

Chronic-moderate ethanol exposure of L(tk-) cells expressing $\alpha 4\beta 3\delta$ GABA_A receptors reduces potency of allopregnanolone potentiation of GABA-evoked inward currents: Possible role of PKC

ID Barrett, SP Kelley. University of Kent, Chatham Maritime, UK

Aim: To investigate the effect of chronic-moderate ethanol (CME) treatment upon direct activation and allosteric modulation of GABA_A receptors, and to assess the sensitivity of these parameters to PKC inhibition in control and ethanol-treated cells.

Methods: L(tk-) cells were exposed to 20mM ethanol in culture media for 14 days prior to induction of stable expression of human recombinant $\alpha 4\beta 3\delta$ receptors using dexamethasone. Concentration-response curves for GABA (1nM - 100 μ M), and allopregnanolone (ALLO; 1nM - 30 μ M) co-applied with 200nM GABA were obtained using whole-cell patch-clamp electrophysiology at a holding voltage of -60 mV. 400nM calphostin C (CphC) or vehicle (DMSO) was administered to cells *via* the pipette solution, which was prepared with 2mM Mg.ATP. SDS-PAGE and western blotting were used to compare levels of whole-cell expression, quantified relative to β -actin, of $\alpha 4$ and δ GABA_A receptor subunits, and several isoforms of PKC (α , γ , δ , and ϵ), in control and CME-treated cells. Data was reported as Mean \pm SEM and significance determined by either one-way ANOVA with Newman-Keuls multiple comparison tests, or two-way, unpaired *t*-tests.

Results: Expression of $\alpha 4$ subunits was reduced 35% ($P < 0.05$) in ethanol-treated cells but the efficacy (control: 9.04 ± 0.93 pA/pF, $n = 41$, CME: 6.39 ± 1.16 pA/pF, $n = 13$, $P > 0.05$) and pEC_{50} (control: 6.18 ± 0.04 , $n = 13$, CME: 6.17 ± 0.04 , $n = 4$, $P > 0.05$) of GABA were unchanged. CphC increased the GABA pEC_{50} relative to control (6.62 ± 0.08 , $n = 3$, $P < 0.001$) but had no effect upon responses at pEC_{20} GABA. Following CME, the potency of GABA was unaltered in the presence of CphC.

The magnitude of ALLO-induced potentiation in control cells (7.34 ± 0.6 fold, $n = 19$) was unchanged by CME (ATP: 7.39 ± 1.0 fold, $n = 9$, $P > 0.05$), and CphC had no significant effect in control or CME-treated cells.

The ALLO pEC_{50} in control cells (6.23 ± 0.05 , $n = 19$) was unaffected by CphC. Following CME, the potency of ALLO was reduced (5.68 ± 0.06 , $n = 9$, $P < 0.001$) but was enhanced in the presence of CphC, which restored potency almost back to control levels (5.94 ± 0.09 , $n = 5$, $P < 0.05$ relative to control).

Expression of the α , γ , δ , and ϵ isoforms of PKC was detected in whole-cell lysates of L(tk-) cells but only PKC α was significantly altered by CME treatment, exhibiting a nearly 4-fold increase (3.9 ± 0.47 fold $P < 0.01$) when compared with that in controls.

Discussion: CME of un-induced L(tk-) cells was sufficient to alter sensitivity of $\alpha 4\beta 3\delta$ receptor function to alterations of the balance of phosphorylation induced by CphC. The increased expression of PKC α after CME may have been directly related to the absence of effect of CphC upon GABA potency. As direct interaction of GABA_A receptors with PKC α has not been determined, the effects observed for potency and efficacy of ALLO following CME may be indicative of changes to the phosphorylation of accessory proteins or other PKC isoforms by PKC α .