Changes in Methylation State of BDNF Gene Induced by Ethanol

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Alcohol addiction is thought to depend on molecular and cellular adaptations in the brain that result from chronic drug exposure (Fitzgerald and Nestler, 1995). This suggests that chronic alcohol abuse involves stable changes in the brain at the molecular and cellular levels responsible for long-lasting alterations in behavior. One of the candidate molecules involved in such mechanisms is brain-derived neurotrophic factor (BDNF), that have been suggested to play a role in reducing some of the behavioural effects of ethanol (McGough et al., 2004). Multiple actions of ethanol on BDNF gene expression and signalling have been described. Nevertheless the considerable complexity within the BDNF gene itself and the multiple mRNAs encoded by up to 9 potential exons is further complicated because of its epigenetic regulation by methylation of BDNF promoters. The knowledge of the promoter that may be differentially regulated during various states of ethanol exposure and a detailed analysis of the effects of different ethanol exposure treatments on mRNA expression is required to understand the biological basis of addiction. This will lead to more effective treatments and eventually to cures and preventive measures to treat alcohol addiction. The aim of this work was to evaluate the effects of ethanol on CpG islands of BDNF exon IX gene in regulating its expression, both 'in vivo' and 'in vitro'. Rat cerebellar granule cells in culture were exposed to acute ethanol, chronic ethanol or ethanol withdrawal. Our results demonstrate that ethanol exposure increases, in a dose dependent manner, the abundance of BDNF exon IX transcript in the three different treatment conditions. We then tested the ability of acute ethanol to alter exon IX methylation by using MSP PCR. Similarly to the effect induced by the two DNA methylation inhibitors, zebularine and RG-108, exposure of cultured neurons to 100 mM ethanol for 3h significantly increased the unmethylated state of BDNF exon IX that was about 2.5 folds grater than the methylated state. This epigenetic action of ethanol was observed also in the hippocampus of male rats treated with increasing doses of ethanol (0.8; 1.6 or 3.0 g/kg; i.p.) tested 1; 3 and 5h after injection. Ethanol induced a significant time and dose dependent increase in BDNF exon IX unmethylated DNA levels, compared with control, which showed a positive correlation with BEC. The increased unmethylated state was accompanied by a corresponding increase in the abundance of BDNF exon IX transcript. The increase in mRNA was dose dependent with the maximal effect at 3.0 g/kg 3h after injection. These results provide the first evidence for an alternative way of ethanol to alter BDNF gene expression. Thus, ethanol induced changes in CpG DNA methylation of BDNF gene seems to be an additional mechanism to implement homeostatic protective actions to prevent adverse effects of ethanol. The ability of ethanol to induce up regulation of BDNF may play a pivotal role and could result from a number of intracellular responses that include the epigenetic mechanism here described.

Fitzgerald and Nestler. (1995). Clinical Neuroscience 3(3):165-73.
McGough et al. (2004). Journal of Neuroscience 24(46):10542-52.