Investigating GABA-A receptor pore conformations using disulfide trapping

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We previously employed a disulfide trapping approach in an attempt to determine how the pore-lining second transmembrane domains (M2) of γ-aminobutyric acid type A receptors (GABAARs) move to open the channel. The M2 domain T6′ residue lines the pore, and we showed that α1/β1T6′C receptors form 6′ cysteine-mediated disulfide bonds in the closed state. However, because GABA induced fast desensitization, investigating dimer formation in the open state was not possible. The present study addressed this by using the non-desensitising agonist, ivermectin, to induce a stable open state, thereby allowing comparison of M2 domain orientations in closed and open states. Patch-clamp electrophysiology and Western-blotting were both performed on GABAARs expressed in HEK293 cells. Whereas unmutated GABAARs were not locked open by ivermectin, α1/βT6′C GABAARs were locked open *via* disulfide bond formation. This was confirmed using both electrophysiology (n > 10 cells) and Western blot (n = 3). Also, a reducing agent, dithiothreitol, reduced the closed-state dimer but not the open-state dimer (n = 5 cells each). Moreover, the closed state dimer needed to be reduced to enable formation of the open state dimer. We propose that, in both the closed and open states, β subunit 6′ cysteines move into sufficiently close proximity for disulfide formation *via* large random motions that appear to be a unique feature of β subunits. Because cross-linking of adjacent β subunits prevents the channels from both opening and closing, a movement of adjacent subunits relative to one another must be essential for channel gating. Our results place constraints on the closed and open state structures of the GABAAR pore and provide evidence for the relative movement of β subunits during gating.