Reimann, 1983; Schlicker et al., 1985); these neurotransmitters included norepinephrine, dopamine, and 5-hydroxytryptamine.

In the present study, we tested GBP for effects in an in vitro superfusion model of stimulation-evoked neurotransmitter release using rat neocortical slices prelabeled with \(^{3}\text{H}\)norepinephrine (NE). The variables of interest were stimulus type (i.e., electrical, K\(^+\), veratridine), stimulus intensity, concentration dependence, onset of action, and reversibility of action. In some experiments, pregabalin [PGB; CI-1008, S(\(+\))-3-isobutylgaba, S(\(+\))-4-amino-3-(2-methyl-

Received for publication June 20, 2000.

\(^1\)Preliminary reports on this work were presented at the 2nd Meeting of European Neuroscience, Strasbourg, France, September 24–28, 1996; the 26th Society for Neuroscience Congress, Washington, DC, November 16–21, 1996; and the 28th Society for Neuroscience Congress, Los Angeles, CA, November 7–12, 1998.

ABBREVIATIONS: GBP, gabapentin; VSCC, voltage-sensitive calcium channels; NE, norepinephrine; PGB, pregabalin; R-IBG, R(\(–\))-3-isobutylgaba; N.S., not significant.
propyl)butanoic acid] (Fig. 1) and its enantiomer \(R-(−)-3\)-isobutylgaba (R-IBG) were evaluated for comparison to GBP. PGB has a pharmacology similar to that of GBP, including nanomolar displacement of \[^3H\]GBP binding to rat neocortical membranes (Bryans and Wustrow, 1999) and clinical efficacy at lower doses in epilepsy, diabetic neuropathy, and anxiety disorders (Abou-Khalil et al., 1999; A. C. Pande and R. M. Poole, personal communication).

The experiments were designed to characterize the modulatory effects of GBP and PGB on neurotransmitter release. The results presented here may offer a rationale for the clinical findings with these drugs, and facilitate the design of additional experiments directed at mechanism of action.

### Experimental Procedures

**Subjects.** Male rats (Sprague-Dawley, 200–220 g; Charles River Laboratories, Wilmington, MA) were housed in an Association for the Assessment and Accreditation of Laboratory Animal Care International-accredited facility according to the standards outlined in the Guide for the Use and Care of Laboratory Animals. The macroenvironment was controlled to provide a temperature of 23 ± 3°C, a relative humidity of 43%, and 12-h light/dark cycles. Animals had ad libitum access to food and water, and were maintained for a minimum of 5 days before sacrifice by decapitation. The brains were removed by blunt dissection and placed in ice-cold buffer until slice preparation.

**Superfusion Model of Neurotransmitter Release.** Neocortical slices (0.4 mm thick, 5 mm diameter) were prepared using a vibrating microslicer (DTK-1000; Dosaka EM Co., Kyoto, Japan), and incubated for 30 min at 37°C in a modified Krebs-Henseleit buffer (121 mM NaCl, 1.8 mM KCl, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 25 mM NaHCO₃, 1.2 mM KH₂PO₄, 11 mM glucose, 0.57 mM ascorbic acid, 0.03 mM EDTA; saturated with 95% O₂, 5% CO₂; pH 7.4) containing \[^3H\]NE (0.1 nM), and the additional experiments directed at mechanism of action.

### Results

In the first series of experiments to confirm and extend published results (Schlicker et al., 1985), GBP (3–300 μM) was tested to inhibit \[^3H\]NE release evoked by electrical stimuli (Table 1). At the standard stimulation frequency of 3 Hz, GBP caused a significant concentration-dependent decrease of \[^3H\]NE release ranging from 10 to 20%. For comparison, PGB (100 μM) reduced this electrically evoked release by 22% (Table 2), whereas R-IBG (100 μM) was practically inactive (nonsignificant 9% inhibition). A nominally calcium-free buffer prevented \[^3H\]NE release to an almost undetectable level (98% inhibition), and the N-type VSCC antagonist \(\omega\)-conotoxin GVIA (0.1 μM) reduced \[^3H\]NE release by 71% (Table 2). The inhibition of electrically evoked release by GBP was effectively eliminated by lower and higher stimulation frequencies of 0.3 and 30 Hz (Table 1).

In the second series of experiments, the effect of GBP (3–300 μM) was evaluated on K⁺-evoked \[^3H\]NE release (Table 1). At the standard K⁺ concentration of 25 mM, GBP significantly reduced \[^3H\]NE release in a concentration-dependent manner with a range of 9 to 31%. PGB (100 μM) and R-IBG (100 μM) also attenuated this K⁺-evoked \[^3H\]NE release by 34 and 24%, respectively (Table 2). The absence of buffer calcium prevented \[^3H\]NE release (96% inhibition), and \(\omega\)-conotoxin GVIA (0.1 μM) caused a 73% reduction of \[^3H\]NE release (Table 2). A lowering of the K⁺ stimulus concentration to 15 mM affected a robust concentration-dependent inhibition of \[^3H\]NE release by GBP, ranging from 22 to 47% (Table 1). A higher concentration of K⁺ (i.e., 50 mM), however, attenuated the effect of GBP; the inhibitions ranged from 2 to 13% with only this latter percentage (at 300 μM) being significant. [Note that an alternative approach to determine the effects of drugs on neurotransmitter release is...
TABLE 1
Effects of gabapentin (3–300 μM) on stimulation-evoked [3H]norepinephrine release from rat neocortical slices
Electrical stimuli consisted of 90 2-ms monophasic pulses. The duration of the K⁺ stimulus was 2 or 4 min (15 mM). Gabapentin was present in the buffer 15 min before stimulation (S₁). Values given represent tritium overflow [S₁ (%)] as X ± S.E. (n ≥ 6).

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Electrical (Hz)</th>
<th>Gabapentin (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3</td>
<td>17.6 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>14.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>17.6 ± 0.8</td>
</tr>
<tr>
<td>K⁺ (mM)</td>
<td>15</td>
<td>14.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>38.9 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>83.7 ± 3.7</td>
</tr>
</tbody>
</table>

Analysis of variance of S₁ values for electrical and K⁺ stimuli: 0.3 Hz, F(3,31) = 1.19 (P = .33, N.S.); 3 Hz, F(3,32) = 4.92 (P < .01); 30 Hz, F(3,32) = 1.19 (P = .33, N.S.); 15 mM, F(3,56) = 7.43 (P < .001); 25 mM, F(3,38) = 10.4 (P < .001); 50 mM, F(3,26) = 3.25 (P < .05). A significant difference from the control value is indicated by asterisks (*P ≤ .05, **P ≤ .01).

TABLE 2
Effects of pregabalin (100 μM), R(-)-3-isobutylgaba (100 μM), α-conotoxin GVIA (0.1 μM), and buffer calcium omission (0 mM) on stimulation-evoked [3H]norepinephrine release from rat neocortical slices
The electrical stimulus consisted of 90 2-ms monophasic pulses. The duration of the K⁺ stimulus was 2 min. Substances (or an absence of buffer calcium) were present in the buffer 15 min before stimulation (S₁). Values given represent tritium overflow [S₁ (%)] as X ± S.E. (n ≥ 6).

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Control</th>
<th>Gabapentin (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>19.0 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>36.6 ± 1.8</td>
</tr>
<tr>
<td>K⁺ (25 mM)</td>
<td>100 μM</td>
<td>17.3 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>α-Conotoxin GVIA 0.1 μM</td>
<td>5.5 ± 0.4**</td>
</tr>
<tr>
<td></td>
<td>Calcium 0 mM</td>
<td>0.4 ± 0.3**</td>
</tr>
</tbody>
</table>

A significant difference from the control value is indicated by asterisks (*P ≤ .05, **P ≤ .01).

The absolute difference between control and drug S₁ values. Using this measure across stimulus conditions in Table 1, the magnitude of concentration-dependent inhibition by GBP was greatest using the 25 mM K⁺ stimulus (i.e., range from 4 to 12%).

A direct comparison of the inhibitory effects of GBP (3–300 μM) in response to an electrical (3 Hz) stimulus and a K⁺ (15 mM) stimulus indicated an approximately 2-fold greater inhibition of the K⁺-evoked [3H]NE release (Fig. 2). In other experiments, the effects of GBP (100 μM) and PGB (100 μM) were evaluated on [3H]NE release evoked by the sodium channel activator veratridine (Table 3); this release was not altered by either drug, but was eliminated by the sodium channel blocker tetrodotoxin (97% inhibition).

A detailed analysis of the inhibition of K⁺ (25 mM)-evoked [3H]NE release by GBP (100 μM) and PGB (100 μM) is presented as fractional rates over the course of typical experiments (Fig. 3, A and B); the inhibitions were 42 and 32%, respectively. Complete concentration-effect relationships were generated for both GBP (0.3–1000 μM) and PGB (1–1000 μM) (Fig. 4, A–C); the IC₅₀ values were 8.9 and 11.8 μM, respectively, with submaximal inhibitions of 33.3 and 39.8% (at 0.1–1 mM). [Note that an IC₅₀ value for GBP was not determined from results using an electrical stimulus (i.e., 3 Hz; Table 1); this decision was based on the relatively small range of inhibition.] The variable inhibition of K⁺ (25 mM)-evoked [3H]NE release by R-IBG (1–1000 μM), being consistently less than that of PGB at equimolar concentrations (e.g., Table 2), precluded establishment of a concentration-effect relationship and determination of an IC₅₀ value (data not shown).

In the third series of experiments, the onset and reversibility of GBP to inhibit K⁺ (25 mM)-evoked [3H]NE release was assessed by varying the slice exposure and washout times before stimulation (Fig. 5, A and B). When GBP (1–100 μM) was present in the buffer for only 5 min before stimula-

---

**Fig. 2.** Comparative effects of gabapentin (3–300 μM) to inhibit [3H]norepinephrine release evoked from rat neocortical slices by an electrical stimulus (90 2-ms monophasic pulses of 3 Hz) and a K⁺ stimulus (15 mM for 4 min). Values given represent inhibition (%) as X ± S.E. (n ≥ 9) relative to the respective mean control S₁ values of 14.8% (n = 9) and 14.3% (n = 15) as listed in Table 1. Comparison of the two stimulus conditions was chosen on the basis of similar control S₁ values. A significant difference between stimulus-dependent inhibitions at each concentration of gabapentin is indicated by asterisks (*P ≤ .05, **P ≤ .01; N.S.).
tion, there was not a significant inhibition of [3H]NE release. Longer slice exposure times to GBP, either the standard 15 or 60 min, effected both time- and concentration-dependent inhibitions (e.g., 10 μM, 41% inhibition for 60 min versus 21% inhibition for 15 min). A washout period between slice exposure to GBP (30 μM) and K+ stimulation indicated a time-dependent reversal of [3H]NE release inhibition by GBP (i.e., inhibitions of 25% for 0 min washout, 13% for 15 min washout, and 0% for 30 min washout).

In the final series of experiments, the previously described similar inhibitions of K+ (25 mM)-evoked [3H]NE release by GBP and PGB (Figs. 3 and 4) suggested comparable, if not identical, mechanisms of action. A direct test of this hypoth-

![Fig. 3. Effects of gabapentin (100 μM) (A) and pregabalin (100 μM) (B) on the basal tritium outflow and K+ -evoked tritium overflow from representative experiments using rat neocortical slices prelabeled with [3H]norepinephrine. Fractional rates for slices used in each experiment are based on 5-min fractions beginning 45 min after the start of superfusion. The K+ stimulus was 25 mM for 2 min, and occurred 70 min after the start of superfusion. The drugs were present in the buffer 15 min before stimulation. Results are given as X ± S.E. (n = 3). In A, control values were 3.52 ± 0.16 pmol of tritium/slice (at the start of fraction collection), tritium overflow [S1 (%)] of 43.5 ± 2.8, and basal tritium outflow (b1) of 1.70 ± 0.05 (5 min −1); and gabapentin values were 3.02 ± 0.37 pmol of tritium/slice, tritium overflow [S1 (%)] of 25.3 ± 2.6, and basal tritium outflow (b1) of 1.75 ± 0.05. In B, control values were 4.34 ± 0.12 pmol of tritium/slice, tritium overflow [S1 (%)] of 40.2 ± 2.8, and basal tritium outflow (b1) of 1.38 ± 0.03; and pregabalin values were 4.38 ± 0.18 pmol of tritium/slice, tritium overflow [S1 (%)] of 27.5 ± 2.6, and basal tritium outflow (b1) of 1.49 ± 0.03.

![Fig. 4. Concentration-effect relationships of gabapentin (0.3–1000 μM) (A) and pregabalin (1–1000 μM) (B) to inhibit K+ (25 mM, 2 min)-evoked [3H]norepinephrine release from rat neocortical slices. Values given represent tritium overflow [S1 (%)] as X ± S.E. (n ≥ 6). Analysis of variance of S1 values gave F(8,70) = 9.56 (P ≤ .001) and F(7,132) = 8.34 (P ≤ .001), respectively. A significant difference from the control value is indicated by asterisks (*P ≤ .05, **P ≤ .01). The transformed data (C) from A and B depict inhibition (%) relative to the respective mean control S1 values of 38.9% (n = 21) and 38.4% (n = 49). The corresponding IC50 values were 8.9 (2.9–27.5) μM and 11.8 (4.4–31.9) μM, and the maximal inhibitions were 33.3 (28.2–38.5)% and 39.8 (33.5–46.1)%.

esis was accomplished by having one drug (at a saturating concentration of 100 μM) present in the buffer throughout superfusion, and adding the other drug (100 μM) 15 min
before the K⁺ stimulation. If the mechanisms of action of the two drugs are identical, then there should not be any additional inhibition. If, however, an additional inhibition is observed, then this would suggest different mechanisms exist for these compounds to inhibit [³H]NE release. Neither GBP nor PGB produced any further inhibition in the presence of the other drug (Fig. 6A). [Note that ω-conotoxin GVIA (0.1 μM), in contrast, was as effective to decrease [³H]NE release by ~75% in the presence of either drug as in their absence (cf. Fig. 6 legend; Table 2).] An alternative test of similar action of two drugs (at nonsaturating concentrations) relies on the assumption that identical mechanisms contribute to an additive rather than a synergistic effect in a biological system. Using a minimally effective concentration (3 μM) of GBP and PGB (as extrapolated from Fig. 4), a combination of these two agents (being equivalent to 6 μM) did not produce a significant reduction of [³H]NE release (Fig. 6B).

In all experiments, none of the test substances altered basal [³H]NE release (e.g., Fig. 3).

Discussion

The present study indicates that both 3-substituted γ-aminobutyric acids, GBP and PGB, are effective to inhibit electrically (3 Hz) evoked, calcium-dependent [³H]NE release from rat neocortical slices. These findings, under conditions simulating action potential-mediated neurotransmitter release, agree with previous reports of GBP (100 μM) causing modest reductions (viz., ≤20% of control values) of electrically (3 Hz) evoked release of [³H]catecholamines and 5-[³H]hydroxytryptamine from mammalian brain slices (Reimann, 1983; Schlicker et al., 1985). The inhibition of neurotransmitter release by GBP and PGB presumably reflects an interaction of these drugs with the extracellular domain of the auxiliary α₂β subunit of neuronal VSCC (Gee et al., 1996; Wang et al., 1999), and the resultant attenuation of depolarization-induced Ca²⁺ influx subserving the release process (Meder and Dooley, 2000).

Several findings relate to and characterize the inhibition of electrically evoked neocortical [³H]NE release by these drugs. First, the presence of a high density of [³H]GBP binding sites and mRNA expression level of the α₂β-1 isofrom subunit exists in the rodent neocortex (Hill et al., 1993; Klugbauer et al., 1999). Second, the effective concentrations of GBP and PGB are within or approximate the estimated therapeutic range in plasma and/or brain tissue of 1 to 100 μM (Ben-Menachem et al., 1992; Welty et al., 1993; D. F. Welty, personal communication). Third, the stereoselective effects of PGB and its less active enantiomer R-IBG are consistent with the different potencies of these compounds in both the [³H]GBP binding assay and in vivo tests (Bryans and Wustrow, 1999). Fourth, a comparatively greater magnitude of release inhibition occurs with a saturating concentration of the N-type VSCC antagonist ω-conotoxin GVIA, emphasizing a distinctly different mode/site of action of GBP and PGB from such (relatively large and potent) peptide blockers, which target the α₁ subunits of VSCC (Ellinor et al., 1994). Finally, a significant decrease of electrically evoked [³H]NE release by GBP is a function of stimulation frequency: relatively minor yet significant inhibition present at the standard frequency of 3 Hz, being absent at lower and higher frequencies (viz., 0.3 and 30 Hz). [Note that locus ceruleus noradrenergic neurons typically show spontaneous discharges varying from 0 to 20 Hz in the conscious rat and monkey (Foote et al., 1983).]

Previous investigations have not directly compared the effects of GBP or PGB on neurotransmitter release evoked by different stimuli under the same set of experimental conditions. In this study, saturating concentrations (i.e., 0.1–1 mM) of both drugs produced greater inhibition (viz., >30% of control values) of [³H]NE release using a 15 or 25 mM K⁺ stimulus than a 3 Hz electrical stimulus. The characterization of K⁺(25 mM)-evoked [³H]NE release, however, gave practically identical results to those obtained with the electrical stimulus; specifically, both types of [³H]NE release exhibited absolute dependence on buffer calcium and marked sensitivity to ω-conotoxin GVIA. Additionally, stereoselective effects are again evident for PGB and R-IBG even though the
latter also causes a significant inhibition in the presence of the K\(^+\) stimulus.

A change in K\(^+\) stimulus concentration, analogous to a change in electrical stimulation frequency, altered the inhibitory effects of GBP. The most robust inhibition occurred at low K\(^+\) concentrations (viz., 15 and 25 mM) with less effect at a higher concentration (viz., 50 mM). These observations, for both electrical and K\(^+\) stimuli, indicate that an increased stimulus intensity attenuates or prevents the modulatory effects of GBP on \([3H]NE\) release. A similar phenomenon has also been demonstrated for \(\omega\)-conotoxin GVIA (Keith et al., 1993). Such findings emphasize that pharmacological modulation of calcium-dependent neurotransmitter release is dependent on the stimulus parameters evoking this release.

In contrast to the inhibitory effects of GBP and PGB when using the previous two stimuli, neither drug reduced veratridine-evoked \([3H]NE\) release. Although this type of tetrodotoxin-sensitive release was of comparable magnitude, its marked sodium dependence may preclude modulation by GBP and PGB. These drugs, which may preferentially target VSCC function (Stefani et al., 1998; Meder and Dooley, 2000), appear more effective to modulate neurotransmitter release requiring only partial or intermittent sodium channel activation.

The dynamic range of GBP and PGB to inhibit K\(^+\) (25 mM)-evoked \([3H]NE\) release, in contrast to electrically (3 Hz) evoked release, permitted construction of classical concentration-effect relationships. These similar relationships can be described as 1) occurring over two log units; 2) having IC\(_{50}\) values of \(\sim10\) \(\mu\)M consistent with the estimated therapeutic concentration range (Ben-Menachem et al., 1992; Welty et al., 1993; D.F. Welty, personal communication); and 3) reflecting a modulation of the release process (viz., submaximal inhibitions of 30–40\% at saturating drug concentrations).

The inhibitory effect of GBP on K\(^+\) (25 mM)-evoked \([3H]NE\) release increases with slice exposure time, and reverses upon washout. These findings are in general agreement with the known association and dissociation kinetics of \([3H]GBP\) binding to rat neocortical membranes (Suman-Chauhan et al., 1993); additional variables may, however, contribute to the onset and reversibility of action in a superfused brain slice [e.g., passive diffusion, system L amino acid transport (Su et al., 1995)]. Such variables may also account for the relative potency difference of at least a 100-fold between IC\(_{50}\) or KD values from these two in vitro assays (viz., \([3H]NE\) release: GBP IC\(_{50}\) = 8.9 \(\mu\)M, PGB IC\(_{50}\) = 11.8 \(\mu\)M; \([3H]GBP\) binding: KD = 38 \(\mu\)M, GBP IC\(_{50}\) = 80 \(\mu\)M, PGB IC\(_{50}\) = 37 \(\mu\)M (Suman-Chauhan et al., 1993; Bryans and Wustrow, 1999)).

A common mechanism of action for GBP and PGB is suggested by similar IC\(_{50}\) values in the \([3H]GBP\) binding assay (Suman-Chauhan et al., 1993; Bryans and Wustrow, 1999), and by the practically identical concentration-effect relationships to inhibit K\(^+\) evoked \([3H]NE\) release. This hypothesis gained further support by the demonstration that the inhibitory effect of each drug on K\(^+\)-evoked \([3H]NE\) release was mutually occluded by the presence of the other one. Moreover, a combination of these drugs at marginally active concentrations did not result in a nonadditive or synergistic reduction of \([3H]NE\) release. These various observations consequently point to similar, if not identical, mechanisms of action of GBP and PGB to inhibit stimulation-evoked \([3H]NE\) release.

The preferential effects of GBP and PGB to inhibit \([3H]NE\) release evoked by K\(^+\) stimuli, relative to electrical stimuli, may be pertinent to the clinical efficacy and broad therapeutic index of these drugs. In this regard, various preclinical studies have implicated NE in epileptiform discharges and neuronal excitability (Lacaille and Harley, 1985; Rutecki, 1995), nociceptive processing (Devor et al., 1994; Aston-Jones et al., 1999; Martin et al., 1999), stress and anxiety responses (Aston-Jones et al., 1999), and motor abnormalities (Adams and Foote, 1988). Administration of GBP (or PGB) may decrease the release of NE and other relevant neurotransmitters in such conditions, and especially those in which excessive neuronal activity leads to elevated \([K^+]_o\) (Svoboda et al., 1988; Rutecki, 1995; Jensen and Yaari, 1997). This hypothesis offers at least a partial explanation for the known efficacy of this drug in seizure models and epilepsy (Chadwick et al., 1998; Bryans and Wustrow, 1999), neuropathic pain states (Field et al., 1997; Rosenberg et al., 1997; Pan et al., 2000).
1999), anxiety models and social phobia (Bryans and Wustrow, 1999; Pande et al., 1999), and dystonia (Chudnow et al., 1997; Richter and Loscher, 1999).

The modest in vitro reductions of electrically evoked [3H]NE release by GBP and PGB may translate into in vivo decreases of action potential-mediated release of this neurotransmitter. Although some increased decreases cannot be excluded from contributing to the therapeutic effects of these drugs, there is the possibility that this change in neuronal activity underlies some of the side effects. Specifically, the most common clinical adverse effects of GBP and PGB are reported to be somnolence and dizziness (Abou-Khalil et al., 1999; Bryans and Wustrow, 1999), behavioral changes seen also in rats as sedation and ataxia at high doses of these agents (Field et al., 1997). These pharmacological effects mirror physiological states of low arousal, including sedation and sleep, which are associated with attenuated locus ceruleus activity and concomitant NE release (Foote et al., 1983; Aston-Jones et al., 1999). One implication of these observations is the potential clinical utility of GBP and PGB to treat sleep disorders.

Recent clinical studies indicate that GBP does not have a significant impact on cognitive processes (Dodrill et al., 1999; Meador et al., 1999). Therapeutic doses of GBP (or PGB) appear, therefore, to not overly compromise the intrinsic role of the locus ceruleus in goal-directed behaviors, especially considering the importance of this system in attention and reaction to environmental stimuli (Foote et al., 1983; Aston-Jones et al., 1999).

In summary, GBP and PGB are especially effective to reduce [3H]NE release evoked by increases in [K+]o. This stimulus causes mild and prolonged depolarizations, which differ considerably from the train of action potentials and repetitive, brief depolarizations of an electrical stimulus. Certain pathological states may, therefore, have as a common attribute altered neurophysiological conditions that approximate those imposed by a K+-stimulus, thereby conferring sensitivity to a GBP- or PGB-mediated reduction of excessive neurotransmitter release. Such an attenuation of neurotransmitter release by these drugs is analogous to decreasing stimulus intensity or reducing the calcium influx necessary for the release process.

The superfusion model of K+-evoked, calcium-dependent [3H]NE release may have utility as an in vitro functional assay to detect compounds with pharmacological properties similar to GBP and PGB. This conclusion is based on a consideration of the effective concentrations and submaximal inhibitions of these drugs as a function of stimulus parameters.

Acknowledgment

We thank Dr. W. Meder for a critical review of this manuscript.

References


Send reprint requests to: David J. Dooley, Ph.D., Department of Neuroscience Therapeutics, Pfizer Global Research & Development, 2800 Plymouth Rd., Ann Arbor, MI 48105. E-mail: david.dooley@pfizer.com