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Striatal dopaminergic terminals in type 1 and type 2 alcoholics measured with [^{3}H]dihydrotetrabenazine and human whole hemisphere autoradiography

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Abstract

A number of studies have pointed to the importance of dopamine system in the context of alcoholism. Previous studies have shown lower dopamine transporter levels on late-onset Cloninger type 1 alcoholics. However, whether this lower level is due to a lower level of dopamine transporter protein or a lower level of dopaminergic nerve terminals remains unclear. The aim of this study was to compare putative alterations of dopaminergic terminals in caudate, putamen and nucleus accumbens of type 1 and type 2 alcoholics and healthy controls by using [^{3}H]dihydrotetrabenazine as a radioligand in postmortem human whole hemisphere autoradiography. We compared the present results with the findings of our earlier studies on the dopamine transporter in these same subjects, demonstrating that alcoholics do not differ significantly from controls in striatal [^{3}H]dihydrotetrabenazine binding. Although type 1 alcoholics have been reported to have up to 36% lower striatal dopamine transporter levels than controls, the results suggest that the density of their dopaminergic nerve terminals is not altered.

Keywords: Alcohol-induced disorders; Ethanol-induced nervous system disorders; Dopamine; Human brain; Transporters

1. Introduction

Ethanol acutely increases brain dopamine (DA) activity, and animal microdialysis studies have shown an induction of DA release in the dorsal (caudate and putamen) and ventral striatum (nucleus accumbens) after acute ethanol exposure (Di Chiara and Imperato, 1988). The dopamine transporter (DAT) is a plasma membrane protein that transports released DA back into DA nerve terminals. The dopaminergic response to alcohol in striatum is very heterogeneous between individuals, as reported in an *in vivo* positron emission tomography (PET) study (Yoder et al., 2007). PET and single photon emission tomography (SPET) studies and *in vitro* human autoradiographic studies have consistently indicated that levels of striatal DAT are lower than control values in Cloninger

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type 1 alcoholics (Repo et al., 1999; Tiihonen et al., 1995; Tupala et al., 2000, 2001a,b, 2003a,b), who are characterized by late onset, social dependency and anxiety (Cloninger, 1995). By contrast, type 2 alcoholics characterized by early onset, impulsive and aggressive behaviour, and social hostility (Cloninger, 1995) seem to have a serotonergic deficit with normal DAT density in the striatum (Tiihonen et al., 1995). In one PET study with no division into Cloninger types, no alteration in DAT density was observed in alcoholics (Volkow et al., 1996). However, whether the lower DAT level among type 1 alcoholics is actually due to lower DAT protein in the nerve terminals or a lower level of dopaminergic terminals in the striatum is not known.

Type 2 vesicular monoamine transporter (VMAT2) transports monoamine neurotransmitters (DA, serotonin, noradrenaline) from the cytoplasm into synaptic storage vesicles, and is in the striatum predominantly (~95%) localised to DA nerve terminals (Da Silva et al., 1993). [^{3}H]Dihydrotetrabenazine ([^{3}H]DTBZ) labels VMAT2 and animal studies demonstrate that changing the synthesis, turnover or release of DA does not result in regulation of this binding site (Naudon et al., 1994; vander Borght et al., 1995). Hence, this ligand has been considered as a useful mark of the integrity of the dopaminergic system (Sossi et al., 2007), and considered to permit quantitative assessment of the density of striatal dopaminergic presynaptic nerve terminals. Other studies have suggested that substances acting directly and potently on the dopaminergic system may modulate the VMAT2 density (Zucker et al., 2001; Schwartz et al., 2007). Despite that, the results obtained with ([^{3}H]DTBZ) binding autoradiography provide estimates of the presynaptic integrity of the dopaminergic system, which can be contrasted with previous findings of decreased DAT density in alcoholics.

Whole hemisphere autoradiography provides high resolution images corresponding to *in vivo* (i.e. PET and SPET) studies, and enables detailed study of various brain structures. In the present study we tested three hypotheses. First, we studied whether the [^{3}H]DTBZ binding to VMAT2 in the striatum at the level of the caudate and the putamen, and the level of the nucleus accumbens) differs between the alcoholic subgroups and controls. Second, we compared our previous results on DAT from the same subjects (Tupala et al., 2000, 2001a,b, 2003a,b) to the present VMAT2 results. Third, because we have previously shown striatal DAT and DA 2 (D_2) receptor binding to correlate between the ventral and dorsal striatum and a similar striatum vs. extrastriatum correlation has been shown in one SPET study in type 1 alcoholics, but not in controls (Repo et al., 1999; Tupala et al., 2003a), we also evaluated the correlations of [^{3}H]DTBZ binding to VMAT2 between the striatal structures. This test is aimed to evaluate whether the relative individual densities of dopaminergic nerve terminals are maintained in different brain areas in these three groups.

2. Experimental procedures

The brain sampling, diagnostics, study subjects and cryosectioning have been described in detail previously (Tupala et al., 2001a,b, 2003a,b).

2.1. Study subjects

The human brain left hemispheres used were obtained during clinical necropsy at the Department of Forensic Medicine, University of Oulu, Finland, and the Department of Forensic Medicine, University of Kuopio, Finland. The Ethics Committee of the University of Oulu and the National Institute of Medicolegal Affairs, Helsinki, Finland, approved the study. Medical records on the cause of death, previous diseases and medical treatments of controls and alcoholics were collected. Alcoholism was coded according to DSM-IV criteria (American Psychiatric Association, 1987), and sub-classified as type 1 or type 2, according to Cloninger (1995). The kappa coefficient of diagnostic agreement subjects was 0.9; i.e., one type 2 alcoholic was diagnosed as a type 1 alcoholic by the second physician. Otherwise, diagnoses were unanimous. Subjects having psychotic disorders or any neurological diseases (such as epilepsy) or taking medication that could affect the CNS (such as neuroleptics or antidepressants including SSRIs), or using substances with a direct effect on the dopaminergic system (such as psychostimulants or opioids) were excluded. A history of tobacco smoking based only on medical records was considered unreliable and was not included in the final criteria.

The study groups consisted of 17 alcoholics further classified as nine type 1 alcoholics (7 males, 2 females; mean age = 52.7 years; mean post-mortem delay = 11.9 h, S.D. = 4.5 h), eight type 2 alcoholics (males: mean age = 34.6 years; mean post-mortem delay = 14.1 h, S.D. = 3.4 h), and 10 controls (8 males, 2 females; mean age = 53.5 years; mean post-mortem delay = 14.8 h, S.D. = 9.2 h) free of psychiatric diagnosis. Alcoholism among these subjects was judged to be severe based on frequent admissions to emergency rooms and doctors' appointments due to alcohol-related problems, and the diagnosis of alcoholism itself was not a difficult task even without interviews. Eight of the nine type 1 alcoholics had alcohol in their blood at the time of death, and one alcoholic had had an abstinence period of 10 h. One of the controls had a small amount of