Increasing the surface density of recombinant $GABA_A$ receptors increases channel conductance

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Major inhibitory signals in the central nervous system are carried via the neurotransmitter g-amino butyric acid (GABA). $GABA_A$ receptors are chloride-selective ion channels located in clusters at the synapse of post-synaptic neurons and extra-synaptically on soma.

The conductance of neuronal $GABA_A$ receptors varies between 10 and 90pS[(1)]. We have reported mean single channel conductances of 20.4±1.4pS from recombinant receptors in L929 cells transfected with α1, β1 & γ2S $GABA_A$ subunits using the transfection reagent lipofectin (Sigma)[(2)]. However co-transfection of the same $GABA_A$ subunits with GABARAP, a GABA receptor associated protein known to cluster $GABA_A$ receptors, showed higher single channel conductance of 60.7±4.3pS[(3)].

In an effort to increase the efficiency of transfection rates in L929 cells, we tested the reagent FuGENE6. Using FACS analysis, we found consistently higher transfection rates using FuGENE6 (6-14%) compared to lipofectin (2-8%). Cross-section histograms from confocal images of fluorescently labeled receptors in the plasma membrane indicated a much higher flourescence intensity in cells transfected using FuGENE6 than with lipofectin. Moreover, surface receptors from FuGENE6 transfected cells showed aggregation of receptors in large clusters.

Patch clamp studies showed significantly higher single channel conductances, 36.4±2.4pS (-Vp+60mV, n=18) in cell-attached patches from cells transfected using FuGENE6 compared to those obtained using lipofectin ($p<0.0001$, student's t-test).

Our results suggest a correlation with higher receptor density in the plasma membrane and the occurrence of high conductance channels. It is possible that these receptors may open and close synchronously by virtue of their close proximity. This hypothesis is supported by our previous finding that $GABA_A$ receptors co-transfected with GABARAP result in receptor clustering in the plasma membrane and show high channel conductances.[(3)].

(1) Gray, R. and Johnston, D. (1985) Journal of Neurophysiology, 54: 134-142
(2) Wang, H., Bedford, F.K. et al. (1999). Nature 397: 69-72.
(3) Everitt A.B., Luu T., Cromer B., Tierney M.L., Birnir B., Olsen R.W., Gage P.W. (2004) Journal of Biological Chemistry. 279: 21701-21706.