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Context-Dependent Modulation of $\alpha\beta\gamma$ and $\alpha\beta\gamma$ GABA_A Receptors by Penicillin: Implications for Phasic and Tonic Inhibition

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Summary

Penicillin, an open-channel blocker of GABA_A receptors, was recently reported to inhibit phasic, but not tonic, currents in hippocampal neurons. To distinguish between isoform-specific and context-dependent modulation as possible explanations for this selectivity, the effects of penicillin were evaluated on recombinant GABA_A receptors expressed in HEK293T cells. When co-applied with saturating GABA, penicillin decreased peak amplitude, induced rebound, and prolonged deactivation of currents evoked from both synaptic and extrasynaptic receptor isoforms. However, penicillin had isoform-specific effects on the extent of desensitization, reflecting its ability to differentially modulate peak (non-equilibrium) and residual (near-equilibrium) currents. This suggested that the context of activation could determine the apparent sensitivity of a given receptor isoform to penicillin. To test this hypothesis, we explored the ability of penicillin to modulate synaptic and extrasynaptic isoforms that were activated under more physiologically relevant conditions. Interestingly, while currents evoked from synaptic isoforms under phasic conditions (transient activation by a saturating concentration of GABA) were substantially inhibited by penicillin, currents evoked from extrasynaptic isoforms under tonic conditions (prolonged application by a sub-saturating concentration of GABA) were minimally affected. We therefore concluded that the reported inability of penicillin to modulate tonic currents could not simply be attributed to insensitivity of extrasynaptic receptors, but rather, reflected an inability to modulate these receptors in their native context of activation.

Keywords

ion channel; ligand-gated; Cys loop; kinetics; Markov; modeling; open-channel block; desensitization; deactivation; rebound

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Introduction

GABA_A receptors are heteropentameric ligand-gated chloride channels responsible for the majority of fast inhibitory neurotransmission in the mammalian brain (Olsen and Macdonald, 2002; Luscher and Keller, 2004). Seven subunit families (α , β , γ , δ , ϵ , θ , and π) are known to exist (Olsen and Macdonald, 2002; Luscher and Keller, 2004), with $\alpha\beta\gamma$ and $\alpha\beta\delta$ isoforms representing the majority of neuronal GABA_A receptors (McKernan and Whiting, 1996). In general, $\alpha\beta\gamma$ receptors are targeted to the synapse (though some γ subunit-containing receptors may also be found extrasynaptically), while $\alpha\beta\delta$ receptors are targeted to extra- or perisynaptic areas (Essrich et al., 1998; Nusser et al., 1998; Wei et al., 2003; Semyanov et al., 2004; Sun et al., 2004).

Following release from pre-synaptic vesicles, a nearly-saturating concentration of GABA activates post-synaptic GABA_A receptors before being cleared rapidly by diffusion and reuptake. This transient activation, thought to occur in the sub-millisecond time scale (Mozrzymas, 2004), gives rise to inhibitory post-synaptic currents (IPSCs) and is termed “phasic” inhibition. Increasing evidence, however, suggests that GABA may escape from synapses, allowing for activation of extrasynaptic receptors (Glykys and Mody, 2007). The persistent increase in chloride conductance resulting from prolonged exposure to sub-saturating concentrations of ambient GABA gives rise to “tonic” inhibition and represents an important alternate mode of GABAergic transmission, one that is less spatially and temporally constrained (Mody and Pearce, 2004; Farrant and Nusser, 2005). Understanding how pharmacological agents differentially modulate these two modes of neuronal inhibition is of increasing interest, as they may play distinct roles in the pathogenesis of neurological disorders such as epilepsy (Peng et al., 2004; Macdonald et al., 2006).

Penicillin is a known convulsant that reduces GABA-evoked currents (Macdonald and Barker, 1977; Horne et al., 1992; Katayama et al., 1992; Tsuda et al., 1994; Behrends, 2000; Sugimoto et al., 2002) and blocks spontaneous activity of GABA_A receptors (Krishek et al., 1996; Tierney et al., 1996; Lindquist et al., 2004). This negative modulation is thought to be mediated by open-channel block, as single channel studies have reported decreased open duration with increased channel opening frequency and burst length (Neher and Steinbach, 1978; Chow and Mathers, 1986; Twyman et al., 1992). Interestingly, electrophysiological studies in hippocampal neurons have found that penicillin inhibits phasic, but not tonic, currents (Yeung et al., 2003). Given that different receptor isoforms are thought to mediate phasic and tonic inhibition (Farrant and Nusser, 2005), and subunit composition is known to influence receptor pharmacology (Wohlfarth et al., 2002; Feng and Macdonald, 2004; Feng et al., 2004), the most parsimonious explanation for this observation is that extrasynaptic isoforms are simply insensitive to penicillin (isoform-specific modulation).

Alternatively, it is possible that both synaptic and extrasynaptic isoforms are sensitive to penicillin, but that its effects are highly dependent on the context of receptor activation (context-dependent modulation). Unlike tonic currents, which are mediated by receptors activated under conditions of near-equilibrium by low concentrations of GABA, phasic currents are mediated by receptors activated under conditions of extreme non-equilibrium by high concentrations of GABA. As a result, the distribution of receptors among various states in the gating scheme is likely to be markedly different, and depending on the relationship between the kinetics of open-channel block and those of receptor gating, could give the appearance of selectivity for receptors activated under phasic, but not tonic, conditions. Indeed, sensitivity to the context of receptor activation, both in terms of exposure duration and agonist concentration, has previously been described for open-channel blockers of nicotinic acetylcholine and NMDA-type glutamate receptors (Ascher, et al., 1978; Marty, 1978; Lipton, 2006).

To distinguish isoform-specific and context-dependent modulation as potential mechanisms underlying the reported ability of penicillin to selectively inhibit phasic currents, its effects were evaluated on currents evoked from recombinant synaptic and extrasynaptic GABA_A receptor isoforms expressed in HEK293T cells. In addition, simulations using simple models of GABA_A receptor function explored the relationship between the microscopic kinetics of open-channel block and the macroscopic properties of currents evoked under phasic or tonic conditions. The results demonstrated that open-channel block can have complex effects on macroscopic current properties, and interestingly, that these effects are highly dependent on the context of receptor activation.

Methods

Cell culture and expression of recombinant GABA_A receptors

Human embryonic kidney (HEK293T) cells were cultured in Dulbecco's Modified Eagle Medium (Invitrogen, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Invitrogen), 100 i.u./ml penicillin, and 100 µg/ml streptomycin (Life Technologies, Grand Island, NY, USA) and maintained at 37°C in humidified 5% CO₂/95% air. Cells were seeded at moderate density in 60-mm culture dishes (Corning Glassworks, Corning, NY, USA) and transfected 24 hr later with 2 µg per cDNA encoding rat α (α 1, α 4, α 5, or α 6), β 3, and either γ 2L or δ GABA_A receptor subunits using a modified calcium phosphate precipitation method (Feng et al., 2004). Two µg of pHOOK (Invitrogen, Carlsbad, CA, USA), which encodes for the surface antibody sFv, were co-transfected for immunomagnetic selection 24 hrs later (Greenfield et al., 1997). Following selection, cells were re-plated in 35-mm culture dishes and currents were recorded 24 hr later using standard patch clamp techniques. Collagen-coated dishes were used for macropatch recordings.

Electrophysiology

Patch-clamp recordings were obtained at room temperature with cells bathed in an external solution composed of (in mM) 142 NaCl, 1 CaCl₂, 6 MgCl₂, 8 KCl, 10 glucose, and 10 HEPES (pH 7.4). Recording electrodes were pulled using a P-87 Flaming Brown Micropipette Puller (Sutter Instruments, San Rafael, CA, USA), fire polished on an MF-9 microforge (Narishige, Tokyo, Japan), and filled with an internal solution consisting of (in mM) 153 KCl, 1 MgCl₂, 10 HEPES, 2 MgATP, and 5 EGTA (pH 7.3). Open-tip resistances of the recording electrodes were typically 1.0–1.6 M Ω . Whole-cell or macropatch currents were recorded under voltage-clamp mode at –20 mV and –50 mV for $\alpha\beta\gamma$ and $\alpha\beta\delta$ receptors, respectively, using an Axopatch 200A amplifier (Molecular Devices, Foster City, CA, USA), low-pass filtered at 2 kHz using the internal 4-Pole Bessel filter of the amplifier, digitized with the Digidata 1322A data acquisition system (Molecular Devices), and stored for offline analysis. Both GABA and penicillin G (benzylpenicillin, sodium salt) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and prepared as stock solutions. Working solutions were made on the day of the experiment by diluting stock solutions with external solution. Drugs were gravity-fed using 4-barrel square glass tubing connected to a Perfusion Fast-Step system (Warner Instruments, Hamden, CT, USA). The 10–90% rise times of open electrode tip liquid junction currents were consistently <2.0 ms. Penicillin was pre-applied for 1.5 sec prior to co-application with GABA. 4 sec pulses of 1 mM GABA were used to evaluate macroscopic current kinetics, 5 ms pulses of 1 mM GABA were used to mimic phasic activation, and 30 sec pulses of 1 µM GABA were used to mimic tonic activation. Consecutive drug applications were separated by at least 45 sec in external solution to allow for complete GABA and penicillin unbinding.

Data analysis and simulations

Data were analyzed offline using Clampfit 8.1 (Molecular Devices). The extent of current inhibition by penicillin (% of GABA current) was determined by dividing the current evoked

during co-application of GABA and penicillin by the current evoked by GABA alone. The extent of desensitization, defined as the loss of macroscopic current in the continued presence of agonist (also commonly referred to as “sag”), was quantified by dividing the amount of current lost (residual current subtracted from peak current) by the peak current amplitude. The time course of deactivation was fit using the standard Levenberg-Marquardt method to the form $\sum a_n e^{(-t/\tau_n)}$, where n is the number of the exponential components, a is the relative amplitude, t is time, and τ is the time constant. A weighted τ in the form of $(a_1 \tau_1 + a_2 \tau_2) / (a_1 + a_2)$ was calculated for comparison of deactivation time courses. The net charge transfer of a pulse was defined as the area between the adjusted baseline and the current. Data were reported as mean $\pm$ SEM. A paired Student's t test was performed to compare individual current features before and after penicillin co-application. An unpaired Student's t test was used to compare current features. Statistical significance was taken as $p < 0.05$.

Kinetic simulations were carried out using Berkeley Madonna 3.1 (www.berkeleymadonna.com), a differential equation solving program, using the fourth order Runge-Kutta method with a time interval of 10 μ s. Analyses of simulated currents were identical to those of real currents.

Results

Penicillin altered the kinetic properties of currents evoked from synaptic and extrasynaptic GABA_A receptor isoforms

To explore the effects of penicillin on the macroscopic current properties of synaptic and extrasynaptic GABA_A receptors, a saturating concentration of GABA (1 mM) was co-applied with penicillin (1 mM) for 4 sec using rapid solution exchange to lifted HEK293T cells transiently transfected with α ($\alpha 1$, $\alpha 4$, $\alpha 5$, or $\alpha 6$), $\beta 3$, and either $\gamma 2L$ or δ subunits. These eight subunit combinations were chosen as a representative sample of receptor isoforms thought to be expressed in synaptic or extrasynaptic areas (Wisden et al., 1992; Saxena and Macdonald, 1994; McKernan and Whiting, 1996; Sur et al., 1999; Brunig et al., 2002; Poltl et al., 2003; Caraiscos et al., 2004; Peng et al., 2004; Glykys et al., 2007). For the purposes of this study, only $\alpha 1\beta\gamma$ and $\alpha 6\beta\gamma$ subunit combinations were considered “synaptic”; the remaining subunit combinations were considered “extrasynaptic”. Note that although the $\alpha 4\beta\gamma$ isoform has been detected in synapses under certain conditions (Hsu et al., 2003; Liang et al., 2006; Zhang et al., 2007), its role in normal synaptic physiology remains unclear.

Penicillin altered the kinetic properties of currents evoked from $\gamma 2L$ subunit-containing receptors (Figure 1A–D). Peak currents were substantially decreased for each isoform ($\alpha 1\beta 3\gamma 2L$: $47.1 \pm 8.1\%$ of control, $n = 6$, $p < 0.01$; $\alpha 4\beta 3\gamma 2L$: $41.8 \pm 3.8\%$ of control, $n = 12$, $p < 0.001$; $\alpha 5\beta 3\gamma 2L$: $37.7 \pm 3.6\%$ of control, $n = 8$, $p < 0.01$; $\alpha 6\beta 3\gamma 2L$: $54.3 \pm 6.7\%$ of control, $n = 10$, $p < 0.05$) (Figure 1E), as were residual currents ($\alpha 1\beta 3\gamma 2L$: $32.3 \pm 4.4\%$ of control, $p < 0.01$; $\alpha 4\beta 3\gamma 2L$: $59.0 \pm 4.0\%$ of control, $p < 0.01$; $\alpha 5\beta 3\gamma 2L$: $44.9 \pm 4.3\%$ of control, $p < 0.01$; $\alpha 6\beta 3\gamma 2L$: $55.3 \pm 5.3\%$ of control, $p < 0.05$). While the extent of desensitization increased for $\alpha 1\beta 3\gamma 2L$ receptors compared to control ($55.5 \pm 3.3\%$ vs $68.7 \pm 2.6\%$, $p < 0.01$), it decreased for $\alpha 4\beta 3\gamma 2L$ ($63.3 \pm 3.1\%$ vs $46.7 \pm 4.6\%$, $p < 0.01$) and $\alpha 5\beta 3\gamma 2L$ ($51.1 \pm 3.7\%$ vs $41.7 \pm 5.4\%$, $p < 0.01$) receptors, and was unchanged for $\alpha 6\beta 3\gamma 2L$ receptors ($57.3 \pm 3.9\%$ vs $55.3 \pm 3.7\%$, $p > 0.05$) (Figure 1F). Despite the variable effects on desensitization, currents evoked from $\gamma 2L$ subunit-containing receptors had prolonged deactivation in the presence of penicillin compared to control currents ($\alpha 1\beta 3\gamma 2L$: 345.8 ± 53.2 ms vs 504.6 ± 51.7 ms, $p < 0.05$; $\alpha 4\beta 3\gamma 2L$: 268.5 ± 34.2 ms vs 668.2 ± 201.8 ms, $p < 0.05$; $\alpha 5\beta 3\gamma 2L$: 617.7 ± 92.6 ms vs 755.3 ± 144.7 ms, $p < 0.05$; $\alpha 6\beta 3\gamma 2L$: 432.4 ± 32.4 ms vs 946.4 ± 116.8 ms, $p < 0.01$) (Figure 1G) and exhibited rebound currents upon penicillin washout (Figure 1A–D).

Penicillin also altered the kinetic properties of currents evoked from δ subunit-containing receptor isoforms (Figure 2A–D). As with γ 2L subunit-containing receptors, peak currents were reduced (α 1 β 3 δ : $78.3 \pm 7.3\%$ of control, $n = 9$, $p < 0.05$; α 4 β 3 δ : $54.2 \pm 1.2\%$ of control, $n = 6$, $p < 0.01$; α 5 β 3 δ : $69.6 \pm 5.2\%$ of control, $n = 5$, $p = 0.07$; α 6 β 3 δ : $69.3 \pm 4.3\%$ of control, $n = 6$, $p < 0.05$) (Figure 2E). Residual currents, however, were only decreased for α 4 β 3 δ and α 6 β 3 δ receptors (α 1 β 3 δ : $95.6 \pm 3.0\%$ of control, $p > 0.05$; α 4 β 3 δ : $65.8 \pm 2.6\%$ of control, $p < 0.01$; α 5 β 3 δ : $101.7 \pm 3.9\%$ of control, $p > 0.05$; α 6 β 3 δ : $65.0 \pm 4.6\%$ of control, $p < 0.05$). The higher sensitivity of peak compared to residual currents caused the extent of desensitization to decrease for the majority of δ subunit-containing receptors (α 1 β 3 δ : $24.8 \pm 6.5\%$ vs $7.4 \pm 1.7\%$, $p < 0.05$; α 4 β 3 δ : $53.4 \pm 2.1\%$ vs $43.5 \pm 2.5\%$, $p < 0.01$; α 5 β 3 δ : $36.2 \pm 4.4\%$ vs $6.8 \pm 0.5\%$, $p < 0.01$), with only α 6 subunit-containing receptors having a slightly increased extent of desensitization ($44.7 \pm 3.9\%$ vs $48.0 \pm 4.1\%$, $p < 0.05$) (Figure 2F). With the exception of α 5 subunit-containing receptors, deactivation was prolonged for all δ subunit-containing receptors (α 1 β 3 δ : 125.3 ± 10.5 ms vs 214.5 ± 19.5 ms, $p < 0.01$; α 4 β 3 δ : 117.8 ± 13.5 ms vs 641.0 ± 112.4 ms, $p < 0.01$; α 5 β 3 δ : 345.7 ± 87.4 ms vs 407.9 ± 100.4 ms, $p > 0.05$; α 6 β 3 δ : 449.1 ± 80.9 ms vs 975.7 ± 147.7 ms, $p < 0.05$) (Figure 2G). In addition, all δ subunit-containing receptors exhibited rebound currents upon penicillin washout (Figure 2A–D). Note that α 5 β 3 δ receptors were expressed poorly in HEK293T cells, and we therefore obtained currents from only 5 cells.

In summary (Table 1), penicillin reduced peak current amplitudes, prolonged deactivation, and induced rebound currents for both synaptic and extrasynaptic GABA_A receptor isoforms, suggesting that the reported inability of penicillin to modulate tonic inhibition in previous studies (Yeung et al., 2003) could not simply be attributed to insensitivity of extrasynaptic receptors. However, the finding that the extent of desensitization was decreased for most extrasynaptic receptor isoforms (the result of peak currents having higher sensitivity than residual currents) raised the possibility that these isoforms might simply appear insensitive to penicillin when activated under near-equilibrium conditions. Thus, we next explored the possibility that the “context” of receptor activation was responsible for the previously reported selectivity of penicillin for phasic currents.

Currents evoked from synaptic GABA_A receptor isoforms in the context of phasic activation were inhibited significantly by penicillin

To investigate its potential effects on phasic inhibition, penicillin (1 mM) was co-applied with a brief pulse (5 ms) of saturating GABA (1 mM) to the synaptic α 1 β 3 γ 2L and α 6 β 3 γ 2L GABA_A receptor isoforms. These experimental conditions are thought to mimic those of phasic activation, as the resulting currents have kinetic properties similar to those of IPSCs (Jones and Westbrook, 1995). Since the slow solution exchange associated with using lifted whole-cells could limit resolution of peak currents, an important consideration given the rapid activation of synaptic isoforms, these recordings were performed on excised outside-out patches.

Compared to currents evoked by brief pulses of GABA alone, co-application of GABA with penicillin reduced the peak current amplitude of α 1 β 3 γ 2L receptors ($62.3 \pm 3.3\%$ of control, $n = 5$, $p < 0.01$) (Figure 3A, C). In addition, penicillin accelerated the weighted deactivation time course from 64.7 ± 4.6 ms to 25.9 ± 5.4 ms ($p < 0.001$), an unexpected finding given that deactivation was significantly prolonged following longer applications (Figure 1G). Combined, these effects caused a substantial decrease in the net charge transfer following an individual GABA pulse ($23.7 \pm 1.8\%$ of control, $p < 0.05$) (Figure 3D). Penicillin also reduced the peak current amplitude ($41.6 \pm 3.9\%$ of control, $n = 11$, $p < 0.001$) (Figure 3B, C) and accelerated deactivation (109.8 ± 13.5 ms vs 72.5 ± 9.5 ms, $p < 0.05$) of α 6 β 3 γ 2L receptors. As with α 1 β 3 γ 2L receptors, these effects combined to substantially decrease the net charge transfer following an individual brief GABA pulse ($45.1 \pm 7.3\%$ of control, $p < 0.05$) (Figure 3D).

Currents evoked from extrasynaptic GABA_A receptor isoforms in the context of tonic activation were inhibited minimally by penicillin

To investigate its potential effects on tonic inhibition, penicillin (1 mM) was co-applied with a long pulse (30 sec) of sub-saturating GABA (1 μ M) to the extrasynaptic $\alpha 4\beta 3\delta$, $\alpha 4\beta 3\gamma 2L$, and $\alpha 5\beta 3\gamma 2L$ receptor isoforms. This subset of receptor isoforms is thought to mediate the majority of the tonic current in hippocampal neurons (Wisden et al., 1992; Caraiscos et al., 2004; Chandra et al., 2006), which was found to be penicillin insensitive (Yeung et al., 2003). Prolonged drug application allowed for currents to reach pseudo-equilibrium, as would be expected for currents mediating tonic inhibition.

For $\alpha 4\beta 3\delta$ receptors, the effects of penicillin were surprisingly limited to the peak current (Figure 4A). Indeed, while peak currents were reduced in the presence of penicillin ($74.8 \pm 2.9\%$ of control, $n = 10$, $p < 0.01$), equilibrium currents were essentially unchanged ($93.7 \pm 4.1\%$ of control, $p > 0.05$). The higher sensitivity of peak currents (peak vs equilibrium, $p < 0.01$) caused the extent of desensitization to decrease from $40.0 \pm 1.2\%$ to $24.7 \pm 2.8\%$ ($p < 0.01$). Penicillin also preferentially modulated peak currents of $\alpha 4\beta 3\gamma 2L$ (Figure 4B; peak: $72.3 \pm 3.9\%$ of control, $n = 10$, $p < 0.01$; equilibrium: $85.0 \pm 3.0\%$ of control, $p < 0.01$; peak vs equilibrium, $p < 0.05$) and $\alpha 5\beta 3\gamma 2L$ (Figure 4C; peak: $58.1 \pm 4.2\%$ of control, $n = 12$, $p < 0.01$; equilibrium: $77.8 \pm 4.7\%$ of control, $p < 0.01$; peak vs equilibrium, $p < 0.01$) receptors. As a result, the extent of desensitization was decreased from $34.9 \pm 3.3\%$ to $23.6 \pm 2.4\%$ ($p < 0.001$) and from $35.4 \pm 3.9\%$ to $14.2 \pm 3.2\%$ ($p < 0.001$) for $\alpha 4\beta 3\gamma 2L$ and $\alpha 5\beta 3\gamma 2L$ receptor currents, respectively.

Thus, while peak currents were significantly reduced for each of these extrasynaptic receptor isoforms, equilibrium currents were less affected (Figure 4D, E). Interestingly, while peak currents were also preferentially modulated for this subset of receptor isoforms in the context of 4 second pulses of saturating GABA (i.e., decreased extent of desensitization; Figure 1F, 2F), the combination of extending the duration of GABA exposure and lowering the GABA concentration rendered equilibrium currents even less sensitive to penicillin. This suggested that the reported selectivity of penicillin for phasic currents resulted not from an inability to modulate extrasynaptic receptors, but rather, from an inability to modulate these receptors in their native “context” of activation.

Simple kinetic models qualitatively reproduced the effects of open-channel block on GABA_A receptor macroscopic current properties

The experimental findings in the previous sections demonstrated that open-channel block could have complex effects on macroscopic current properties. Although penicillin decreased peak current amplitudes and induced rebound currents for both synaptic and extrasynaptic receptor isoforms, its effects on desensitization were isoform-specific. In addition, while penicillin prolonged deactivation following long pulses, it accelerated deactivation following brief pulses. Moreover, while currents evoked under phasic conditions were particularly vulnerable to penicillin, those evoked under tonic conditions were relatively insensitive. To determine the kinetic basis for these findings, the effects of open-channel block were evaluated on macroscopic current properties using Markov models of GABA_A receptor function.

Although comprehensive kinetic models have been proposed that account for both the microscopic and macroscopic properties of GABA_A receptors (Haas and Macdonald, 1999; Lema and Auerbach, 2006; Lagrange et al., 2007), simple models are often sufficient to illustrate the salient features of GABA-evoked currents. Two such models, proven useful for investigating the relationship between microscopic gating and macroscopic currents, are shown in Figure 5 (panels A1 and B1; Katz and Thesleff, 1957; Jones and Westbrook, 1996; Bianchi et al., 2007). Since penicillin is thought to act via open-channel block (Twyman et al., 1992),

its effects were modeled by reversibly connecting a non-conducting state to the open state (Figure 5A1, B1; dashed boxes) (Adams, 1977; Ruff, 1977; Ascher et al., 1978; Marty, 1978; Neher and Steinbach, 1978). Receptors entered this “blocked” state via a concentration-dependent blocking rate (k_+) and returned via a concentration-independent unblocking rate (k_-), with k_- being taken as the inverse of the brief closed time associated with penicillin block and k_+ being chosen to account for the concentration-dependent increase in burst length (Neher and Steinbach, 1978; Twyman et al., 1992).

For both “linear” and “branched” models, simulated co-application of 1 mM GABA with 1 mM penicillin generated currents with smaller peaks, more desensitization, prolonged deactivation, and rebound upon washout, compared to currents evoked by GABA alone (Figure 5A2, B2, A3, B3). With the exception of the effect on desensitization, these results were qualitatively similar to our experimental observations and consistent with the predicted effects of open-channel block on macroscopic currents (Adams, 1977; Ruff, 1977; Ascher et al., 1978; Marty, 1978). For example, since penicillin blockade was modeled by adding a non-conducting state to the gating scheme, receptor occupancy in all other states decreased, the result being smaller amplitude currents (Figure 5A2, B2). In addition, since GABA unbinding could only occur from C2, adding the B state increased receptor mean bound time. Much like D states, this allowed for additional openings prior to GABA unbinding, which prolonged deactivation (Figure 5A2, B2) (Jones and Westbrook, 1995; Bianchi et al., 2007). Rebound currents upon blocker washout were also expected, the result of surging O state occupancy following blocker unbinding (Figure 5A3, B3).

Blocking and unblocking rates played distinct roles in modulating the extent of desensitization

Although the previous simulations qualitatively reproduced many of our experimental observations, it was unclear how open-channel block could support decreased or unchanged extents of desensitization (Figures 1F, 2F). Indeed, since the B state was arranged in series with the O state, just as the D state was arranged in the linear model (Figure 5A1), desensitization was expected to increase, as receptors could now close via an additional route. Support for the idea that the B state could serve as an additional D state came from the observation that currents continued to decline after reaching peak in the absence of the D state (Figure 5A4, B4; dashed traces), but not in the absence of both D and B states (Figure 5A4, B4; solid traces). However, it has previously been demonstrated that the effect of open-channel block on the relaxation of macroscopic currents depends on the relationship between the microscopic rates of blockade and those of channel gating (Ascher et al., 1979). We therefore systematically evaluated the relationship between channel blocking and unblocking rates and the extent of macroscopic desensitization (Figure 6A1–A4).

Using the default blocking rate ($k_+ = 550 \text{ ms}^{-1}$) with the linear model (Figure 5A1), decreasing the unblocking rate (k_-) progressively increased desensitization (Figure 6A1). This corresponded to increased desensitization for the lowest unblocking rate ($k_- = 0.01 \text{ ms}^{-1}$) compared to the GABA control current (dashed line), but slightly decreased desensitization for the highest unblocking rate ($k_- = 1.0 \text{ ms}^{-1}$). This same trend was observed in the context of a higher blocking rate ($k_+ = 5500 \text{ ms}^{-1}$), though the effect was substantially more pronounced (Figure 6A2). Conversely, increasing the blocking rate in the context of the default unblocking rate ($k_- = 0.1 \text{ ms}^{-1}$) only increased desensitization (Figure 6A3), while increasing the blocking rate in the context of a larger unblocking rate ($k_- = 1.0 \text{ ms}^{-1}$) only decreased desensitization (Figure 6A4). Thus, blocking and unblocking rates played different roles in determining the sensitivity of desensitization to open-channel block: *the unblocking rate determined the direction of change (increased, decreased, or unchanged) while the blocking rate served mainly to modulate the magnitude of the change*. Similar patterns were observed

with the branched model (not shown), indicating that this phenomenon did not depend on D state connectivity.

Blocked states decreased the extent of macroscopic desensitization by competing with existing desensitized states

While the previous simulations demonstrated that both increased and decreased (or even unchanged) extents of desensitization were possible with open-channel block depending on the value assigned to the unblocking rate, the kinetic basis for this observation was unclear. One possibility was that certain combinations of blocking and unblocking rates rendered the B state “non-desensitizing” (i.e., unable to support macroscopic desensitization despite being arranged like a “linear” D state). Indeed, previous kinetic studies have demonstrated that simple models containing O and D states in linear arrangement support macroscopic desensitization only when specific microscopic conditions are met. Specifically, macroscopic desensitization can only occur when the channel opening rate (β) is greater than the resensitization rate (ρ) (Bianchi et al., 2007). Thus, addition of non-desensitizing B states (i.e., those whose unblocking rate (k_-), which is analogous to the resensitization rate (ρ), is less than β), would be expected to decrease desensitization by decreasing the contribution of the existing D state to the macroscopic current. To test this hypothesis, blocking and unblocking rates were co-varied, and the extent of desensitization supported in the presence of both B and D states (Figure 6B1, C1) was compared to that supported by the B state alone (Figure 6B2, C2).

By comparing the results in Panels B1 and C1 with those in Panels B2 and C2 (Figure 6), a simple explanation was provided for the ability of open-channel block to both increase and decrease desensitization. This was best illustrated by the curves corresponding to the highest unblocking rate (Figures B1, C1; $k_- = 1.0 \text{ ms}^{-1}$). When the blocking rate was low ($k_+ = 100 \text{ ms}^{-1}$), desensitization was nearly identical to that of the control current (dashed line). This reflected low B state occupancy, as transitions into this state were infrequent (due to the low blocking rate) and short-lived (due to the high unblocking rate). In other words, the models behaved as if the B state did not exist. At the other extreme, however, when the blocking rate was high ($k_+ = 10000 \text{ ms}^{-1}$), receptors in the O state were far more likely to transition into the B than into the D state. In this case, the model behaved as if the D state did not exist, and the extent of desensitization was determined primarily by the relationship between entry and exit rates from O and B states. However, because this particular unblocking rate ($k_- = 1.0 \text{ ms}^{-1}$) was equal to the channel opening rate ($\beta = 1.0 \text{ ms}^{-1}$), the B state could not support desensitization, independent of the magnitude of the blocking rate (Figure 6B2, C2; bottom curves) (Bianchi et al., 2007). Thus, increasing the blocking rate shifted the behavior of the kinetic model from that of the macroscopically *desensitizing* C-O-D arrangement towards that of the macroscopically *non-desensitizing* C-O-B arrangement, resulting in progressively less desensitization. Conversely, in cases where the C-O-B sub-scheme supported more desensitization than the C-O-D sub-scheme (e.g., compare Figure 6B2, C2 when $k_- = 0.01 \text{ ms}^{-1}$ to the control extent of desensitization in Figure 6B1, C1), increasing the blocking rate increased desensitization (Figure 6B1, C1; top curve). *Therefore, whether desensitization was increased, decreased, or unchanged following addition of the blocker depended simply on the relative contributions of the desensitized and blocked states to the macroscopic current.*

Although this suggested that penicillin had isoform-specific effects on desensitization because of differences in blocking and/or unblocking rates, it should be emphasized that, in principle, blocking and unblocking rates could have been similar for all isoforms, with variable effects being observed due to differences in channel gating (Haas and Macdonald, 1999; Lagrange et al., 2007). Indeed, preliminary simulations demonstrated that the effects of open-channel block depended on both the relative stability and connectivity of O and D states, as increased,

decreased, and unchanged extents of desensitization were all theoretically possible for any given combination of blocking and unblocking rates (Supplemental Figure 1).

Open-channel block could selectively modulate either peak or residual currents

The simulations in the previous sections confirmed that open-channel block could decrease the extent of desensitization, as observed experimentally for the majority of receptor isoforms. However, for certain receptor isoforms (e.g., $\alpha 1\beta 3\delta$ and $\alpha 5\beta 3\delta$), this decrease was mediated almost entirely by inhibition of peak currents; residual currents were essentially unaffected (Figure 2). To explore this phenomenon, the sensitivity of peak and residual currents to open-channel block was systematically evaluated. Each set of simulations was performed with the linear model in the context of either increased (Figure 7A1, A2) or decreased (Figure 7B1, B2) D state stability (defined as δ/p), allowing for an expanded view of the kinetic parameter space.

There was a striking difference between the relative contribution of blocking and unblocking rates to peak and residual currents. While there was no limit to the ability of open-channel block to inhibit residual currents (indeed, an infinitely low unblocking rate would simply cause the blocked state to become absorbing, driving open state occupancy to zero) (Figure 7A2, B2), peak currents were primarily sensitive to changes in the blocking rate (Figure 7A1, B1). This reflected the fact that current rise times were rapid (~ 1 ms) for both high and low D state stability conditions (Figure 7C1, D1; solid traces). As a result, decreasing the unblocking rate below 1.0 ms^{-1} had progressively less effect on peak current, as this supported dwell times in the B state > 1 ms, and therefore, permitted channel reopening (on average) only after currents reached peak. Thus, there was a limit to the ability of open-channel block to inhibit peak currents, which was determined by the relationship between the macroscopic rate of current activation (in the absence of blocker) and the microscopic blocking rate.

Under certain conditions, the differential sensitivity of peak and residual currents to open-channel block allowed phasic and tonic currents to appear selectively modulated. For example, when the blocking rate was low ($k_+ = 100$), peak currents were always $\geq 90\%$ of control, the result of transitions into the B state being infrequent during current activation (Figure 7A1, B1). However, when combined with the lowest unblocking rate ($k_- = 0.01$), substantial reductions were observed in the residual current (Figure 7A2, B2). This effect was more apparent with low D state stability (compare Figure 7A2 to Figure 7B2), as this allowed for higher B state fractional occupancy with any given combination of blocking and unblocking rates (not shown). Thus, blockers with low blocking and even lower unblocking rates (i.e., slowly equilibrating but relatively high equilibrium occupancy) would have limited capacity to modulate early (non-equilibrium) phases of current activation but high capacity to modulate later (near-equilibrium) phases, giving the appearance of selectivity for receptors activated under tonic conditions (Figure 7C1, C2).

Conversely, certain combinations of rate constants allowed open-channel block to appear selective for peak currents. For example, when the blocking rate was increased by two orders of magnitude ($k_+ = 10,000$), peak currents were reduced to $\sim 20\%$ of control (Figure 7A1, B1). However, when combined with the largest unblocking rate ($k_- = 1.0$), residual currents were inhibited less than peak currents (Figure 7A2, B2). This effect was substantially more pronounced under conditions of increased D state stability (Figure 7A2), where $\sim 60\%$ of the residual current remained. In fact, when D state stability was increased by an additional order of magnitude, this same combination of blocking and unblocking rates had almost no effect on residual current, despite causing a similar reduction in peak current (not shown). Thus, blockers with high blocking and unblocking rates (i.e., rapidly equilibrating but relatively low equilibrium occupancy) would have limited capacity to modulate later (near-equilibrium) phases of current activation but high capacity to modulate early (non-equilibrium) phases,

giving the appearance of selectivity for receptors activated under phasic conditions (Figure 7D1, D2).

Decreasing the GABA concentration further decreased the sensitivity of residual currents to open-channel block

Our experimental results demonstrated that residual currents were less sensitive to penicillin when evoked by sub-saturating concentrations of GABA. For example, while the residual currents of $\alpha 4\beta 3\delta$ receptors were inhibited ~40% by penicillin when evoked by 1 mM GABA (Figure 2), those evoked by 1 μ M GABA were inhibited <10% (Figure 4). This concentration-dependence of open-channel block has also been observed with acetylcholine receptors, and has been attributed to the fact that the effectiveness of open-channel block depends on channel open probability (Ascher et al., 1978; Marty, 1978). To illustrate this phenomenon, the ability of 1 mM penicillin to modulate currents evoked by prolonged co-applications (30 s) of GABA was simulated over a range of GABA concentrations (Figure 8). Decreasing the GABA concentration increased equilibrium occupancy of the unliganded state (C1) (Figure 8A) and decreased occupancy of the liganded states (not shown). In addition, increasing B state stability left-shifted the concentration-occupancy curve (Figure 8A), reflecting the decreased GABA EC₅₀ (Neher and Steinbach, 1978). Interestingly, while decreasing the GABA concentration did not alter the ratio of O to B state occupancies (Figure 8B), it substantially decreased the sensitivity of residual currents to open-channel block (Figure 8C). This occurred even when B state occupancy was substantially higher than O state occupancy (e.g., when $k+[B]/k- = 10$; Figure 8C, D). Similar behavior was observed using the branched model (not shown) and comprehensive kinetic models of $\alpha 1\beta 3\gamma 2L$ and $\alpha 4\beta 3\gamma 2L$ receptors (Lagrange et al., 2007) (Supplemental Figure 2), indicating that this effect was independent of the number and connectivity of O states.

Discussion

Penicillin produced context-dependent modulation of GABA_A receptor currents

Preferential modulation of phasic or tonic inhibition is generally attributed to synaptic and extrasynaptic receptor isoforms having different sensitivities to a given pharmacological agent (Nusser and Mody, 2002; Stell and Mody, 2002; Farrant and Nusser, 2005). The observation that penicillin selectively inhibited phasic currents has thus been suggested to reflect specificity for synaptic isoforms (Yeung et al., 2003). However, we found that penicillin inhibited GABA currents evoked from both synaptic and extrasynaptic isoforms, indicating that isoform-specificity was insufficient to account for the apparent insensitivity of tonic currents. We therefore explored the possibility that the markedly different contexts of receptor activation associated with phasic and tonic inhibition were responsible for their different sensitivities to penicillin. Consistent with this hypothesis, we found that currents evoked by prolonged exposure to sub-saturating GABA (i.e., tonic activation) were relatively insensitive to penicillin, while those evoked by brief pulses of nearly-saturating GABA (i.e., phasic activation) were highly sensitive. Although we did not evaluate the effects of penicillin on every known GABA_A receptor isoform, the results were nonetheless consistent with the idea that the context of receptor activation, and not the subset of receptor isoforms present in synaptic and extrasynaptic compartments, was responsible for the reported selectivity of penicillin for phasic currents.

Penicillin had variable effects on GABA_A receptor macroscopic current properties

Penicillin has previously been demonstrated to negatively modulate GABA_A receptor currents (Macdonald and Barker, 1977; Horne et al., 1992; Katayama et al., 1992; Tsuda et al., 1994; Behrends, 2000; Sugimoto et al., 2002) by an open-channel block mechanism (Twyman et al., 1992). Consistent with these studies, we found that penicillin decreased peak current

amplitudes for both synaptic and extrasynaptic receptor isoforms when evoked by saturating GABA. This suggested that phasic currents would always be inhibited by penicillin, regardless of which subset of receptor isoforms is localized to synapses. Penicillin is therefore likely to have widespread effects on phasic inhibition, which may underlie its potent pro-convulsant properties and ability to produce epileptiform activity *in vitro* (Schneiderman et al., 1994; Uysal et al., 1996; Schneiderman 1997; Jimenez et al., 2000; Shen and Lai, 2002).

Interestingly, penicillin had variable effects on the extent of desensitization, with increased, decreased, and even unchanged extents being observed with different receptor isoforms. Although this result was unexpected for a simple open-channel block mechanism, particularly since the blocked state provided an additional route for channel closure, our simulations emphasized that macroscopic current shape does not specify uniquely the precise arrangement of states in the microscopic gating scheme (Mozrzymas et al., 2003; Bianchi et al., 2007). In other words, the fact that individual receptors must open before becoming blocked should not be taken to mean that the resulting macroscopic currents will be affected only after they have reached peak. Much like desensitized states, blocked states can influence both peak and residual currents, and depending on the relationship between the kinetics of block and those of channel gating, can have markedly different effects on each of these current properties, causing the extent of desensitization to change. While the physiological significance of altered desensitization remains unclear (since prolonged exposure to high concentrations of GABA is not thought to occur *in vivo*), increasing evidence suggests that the shape of desensitization influences the pattern of current responses during repetitive stimulation (Bianchi and Macdonald, 2002; Pugh and Raman, 2005; Lagrange et al., 2007).

Although the effects on desensitization were highly variable, most receptor isoforms displayed prolonged deactivation in the presence of penicillin (in the case of decreased desensitization, this provided a novel experimental example of desensitization-deactivation “uncoupling”; Bianchi et al., 2007). However, penicillin accelerated deactivation following brief pulses, a finding consistent with the reported ability of penicillin to accelerate IPSC decay in hippocampal neurons (Mtchedlishvili and Kapur, 2005). While this behavior was also observed during our kinetic simulations (Figure 7C2), it should be noted that only the fast phase of deactivation was actually accelerated by open-channel block. Similar to the effect of stabilizing desensitized states (Bianchi et al., 2007), the accelerated fast phase was accompanied by a markedly prolonged slow phase (Figure 7C2; note that traces corresponding to deactivation in the presence of blocker did not return to baseline), causing the weighted deactivation time course to actually be prolonged (not shown). Slow phases, however, may be difficult to resolve experimentally above background noise if they are relatively low amplitude, giving the appearance of accelerated deactivation.

Thus, while the effects of penicillin on macroscopic current properties were complex, our simulations using simple models of GABA_A receptor function demonstrated that these effects were entirely consistent with simple open-channel block. In addition, the simulations exposed a striking similarity between the roles played by blocked and desensitized states in shaping the macroscopic current, suggesting that open-channel blockers could be useful experimental tools for exploring the relationship between microscopic and macroscopic desensitization. Unlike native desensitized states, the entry rate into the blocked state can be precisely controlled simply by varying the blocker concentration, the orientation of the blocked state with respect to the open state is always known, and the exit rate from the blocked state can be unambiguously determined with single channel analysis.

Receptor activation by low concentrations of GABA contributed to the apparent insensitivity of tonic currents to penicillin

For an open-channel blocker to alter the extent of macroscopic desensitization, it must, by definition, have different effects on peak and residual currents. This implies, however, the capacity to differentially modulate phasic and tonic inhibition. For example, preferential inhibition of peak currents predicts that non-equilibrium phases of receptor activation will be more sensitive than near-equilibrium phases, meaning that phasic inhibition should be affected more than tonic inhibition. The observation that penicillin decreased the extent of desensitization for most receptor isoforms was therefore consistent with the lower sensitivity of tonic currents (Yeung et al., 2003). However, it should be noted that only a small reduction was reported in the root-mean-square noise of hippocampal tonic currents; their magnitude was actually unchanged (Yeung et al., 2003; Mtchedlishvili and Kapur, 2005). In contrast, residual currents from most extrasynaptic isoforms were still inhibited substantially by penicillin when evoked by a nearly-saturating concentration of GABA (Figures 1, 2).

This suggested that the near-equilibrium conditions that characterize tonic inhibition were only partly responsible for the apparent insensitivity of extrasynaptic receptors to penicillin. Interestingly, residual currents were considerably less sensitive when evoked by a sub-saturating concentration of GABA (compare residual currents in Figures 1 and 2 to those in Figure 4). While this could potentially have been explained by lower affinity of the blocker for mono-liganded compared to di-liganded open states, single channel studies have reported that penicillin actually had the highest affinity for the mono-liganded open state (Twyman et al., 1992). Alternatively, this may simply have reflected the decreased effectiveness of open-channel block at lower agonist concentrations, which decrease channel open probability (Ascher et al., 1978; Marty, 1978). Indeed, our simulations illustrated that this phenomenon was independent of the specific connectivity or number of open and desensitized states in the gating scheme, indicating that sufficiently low GABA concentrations would render tonic currents minimally sensitive to penicillin regardless of which receptor isoforms comprised the extrasynaptic pool.

Thus, our results demonstrated that the “context” of receptor activation, both in terms of time course of exposure and agonist concentration, was an important determinant of the sensitivity of GABA_A receptor currents to penicillin. While it remains unclear whether such context-dependent modulation is possible with other known GABA_A receptor modulators, preliminary simulations suggested that this behavior was not unique to open-channel block. For example, phasic and tonic currents could also have markedly different sensitivities to modulators that “block” non-conducting states, and even to modulators that stabilize open states (not shown). The latter are of particular interest, as the ability to selectively enhance equilibrium or non-equilibrium currents would have important therapeutic implications for disease states caused by impairments in tonic or phasic inhibition, respectively.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Penicillin altered the macroscopic current properties of $\gamma 2L$ subunit-containing GABA_A receptors

A–D, Whole-cell currents were evoked by 1 mM GABA either alone (left) or in the presence of 1 mM penicillin G (PG) (right) for $\alpha 1\beta 3\gamma 2L$, $\alpha 4\beta 3\gamma 2L$, $\alpha 5\beta 3\gamma 2L$, and $\alpha 6\beta 3\gamma 2L$ receptors, respectively. The solid line above each trace indicates the duration of GABA application. The dashed line denotes the duration of penicillin application. E, Penicillin decreased peak current amplitudes. F, Penicillin had variable effects on the extent of desensitization. G, Penicillin increased the weighted time constant (τ) of deactivation. Error bars denote SEM.

+ The peak current evoked by co-application of GABA and penicillin was significantly reduced compared to the GABA control current at $p < 0.05$; ++ $p < 0.01$; +++ $p < 0.001$.

* Significantly different from GABA control current desensitization or deactivation at $p < 0.05$;
** $p < 0.01$.

Figure 2. Penicillin altered the macroscopic current properties of δ subunit-containing GABA_A receptors

A–D, Whole-cell currents were evoked by 1 mM GABA either alone (left) or in the presence of 1 mM penicillin G (PG) (right) for $\alpha 1\beta 3\delta$, $\alpha 4\beta 3\delta$, $\alpha 5\beta 3\delta$, and $\alpha 6\beta 3\delta$ receptors, respectively. The solid line above each current trace indicates the duration of GABA application. The dashed line denotes the duration of penicillin application. E, Penicillin decreased peak current amplitudes. F, Penicillin had variable effects on the extent of desensitization. G, Penicillin increased the weighted time constant (τ) of deactivation. Error bars denote SEM.

+ Significantly reduced compared to the GABA control current at $p < 0.05$; ++ $p < 0.01$

* Significantly different from GABA control current desensitization or deactivation at $p < 0.05$;
** $p < 0.01$.

Figure 3. Penicillin substantially inhibited currents evoked from synaptic GABA_A receptor isoforms under phasic conditions

A, B, Macropatch currents were evoked by a brief (5 ms) application of 1 mM GABA either alone (left) or in the presence of 1 mM penicillin G (PG) (right) for $\alpha 1\beta 3\gamma 2L$ and $\alpha 6\beta 3\gamma 2L$ receptors, respectively. The solid arrow above each current trace indicates the brief duration of GABA application. The dashed line denotes the duration of penicillin application. C, Penicillin decreased peak current amplitudes. D, Penicillin decreased total charge transfer. Error bars denote SEM.

* Significantly different from GABA control current at $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 4. Penicillin had minimal effect on currents evoked from extrasynaptic GABA_A receptor isoforms under tonic conditions

A–C, Whole-cell currents were evoked by a long pulse (30 sec) of 1 μ M GABA either alone (left) or in the presence of 1 mM penicillin G (PG) for $\alpha 4\beta 3\delta$, $\alpha 4\beta 3\gamma 2L$, and $\alpha 5\beta 3\gamma 2L$ receptors, respectively. The solid line above each current trace indicates the duration of GABA application. The dashed black line denotes the duration of penicillin application. The dashed gray line indicates the amount of control steady-state current. D, Penicillin reduced peak current amplitudes. E, Penicillin had limited effect on residual current amplitudes. Error bars represent SEM.

** Significantly different from GABA control peak or steady state current amplitude at $p < 0.01$.

Figure 5. The effects of penicillin-mediated open-channel block on macroscopic currents were simulated using simple kinetic models

A1, 4-state kinetic model, with closed (C), open (O), and desensitized (D) states in “linear” arrangement, was used to generate simulated GABA currents. Agonist binding occurs between C_1 and C_2 . Open-channel block was simulated by adding a blocked state (B) to the O state (dashed box). Rate constants were: $k_{on} = 7000$, $k_{off} = 1.0$, $\beta = 1.0$, $\alpha = 0.3$, $\delta = 0.3$, $\rho = 0.1$, $k_+ = 550$, $k_- = 0.1$. All rates had units of ms^{-1} , except for the agonist binding (k_{on}) and blocker binding (k_+) rates, which were multiplied by the concentration of GABA [G] and blocker [B], respectively, and thus had units of $ms^{-1}M^{-1}$. B1, 4-state kinetic model, with O and D states in “branched” arrangement, was used to generate simulated GABA currents. Rate constants were:

$k_{on} = 7000$, $k_{off} = 1.0$, $\beta = 1.0$, $\alpha = 0.6$, $\delta = 0.6$, $\rho = 0.1$, $k_+ = 550$, $k_- = 0.1$. Note that while the original single channel studies assigned different blocking and unblocking rates for each O state (Twyman et al., 1992), the averaged values of k_+ and k_- were used here since linear and branched models contained a single O state. The remaining rate constants were chosen to qualitatively reproduce the macroscopic current properties of $\alpha\beta\gamma$ receptors (Haas and Macdonald, 1999; Lagrange et al., 2007). A2, B2, Currents were simulated from linear (Panel A2) and branched (Panel B2) models to 100 ms pulses of 1 mM GABA either in the presence (dashed traces) or absence (solid traces) of 1 mM penicillin G (PG). A3, B3, Currents were simulated from linear (Panel A3) and branched (Panel B3) models to 100 ms pulses of 1 mM GABA and 1 mM penicillin, with washout of penicillin occurring either simultaneously or 50 ms after GABA washout. Note the rebound currents upon penicillin washout, which became smaller in amplitude if deactivation was permitted before washout. A4, B4, Currents (scaled to peak) were simulated from linear (Panel A4) and branched (Panel B4) models to 100 ms pulses of 1 mM GABA either with (solid traces) or without (dashed traces) 1 mM penicillin when the D state entry rate was set to zero.

Figure 6. The extent of macroscopic desensitization was differentially sensitive to blocking and unblocking rate constants

A, Currents (normalized to peak) were simulated for the linear model to 100 ms co-applications of 1 mM GABA and 1 mM penicillin G (PG). In panels A1 and A2, the unblocking rate was varied in the context of either the default blocking rate (Panel A1; $k^+ = 550$) or a higher blocking rate (Panel A2; $k^+ = 5500$). In panels A3 and A4, the blocking rate was varied in the context of either the default unblocking rate (Panel A3; $k^- = 0.1$) or a higher unblocking rate (Panel A4; $k^- = 1.0$). Horizontal dashed lines indicate the residual current remaining in the absence of penicillin. B1–2, C1–2, The extent of desensitization was evaluated for simulated currents evoked by 100 ms pulses of 1 mM GABA to linear (Panels B1 and B2) and branched (Panel

C1 and C2) models over a range of blocking ($k_+ = 100, 200, 300, 600, 1000$) and unblocking ($k_- = 0.01, 0.02, 0.03, 0.06, 0.1, 0.2, 0.3, 0.6, 1.0$) rates in the presence of 1 mM penicillin. Dashed horizontal lines indicate the extent of desensitization in the absence of penicillin. In Panels B2 and C2, the D state entry rate was set to zero. Note that under these conditions, macroscopic desensitization was not observed for the highest unblocking rate tested ($k_- = 1.0 \text{ ms}^{-1}$), independent of the magnitude of the blocking rate.

Figure 7. Peak and residual currents had different sensitivities to blocking and unblocking rate constants

A, B, Peak (Panels A1 and B1) and residual (Panels A2 and B2) currents had different sensitivities to open-channel block in the context of increased (Panels A1 and A2) or decreased (Panels A2 and B2) D state stability. Increased stability was achieved by increasing δ and decreasing ρ two-fold each, while decreased stability was achieved by decreasing δ and increasing ρ two-fold each. C, Currents were simulated with either 100 ms (Panel C1) or 1 ms (Panel C2) pulses of 1 mM GABA in the presence of 1 mM penicillin G (PG) in the context of low blocking ($k^+ = 100$) and low D state stability ($\delta/\rho = 0.75$). D, Currents were simulated with either 50 ms (Panel D1) or 1 ms (Panel D2) pulses of 1 mM GABA in the presence of 1

mM penicillin in the context of high blocking ($k_+ = 10000$) and high D state stability ($\delta/\rho = 12$). Responses simulated with 1 mM GABA alone are shown in black. Peak currents were not normalized. All simulations were performed with the linear model (Figure 5A1; see figure legend for rate constants).

Figure 8. Decreasing the GABA concentration decreased the sensitivity of residual currents to open-channel block

A, The equilibrium fractional occupancy of the unbound state (C_1) increased as the GABA concentration and B state stability (defined as the ratio of the effective blocking rate, k_+ [Blocker], to the unblocking rate, k_-) decreased. B, The ratio of O state occupancy to B state occupancy was unchanged across GABA concentrations for any given B state stability. C, The sensitivity of residual currents to open-channel block decreased with decreasing GABA concentration and B state stability. D, Currents were simulated with 1 mM GABA (left) and 1 μM GABA (right) either alone (solid traces) or in the presence of 1 mM penicillin G (PG; dashed traces) when B state stability was high ($k_+[\text{PG}]/k_- = 10$). Currents evoked by GABA alone (black traces) were scaled to the amplitude of residual currents. All simulations were performed with the linear model (Figure 5A; see legend for rate constants).

Table 1

Summary of the changes in macroscopic current properties for synaptic and extrasynaptic GABA_A receptor isoforms (4 sec application of 1 mM GABA vs co-application of 1 mM GABA and 1 mM penicillin)

	I_{peak} amplitude	Desensitization	Deactivation
$\alpha 1\beta 3\gamma 2L$	↓	↑	↑
$\alpha 1\beta 3\delta$	↓	↓	↑
$\alpha 4\beta 3\gamma 2L$	↓	↓	↑
$\alpha 4\beta 3\delta$	↓	↓	↑
$\alpha 5\beta 3\gamma 2L$	↓	↓	↑
$\alpha 5\beta 3\delta$	NS	↓	NS
$\alpha 6\beta 3\gamma 2L$	↓	NS	↑
$\alpha 6\beta 3\delta$	↓	↑	↑

↑/↓, denotes increased or decreased peak current amplitude, extent of desensitization, or weighted deactivation time constant; NS, not significantly different from the GABA control current.