GENETICS AND CELL BIOLOGY

5-HT\textsubscript{1A} Receptors in the Frontal Cortical Brain Areas in Cloninger Type 1 and 2 Alcoholics Measured by Whole-Hemisphere Autoradiography

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(Received 10 April 2008; first review notified 24 June 2008; in revised form 11 August 2008; accepted 2 October 2008; advance access publication 22 November 2008)

Abstract — Aims: The Cloninger type 1 alcoholics are prone to anxiety, and in many cases patients have begun to use alcohol in order to relieve their anxiety. We have previously reported a decrease of the serotonin transporter density in the perigenual anterior cingulate cortex (pACC) in type 1 alcoholics. The 5-HT\textsubscript{1A} receptors are the binding sites for anxiolytic drug buspirone. We aimed to investigate the alteration in the density of 5-HT\textsubscript{1A} receptors, that may also alter the effect of serotonin in the pACC in alcoholics. Methods: The density of the serotonin receptor 5-HT\textsubscript{1A} among Cloninger type 1 and 2 alcoholics (nine and eight subjects, respectively) and 10 control subjects were determined by postmortem whole-hemisphere autoradiography with WAY-100635. Results: Substantially sparser 5-HT\textsubscript{1A} (by \(\sim 31\%\), \(P = 0.010\)) density was observed in the pACC of alcoholic subjects in relation to non-alcoholic comparison subjects. In a secondary analysis for the difference between the alcoholic subtypes and controls, the 5-HT\textsubscript{1A} density was decreased significantly by \(\sim 32\%\) \(P = 0.015\) in the upper level of pACC in type 1 alcoholics. Conclusions: The detected decrease of 5-HT\textsubscript{1A} receptor density on the pACC suggests further that the serotonergic system is defected in the so-called affect region, especially in the type 1 alcoholics.

INTRODUCTION

The alcoholics are a heterogeneous group of people who present a wide spectrum of problems in regulation of emotions, ranging from anxiety to aggressive behaviour. The Cloninger type 1 alcoholism is characterized by an anxiety-prone temperament with no increased impulsive aggression (Cloninger, 1995). In contrast, type 2 alcoholism (\(~20\%\) of alcoholics) is more strongly correlated with heredity and characterized by teenage-onset of antisocial behaviour. The character of type 2 alcoholics is socially hostile (i.e. poorly cooperative, antisocial, vengeful), and they tend to be unusually impulsive, risk taking and prone to violent offending (Tiihonen \textit{et al.}, 1993). Cloninger type 1 alcoholics are reported to have dopaminergic defect, as reviewed by Tupala and Tiihonen (2004), but they also have decreased serotonin transporter density in the perigenual anterior cingulate cortex (pACC) (Mantere \textit{et al.}, 2002), which is important for the regulation of emotions and planning of actions. The serotonin transporter density alone may not fully reflect the serotonergic activity in the area. Balanced pre- and post-synaptic receptor densities in brain areas are also required for normal function of the serotonergic system. Alterations in the 5-HT\textsubscript{1A} density in brain have been associated earlier with many psychiatric disorders. The 5-HT\textsubscript{1A} density was found to be decreased in depression with or without serotonergic medication in a PET study (Sargent \textit{et al.}, 2000) and to be decreased in panic disorder (Neumeister \textit{et al.}, 2004). In contrast to those findings, increased \([^{11}C]\text{WAY-100635}\) binding potential has been reported in several cortical areas in women with bulimia (Tiihonen \textit{et al.}, 2004) and in cingulate gyrus in women recovered from anorexia nervosa (Bailer \textit{et al.}, 2005). The 5-HT\textsubscript{1A} agonist buspirone demonstrates anxiolytic effect in rat experiments, although they cause reduction of activity in rats (Haller \textit{et al.}, 2001), and in human they act as anxiolytes (Malec \textit{et al.}, 1996). It is important to obtain information of the densities of the 5-HT\textsubscript{1A} receptors in the brains in alcoholics. The literature suggests that the serotonergic response through post-synaptic 5-HT\textsubscript{1A} receptors may be decreased in alcoholics (Pinto \textit{et al.}, 2002).

There is currently not enough data of the 5-HT\textsubscript{1A} receptor densities or their function in alcoholics, especially not in the context of the neurochemical classification of alcoholics into types 1 and 2 with different behavioural and neurochemical background. The aim of the study was to evaluate the putative alterations in the 5-HT\textsubscript{1A} densities in alcoholics by postmortem autoradiography with tritium-labelled selective 5-HT\textsubscript{1A} receptor antagonist WAY-100635. A secondary aim was to evaluate whether the putative alterations in the 5-HT\textsubscript{1A} are observed in both Cloninger type 1 and 2 alcoholics, since only type 1 alcoholics are reported to have significantly decreased serotonin transporter density in the studied brain areas.

EXPERIMENTAL PROCEDURES

The brain sampling, diagnostics, study subjects and cryosectioning have been described in detail previously (Tupala \textit{et al.}, 2001; Mantere \textit{et al.}, 2002) and summarized below. The brain slices were incubated with radioactively labelled 5-HT\textsubscript{1A} receptor-binding ligands, with or without unlabelled displacer, as described below.

\textit{Study subjects}

Human brain’s 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

\[\text{Deceased.}\]
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 were collected. Alcoholism was coded according to DSM-IV criteria (APA, 1994) and sub-classified as type 1 or 2, according to Cloninger (1995). Two physicians reviewed data from medical records and anamnestic data, including police and criminal records. The most important criteria for defining the two groups of alcoholics were early onset (in teenage or before the age of 25) of alcohol abuse and documented severe antisocial behaviour among type 2 alcoholics. The Cohen’s kappa coefficient (Cohen, 1960) 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 direct effect on the dopaminergic system (such as psychostimulants or opioids) were excluded. A history of tobacco smoking history, 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 9 type 1 alcoholics (7 males, 2 females; mean age 52.7 years; postmortem delay 11.9 ± 4.5 h; mean ± SD), 8 type 2 alcoholics (males; mean age 34.6 years; postmortem delay 14.1 ± 3.4 h; mean ± SD) and 10 controls (8 males, 2 females; mean age 53.5 years; postmortem delay 14.8 ± 9.2 h; mean ± SD) free of psychiatric diagnosis. Alcoholism among these subjects was severe judging by 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. Eight of the nine type 1 alcoholics had alcohol in their blood at their time of death, and one alcoholic had 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, three had traces of benzodiazepines and one was positive for cannabinoids. One had had an abstinence period of 5 days and one of 3–7 days. The mean alcohol concentration in the blood was 0.20 ± 0.17% and 0.19 ± 0.14% in the type 1 and 2 alcoholics, and the two groups did not differ in this respect [F(0.174), P = 0.90, independent samples t-test]. All subjects died of sudden causes. Evaluation of the duration of heavy alcohol use, family history of alcohol misuse or smoking based only on medical records was considered unreliable and was not included in the analysis.

**RESULTS**

Binding values in all groups and in all brain areas were normally distributed (data not shown). The results of the selective \[^{3}H\]WAY-100635 binding to 5-HT\textsubscript{1A} are presented in Table 1. The 5-HT\textsubscript{1A} density was moderate in the cerebral cortex. 5-HT\textsubscript{1A} was observed to be expressed mainly in the outmost neuronal layers, as seen in Fig. 1. The 5-HT\textsubscript{1A} receptor binding was decreased in alcoholics compared to the controls in the lower and upper level of pACC (−37% and P = 0.036, −31% and P = 0.01, respectively) with large effect sizes (1.13–1.61).
In a secondary analysis, the difference in the 5-HT1A density was tested between the alcoholic subtypes and controls, and the multiple tests were corrected with Bonferroni. The 5-HT1A density was observed to be decreased significantly by −32% (P = 0.015) in the upper level of pACC in type 1 alcohols only. There was no significant correlation between the age and the 5-HT1A density in the pACC. If anything, there was a minor trend towards positive correlation in both lower and upper level of pACC in the type 1 alcohols only (R = 0.42, P = 0.51, and R = 0.63, P = 0.069, respectively), but not in the controls (R = −0.05, and −0.18).

The 5-HT1A densities between the different brain areas were compared using the Pearson correlation. There was a significant correlation between the 5-HT1A densities in the upper level of pACC and superior frontal gyrus in type 1 alcohols only (R = 0.74, P = 0.013), but not in other groups (Fig. 2). The difference between the groups was not significant (R² = 2.85, P = 0.24). The 5-HT1A densities in the upper and lower levels of pACC did not correlate significantly in any of the subject groups, and the differences between the subject groups were not significant (R² = 1.31, P = 0.52). There was, however, a trend towards positive correlation between 5-HT1A densities in lower and upper pACC in type 1 alcohols only (R = 0.60, P = 0.064), but not in other groups.

**Correlation between the 5-HT1A and SERT densities in the frontal cortical areas**

In order to further study the serotonergic system in frontal cortical regions in the alcoholic forebrain, the 5-HT1A binding densities in the pACC and other frontal cortical regions were compared to the SERT binding in the same brain areas published previously (Mantere et al., 2002). The Spearman correlation coefficient was used in the comparison. There was a trend towards positive correlation between the 5-HT1A binding and SERT binding in pACC in both the alcoholic types, but not in controls (Fig. 3). No significant correlations between the 5-HT1A and SERT densities were observed when comparing data from individual regions.

**DISCUSSION**

The distribution of [3H]WAY-100635 binding to the 5-HT1A receptors in human postmortem brains was in line with the...
The correlation of $[^3]H$WAY-100635 binding to 5-HT$_{1A}$ between the upper level of pACC and the superior frontal gyrus, which may suggest a global nature of the decrease of 5-HT$_{1A}$ receptors in the cortical areas of the type 1 alcoholics.

The correlations between the normalized $z$-scores of the $[^3]H$WAY-100635 binding to 5-HT$_{1A}$ and $[^3]H$citalopram binding to SERT from the publication of Mantere and co-workers (2002), in the pACC. The units are normalized $z$-scores.

The mean age and age range could not be perfectly matched between the type 2 alcoholic and the two other groups. The difference in the mean ages between the subjects was taken into account in the statistical analysis by using age as a covariate. There was no correlation between the age and the 5-HT$_{1A}$ density in any of the studied brain areas, although correlations between the age and monoamine receptor densities have been observed previously. For example, the dopaminergic D2 receptors decline rapidly with age from the moderately high levels observed around the age of 20 in the...
type 2 alcoholics only (Tupala et al., 2004) in the pACC in these same subjects. The dissimilar age profiles may suggest that the regulation of the serotonergic and dopaminergic receptor densities in the same brains may be relatively independent from each other. The type 2 alcoholics may have had shorter exposure than the rest of the index subjects but, as by definition, the trend for the age of onset was also much younger in the type 2 alcoholics, which diminishes the difference of exposure times to ethanol. The onset of type 2 alcoholism is typically in teenage and type 1 alcoholism after 25 years of age (Cloninger, 1995), and the life expectancy of type 2 alcoholics is markedly shorter than in the normal population due to the fact that antisocial personality shortens the life expectancy (Repo-Tiihonen et al., 2001).

The observed decreased 5-HT1A density in type 1 alcoholics is not a totally unexpected finding, considering that 5-HT1A partial agonist buspirone is used to treat anxiety (Argyropoulos et al., 2000). Buspirone has been reported to decrease alcohol consumption in animal experiments (Collins and Myers, 1987) but has clinical effect on drinking only in patients with comorbid anxiety (Malec et al., 1996), which by definition resemble the core definition of the Cloninger type 1 alcoholism. The present observation for the decreased 5-HT1A in alcoholics is in line with the body of literature, suggesting serotonergic defects in alcoholics. The present result is highly similar to our previous observations of the decrease of the SERT in the pACC (Mantere et al., 2002) in the same subjects, i.e. decrease is observed especially in the type 1 alcoholics. The results are from the single set of subjects, but consistent, and in line with the body of the literature. However, it should be noted that in a recent PET imaging study (Brown et al., 2007), no difference between the serotonin transporter densities between the controls and abstinent alcoholics was observed, possibly due methodological issues. Both violent patients and alcoholics have been suggested to have serotonergic defect (Virkkunen et al., 1994; Tiihonen et al., 1997; Frankle et al., 2005). Despite that, the 5-HT1A densities were not significantly different in the type 2 alcoholics compared to controls, although the observed large effect size in the present preliminary data suggests that in a larger set the result may reach significance.

The serotonin system modulates the activity of inhibitory areas in the prefrontal cortex and related areas such as the anterior cingulate cortex (Hariri et al., 2002; Heinz et al., 2005). The neurophysiologists have suggested that the 5-HT1A is expressed also in glutamatergic neurons, also in pACC, possibly regulating the glutamatergic activity on the region (Czyrak et al., 2003). That may have a major impact on the excitatory transmission on the area. The activity of the prefrontal cortex is also regulated by the amygdala (Garcia et al., 1999) that also plays a role in the function of selective attention through the anterior cingulate cortex (Franken 2003). It has been reported that a serotonergic defect in the amygdala-anterior cingulate cortex axis may impair the regulation of emotional responses (Heinz et al., 2005) and may also be linked on the mechanisms of depression (Pezawas et al., 2005). The 5-HT1A has evolutionally conserved aggression-reducing effect even in animal models organisms very distinct from human (Clotfelter et al., 2007). The density of 5-HT1A has an impact on the reactivity of amygdala (Fisher et al., 2006), and we have previously reported a distinct correlation between the SERT densities in both prefrontal cortical areas and in amygdala in type 2 alcoholics (Mantere et al., 2002; Storvik et al., 2007). The present results and the recent literature suggest that although in the pACC there was no greater decrease in the type 2 alcoholics, the function of 5-HT1A in amygdala should be studied further.

Considering the putative mechanisms behind the observed results, it does not seem likely that the 5-HT1A densities were decreased by a mechanism related to the monoaminergic release during acute ethanol intoxication, since even specific serotonergic drugs in clinical doses are not found to have any effect on the 5-HT1A densities, as reported in a recent PET study (Moses-Kolko et al., 2007). In addition, the 5-HT1A density did not decrease with age in the type 1 alcoholics, suggesting that prolonged alcohol abuse does not decrease the density further. No effect is caused either by reduced serotonergic activities caused by lesions, as reviewed recently (Drevets et al., 2007). The type 5-HT1A densities may be decreased in type 1 alcoholics either by genetic background, or they may be secondary and caused by the dopaminergic defect observed in this group (Tiihonen et al., 2004; Tupala et al., 2004).

There may also be a global factor decreasing the 5-HT1A density in the alcoholics. For the putative mechanism for the observed decrease of the 5-HT1A densities in the type 1 alcoholics and the similar trend in the type 2 alcoholics, one possibility clue is provided by the suggested effects of glucocorticoid hormones, as reviewed by Drevets and co-workers (2007). We hypothesize that the key may be the defected regulation of hypothalamus, since it regulates endocrinological activities. We have previously reported a trend towards a decrease of the SERT density in the hypothalamus in alcoholics (Storvik et al., 2008). In addition, the SERT densities in the hypothalamus and in the amygdala correlate significantly (Storvik et al., 2008). The serotonergic defects previously observed in the type 2 alcoholics may lead to the observed trend towards the decrease in the 5-HT1A densities due to dysregulated hormonal responses. The effect may even be bidirectional, as the reactivity of the amygdala correlates negatively with the 5-HT1A density on that area (Fisher et al., 2006). Unfortunately, we have no data from the amygdala to discuss. All this suggests that the issue should be studied further, since the stress and drinking are associated even in the clinical level (Field and Powell, 2007). Naturally, if real, the observed decreases in the 5-HT1A densities may be related to yet unknown factors, such as genetic background, and may be linked to polymorphisms.

The response to ‘anti-addiction’ medication is different between the type 1 and 2 alcoholics (Kiefer et al., 2005, 2008). The present data of the observed decrease of 5-HT1A density in type 1 alcoholics may help to explain why buspirone and other 5-HT1A agonist anxiolytes have beneficial effects only in a subset of alcoholics that are prone to anxiety and are initially addicted by the anxiolytic effect of alcohol. This and previous studies from the same set of subjects (Mantere et al., 2002; Storvik et al., 2006) have suggested that the serotonergic system is differentially affected in type 1 and 2 alcoholics. The present correlation analysis suggests similarities in the intercorrelation between of the SERT and 5-HT1A both in type 2 and type 1 alcoholics in pACC. In addition, the correlations between the brain areas were different between the alcoholic types, thus giving support to the model of Cloninger (1995). We hypothesize that the observed significant decrease of 5-HT1A and SERT (Mantere et al., 2002) in type 1 alcoholics are secondary and related to the dopaminergic defect in those
patients (Tupala et al., 2004; Tupala and Tiihonen, 2004) and that the observed trend towards the decrease of 5-HT1A density in type 2 alcoholics have other aetiology. As a conclusion, the results suggest that the serotonergic system is affected in both type 1 and 2 alcoholics, in a type-specific manner. The 5-HT1A density is decreased in type 1 alcoholics. That may have a role in the aetiology of anxiety and alcoholism, and may help to explain the effects of buspirone in the type 1 anxiety-prone alcoholics. However, these results must be considered as preliminary and further studies with larger sample sizes should allow more definitive conclusions.

Acknowledgements — We wish to thank Pirkko Räisänen, MD, PhD, for her help with the diagnostics and Pirjo Halonen, MSc, and Vesa Kivistm, MSc, for their help with the statistical analyses. We also thank Terttu Särkioja, MD, PhD, and Kari Karkola, MD, PhD, for providing the brains for this study.

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