$GABA_A$ αβ receptors open spontaneously when the conserved M2 leucine 9′ residue is mutated to a threonine

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Inhibitory neurotransmission in the central nervous system of the brain is largely mediated by the γ-aminobutyric acid type A ($GABA_A$) receptor. This pentameric receptor is selectively permeable to chloride ions when activated by agonist. The simplest functional $GABA_A$ receptor is composed of α and β subunits. Each subunit has 4 transmembrane α-helices, of which the second transmembrane helix (M2) from all 5 subunits forms the pore. At the 9′ position of the M2 is a leucine residue that is conserved in all ligand-gated ion channels.

A single-channel study was performed to examine the effect of substituting the conserved leucine 9′ residue (L9′) to a threonine in the M2 domain in $GABA_A$ αβ receptors. Site-directed mutagenesis was performed on the human α1 and β1 subunit cDNAs. L929 mouse fibroblasts were transfected with either wild type or mutant $GABA_A$ α and β subunits and green fluorescent protein (GFP) plasmids. Successfully transfected cells showed bright green fluorescence and were targeted for single-channel outside-out patch-clamp recordings.

Wild type $GABA_A$ αβ receptors showed single-channel activity when activated by agonist. By contrast, αβ receptors that contained the L9′T substitution in either the α, β or both subunits had spontaneous channel activity. The single-channel activity recorded from wild type αβ receptors in the presence of 1 μM GABA consisted predominantly of brief open time events with a main single-channel conductance of 15 pS. Single-channel activity was recorded from mutant α(L9′T)β and αβ(L9′T) receptors in the absence of agonist. The spontaneous single-channel activity from these two mutant combinations had significantly longer open time events compared to wild type channels, and there was no change in the single-channel conductance. Application of GABA to the mutant α(L9′T)β and αβ(L9′T) receptors led to an increase in single-channel activity. In contrast to the behaviour of α(L9′T)β and αβ(L9′T) receptors, outside-out recordings from the mutant α(L9′T)β(L9′T) receptor showed only a spontaneous leak current with no single-channel closures. The application of penicillin to this spontaneous leak induced very brief closures.

The data suggest that in wild type αβ receptors the functional role of the L9′ residue is to stabilise the closed state of the channel. When mutated to a threonine the equilibrium of the receptor is pushed towards the open state as revealed by the ability of mutant receptors to now open spontaneously.