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Benzodiazepine modulation of $GABA_A$ receptor opening frequency depends on activation context: A patch clamp and simulation study

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Summary Benzodiazepines (BDZs) are $GABA_A$ receptor modulators with anxiolytic, hypnotic, and anticonvulsant properties. BDZs are understood to potentiate $GABA_A$ receptor function by increasing channel opening frequency, in contrast to barbiturates, which increase channel open duration. However, the *in vitro* evidence demonstrating increased opening frequency involved prolonged exposure to sub-saturating GABA concentrations, conditions most similar to those found in extrasynaptic areas. In contrast, synaptic $GABA_A$ receptors are transiently activated by high GABA concentrations. To determine if BDZ modulation of single-channel opening frequency would be different for BDZ-sensitive receptors activated under synaptic versus extrasynaptic conditions, a combination of patch clamp recording and kinetic modeling was used. Consistent with the original experimental findings, BDZs were found to increase receptor affinity for GABA by decreasing the unbinding rate. While this mechanism was predicted to increase opening frequency under extrasynaptic conditions, simulations predicted that the same mechanism under synaptic conditions would increase the number, but not the frequency, of single-channel openings. Thus, a single mechanism (slower GABA unbinding) can produce differential changes in opening frequency under synaptic versus extrasynaptic conditions. The functional impact of BDZs on $GABA_A$ receptors therefore depends upon the physiological context of receptor activation.

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Introduction

As the predominant source of fast synaptic inhibition in the brain, $GABA_A$ receptors have been the focus of emerging hypotheses not only of seizure pathophysiology, but also of anticonvulsant drug pharmacology. However, their role as the therapeutic target of benzodiazepines (BDZs) remained controversial for some time. While BDZs were observed to enhance $GABA_A$ receptor currents during intracellular recording from cultured neurons (Choh et al., 1977; Macdonald and Barker, 1978), others studies suggested that BDZs were antagonists of $GABA_A$ receptors (Gahwiler, 1976; Mathers, 1987), and still others indicated actions at glycine receptors (Young et al., 1974). Not until the development of single-channel recording (Colquhoun, 1991) was direct evidence presented that BDZs enhanced $GABA_A$ receptor responses (Rogers et al., 1994; Twyman et al., 1989). Specifically, BDZs were reported to increase single-channel opening frequency without altering mean open duration, an effect attributed to an increased affinity of GABA for the receptor (Rogers et al., 1994). Barbiturates, in contrast, were reported to increase the duration of openings without increasing their frequency (MacDonald et al., 1989; Twyman et al., 1989).

However, the *in vitro* electrophysiological evidence demonstrating BDZ-mediated increased opening frequency involved conditions most similar to those occurring in extrasynaptic areas, where receptors are persistently activated by sub-saturating concentrations of ambient GABA (~1 μM or less) (Farrant and Nusser, 2005). Under these conditions, binding of GABA to the receptor is infrequent, and because GABA is always present, receptors are free to repeatedly bind and unbind GABA. Synaptic receptors, in contrast, are transiently activated (~1 ms) by saturating concentrations of GABA (~1 mM) (Jones and Westbrook, 1996), such that receptors are predominantly GABA-bound, and the majority of channel activity (which can last 10—100 s of milliseconds) occurs without the prospect of rebinding. Given that BDZs were reported to increase the affinity of GABA for the receptor, a mechanism that favors GABA-bound receptor conformations, this suggests that the effects of BDZs on opening frequency would be different for receptors activated under synaptic versus extrasynaptic conditions. Although extrasynaptic receptors are enriched for BDZ-insensitive receptor isoforms (i.e., those containing the δ subunit), BDZ-sensitive receptor isoforms (i.e., those containing the γ subunit) occur commonly in extrasynaptic locations as well (Glykys et al., 2008; Glykys and Mody, 2007), and in some cases, are actually more abundant than BDZ-insensitive receptors (Farrant and Nusser, 2005). Therefore, the potential context-dependence of BDZ modulation of $GABA_A$ receptor function remains an important mechanistic issue.

Materials and methods

Cell culture and electrophysiology

HEK293T cells transiently expressing α1, β3, and γ2L $GABA_A$ receptor subunit cDNAs were prepared for patch clamp electrophysiological recordings as described previously (Bianchi and Macdonald, 2001). Briefly, whole cell patch clamp experiments involved gently lift the patched cells from the culture dish. Solutions containing GABA (with or without diazepam) were then applied to these lifted cells using a rapid drug application system composed of 4-barrelled square glass capillary tubing (Hinkle et al., 2003). The solution interface was translated across the cell using a mechanical stepper device (Warner Instruments, USA). Solution exchange times were consistently <400 μs when measured at the open patch electrode, but were slightly slower around the lifted cell.

Kinetic simulations

Simulations of macroscopic currents (100 s of receptors) were conducted using Berkeley Madonna (www.berkeleymadonna.com) software that solves the probability that a receptor will occupy any state of a kinetic model as a function of time. QUB (www.qub.buffalo.edu) software was used for single-channel simulations such that resting (C_u), bound closed (C_b and D), and bound open (O) states could be distinguished via assigning different (arbitrary) current levels ($C_u = 0$ units, C_b and $D = -2$ units, and $O = -6$ units, respectively) to each state. For equilibrium (''extrasynaptic'') simulations, 10 ms after the start of the trial, 1 μM GABA was applied for 1990 ms. 10 such trials constituted a batch. For phasic (''synaptic'') simulations, 10 ms after the start of a trial, 1 mM GABA was applied for 1 ms. Channel openings were then observed for 490 ms. 50 such trials were analyzed per batch, and batches were averaged for analysis. GraphPad Prism (www.graphpad.com) was used for statistical analysis. All data were plotted as mean ± SEM, and statistical significance was taken as $p < 0.05$ using ANOVA followed by Tukey post hoc analysis.

Results

$GABA_A$ receptor macroscopic current properties were predicted to have different sensitivities to changes in GABA affinity

BDZs have been shown to enhance $GABA_A$ receptor currents by increasing the affinity of GABA for the receptor (which can involve decreasing the unbinding or increasing the binding rate constants) (Twyman et al., 1989), causing a ''left shift'' of the GABA concentration—response curve (Ghansah and Weiss, 1999). This mechanism predicts that BDZs should increase peak current amplitude only when receptors are activated by sub-saturating concentrations of GABA. Once receptors are activated by a saturating GABA concentration, no further increase in peak current should be possible through increasing agonist binding affinity (Ghansah and Weiss, 1999). Furthermore, desensitization (the loss of current in the continued presence of agonist) should not be affected by BDZs during application of a saturating concentration of GABA, again because binding sites are already saturated (Bianchi et al., 2007). In contrast, the sensitivity of deactivation (the process by which currents return to baseline following GABA washout) to BDZs should depend on whether BDZs increased affinity by increasing the binding rate or decreasing the unbinding rate. Since deactivation occurs in the absence of free GABA, its time-course should be insensitive to changes in the binding rate (defined as the product of the ''true'' binding rate and the GABA concentration, which equals zero during deactivation). In contrast, deactivation should be highly sensitive to slower unbinding, which increases the average time