Correlating functional models of channel activation with subunit specific $GABA_A$ receptors

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$GABA_A$ receptors are the major mediators of rapid inhibitory neurotransmission. They are pentamers, comprised of either identical or homologous subunits, and have a number of important sites at which drugs act to modulate their activity. Many sedative and anaesthetic drugs, including benzodiazepines, alcohol, barbiturates, propofol, ether and alkane anaesthetics as well as neurosteroids, achieve channel modulation. GABA is thought to bind at the β-α interfaces within the extracellular domain of the channel, whereas benzodiazepines bind at the homologous γ-α interface. The predominant isoform of $GABA_A$ receptor in the mammalian nervous system is the α1β2γ2 channel, but there are also other significant subtypes, including α2 and α3-containing channels. For instance, inhibitory synapses on thalamocortical neurons found in the ventrobasal nucleus of the thalamus are populated by α1β2γ2 $GABA_A$ receptors, whereas α3β3γ2 $GABA_A$ receptors are found at synapses in the neighbouring reticular nucleus of the thalamus. Inhibitory synaptic currents recorded from both of these thalamic regions have demonstrated that the rising phase of the currents at both synapses develop at a similar rate, but the time course of the current decay at α3β3γ2 synapses is relatively slow. This suggests that the two channel types have different kinetic properties. Furthermore, there is evidence that the benzodiazepine-like hypnotic drugs, zolpidem and eszopiclone modulate these two channels differentially, again suggesting inherent functional differences between the two.

We investigated α1β2γ2 and α3β3γ2 $GABA_A$ receptors, transiently expressed in HEK293 cells, using patch-clamp electorphysiology. Single channel currents were recorded in outside-out patches at a clamped potential of −70 mV and in the presence of a broad range of concentrations of GABA, zolpidem and eszopiclone. Activations from individual ion channels were isolated from the records and analysed within the framework of kinetic mechanisms. This method enabled us to obtain information regarding ligand binding and the downstream, conformational events that constitute channel activation.

α3β3γ2 receptors exhibited channel activations that were of longer duration than α1β2γ2 receptors, over the entire range of GABA concentrations tested, including at 2 μM GABA, which elicits relatively brief bursts of channel activity. This observation likely accounts for the slower deactivation rate of synaptic currents mediated by α3β3γ2 channels. Zolpidem and eszopiclone also increased the duration of single channel activations in both channels. Fitting a reaction mechanism to both $GABA_A$ receptor isoforms enabled us to obtain estimates of the binding affinity of the channels for GABA. Single channel bursts of activity that showed evidence of modulation could be discerned in the records in the continuous presence of low GABA (2 μM) and modulating drug (1 μM), enabling us to obtain binding affinity estimates for zolpidem and eszopiclone. The binding data obtained by fitting single channel records to mechanisms were compared to ligand docking simulations using homology models of both channels. In addition, our mechanisms described how zolpidem and eszopiclone lengthen the active periods of individual channels and demonstrate that single channel currents of α1β2γ2 and α3β3γ2 $GABA_A$ receptors can be accurately described by a common functional mechanism. This suggests that these channels undergo similar structural changes when activated and modulated by ligands.