Supplemental Data

Article Title: Characterization of two mutations, M287L and Q266I, in the α1 glycine receptor subunit that modify sensitivity to alcohols

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Journal name: The Journal of Pharmacology and Experimental Therapeutics

Table A. Parameters of glycine concentration-response curves in *Xenopus* oocytes. EC_{50}, effective concentration 50, n_H, Hill coefficient and n, number of oocytes per group. Data is presented as mean (95% confidence intervals) or mean ± S.E.M. *p< 0.05 versus corresponding WT. The two α1 wild-type (WT) curves were determined on different occasions (same batches as the corresponding mutants), and they are not significantly different.

	α1 WT (n= 6)	**α1(M287L)** (n= 5)
EC_{50} (μM)	201 (167 to 244)	664 (568 to 776)*
n_H	1.6 ± 0.2	1.4 ± 0.1
	α1 WT (n= 7)	**α1(Q266I)** (n= 8)
EC_{50} (μM)	136 (107 to 172)	227 (207 to 248)*
n_H	2.0 ± 0.4	2.2 ± 0.2

Table B. Drug effects on GlyR expressed in *Xenopus* oocytes. Glycine (EC_5) was co-applied with the drug; no pre-application was used, except pentylenetetrazole (1-min pre-application). The concentrations for ketamine, pentobarbital and flurazepam were chosen based on the levels present in brain, capable of inducing loss of righting reflex (Cohen et al., 1973; Miller et al., 1988; Franks and Lieb, 1994). The concentration for pentylenetrazol was approximately the level that induces seizures (Yonekawa et al., 1980). Data shown is the change in the control (EC_5) glycine response, expressed as a percentage of the control glycine response. Data is presented as mean ± S.E.M. (number of oocytes tested). Statistical analysis: One-Way ANOVA, Dunnett's Multiple Comparison Test for post-hoc analysis; * $p < 0.05$ versus WT.

	α1 WT	**α1(M287L)**	**α1(Q266I)**
Ketamine (365 µM)	-20 ± 3 (5)	-17 ± 6 (5)	-27 ± 3 (4)
Pentobarbital (50 µM)	11 ± 1 (4)	52 ± 6* (4)	-8 ± 5* (4)
Flurazepam (25 µM)	-1 ± 3 (4)	-3 ± 1 (4)	-7 ± 3 (4)
Pentylenetetrazole (6 µM)	25 ± 10 (4)	5 ± 3 (4)	14 ± 3 (4)
GABA (1 mM)	-9 ± 4 (4)	-23 ± 1* (4)	-11 ± 2 (4)
Glutamate (1 mM)	-9 ± 4 (4)	-10 ± 2 (4)	-11 ± 6 (4)

Table C. Parameters from binding experiments in homogenates of brain stem and spinal cord from WT ($\alpha1^{M/M}$ and $\alpha1^{Q/Q}$) and heterozygous knockin ($\alpha1^{M/L}$ and $\alpha1^{Q/I}$) mice. B_{MAX} (fmol/mg protein) is the maximal number of binding sites, K_D (nM) is the affinity constant, n_H is the Hill coefficient and IC_{50} (μM) is the inhibitory concentration 50. Data was analyzed using t-test: # p= 0.08, * p< 0.05, ** p< 0.01, *** p< 0.0001 versus corresponding WT. N.D., not determined.

	$\alpha1^{M/M}$	**$\alpha1^{M/L}$**	**$\alpha1^{Q/Q}$**	**$\alpha1^{Q/I}$**
[^{3}H]-Strychnine binding				
Saturation binding experiments				
B_{MAX}	1408 ± 45 (4)	1425 ± 96 (4)	1338 ± 72 (7)	1530 ± 120 (7)
K_D	4.31 ± 0.57 (4)	4.62 ± 0.38 (4)	3.9 ± 0.5 (7)	4.2 ± 1.1 (7)
n_H	1.10 ± 0.07 (4)	1.05 ± 0.06 (4)	1.11 ± 0.11 (7)	1.17 ± 0.11 (7)
Glycine competitive binding experiments				
IC_{50}	23.7 ± 2.2 (3)	37.5 ± 1.7 ** (3)	20.4 ± 2.5 (4)	36.2 ± 4.4 * (4)
n_H	-1.11 ± 0.07 (3)	-1.42 ± 0.16 (3)	-1.12 ± 0.06 (4)	-1.11 ± 0.04 (4)
Taurine competitive binding experiments				
IC_{50}	100.5 ± 1.6 (3)	178.3 ± 3.6 *** (3)	149 ± 25 (4)	231 ± 31# (4)
n_H	-1.37 ± 0.08 (3)	-1.60 ± 0.11 (3)	-1.64 ± 0.17 (4)	-1.37 ± 0.06 (4)
[^{3}H]-Flunitrazepam binding				
Homologous competitive binding experiments				
B_{MAX}	N.D.	N.D.	1346 ± 37 (3)	1543 ± 123 (3)
K_D	N.D.	N.D.	2.48 ± 0.06 (3)	2.70 ± 0.24 (3)

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