Amino acid substitutions in the human homomeric β_3 $GABA_A$ receptor that enable activation by GABA

Carla Gottschald Chiodi[1], Daniel T. Baptista-Hon[2], William N. Hunter[1] and Tim G. Hales[2]

[1]Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee, DD1 5EH, UK.
[2]The Institute of Academic Anaesthesia, Division of Systems Medicine, School of Medicine, Ninewells Hospital, University of Dundee, Dundee, DD1 9SY, UK.

Running title: Only two amino acid substitutions enable GABA-mediated activation of human homomeric β_3 $GABA_A$ receptor

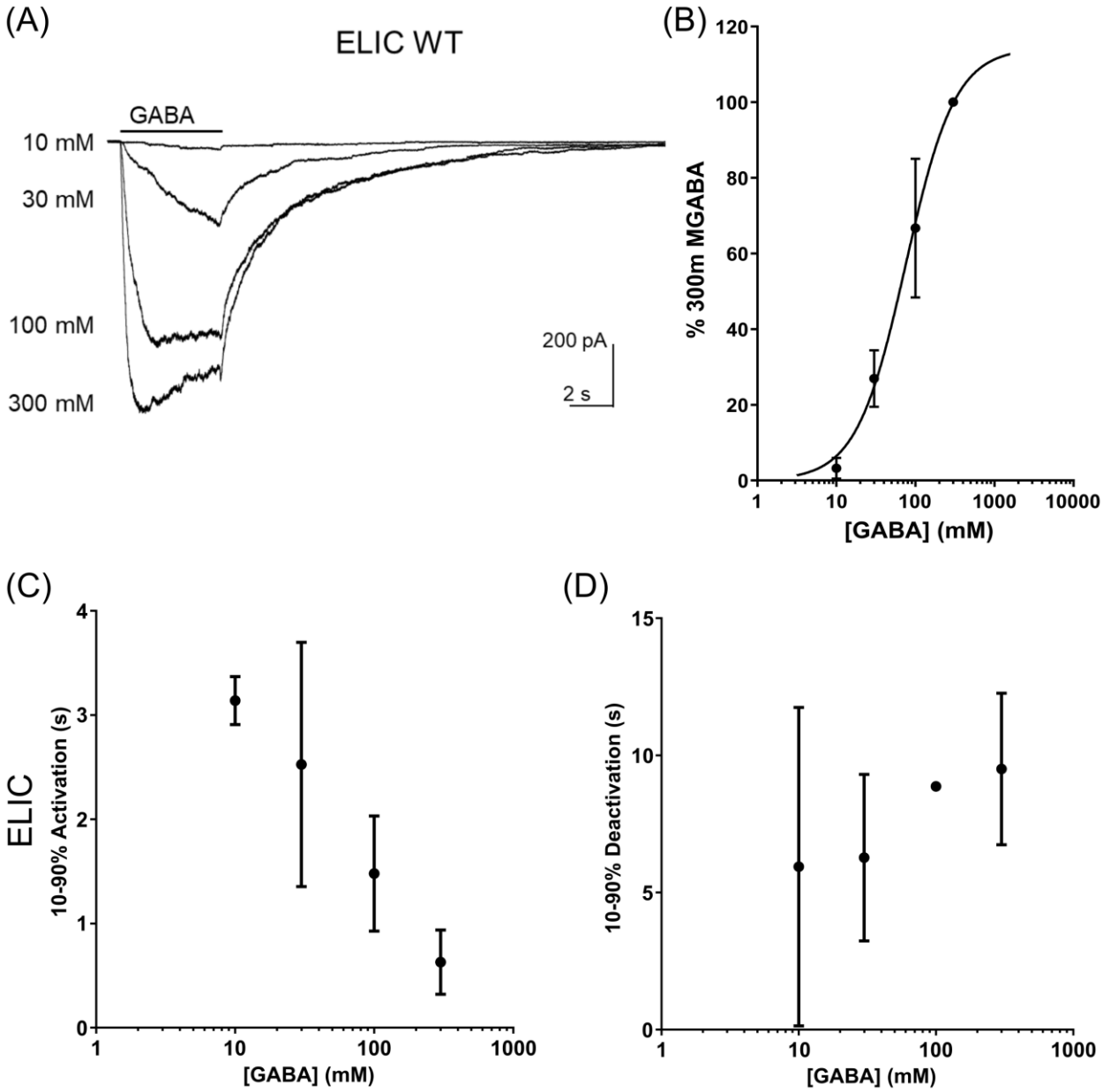
To whom correspondence should be addressed: T. G. Hales: Institute of Academic Anaesthesia, Division of Systems Medicine, Ninewells Hospital, University of Dundee, Dundee, DD1 9SY, UK. Email: t.g.hales@dundee.ac.uk

---

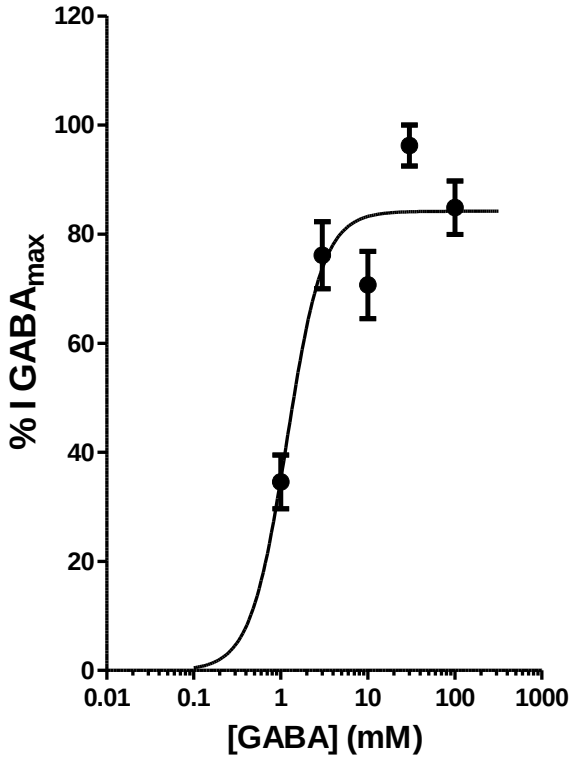
SUPPORTING INFORMATION

Supplementary Table S1. Summary of EC_{50} and current densities values. Mean ± SD EC_{50} values obtained from logistic function fit parameters of individual experiments. Mean ± SD current densities evoked by peak concentrations of GABA. No significant differences between $GABA_AR$ β_3 C1 and the mutants were observed (one-way ANOVA *post hoc* Dunnett's; EC_{50} P = 0.4556, F (3,12) = 0.9311; current densities P = 0.5714, F (3,12) = 0.6973). n = number of experiments.

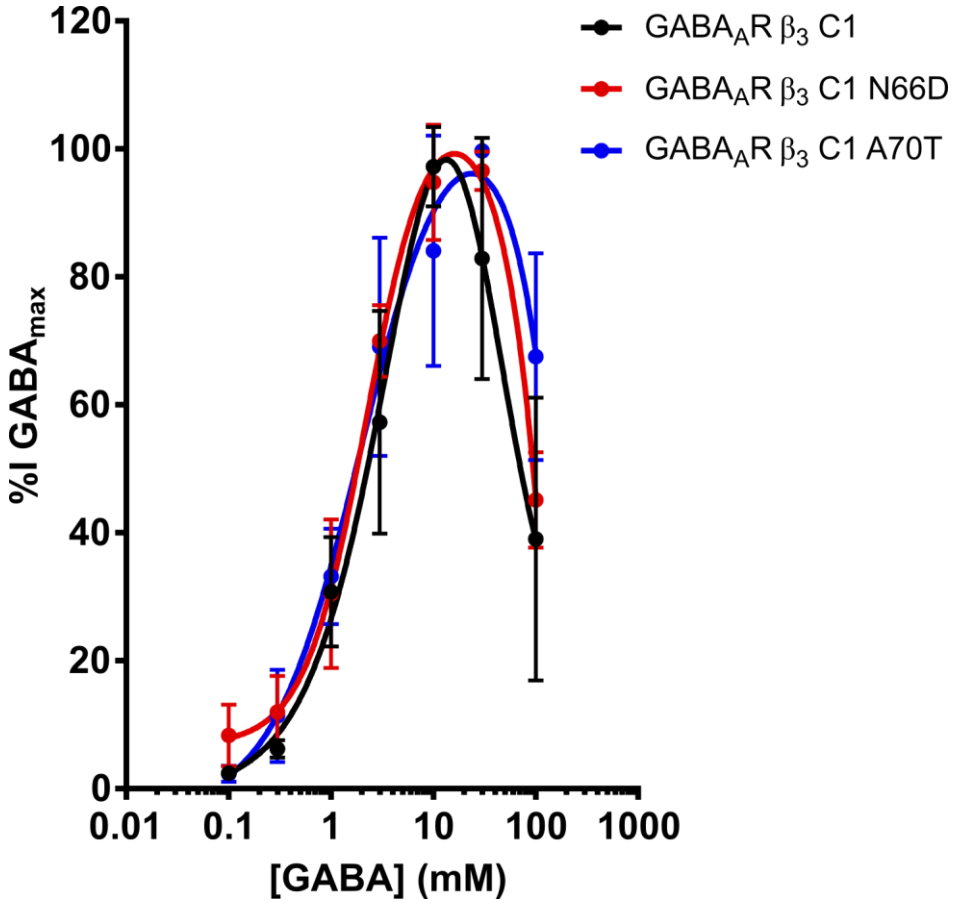
Receptor	EC_{50} (mM)	Current density (pA/pF)	n
$GABA_AR$ β_3 C1	2.9 ± 2.1	-15.7 ± 12.5	4
$GABA_AR$ β_3 C1 N66D	2.3 ± 1.3	-16.1 ± 11.2	4
$GABA_AR$ β_3 C1 A70T	1.8 ± 1.4	-11.2 ± 3.3	4
$GABA_AR$ β_3-cryst+β_3 C1	1.3 ± 0.46	-8.1 ± 6.6	4



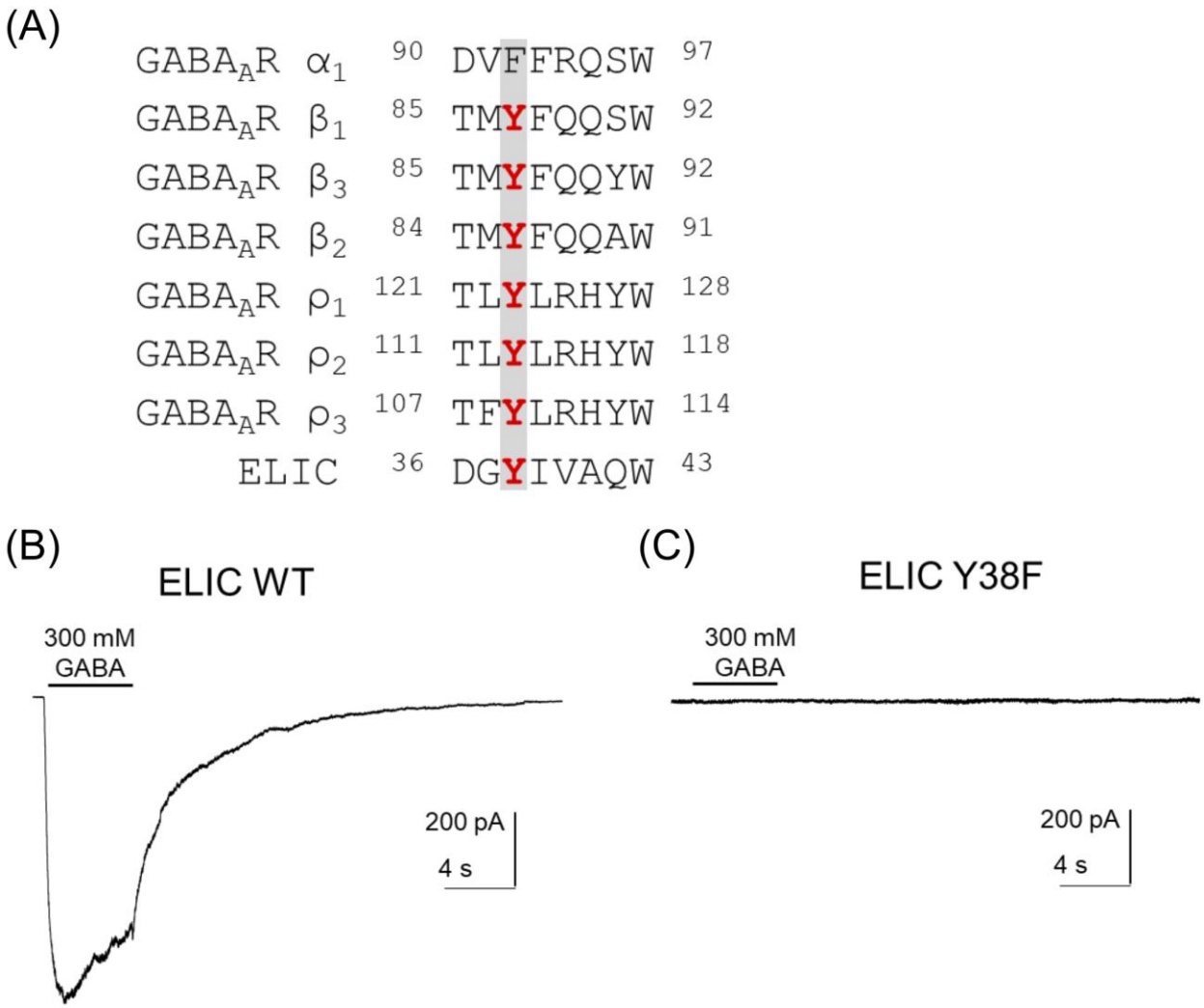
Supplementary Figure S1. GABA-evoked currents mediated by ELIC and kinetics. (A) Examples of currents mediated by ELIC WT, evoked by increasing concentrations of GABA. The bar indicates GABA application (5 s). (B) Concentration-response curve obtained using the percentage of the maximum amplitude recorded for each cell (n = 3). Logistic equations were fitted to the data points using GraphPad Prism. (C) Mean of 10-90% rise time of current activation mediated by ELIC WT indicates the activation is faster when the concentration of GABA increased. (D) Mean of 10-90% time of current deactivation mediated by ELIC WT suggests a slow deactivation and independent of the ligand concentration.



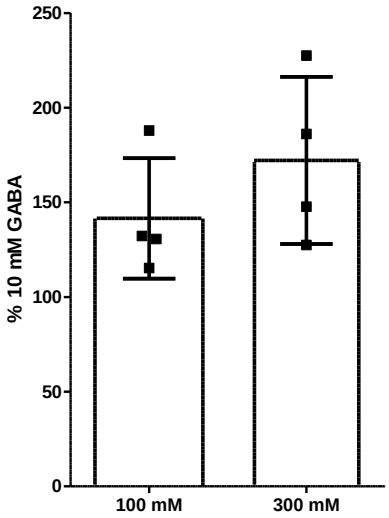
Supplementary Figure S2. Heteromeric β_3-cryst/β_3 C1 heteromeric GABA$_A$Rs do not display GABA-mediated inhibition. Figure shows the concentration-response relationship of β_3-cryst/β_3 C1 heteromeric GABA$_A$Rs obtained using the percentage of the maximum amplitude recorded for each cell (n = 4). A logistic equation was fitted to the data points using GraphPad Prism. The mean EC_{50} as well as the current density, obtained from each individual cell, is summarized in Supplementary Table 1.



Supplementary Figure S3. Loop G substitutions in β_3 C1 GABA$_A$R did not affect GABA potency. Concentration-response curves obtained using the percentage of the maximum amplitude recorded for each cell (n = 4). Logistic equations were fitted to the data points using GraphPad Prism. The substitutions in loop G did not affect GABA potency. GABA at high concentrations was still inhibiting the channel (N66D IC_{50} 229.6 mM and A40T IC_{50} 11.0 mM). A summary of the data is in Supplementary Table S1.



Supplementary Figure S4. Importance of Tyr in homomeric receptors activated by GABA. (A) Amino acid sequence alignment shows the Tyr is conserved in all pLGIC subunits that form homomeric GABA-activated receptors. (B) Example of currents evoked by 300 mM GABA mediated by ELIC WT. (C) No current was evoked by 300 mM GABA mediated by ELIC Y38F, suggesting the tyrosine in this position is important for receptor activation. The bar indicates GABA application (5 s).



Supplementary Figure S5. Supramaximal concentrations of GABA did not inhibit currents mediated by β_3 C1 F87Y GABA$_A$Rs. Bar graph shows mean current amplitude evoked by 100 mM or 300 mM GABA, normalized to that evoked by 10 mM GABA, at β_3 C1 F87Y GABA$_A$Rs. There is no significant difference in GABA-evoked current amplitude at these concentrations (P = 0.3032, n = 4, t-test). These data indicate that the F87Y substitution abolished the inhibitory component at β_3 C1 F87Y GABA$_A$Rs.