The Cys-loop's role in ligand-binding and channel-gating in the $GABA_A$ receptor

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The Cys-loop is conserved amongst members of the ligand-gated ion channel (LGIC) family. Within this loop there is a conserved motif referred to as the XPZD motif which sits at the bottom of the Cys-loop at positions 8 to 11 where the conserved cysteines are designated positions 1 and 15. The 4 residues that comprise the XPZD motif in the $GABA_A$ receptor are either conserved throughout the LGIC family or the chemical nature of the residue is conserved. The recently solved crystal structure of an acetylcholine binding protein places the Cys-loop at the junction between the extracellular ligand-binding and transmembrane domains (Brejc *et al*., 2001). We hypothesise that the Cys-loop may play a role in linking ligand-binding to channel-gating in the $GABA_A$ receptor. To test this hypothesis we are mutating residues in the conserved XPZD motif within the Cys-loop of $GABA_A$ receptor subunits and examining their ability to function as ion channels.

Mouse L929 cells were transfected with combinations of wild type (WT) ($\alpha1\beta1\gamma2s$) and mutant GABA subunit cDNAs. When the invariant proline residue in the Cys-loop was replaced with alanine (P9′A) in the α or β subunit and co-expressed with the remaining WT subunits, the whole cell response to GABA was reduced. The reduced response could be caused by fewer receptors in the membrane as a result of lower expression, impaired assembly or defective trafficking of receptors or the reduction could be a result of changes in channel properties such as kinetics. To determine the cause of the reduced response, immunofluorescence and radioactive muscimol binding studies are being used to quantitate and compare the presence of mutant receptors in the membrane with the WT receptor and secondly, single channel currents are being recorded to examine if the reduced response is caused by changes in channel kinetics.

The response of $GABA_A$ receptors to agonists can be potentiated by drugs such as diazepam, pentobarbitone and etomidate. The effects of these drugs were tested on $GABA_A$ receptor mutants and compared with the effects on WT $GABA_A$ receptors. Responses were generated with 1μM GABA followed by potentiation with 1μM diazepam, 100 μM etomidate or 50 μM pentobarbitone. The potentiated response to each drug was less than the WT response when proline was mutated to alanine at 9′ in the Cys-loop in either α or β subunits.

The (P9′A) mutation in the Cys-loop of $GABA_A$ subunits reduces the whole cell current in response to GABA and changes responses to various drugs. Further studies are being undertaken to test the role of the Cys-loop in these fundamental actions of the ion channel.

Brejc, K., van Dijk, W. J., Klaassen, R.V., Schuurmans, M., van Der Oost, J., Smit, A.B. & Sixma, T.K. (2001) *Nature,* **411,** 269-276.