European Neuropsychopharmacology (2006) 16, 552–560

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Visualization of the cortical dopamine transporter in type 1 and 2 alcoholics with human whole hemisphere autoradiography

Erkki Tupala [a,b,*], **Pirjo Halonen** [c], **Jari Tiihonen** [a,d]

[a] *Department of Forensic Psychiatry, Niuvanniemi Hospital, University of Kuopio, Kuopio, Finland*
[b] *Department of Pharmacology and Toxicology, University of Kuopio, Kuopio, Finland*
[c] *Kuopio University IT Service Centre, University of Kuopio, Kuopio, Finland*
[d] *Department of Clinical Physiology, Kuopio University Hospital, Kuopio, Finland*

Received 19 May 2005; received in revised form 15 February 2006; accepted 28 February 2006

KEYWORDS
Alcohol-induced disorders;
Ethanol-induced nervous system disorders;
Dopamine;
Autoradiography;
Human brain;
Transporters

Abstract We measured cortical dopamine transporter (DAT) in Cloninger type 1 and 2 alcoholics by using [^{125}I]PE2I as a radioligand in human postmortem whole hemispheric autoradiography, and evaluated the putative correlations of DAT between cortical areas and nucleus accumbens. There was a low, but distinct cortical binding in the cryosections. The mean binding was generally higher in both groups of alcoholics compared to controls, and the results reached statistical significance with a large effect size (1.25) in the temporal cortex of type 2 alcoholics. This is surprising, because several studies have reported lower DAT densities in the striatum among alcoholics compared to controls. Moreover, the density of DAT had a statistically significant positive correlation between temporal cortex and nucleus accumbens in controls, whereas among type 2 alcoholics the correlation was statistically significantly negative, which may suggest some pathology relating to the antisocial behaviour of these alcoholics.

1. Introduction

The neuronal dopamine transporter (DAT) is a presynaptically located protein responsible for the reuptake, and thus, removal of DA from the synaptic cleft. Because DAT is exclusively located on terminals of dopamine (DA) neurons (Boja et al., 1994), this transporter can serve as a marker for the DA neurons in studies of neuropsychiatric diseases and drug abuse. Early direct measurement of [^{3}H]dopamine uptake in animals as well as human in vivo and in vitro studies have indicated that DAT is mainly, but not exclusively confined to the striatum (Iversen and Snyder, 1968; Coyle and Snyder, 1969; Boja et al., 1994; Farde et al., 1994; Hall et al., 1999). These are in agreement with in situ hybridization studies, indicating that the distribution of DAT mRNA in the animal and human brain is almost exclusive to nigrostriatal neurons (Giros et al., 1991; Hurd et al., 1994). However, DAT and DAT mRNA has also been found in

* Corresponding author. University of Kuopio, Department of Forensic Psychiatry, Niuvanniemi Hospital, FIN-70240 Kuopio, Finland. Tel.: +358 50 55 97 317; fax: +358 17 203 494.
E-mail address: erkki.tupala@niuva.fi (E. Tupala).

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doi:10.1016/j.euroneuro.2006.02.008

extrastriatal regions like hypothalamus and amygdala (Meister and Elde, 1993; Staley et al., 1994; Revay et al., 1996), and animal studies on rodents (Mundorf et al., 2001) and non-human primates (Lewis et al., 2001) have been able to detect DAT in cortical areas. In addition, a recent positron emission tomographic (PET) study reported low DAT densities in the orbitofrontal and dorsolateral prefrontal cortices among chronic users of metamphetamine (Sekine et al., 2003) and one postmortem study has reported low densities of DAT in the prefrontal cortex of chronic users of cocaine (Hitri et al., 1994), which are drugs that as well as ethanol, exert their rewarding effects through activating the mesolimbocortical DA system (Koob and Bloom, 1988; Self and Nestler, 1995; Koob and Le Moal, 1997). However, to our knowledge, this is the first study on cortical DAT among alcoholics.

In vivo PET and single photon emission tomographic (SPET) studies have indicated lower striatal DAT binding and higher presynaptic DA function in Cloninger type 1 alcoholics (characterized by late onset, social dependency, low novelty seeking and anxiety) (Cloninger et al., 1981; Cloninger, 1995; Repo et al., 1999; Tiihonen et al., 1995, 1998; Laine et al., 1999), whereas type 2 alcoholics (characterized by early onset, impulsive and antisocial behaviour) have been suggested to have a serotonergic deficit (Cloninger et al., 1981; Cloninger, 1995). In addition, we have shown low DAT densities in the nucleus accumbens (NAC) and dorsal striatum of alcoholics by using human postmortem whole hemisphere autoradiography (Tupala et al., 2000, 2001a,b, 2003a,b,c). However, there is also evidence that type 2 alcoholics may have even higher striatal DAT binding and heterogeneity, thus suggesting an overfunction of the DA system in this patient group (Tiihonen et al., 1995; Kuikka et al., 1998).

Although there are several studies on striatal DAT among alcoholics, no reports on cortical DAT have been presented to date. The aim of this study was to measure DAT densities in the anterior cingulate, frontal and temporal cortical areas of eight type 1 alcoholics, eight type 2 alcoholics and 10 controls by using [^{125}I]*N*-(3-iodoprop-2E-enyl)-2-carbomethoxy-3-(4′-methylphenyl)nortropane) ([^{125}I]PE2I) as a radioligand for human postmortem whole hemisphere autoradiography (WHA). This high affinity radioligand has proven to be an extremely selective DAT ligand with low affinity for other monoamine transporters (30 and >60 fold selectivity over serotonin and noradrenaline transporters, respectively) (Emond et al., 1997). This makes [^{125}I]PE2I superior to old cocaine derivatives like [^{125}I]β-CIT, frequently used in previous studies and it has been used successfully in both in vitro and in vivo imaging of DAT (Hall et al., 1999; Kuikka et al., 1998; Tupala et al., 2000, 2001a,b, 2003a,b,c). Because we have previously detected up to 39% lower DAT densities in subcortical areas of alcoholics (Tupala et al., 2000, 2001a, 2003a), we tested whether this is the case also in the cortical areas. In addition, because we have shown dopamine binding sites to correlate between different brain areas among alcoholics, but not in the controls (Tupala et al., 2001a, 2003a), we compared the present results to our previous studies on DAT in the NAC (Tupala et al., 2000, 2001b, 2003b,c).

2. Experimental procedures

The procedure has been described in detail previously (Hall et al., 1994; Tupala et al., 2001a, 2003c).

2.1. Brain sampling

Human brains 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. The brains did not exhibit any gross morphological abnormalities as evaluated from a series of cresyl violet (Nissl) stained sections.

2.2. Diagnostics

Two physicians made diagnoses independently of each other. Mental disorders were coded according to DSM-III-R diagnostic criteria (American Psychiatric Association, 1987) and alcoholics were subclassified as type 1 or 2 according to Cloninger (Cloninger et al., 1981; Cloninger, 1995). Kappa coefficient of agreement regarding the 26 subjects (Cohen, 1960) was 0.9, i.e. one type 2 alcoholic included in the study was diagnosed as a type 1 alcoholic by the second diagnostician. Otherwise diagnoses were unanimous. Subjects having any central nervous system diseases (such as epilepsy or psychotic disorders), or taking medication that affects the central nervous system (such as neuroleptics or antidepressants), were excluded.

2.3. Study subjects

All 26 cases were Caucasians. The study groups consisted of eight type 1 alcoholics (7 males, 1 female; age-range 39—76 years; mean age 52.9 ± 13.2 years; postmortem delay 12.0 ± 4.8 h; mean ± SD), eight type 2 alcoholics (males; age-range 18—49 years; mean age 34.6 ± 12.2 years; postmortem delay 14.1 ± 3.4 h; mean ± SD) and 10 controls (8 males, 2 females; age-range 36—77 years; mean age 53.5 ± 10.6 years; postmortem delay 14.8 ± 9.2 h; mean ± SD) free of psychiatric diagnosis. Postmortem delays between the groups were not significantly different (P = 0.62—0.98, Scheffe's test for multiple comparisons, two-tailed). Alcoholism among alcoholic subgroups was severe as judged on the basis of the frequent admissions to emergency stations and doctors' appointments due to alcohol-related problems and the diagnosis of alcoholism itself was not a difficult task even without interviews. Seven of the eight type 1 alcoholics had alcohol in their blood at the time of death, and one had an abstinence period of 10 h. One of the controls had a small amount of alcohol in his blood at the time of death (0.036%). Two of the type 1 alcoholics had traces of diazepam in their blood samples. Six type 2 alcoholics had alcohol in their blood at the time of death. One had abstinence period of 3—7 days and one of 5 days. Three had traces of benzodiazepines in their blood and one was positive for cannabinoids. Mean alcohol