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Increased Tryptophan Hydroxylase Immunoreactivity in the Dorsal Raphe Nucleus of Alcohol-Dependent, Depressed Suicide Subjects is Restricted to the Dorsal Subnucleus

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Abstract

Considerable evidence suggests that alcoholics with co-occurring depressive disorder are at greater risk for developing psychosocial problems particularly suicidal behavior. Moreover, dysfunction in serotonin (5-HT) neurotransmission has been implicated in depression, suicide and alcoholism. In the present study, we measured the levels of tryptophan hydroxylase (TPH), the main synthetic enzyme of 5-HT synthesis, in specific nuclei of the dorsal raphe (DR) in depressed suicide victims with alcohol dependence and matched psychiatrically normal controls. TPH immunoreactivity (IR) was quantified in frozen tissue sections containing the DR from 8 suicide victims with a diagnosis of major depression and alcohol dependence, and 8 psychiatrically normal control subjects by using immunohistochemical methods. We found that the levels of TPH-IR were significantly increased by 46% in the dorsal subnucleus of the DR in depressed suicide victims with alcohol dependence when compared with controls. In contrast, TPH-IR levels did not significantly differ in the other DR subnuclei between depressed, alcoholic suicide subjects, and controls. Our results indicate that abnormalities in 5-HT biosynthesis in the brain of depressed alcoholic suicide subjects are restricted within distinct regions of the DR.

Keywords

alcohol dependence; brainstem; human; mood disorders; postmortem; immunohistochemistry

INTRODUCTION

Abnormalities in serotonin (5-HT) neurotransmission have been associated with major depression, alcoholism, and suicidal behavior. For example, decreased cerebrospinal fluid levels of the 5-HT metabolite, 5-hydroxyindolacetic acid (5-HIAA), have been found in individuals with early onset alcoholism (Fils-Aime et al., 1996). Postmortem studies report reduced 5HT_{1A} receptor binding sites in the prefrontal cortex of alcoholic suicides and alcoholic nonsuicides (Underwood et al., 2004). Also, serotonin transporter (SERT) binding sites are decreased in the cingulate cortex of alcoholics (Mantere et al., 2002).

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One serotonergic substrate that is particularly important is the rate-limiting enzyme for 5-HT biosynthesis, tryptophan hydroxylase (TPH). TPH is abundantly expressed in neurons of the dorsal raphe (DR) and median raphe (MR) in the brainstem. However, very few postmortem studies have examined the DR serotonin system in alcoholic subjects. A loss of TPH-immunoreactive (IR) neurons was found in the DR of chronic alcoholic subjects with Korsakoff's syndrome (Halliday et al., 1993). Several studies have associated TPH gene polymorphisms with suicidal behavior and alcoholism (Lalovic and Turecki, 2002; Nielsen et al., 1998), but no studies have investigated the biosynthetic integrity of TPH in the DR nucleus of depressed alcoholic subjects.

The present study was designed to quantify the regional levels of TPH-immunoreactivity in tissue sections containing the DR from suicide victims with a confirmed diagnosis of major depression and alcohol dependence, and in matched psychiatrically normal control subjects by using immunoautoradiographic methods.

All procedures in our study were approved by the Douglas Hospital Research Centre, McGill University IRB, with signed informed consent obtained from next of kin. Eight subjects, who died by suicide and had a diagnosis of major depression and alcohol dependence, were each matched with one psychiatrically normal control subject for gender and as closely as possible for age and postmortem interval (PMI). Human brain tissue was obtained from the Quebec Suicide Brain Bank (QSBB), which has a collaboration with the Quebec Coroner's Office.

Brains were collected at autopsy and immediately transported on ice to the QSBB for dissection. The subject groups consisted of 8 major depressed, alcohol-dependent suicide subjects (mean age 44.00 ± 18 years; mean PMI 25.25 ± 7.5 h; mean pH 6.42 ± 0.21), and 8 normal control subjects without history of alcohol dependence (mean age 41 ± 16 years; mean PMI 26 ± 4.4 h; mean pH 6.31 ± 0.28). The PMI was defined as the interval between death and freezing of the brain tissue. The PMI of the suicide subjects were estimated from the coroner examiners and may not well match with those of the control subjects. Of the 8 suicide subjects, 5 had negative toxicological results, 2 were positive for alcohol, and 1 was positive for alcohol and cocaine. No subjects had positive toxicological results for antidepressants or other medications. The suicidal subjects died primarily by hanging (6 out of 8), whereas controls died in work-related accidents, myocardial infarction, or were passengers in car accidents. Subjects were psychiatrically characterized by psychological autopsies, which are validated methods to reconstruct psychiatric history by means of extensive proxy-based interviews, as outlined elsewhere (Kim et al., 2003). Briefly, psychological autopsies were carried out using structured interviews SCID I (Spitzer et al., 1992) by trained clinicians with an average of two informants per family. Following the interview, a review of the Coroner's notes and of all relevant medical records was performed, and a case report was written for the purpose of a best-estimate diagnosis. Best consensus DSM-IV. (APA, 1994) axis I diagnosis were made by a panel of clinicians, based on the analysis of the case reports.

Brain tissue and tissue sectioning were processed according to previously described procedures (Bonkale et al., 2004). Slide-mounted tissue sections were selected from one anatomical level of the DR containing the ventrolateral subnucleus as previously described (Baker et al., 1990; Bonkale et al., 2004).

Tissue sections were labeled using a PH8 antibody that binds to a common epitope of phenylalanine hydroxylase, tyrosine hydroxylase, and TPH. However, in human tissue that has been paraffin-embedded and fixed with paraformaldehyde, PH8 antibody detected only TPH in serotonergic raphe neurons (Haan et al., 1987). Recently, a second TPH isoform

(TPH2) was identified (Walther et al., 2003). The PH8 antibody, likely recognizes both TPH1 and TPH2 isoforms. Because TPH2 is exclusively present in the brain, therefore, in all likelihood the TPH-IR detected in the present study corresponds to TPH2 protein.

The immunautoradiography was performed essentially as described earlier (Bonkale et al., 2004). A total of 5 sections of the midbrain were selected at 200 μ m intervals for each subject. In brief, slide sections were fixed in 4% paraformaldehyde for 14 h. Tissue sections were pretreated in PBS with 0.4% Triton X-100, 1.5% normal goat serum, and 0.5% normal human serum for 1 h. Sections were incubated overnight at 4°C with the primary antibody to PH8 (1:10,000; Chemicon, Temecula, CA) diluted in PBS containing 1.5% normal goat serum, 0.05% bovine serum albumin, and 0.4% Triton X-100. The sections were then washed three times for 5 min in PBS containing 0.4% Triton X-100 and then incubated with the [¹²⁵I]-labeled secondary antibody (sheep anti-mouse IgG [¹²⁵I]-JF(ab')₂ fragment; NEN (Boston, MA), specific activity 1320 Ci/mmol, initial concentration 0.5 μ Ci/ml), using a dilution of 1:200 in PBS containing 3% normal goat serum and 0.4% Triton X-100 for 120 min. After washing, tissue sections were rinsed in 70% ethanol and air-dried. The dried tissue sections were apposed to β -max Hyperfilm (Amersham, Arlington Heights, IL) and exposed for 48 h with a set of ¹⁴C standards, which were cross-calibrated to iodinated standards (Amersham). The quantification of the autoradiographic images was accomplished using a personal computer-based image analysis system (the "Microcomputer Imaging Device," MCID; M5) from Imaging Research Inc. (St. Catharines, Ontario, Canada) as previously described (Bonkale et al., 2004).

For statistical analysis, we used Student's *t* test to compare levels of TPH-IR in the individual DR subnuclei between the depressed, alcoholic suicide, and control subject groups. Analysis of covariance (ANCOVA) with PMI and age as covariates were performed. All statistical assumptions were met, and the statistical significance was defined as *P* = 0.05.

The concentration of TPH-IR was significantly increased by 46% in the dorsal subnucleus (DRd) of the DR in alcohol-dependent, depressed suicide subjects when compared with control subjects (*t* = 2.73, *df* = 14, *P* = 0.016, Fig. 1). Although TPH-IR concentrations appeared elevated in the other DR subnuclei of the ventral (DRv), ventrolateral (DRvl), and interfascicular (DRif) regions in the alcohol-dependent, depressed subjects relative to the controls, the differences failed to reach statistical significance (DRv, *t* = 0.695, *df* = 14, *P* = 0.499; DRvl, *t* = -0.936, *df* = 14, *P* = 0.365; DRif, *t* = 0.694, *df* = 14, *P* = 0.499). The influence of age and PMI on TPH-IR in the DR was examined, and no significant correlations were found between age or PMI of the subjects and TPH-IR levels in the subnuclei of DR. Representative immunautoradiographic images of TPH-IR concentrations in the specific subnuclei of the DR of a control and depressed alcoholic suicide subject pair are shown in Figures 2A and 2B, respectively.

Our results revealed that depressed alcoholics had a 46% increase of the level of TPH-IR compared with control subjects, which was restricted to the DRd subnucleus. There are anatomical findings showing that the DR is a heterogeneous nucleus with morphologically and functionally distinct subnuclei. The DRd is the largest subnucleus of the human DR but has also the lowest neuronal density (Baker et al., 1990). Although there is overlap of projections from different DR subnuclei in rat brain, the DRd neurons target mainly the amygdala and the substantia nigra, and to lesser extent the hippocampus, striatum, and parietal cortex (Jacobs and Azmitia, 1992). These observations, together with the results of the present study, suggest that the DRd may be specifically involved in abnormalities in 5-HT biosynthesis in the brain of depressed alcoholic suicide subjects.

The results of the present study are interesting given the evidence that chronic alcohol consumption has detrimental effects on serotonergic neurons. For example, rodents chronically treated with ethanol or ethanol-preferring rats exhibit a reduction in the number of TPH-IR neurons in the DR (Casu et al., 2004; Jang et al., 2002). Studies by Halliday and colleagues (1993) quantified the number of TPH-IR neurons in the DR of three alcohol-use groups (Baker et al., 1996). A significant reduction in the number of TPH-IR neurons in the DR of chronic alcoholic subjects with Wernicke's encephalopathy or Korsakoff's psychosis was found, while no significant changes in the number of DR neurons were reported in chronic alcoholic subjects without Wernicke's or Korsakoff's conditions (Baker et al., 1996; Halliday et al., 1993). Two in vivo imaging studies showed significant reductions in SERT binding sites in the midbrain raphe nuclei, but no changes in cortical or forebrain regions of alcohol-dependent subjects (Heinz et al., 1998; Szabo et al., 2004). Given the previous serotonergic alterations reported in rodents and humans following alcohol ingestion, one might have predicted a down-regulation of TPH protein expression in the DR of depressed alcohol-dependent suicide subjects. However, our finding of an increase in TPH-IR in a discrete subnucleus of the DR, the DRd, in the depressed alcohol-dependent suicide subjects may be reflective of a compensatory mechanism to elevate TPH biosynthesis as an attempt to compensate for reduced functional capacity of 5-HT neurotransmission in specific cortical or subcortical terminal fields (Mantere et al., 2002; Underwood et al., 2004).

In conclusion, the increase in TPH-IR levels in the DRd suggests that abnormalities in 5-HT biosynthesis in the brain of depressed alcoholic suicide subjects are restricted within distinct regions of the DR. These results emphasize the importance of further research to investigate the biosynthetic integrity of TPH2 in the brain of psychiatric patients.

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Fig. 1. Scatter plots illustrating the TPH-IR concentrations in the individual subnuclei of the dorsal raphe of alcohol-dependent, depressed suicide ($n = 8$) and control subjects ($n = 8$).

Fig. 2. Representative color-enhanced film immunoautoradiographic image of TPH-IR concentrations in the dorsal raphe of a matched control subject (**A**) and depressed, alcohol-dependent suicide subject (**B**). The red outline delineates boundaries of the DR subnuclei.