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Benzodiazepines modulate GABA_A receptors by reducing a gamma-subunit-mediated inhibition of GABA sensitivity

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Background

Heterologous expression of α_1 , β_2 and $\gamma_{2S}(\gamma_1)$ subunits produces a mixed population of GABA_A receptors containing $\alpha_1\beta_2$ or $\alpha_1\beta_2\gamma_{2S}(\gamma_1)$ subunits. GABA sensitivity (lower in receptors containing γ_1 or γ_{2S} subunits) and the potentiation of GABA-activated chloride currents (I_{GABA}) by benzodiazepines (BZDs) are dependent on $\gamma_{2S}(\gamma_1)$ incorporation [1]. A variable γ subunit incorporation may affect the estimation of I_{GABA} potentiation by BZDs. We propose an approach for estimation of BZD efficiency that accounts for a mixed population of $\alpha_1\beta_2$ and $\alpha_1\beta_2\gamma_{2S}(\gamma_1)$ receptors.

Methods

We investigated the relation between GABA sensitivity (EC_{50}) and BZD modulation by analyzing triazolam-, clonazepam- and midazolam-induced potentiation of I_{GABA} in *Xenopus* oocytes under two-microelectrode voltage clamp.

Results

Plotting EC_{50} versus BZD-induced shifts of GABA concentration-response curves ($\Delta EC_{50}(\text{BZD})$) of oocytes injected with different amounts of α_1 , β_2 and $\gamma_{2S}(\gamma_1)$ cRNA (1:1:1–1:1:10) revealed a linear regression between $\gamma_{2S}(\gamma_1)$ -mediated reduction of GABA sensitivity (EC_{50}) and $\Delta EC_{50}(\text{BZD})$. The slope factors of the regression were always higher for oocytes expressing $\alpha_1\beta_2\gamma_1$ subunit receptors (triazolam: 1.8 ± 0.1 ; clonazepam: 1.6 ± 0.1 ; midazolam: 2.3 ± 0.2) than for oocytes expressing $\alpha_1\beta_2\gamma_{2S}$ receptors (triazolam: 1.4 ± 0.1 ; clonazepam: 1.4 ± 0.1 ; midazolam: 1.3 ± 0.1). Mutant GABA_A receptors ($\alpha_1\beta_2$ -R207C γ_{2S}) with lower GABA sensitivity showed higher drug efficiencies (slope factors: triazolam: 1.1 ± 0.1 ; clonazepam: 1.1 ± 0.1 ; midazolam: 1.2 ± 0.1) whereas higher GABA sensitivity of α_1 -L263S $\beta_2\gamma_{2S}$ mutant receptors was associated with lower efficiency (slope factor: clonazepam: 1.7 ± 0.1).

Conclusions

Regression analysis enabled the estimation of BZD efficiency when variable mixtures of $\alpha_1\beta_2$ and $\alpha_1\beta_2\gamma_{2S}(\gamma_1)$ receptors are expressed and provided new insights into the $\gamma_{2S}(\gamma_1)$ dependency of BZD action. The method for determining the slope of the regression line also allowed the determination of the percentage of $\alpha_1\beta_2\gamma_{2S}$ receptors. Assuming a twofold difference in the single channel conductance of $\alpha_1\beta_2$ and $\alpha_1\beta_2\gamma_{2S}$ receptors, at 70% current ratio of γ_{2S} -containing receptors our mathematical model predicts only about 50% of γ_{2S} subunit incorporation. Our data suggest that BZDs reduce a γ -subunit-mediated inhibition of GABA sensitivity.

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Reference

1. Boileau AJ, Baur R, Sharkey LM, Sigel E, Czajkowski C: **The relative amount of cRNA coding for γ_2 subunits affects stimulation by benzodiazepines in GABA_A receptors expressed in *Xenopus* oocytes.** *Neuropharmacology* 2002, **43**:695–700.