Developing activation mechanisms for $GABA_A$ receptors

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The α1β2γ2 and α3β3γ2 are two synaptic isoforms of α-aminobutyric acid type A ($GABA_A$) receptor. They are found at different synapses, for example in the thalamus, where they mediate different inhibitory postsynaptic current profiles, particularly with respect to the rate of current decay. The kinetic characteristics of both isoforms were investigated by analysing single-channel currents over a wide range of GABA concentrations. α1β2γ2 channels exhibited briefer active periods than α3β3γ2 channels over the entire range of agonist concentrations and had lower intraburst open probabilities at subsaturating concentrations. Activation mechanisms were constructed by fitting postulated reaction schemes to data recorded at saturating and subsaturating GABA concentrations, simultaneously. Reaction mechanisms were ranked according to goodness of fit values to open and shut dwell histograms of single channel activity, and how accurately they simulated ensemble currents. The highest ranked mechanism for both channels consisted of two sequential binding steps, followed by three conducting and three nonconducting configurations. The equilibrium dissociation constant for GABA at α3β3γ2 channels was ~3 μM compared with ~19 μM for α1β2γ2 channels, suggesting that GABA binds to the α3β3γ2 channels with higher affinity. A notable feature of the mechanism was that two consecutive doubly liganded shut states preceded all three open configurations. The lifetime of the third shut state was briefer for the α3β3γ2 channels. The longer active periods, higher affinity, and preference for conducting states are consistent with the slower decay of inhibitory currents at synapses that contain α3β3γ2 channels. The reaction mechanism we describe accurately simulates real macropatch and synaptic currents mediated by the two $GABA_A$ receptor subtypes and may be appropriate for the analysis of other $GABA_A$ receptor isoforms. The mechanism may also be applicable for the rational investigation of the kinetic effects of therapeutic agents that activate and modulate $GABA_A$ receptors, in addition to mutated channels that give rise to disease.