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ORIGINAL INVESTIGATION

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Dopamine transporter and D_2-receptor density in late-onset alcoholism

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Abstract *Rationale:* Late onset type 1 alcoholism has been suggested to be associated with an underlying dopaminergic defect. Therefore, it is relevant to study both postsynaptic D_2-receptor and presynaptic dopamine transporter (DAT) densities among alcoholics. *Objective:* We investigated DAT densities, along with striatal and extrastriatal dopamine D_2-receptor densities, in nine non-violent late-onset male alcoholics, who had no major mental disorder nor antisocial personality disorder (ASPD), and nine healthy controls. *Methods:* [^{123}I]PE2I and [^{123}I]epidepride were used in SPECT imaging. *Results:* DAT occupancy ratios (striatum/cerebellum) were significantly lower among alcoholics than in controls. Extrastriatal D_2-receptor occupancy ratios (temporal pole/cerebellum) were not significantly different between the groups. *Conclusions:* Striatal presynaptic DAT densities are decreased among type 1 alcoholics, and this finding is not associated with recent alcohol abuse.

Key words Alcoholism · Dopamine transporter · [^{123}I]PE2I · [^{123}I]Epidepride · SPECT

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Introduction

Alcoholism that is characterized by late-onset, social dependency and anxiety (type 1) has been suggested to be associated with an underlying dopaminergic deficit, as opposed to the serotonergic deficit seen in early onset type 2 alcoholism, which is characterized by euphoria-seeking and violence (Cloninger 1995). Therefore, it is relevant to study dopamine function in late onset alcoholism, such as postsynaptic D_2-receptor or presynaptic dopamine transporter (DAT) densities. In brain, the neurotransmitter dopamine (DA) is taken up by a specialized carrier protein that is localized on the cell membrane of dopaminergic presynaptic nerve terminals. Radioactive ^{123}I-labelled ligands have been used together with SPECT to visualize DAT in the living human brain (Brucke et al. 1993; Innis et al. 1993; Kuikka et al. 1993a, 1998; Tiihonen et al. 1995). A cocaine analogue, 2β-(carbomethoxy-3β-(4-iodophenyl)tropane, has high affinity to both DA and other monoamine re-uptake sites, whereas *N*-(3-iodoprop-2E-enyl)-2β-carboxymethoxy-3β-(4-methylphenyl)nortropane (PE2I) binds selectively and with high affinity to the DA transporter (Emond et al. 1997). In animal studies, PE2I has been shown to bind in brain areas where DA transporter density is highest (Guilloteau et al. 1998). Recently, this finding was also confirmed in humans (Kuikka et al. 1998).

Findings concerning DAT densities among alcoholics with in vivo studies have remained controversial (Tiihonen et al. 1995; Volkow et al. 1996; Gilman et al. 1998; Laine et al. 1999). It is likely that the sub-type of alcoholism (Cloninger 1987, 1995) may influence the results of these findings, as most studies do not differentiate between the sub-types of alcoholism. We recently reported higher DAT densities among violent type-2 alcoholics when compared with healthy controls, while late-onset type-1 alcoholics had lower densities than healthy controls (Tiihonen et al. 1995). In the studies by Hietala et al. (1994) and Volkow et al. (1996), the striatal postsynaptic D_2-receptor densities were found to be low among alcoholics.

The aim of this study was to investigate DAT densities, along with striatal and extrastriatal dopamine D_2-receptor densities in late-onset non-violent alcoholics and age-matched healthy controls.

Materials and methods

Patients

The procedure was approved by the local ethics committee (Kuopio University Hospital), and all subjects gave their informed consent. Exclusion criteria for all subjects were major mental disorders, traumas and neurological disorders, previous drug abuse or present use of any psychotropic drugs. Nine non-violent male alcoholic patients were recruited with the help of a local rehabilitation center for alcoholics. The patients had not obtained pharmacological treatment for alcoholism, and the period of abstinence had ranged from 1 week to 4 years. Excluding one patient, who had abstained for 4 years, the mean (±SD) length of abstinence had been 43.5±62.36 days (range 7–165 days). All patients met the criteria for alcohol dependence or abuse according to DSM-IV (American Psychiatric Association 1994). They were interviewed by using SCID overview and SCID-II interview concerning antisocial personality disorder (First et al. 1996). The patients had neither histories of mental illness nor antisocial personality disorder (which is commonly associated with type-2 alcoholism). Aside from two arrests for drunken driving, none had committed any criminal offenses. The mean age of alcoholic patients was 51.1±2.7 (mean±SD, range: 45–54 years). Nine healthy males with a mean age of 46.3±4.5, 41–52 years were recruited from health care personnel, to serve as controls. None of them had a history of alcohol dependence or abuse. All control subjects had abstained from alcohol 7 days prior to the SPECT scan.

Imaging procedures

[^{123}I]PE2I

Subjects received 400 mg potassium perchlorate orally 0.5–1.0 h before injection of the tracer, in order to reduce uptake of radioactive ^{123}I by the thyroid. The dose of [^{123}I]PE2I injected varied from 165 to 220 MBq. The injection was administrated slowly into the right antecubital vein in a quiet and dimly lit room. SPECT data were acquired by a MultiSPECT 3 gamma camera with fan beam collimators (Siemens Medical Systems, Inc., Hoffman Estates, Ill., USA) as previously described (Kuikka et al. 1993b). The energy window was centered around the photopeak of ^{123}I (148–170 keV). Over 360° (120° per camera head), 40 views/head were acquired in a 128×128 matrix (pixel size of 2.8 mm). Transversal slices were reconstructed using a filtered back projection technique (Butterworth: order of 8.0 and a cut-off frequency 0.75 cm^{-1}) and corrected for gamma-ray attenuation, with a uniform attenuation coefficient of 0.1 cm^{-1}. The imaging resolution was 7–8 mm (Kuikka et al. 1998). No blood samples were routinely drawn in PE2I studies. The complete study lasted 90 min for each individual.

[^{123}I]Epidepride

On the day after the [^{123}I]PE2I study, the study subject received a 185–220 MBq dose [^{123}I]epidepride (Kuikka et al. 1997), which was administered into the right antecubital vein. Venous blood samples were collected from the left antecubital vein at 3, 10, 30, 60, 120, 180 and 240 min after injection. Plasma was separated and the parent (unchanged) plasma concentration (in percent of dose injected per 100 ml; %/ID/100 ml) and radioactive metabolites of [^{123}I]epidepride were determined by using a gradient HPLC method (Bergström et al. 1997). The camera and computer settings were similar to those in the [^{123}I]PE2I study, and the complete study lasted over 4 h for each individual.

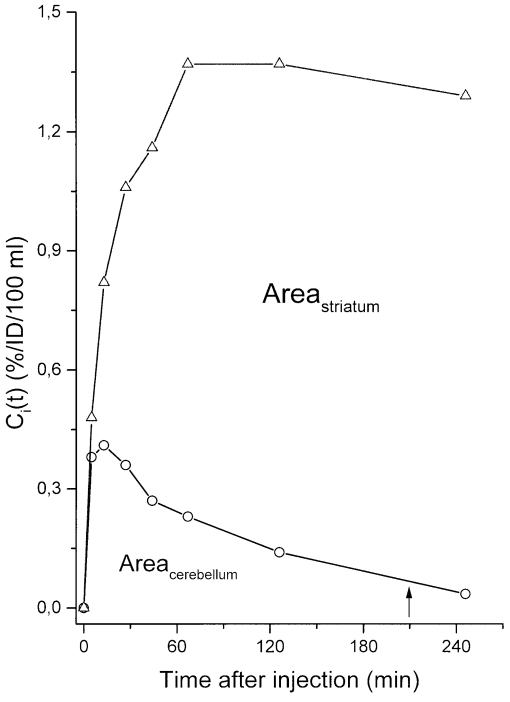

Fig. 1 Regional time-concentration curves of [^{123}I]epidepride in a 41-year-old male. Areas under the curves (from zero to 210 min after injection of the tracer) were used to calculate the striatum-to-cerebellum occupancy ratio

Radiation exposure

The total radiation exposure to each study subject was approximately 8–10 mSv, as given in the effective dosages.

Data analysis

Two slices were consecutively summarized for a total slice thickness of 5.6 mm. Regions of interest were drawn onto the cerebellum (as a reference region=free+non-specific binding) and onto the basal ganglia (free+non-specific+specific binding). In addition, the temporal poles were used as the regions of interest for extrastriatal binding of [^{123}I]epidepride. Regions of interest were measured in three consecutive slices and the average count density in each volume of interest was tabulated and corrected for the radioactive decay of ^{123}I. A cross-calibration between the plasma samples and the regional brain count densities was performed (Kuikka et al. 1997). The cross-calibrated count densities were given in %/ID/100 ml. The technologist who drew the regions of interest and analyzed SPECT data was blind to the diagnosis of the study subjects.

The ratio of the tracer occupancy [O_i (ml/ml)] in the given region (i), relative to that in the cerebellum [O_{CBL} (ml/ml)], was used as indexes of DAT and D_2-receptor availability (Volkow et al. 1996), and estimated as (Kuikka et al. 1997):

$$R_i = O_i/O_{CBL} = \text{area of } ROI_i/\text{area of } ROI_{CBL} \quad (1)$$

where area of ROI_i was the integral of the regional time-concentration curve $C_i(t)$ from zero to 75 min for [^{123}I]PE2I, and 210 min for [^{123}I]epidepride. $Area_{CBL}$ represented the integral of

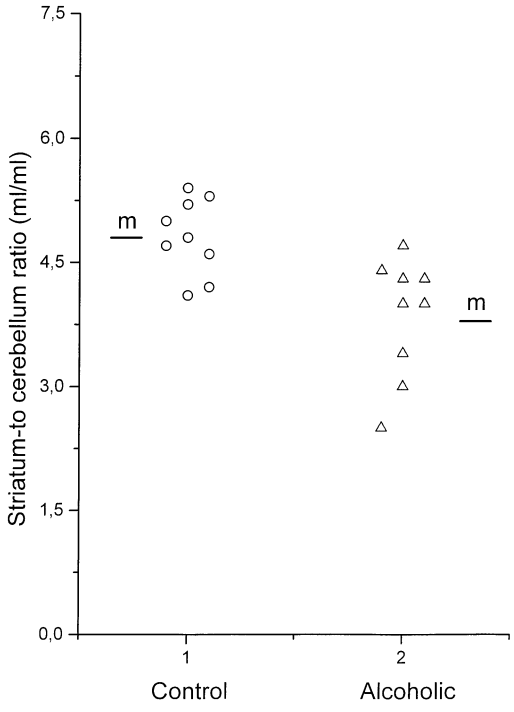

Fig. 2 Scatter plots of the DAT occupancy ratios (striatum-to-cerebellum) in alcoholics and in age-matched healthy males

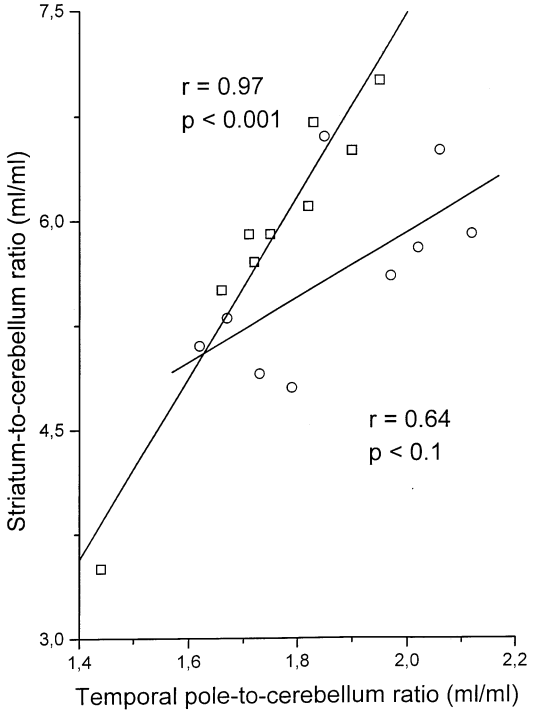

Fig. 3 Linear relationships between the extrastriatal and striatal dopamine D_2-receptor occupancy ratios in age-matched healthy males (○) and in alcoholics (□)

the cerebellar time-concentration curve $C_{cer}(t)$ (Fig. 1), where the regional time-concentration curves were expressed in %/ID/100 ml.

Student's *t*-test and Spearman rank correlation were used in comparison between groups.

Results

In the ^{123}I-PE2I measurements, the DAT occupancy ratios (striatum-to-cerebellum); mean±SD were significantly lower among alcoholics than in controls (3.8±0.7 and 4.8±0.5, respectively, $P<0.005$, Student's *t*-test) (Fig. 2). No significant correlation was found ($r=0.27$, $P=0.51$) between the length (43.5±62.36 days, $n=8$) of sobriety and DAT density.

In the ^{123}I-epidepride measurements, the dopamine D_2-receptor occupancy ratios (striatum-to-cerebellum) were not significantly different between alcoholics and controls (5.9±1.0 and 5.6±0.7, respectively). However, the percentage of the lipophilic metabolite of epidepride in plasma, which was measured after 60 min of injection, was significantly higher among alcoholics than in controls (15.8±5.8% and 5.5±3.9%, respectively, $P<0.005$, Student's *t*-test). The extrastriatal dopamine D_2-receptor occupancy ratios (temporal pole-to-cerebellum) were not significantly different between alcoholics and controls (1.75±0.15 and 1.87±0.17, respectively), although a highly significant ($r=0.97$; $P<0.001$, Spearman rank correlation) correlation existed between the extrastriatal and striatal dopamine D_2-receptor occupancy ratios in alcoholics (Fig. 3).

Discussion

The present findings, visualized by the highly selective radioligand PE2I (Kuikka et al. 1998), are in line with the previous reports on results of lower DAT densities among alcoholics compared with controls (Tiihonen et al. 1995; Gilman et al. 1998; Little et al. 1998). Low DAT density has been considered as a consequence of acute withdrawal after heavy drinking (Laine et al. 1999). However, according to the present study, it was found that the length of the abstinence period explained only 7% of the variance of DAT density. This implies that low DAT density is not only a state marker, but, at least in some extent, also a trait marker for late onset alcoholism. Striatum-to-cerebellum dopamine D_2-receptor occupancy ratios, however, were not found to be lower in alcoholics than in controls, as previously suggested (Hietala et al. 1994; Volkow et al. 1996), nor was any significant difference found in the extrastriatal phase. The present findings indicate that presynaptic DAT density is lower in late-onset non-violent alcoholics than in healthy controls. The decrease in DA reuptake possibly represents a compensation of postsynaptic disturbance in DA transmission among type 1 alcoholics in a similar way as has been suggested concerning presynaptic dopamine synthesis (Tiihonen et al. 1998).

No significant difference was observed in the extrastriatal D_2-receptor occupancy between alcoholics and controls. Because of striatal (unlike extrastriatal) uptake of [^{123}I]epidepride does not achieve true equilibrium, an area method (Eq. 1) was used to calculate the D_2-recep-

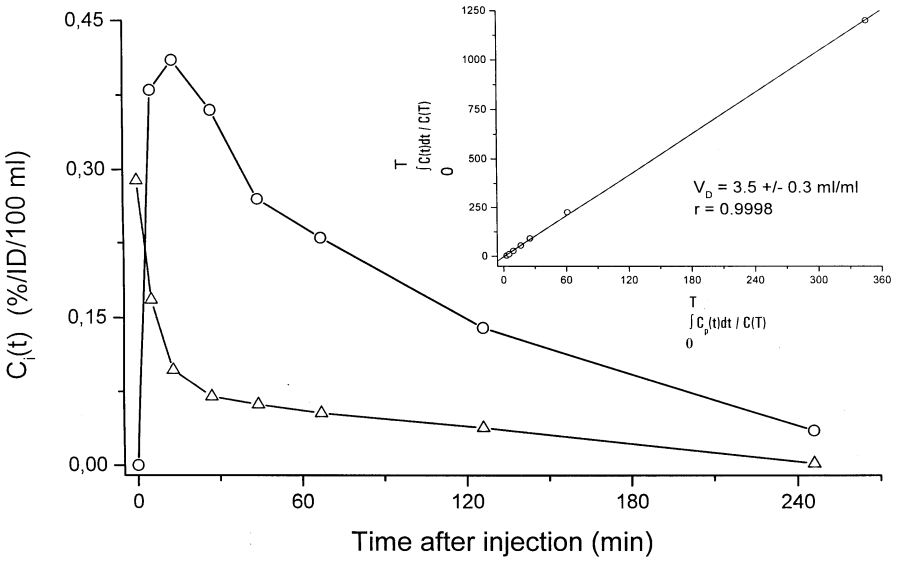

Fig. 4 Time-concentration data from the plasma parent samples, Cp(t), and cerebellar ROIs in a SPECT study using [^{123}I]epidepride. The slope of the linear fit of the Logan-Patlak analysis at pseudo-equilibrium is regarded as an approximate value of the distribution volume (*insert*)

tor occupancy ratios. Therefore, the results concerning striatal D_2-receptors must be interpreted with caution (but not the striatal DAT occupancy that has achieved equilibrium). Moreover, [^{123}I]epidepride is rapidly metabolized, and radioactive lipophilic metabolites may enter into the brain to either bind non-specifically in different brain regions or have affinity to the binding site of [^{123}I]epidepride (Bergström et al. 1997). In the present study, the percentage of the lipophilic metabolites varied between 0 and 22%, which may affect the nonspecific binding of [^{123}I]epidepride and contaminate the count density for the cerebellum. The percentage of lipophilic metabolite was about 3-fold higher in alcoholics than in controls. The amount of lipophilic metabolite is normally less than 10–15%, suggesting that it does not disturb quantitation of the D_2-receptor density at later imaging time points.

The cerebellar and parent plasma time-concentration curves were further analyzed using a Logan-Patlak plot technique (Logan et al. 1990), where the slope of the linear fit at pseudo-equilibrium conditions provided an approximate value of the distribution volume (Fig. 4). Indeed, the distribution volume in the cerebellum was nearly significantly (P<0.1) larger (4.3±1.4 ml/ml) in alcoholics than in healthy males (3.4±1.1 ml/ml), respectively. The results suggest that a true distribution volume of the dopamine D_2-receptor radioligand [^{123}I]epidepride in the striatum might be slightly higher in type 1 alcoholics than in controls, whereas this value is lower for [^{123}I]PE2I. In addition, there was a strong linear dependence of the striatal [^{123}I]epidepride uptake on the temporal uptake in alcoholics (Fig. 3). In sum, the results on epidepride uptake indicate that extrastriatal D_2-receptor density is not decreased among abstinent late-onset alcoholics.

In conclusion, the results suggest that pre-synaptic DAT occupancy is decreased in late onset non-violent alcoholics, and this finding is not considered to be a consequence of recent alcohol abuse.

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