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Decreased $GABA_A$ benzodiazepine binding site densities in postmortem brains of Cloninger type 1 and 2 alcoholics

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ABSTRACT

Ethanol modulates the $GABA_A$ receptor to cause sedative, anxiolytic and hypnotic effects that are qualitatively similar to benzodiazepines and barbiturates. The aim of this study was to explore if $GABA_A$ receptor density is altered in post-mortem brains of anxiety-prone Cloninger type 1 and socially hostile type 2 alcoholic subtypes when compared to controls. The $GABA_A$ binding site density was measured by whole-hemisphere autoradiography with tritium labeled flunitrazepam ([^{3}H]flunitrazepam) from 17 alcoholic (nine type 1, eight type 2) and 10 non-alcoholic post-mortem brains, using cold flumazepam as a competitive ligand. A total of eight specific brain areas were examined. Alcoholics displayed a significantly ($p < 0.001$, bootstrap type generalizing estimating equations model) reduced [^{3}H]flunitrazepam binding site density when compared to controls. When localized, type 2 alcoholics displayed a significantly ($p \leq 0.05$) reduced [^{3}H]flunitrazepam binding site density in the internal globus pallidus, the gyrus dentatus and the hippocampus, whereas type 1 alcoholics differed from controls in the internal globus pallidus and the hippocampus. While previous reports have demonstrated significant alterations in dopaminergic and serotonergic receptors between type 1 and type 2 alcoholics among these same subjects, we observed no statistically significant difference in [^{3}H]flunitrazepam binding site densities between the Cloninger type 1 and type 2 alcoholics.

Introduction

Alcoholics are a heterogenous group that presents a wide spectrum of problems in their regulation of emotion. The Cloninger type 1 alcoholics are characterized by an anxiety-prone temperament with cautious behavior and low novelty seeking. By contrast, type 2 alcoholism (~20% of alcoholics) is correlated with heredity and characterized by teenage-onset antisocial behavior. The character of the type 2 alcoholic is often socially hostile (i.e., poorly cooperative, aggressive, vengeful) and they are often diagnosed with an antisocial personality disorder that is connected to alcohol use disorder. Type 2 alcoholics tend to be impulsive and risk-taking and they often have a criminal record (Tiihonen & Hakola, 1994). This model of alcoholism initially suggested that type 2 alcoholics have serotonergic defects (Cloninger, 1995; Cloninger et al., 1988), and certain serotonergic alterations have been reported for persons with impulsive behavior and violent alcoholics (Bevilacqua et al., 2010; Frankle et al., 2005; Hallikainen et al., 1999; Storvik, Tiihonen, Haukijärvi, & Tupala, 2006; Tiihonen et al., 1997; Virkkunen et al., 1994). Additional serotonergic differences between type 1 and type 2 alcoholics have also been reported (Mantere et al., 2002; Storvik, Häkkinen, Tupala & Tiihonen, 2009; Storvik et al., 2006). Considerable differences in other neurosignaling systems have been reported between type 1 and type 2 alcoholics, especially in the dopaminergic system (Tupala, Hall, Särkioja, Räsänen, & Tiihonen, 2000), and more recently in

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differing endocannabinoid levels (Lehtonen, Tupala, Hyytiä, Tiihonen, & Callaway, 2010).

The inhibitory GABA type A ($GABA_A$) receptor is one of the main targets for ethanol in the central nervous system (CNS), where acute ethanol intake potentiates the action of the $GABA_A$ receptor to cause a form of sedation that is similar to most anxiolytics and hypnotics. During chronic ethanol exposure, neuronal adaptation occurs, which includes changes in GABAergic function with the development of tolerance and dependence. Alcohol withdrawal syndrome includes a variety of symptoms (e.g.; anxiety, insomnia, seizures etc.) that have been linked to GABAergic function (Davies, 2003; Grobin, Matthews, Devaud, & Morrow, 1998; Krystal et al., 2006). Controversy remains over the direct or indirect facilitatory effects of ethanol on $GABA_A$ receptors (Grobin et al., 1998; Krystal et al., 2006). However, mounting evidence for a potent and direct effect for ethanol on $GABA_A$ subgroups, together with the molecular mapping of $GABA_A$ domains (Mihic et al., 1997) implicates the relevance of ethanol's influence on $GABA_A$ receptors.

The $GABA_A$ receptor is a pentamer peptide of five α, β, and γ subunits. $GABA_A$ receptors with $\alpha1$, $\alpha2$, $\alpha3$ or $\alpha5$ subunits have a high affinity for both benzodiazepine agonists and the antagonist flumazenil (Möhler, 2006). Particularly those receptors having $\alpha2\beta3\gamma2$ subunit composition are thought to mediate the anxiolytic action of benzodiazepines (Möhler, 2006). During recent years, several research groups have reported findings that associate the $GABA_A$ receptor subunit coding genes to alcoholism (Loh & Ball, 2000; Long et al., 1998). It has been suggested that certain polymorphisms in the genes GABRA2 and GABRG1 increase the risk for alcohol dependence (Covault, Gelernter, Hesselbrock, Nellissery, & Kranzler, 2004; Covault, Gelernter, Jensen, Anton, & Kranzler, 2008; Edenberg et al., 2004; Enoch et al., 2009; Fehr et al., 2006; Lappalainen et al., 2005). Fehr et al. (2006) found the prevalence of certain GABRA2 gene risk haplotype to be higher among alcoholic patients with a positive family history and early onset of dependence. Enoch, Schwartz, Albaugh, Virkkunen, and Goldman (2006) suggested that the effect of the GABRA2 genetic polymorphism on the risk of alcoholism may be modulated through anxiety. Dick et al. (2009) suggested that polymorphism in GABRA2 may be involved in a general predisposition toward behavioral disinhibition; e.g., impulsivity. These characteristics (i.e.; positive family history, early onset, trait anxiety and impulsivity) also define the subgroups of Cloninger type 1 and 2 alcoholics (Cloninger et al., 1988). Together, these reports suggest that GABAergic function in the CNS may differ between alcoholics and non-alcoholics and also between Cloninger type 1 and type 2 alcoholics.

Neuroimaging studies of recovering alcohol-dependent patients who had been sober for one to three months have shown lower levels of $GABA_A$ receptor densities in several cortical areas when compared to healthy controls (Abi-Dargham et al., 1998; Gilman et al., 1996; Lingford-Hughes et al., 1998). However, contradictory results of higher $GABA_A$ binding densities have also been reported (Jalan et al., 2000). Receptor binding assay studies from post-mortem human brain samples have provided a variety of results; e.g., unchanged binding for benzodiazepine agonists among alcoholics in both frontal and motor cortices and the putamen (Dodd et al., 1996; Freund & Ballinger, 1989a), increased binding in both frontal and motor cortices (Kril et al., 1988) and decreased binding in both the frontal cortex and hippocampus (Freund & Ballinger, 1988, 1989b). Reviewed collectively, these changes in alcoholic $GABA_A$ receptor binding densities vary considerably between studies and studied brain areas when compared to controls. Also, it remains unclear whether or not the results of the neuroimaging studies and receptor binding assay studies reflect the neurotoxic consequences of alcoholism and subsequent neuroadaptive mechanisms during abstinence, hereditary GABAergic disturbances that preceded alcoholism (Davies, 2003) or perhaps something else.

So far, most studies on $GABA_A$ receptor binding have not compartmentalized alcoholics on the basis of suspected etiological groups. We have previously reported many type-specific neurochemical alterations in receptor and transporter densities among Cloninger type 1 and type 2 alcoholics (Lehtonen et al., 2010; Mantere et al., 2002; Storvik et al., 2009; Storvik, Haukijärvi, Tupala, & Tiihonen, 2008; Tupala et al., 2000; Tupala & Tiihonen, 2004), and these results strongly suggest that there may be significant neurochemical differences between these two alcoholic subgroups. $GABA_A$ benzodiazepine binding site densities between Cloninger type 1 and 2 alcoholics have not yet been studied. The aim of the present study was to compare $GABA_A$ receptor binding site densities of Cloninger type 1 and 2 alcoholic subjects with healthy controls by whole-hemisphere autoradiography, which allowed us to study many cortical and subcortical regions at the same time.

Materials and methods

Diagnostics and sample preparation

Post-mortem brain left hemispheres (17 alcoholics and 8 controls) were obtained during clinical autopsy from the Department of Forensic Medicine, University of Oulu, Finland, and two of the non-alcoholic control brains were obtained from the Department of Forensic Medicine, University of Eastern Finland, Kuopio, Finland. The recovery procedure was essentially the same in both locations. This portion of the study was approved by the Ethics Committees of the University of Oulu and the National Institute of Medico-legal Affairs, Helsinki, Finland. A post-mortem analysis for drugs was performed, which included alcohol, as part of the normal necropsic protocol. None of the hemispheres exhibited damage or gross neuroanatomical abnormalities. Medical records concerning the cause of death, previous diseases, and medical treatments for both controls and alcoholics were also collected.

Diagnoses were made by two psychiatrists independently of each other. Medical records' data were available for all 27 subjects. Mental disorders were coded according to DSM-IV criteria (APA, 1994), and alcoholics were sub-classified as type 1 or 2, according to criteria established by Cloninger (Cloninger, 1995; Cloninger et al., 1988). The kappa coefficient of diagnostic agreement for the subjects was 0.9; i.e., one type 2 alcoholic was diagnosed as a type 1 alcoholic by the other physician. Otherwise, diagnoses were unanimous. The most important criteria for defining the two groups of alcoholics were early onset of alcohol abuse (before the age of 25) and documented severe antisocial behavior for the type 2 alcoholics. Subjects having psychotic disorders or any neurological diseases, or those taking medication that could affect the CNS (such as antipsychotics or antidepressants) were excluded. A history of tobacco smoking was considered unreliable and was not included in the final evaluation.

All 27 subjects were Finns. The study groups consisted of nine type 1 alcoholics (seven men and two women; age: mean = 52.7 years, SD = 12.4; post-mortem delay: mean = 11.9 h, SD = 4.5); eight type 2 alcoholics (all men; age: mean = 34.6 years, SD = 12.2; post-mortem delay: mean = 14.1 h, SD = 3.4); and 10 non-alcoholic controls (eight men and two women; age: mean = 53.5 years, SD = 10.7; post-mortem delay: mean = 14.8 h, SD = 9.2). All of the controls were free of a psychiatric diagnosis. Intervals between death and autopsy did not differ significantly between three groups ($p = 0.62–0.98$, Scheffe's test for multiple comparisons, two-tailed). Six of the eight type 2 alcoholic subjects had a criminal record or a history of violent offenses (physical or sexual). Alcoholism in both type 1 and type 2 groups was severe, as judged by frequent