

**P008** Construction and characterisation of a chimeric human  $\alpha 7$  nicotinic acetylcholine / mouse 5HT3 receptor

**P.J. Craig, R. Zwart, S. Bose, R.E. Beattie, E.A. Folly, L.R. Johnson, E. Bell, N.M. Evans, S.G. Volsen, E. Sher, N.S. Millar\* and L.M. Broad**

*Eli Lilly & Company, Lilly Research Centre,  
Erl Wood Manor, Windlesham, Surrey*

*\*Wellcome Laboratory for Molecular Pharmacology,  
Dept Pharmacology, University College London, London*

The neuronal nicotinic acetylcholine receptor  $\alpha 7$  subunit can assemble as a homo-pentamer to form a functional ligand-gated ion channel.

Chimeric  $\alpha 7/5HT3$  receptors have been described previously as tools for investigating the role of the various domains of the receptor. We report on the construction and characterisation of a novel human  $\alpha 7$  / mouse 5HT3 chimera comprising the N-terminal region of the human  $\alpha 7$  nicotinic acetylcholine receptor linked at valine 202 with the transmembrane / C-terminal regions of the mouse 5HT3 receptor.

Expression in *Xenopus* oocytes or HEK293 cells resulted in functional channels that were sensitive to ligands of nicotinic acetylcholine, but not 5HT3 receptors. Currents obtained from oocytes injected with cDNA for the chimera desensitised more slowly than those obtained by injection of wild-type  $\alpha 7$ . The response of both wild-type and chimeric receptors was potentiated by 5OH-indole. Expression in mammalian cells was initially demonstrated by surface  $\alpha$ -bungarotoxin binding and single-cell calcium imaging in transiently transfected HEK 293 cells. Subsequently, stable clones were produced and functional clones selected by assessing agonist induced calcium increase in a FILPR.