Clustering of recombinant $GABA_A$ receptors alters channel properties

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'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 about 30pS and, although their open probability can be increased by modulating drugs, conductance is not enhanced by drugs.

It has been suggested that high channel conductances may represent cooperative openings of clustered channels resulting in an apparent high single channel conductance. We tested this hypothesis in an expression system by co-expressing two proteins known to cluster $GABA_A$ receptors. Rapsyn is a membrane associated protein that plays a crucial role in clustering ACh receptors at the neuromuscular junction, but has also been shown to cluster expressed $GABA_A$ receptors. GABARAP interacts with the $GABA_A$ γ subunit and promotes receptor clustering (Wang *et al.*, 1999).

We co-transfected (lipofectin) $GABA_A$ α5 and β1 subunit cDNAs with or without rapsyn into mouse fibroblast L929 cells. We measured single channel conductance in the cell-attached (c/a) or inside-out (i/o) configurations 24-72 hours later. In the control groups (i.e. $GABA_A$ subunits alone), single channel conductances were within the range 10-35pS. When rapsyn was co-expressed with $GABA_A$ subunits, 4 out of 8 patches showed single channel conductances greater than 40pS. Control patches expressing $GABA_A$ α1, β1 and γ2s subunits alone had a mean conductance of 22.3 ± 1.2pS (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. High and low conductance channel activity was blocked by 100μM bicuculline. The current-voltage relationship of high conducting channels showed outward rectification of the current, similar to that seen in native receptors.

Diazepam can increase both open probability and conductance of $GABA_A$ channels containing the γ subunit. In 5 patches from cells co-transfected with $GABA_A$ 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, diazepam did not increase channel conductance.

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

Our results show that co-expression with "clustering" proteins can change 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.

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