FLUMAZENIL SELECTIVELY PREVENTS THE INCREASE IN α_4 SUBUNIT GENE EXPRESSION AND AN ASSOCIATED CHANGE IN $GABA_A$ RECEPTOR FUNCTION INDUCED BY ETHANOL WITHDRAWAL

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Long-term exposure to ethanol results in the development of tolerance and dependence with the manifestation of alcohol withdrawal syndrome in humans and experimental animals after abrupt ethanol withdrawal. The molecular mechanisms underlying this phenomenon remain unclear. Benzodiazepines are one of the best pharmacological treatments available for the life-threatening condition of alcohol withdrawal syndrome and are clinically effective in ameliorating the symptoms of this syndrome. We have recently shown that benzodiazepines also prevent, in neuronal cell cultures, some of the ethanol withdrawal-induced molecular and functional changes of the γ-aminobutyric acid type A ($GABA_A$) receptors. Using the same in vitro experimental model, in the present work we investigated the effects, on such changes, of the benzodiazepine receptor antagonist flumazenil, a compound able to positively modulate α_4-containing $GABA_A$ receptors, and that has paradoxically been found to be effective in the treatment of ethanol withdrawal syndrome in humans and rodents. Cultured cerebellar granule cells were exposed for 5 days to 100 mM ethanol and to perform withdrawal the medium containing ethanol was replaced with ethanol-free medium or medium containing 10 μM flumazenil; the neurons were then incubated for an additional 6 h. The abundance of the different $GABA_A$ receptor subunit mRNAs was determined with an RNase protection assay, the levels of the corresponding peptides by immunoblot, while receptor function was assayed by whole-cell patch-clamp electrophysiological recordings. We here report that flumazenil prevented both the ethanol withdrawal-induced up-regulation of the α_4 subunit and the increase in its own modulatory action. In contrast, flumazenil did not inhibit ethanol withdrawal-induced decrease in α_1 and δ subunit expression as well as the corresponding decrease in the modulatory action on $GABA_A$ receptor function of both the α_1-selective ligand zaleplon and the δ-containing receptor preferentially acting steroid allopregnanolone. These observations are the first molecular and functional evidence showing a selective inhibition by flumazenil of the up-regulation of α_4 subunit expression elicited by ethanol withdrawal. The mechanism by which flumazenil exerts its selective effects on the expression of the α_4 subunit and related receptor function during ethanol withdrawal needs to be further investigated. However, this in vitro finding is consistent with the clinical and experimental evidences showing the efficacy of flumazenil to abolish ethanol withdrawal syndrome.