GABARAP influences the conductance of recombinant $GABA_A$ channels

A.B. Everitt, M.L. Tierney and P.W. Gage, The John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia.

'Native' $GABA_A$ receptors display distinct electrophysiological properties not always seen in recombinant receptors irrespective of subunit composition. Native channels can have conductances over 40pS (Gray & Johnson, 1985; Smith *et al.*, 1989: Curmi *et al*., 1993). Moreover, the conductance of some channels can be increased by modulating drugs such as diazepam, pentobarbitone and propofol (Eghbali *et al.*, 1997; Guyon *et al.*, 1999; Eghbali *et al.*, 2003). By contrast, conductances of recombinant channels have never exceeded 35pS and, although their open probability can be increased by modulating drugs, channel conductance was not enhanced by drugs.

A $GABA_A$ receptor-associated protein, GABARAP, is an intracellular protein that can interact with the $GABA_A$ γ2 subunit. When co-expressed with $GABA_A$ subunits, GABARAP caused clustering of the receptors and changes in whole-cell current kinetics in QT6 fibroblasts (Wang *et al.*, 1999). Recent observations have shown that over-expression of GABARAP in *Xenopus* oocytes and in neurons increases the level of $GABA_A$ receptors detected at the plasma membrane, indicating that GABARAP is involved in the trafficking of $GABA_A$ receptors (Chen *et al.*, 2005; Leil *et al.*, 2004).

It has been suggested that high channel conductances may represent co-operative openings of clustered channels resulting in an apparent high single channel conductance (Laver & Gage, 1997). We tested this hypothesis in an expression system by co-expressing GABARAP, known to cluster $GABA_A$ receptors, with $GABA_A$ receptor subunits in L929 mouse fibroblasts.

Immunofluorescent studies revealed that co-expression of GABARAP with $GABA_A$ subunits, showed a punctate pattern of staining of surface receptors compared to a diffuse pattern in control cells.

We recorded single channel currents in the cell-attached (c/a) configuration 24-72 hours post transfection. Control patches expressing $GABA_A$ α1, β1 and γ2s subunits alone had a mean conductance of 22.3 ± 1.2 pS (n=15). In 16 out of 25 patches recorded from cells co-transfected with $GABA_A$ α1, β1 and γ2s subunits and GABARAP, single channel conductances were above 40pS (γ = 60.7 ± 4.3pS, n=16). These 'high' conductance channels were never seen in control patches. In the remaining 9 patches, the mean conductance was 29.1 ± 1.9 pS. Both high and low conductance channel activities were blocked by 100μM bicuculline. The current-voltage relationship of high conductance channels showed outward rectification of the current, similar to that seen in native receptors.

Diazepam and pentobarbital have been shown to increase both open probability and conductance of $GABA_A$ channels. In patches from cells co-expressing $GABA_A$ α1, β1 and γ2s subunits and GABARAP, both of these effects were seen irrespective of initial channel conductance. In control patches where GABARAP was not expressed, application of diazepam or pentobarbital increased the channel open probability with no effect on single channel conductance.

Our results show that co-expression with GABARAP has changed the properties of recombinant $GABA_A$ channels. It is possible that clustered receptors may be able to couple and open cooperatively by virtue of their close physical proximity.

Curmi, J.P., Premkumar, L.S., Birnir, B. & Gage, P.W. (1993), *Journal of Membrane Biology,* 136, 273-280.
Eghbali, M., Curmi, J.P., Birnir, B. & Gage, P.W. (1997), *Nature*, 388, 71-75.
Eghbali, M., Gage, P.W. & Birnir, B. (2003), *European Journal of Pharmacology*, 468 (2): 75-82.
Gray, R., Johnston, D. (1985) *Journal of Neurophysiology*, 54: 134-142.
Guyon, A., Laurent, S., Paupardin-Tritsch, D., Rossier, J. & Eugen, D. (1999), *Journal of Physiology,* 516, 719-737.
Smith, S.M., Zorec, R. & McBurney, R.N. (1989) *Journal of Membrane Biology,* 108, 45-52.
Wang, H., Bedford, F.K., Brandon, N.J., Moss, S.J. & Olsen, R.W. (1999), *Nature*, 397, 69-72.
Chen, Z., Chang-Sheung, S., Leil, T.A., Olcese, R. & Olsen, R.W. (2005), *Molecular Pharmacology,* 68(1), 152-159.
Leil, T.A., Chen, Z., Chang-Sheung, S. & Olsen, R.W. (2004), *Journal of Neuroscience*, 24(50): 11429-11438.
Laver, D.R.. & Gage, P.W. (1997), *Prog. Biophsy. Mol. Biol*, 67: 99-140.