PLATELET SEROTONIN UPTAKE IS HIGHER IN EARLY-ONSET THAN IN LATE-ONSET ALCOHOLICS

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(Received 7 December 1999; in revised form 23 March 2000; accepted 28 March 2000)

Abstract — The main objective of this study was to compare platelet serotonin (5-HT) uptake between early-onset alcoholics (EOA) and late-onset alcoholics (LOA). Subjects were 24 dependent male alcoholic in-patients and 21 healthy control subjects. 5-HT uptake was quantified by incubating platelets with [3H]5-HT at various concentrations (0.5 to 1000 nM). 5-HT uptake was higher in EOA, compared to both the LOA and control groups (P < 0.02) at the highest 5-HT concentration (1000 nM). No significant difference was found between LOA and controls or between EOA + LOA and controls. Previous studies have shown that 5-HT uptake was higher in platelets, lymphocytes, and brain of alcoholics vs controls, but our data suggest that higher platelet serotonin transporter function is limited to EOA, not LOA.

INTRODUCTION

The 5-HT (5-hydroxytryptamine or serotonin) neurotransmitter system has been implicated in the pathophysiology of alcoholism, especially in the subgroup of alcoholics with an early onset (Fils-Aime et al., 1996). Many previous studies support the involvement of the serotonergic system in impulsive and violent behaviours that accompany early-onset alcoholism. Recently, the 5-HT1 antagonist ondansetron was reported to reduce drinking in a group of early- (EOA) vs late- (LOA) onset alcoholics (Johnson et al., 1999). This recent study prompted us to look for differences in serotonin function between EOA and LOA. The differences between early- and late-onset alcoholism have proved to be a useful distinction for research studies and were first described by Cloninger et al. (1988).

The 5-HT transporter (SERT) is an important feature of the serotonergic system in that it controls the level of 5-HT in the synapse and other extracellular compartments in the brain (Bunin and Wightman, 1999). Other tissues also express this transporter. The SERT protein, whether in brain, platelets or lymphocytes, has been shown to possess identical amino acid sequences and the same pharmacological sensitivities (Lesch et al., 1993; Villinger et al., 1994). The level of peripheral SERT function in platelets or lymphocytes may prove useful as a reflection of abnormal expression or function of a biochemical system that is important to the behavioural aspects of alcohol drinking.

Previous studies indicate that a difference in platelet 5-HT uptake exists between alcoholic and control subjects. Ernouf et al. (1993) reported that platelet 5-HT uptake was higher in male and female abstinent alcoholics and their male and female children (<14 years) than in controls. Daoust et al. (1991) showed that maximal platelet 5-HT uptake, but not paroxetine binding, was higher in alcoholics than in non-alcoholic subjects. Faraj et al. (1997) showed that 5-HT uptake into peripheral blood lymphocytes was higher in a group of abstinent (2 to 10 years), recovering alcoholics, than in a group of non-alcoholic controls. Rausch et al. (1991) showed that males with a family history positive (FHP; alcoholic fathers) had a significantly higher mean Vmax for platelet 5-HT uptake, compared with those of family history negative (FHN) serving as controls.

Based on these reports, it appears that SERT function differs between alcoholics and non-alcoholics. Therefore, in this study, we compared 5-HT uptake into platelets of non-alcoholic healthy controls with EOA and LOA groups.

MATERIALS AND METHODS

Subjects

Alcoholic subjects were 24 informed male volunteers who were in-patients at the Alcoholic Treatment Unit of the Audie Murphy Memorial Veterans Administration Hospital in San Antonio, TX, USA. All 24 met the DSM-III-R criteria for alcohol dependence (American Psychiatric Association, 1987). All were enrolled on a 4-week treatment programme. EOA (n = 17) and LOA (n = 7) were categorized based on age at which dependent drinking had commenced (EOA ≤25 years, LOA >25 years; according to Johnson et al., in preparation). Alcoholic subjects were required to have an elevated γ-glutamyl transferase (GGT) level, withdrawal symptoms, and/or a blood-alcohol level above 200 mg/dl upon admission to be entered into the study. These subjects had abstained from alcohol for between 2 and 13 days (mean 6.6 days) prior to blood sampling. Alcoholic subjects had been drinking between 84 and 715 g of ethanol daily for at least 30 days. The mean ± SD of daily ethanol consumption was 373 ± 174 g for EOA and LOA combined. There was no statistically significant difference in alcohol consumption between EOA and LOA. Alcohol consumption for control subjects was less than 10 g/day, except for one subject who drank 30 g/day. Only one control subject drank on a daily basis. The age range for alcoholic subjects was 24 to 70 years with a mean ± SD of 40 ± 9.9 years. Controls were 21 healthy male volunteers, recruited from staff at the medical school, with an age range of 25 to 50 years, mean 34.7 ± 8.31 years. The means of the current ages of controls, EOA, LOA, and EOA + LOA did not differ statistically (P < 0.05). Control and alcoholic subjects

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were administered the SCID Structured Interview for Alcohol Dependence (Spitzer et al., 1989) and the Hamilton (1960) Depression Rating Scale (24 questions). On the SCID interview, control subjects scored between 11 and 14 and alcoholics scored between 33 and 36 (minimum score 11, maximum score 36). For the Hamilton scale, eight of 24 alcoholic subjects scored at 14 or higher and all control subjects were in the normal range. None of the control subjects exhibited depressive symptoms.

**Preparation of platelet suspension and determination of platelet \([^{3}H]5\text{-HT}\) uptake**

Platelet suspensions were prepared as described in Javors et al. (1990). Blood was drawn from an arm vein with a Butterfly infusion set (21 gauge, 0.75 in, needle) into 60-ml polypropylene syringes containing 10 ml of acid–citrate–dextrose (ACD) buffer and 120 U of heparin for every 50 ml of blood. ACD buffer contained 85 mM sodium citrate, 62.2 mM citric acid, and 110 mM dextrose, pH 4.9. The blood was then placed in polystyrene test tubes and centrifuged at 62.2 mM citric acid, and 110 mM dextrose, pH 4.9. The blood was then placed in polystyrene test tubes and centrifuged at 150 g for 20 min at 23°C in a Beckman TJ-6 centrifuge, using a swinging bucket rotor. With a polypropylene Pasteur pipette, we aspirated the platelet rich plasma (PRP), carefully avoiding the Buffy coat, added prostaglandin I-2 (PGI-2) (300 ng/ml) to prevent loss of platelets during centrifugation, then centrifuged the PRP at 850 g for 10 min at 23°C to pellet the platelets. The platelet-poor plasma was discarded and the platelet pellet carefully resuspended in platelet buffer (137 mM sodium chloride, 2 mM potassium chloride, 1 mM magnesium chloride, 5.5 mM glucose, 5.0 mM HEPES, pH 7.4), then the platelet suspension was incubated at 37°C until it had the characteristic pearlescent appearance that indicates discoid, unactivated platelets. We determined the platelet count in this sample with a Coulter counter model S-plus VI and adjusted the count to 2 x 10^8 platelets/ml with the addition of platelet buffer.

For \([^{3}H]5\text{-HT}\) uptake, aliquots of a platelet suspension (2 x 10^8 platelets/ml) were incubated with \([^{3}H]5\text{-HT}\) (specific activity 28.2 Ci/mmol) at several concentrations (0.5, 1, 2, 4, 8, 16, 32, 64, 125, 250, 500, and 1000 nM). The uptake was initiated with \([^{3}H]5\text{-HT}\), the incubation proceeded for 1 min, and then the uptake was quenched by the addition of 5 ml of platelet buffer containing 300 ng/ml of PGI-2 and immediate filtration through Whatman GF-B filters. Assay tubes and filters were washed with an additional 5 ml of platelet buffer with PGI-2. The filters were air dried, placed in 8 ml Beckman Ready-Solv HP scintillation counting fluid, and counted for \([^{3}H]5\text{-HT}\). Platelet 5-HT uptake was expressed as counts per minute (cpm) and represents total uptake (specific and non-specific). This assay was performed the same day as the blood was drawn.

**Statistics**

Analysis of variance (ANOVA) for repeated measures was used to analyse group differences among EOA, LOA, and controls with 5-HT concentration as a repeated measures factor. 5-HT uptake was studied in detail by pairwise comparison of concentration and group means using the ANOVA sources of variability for both between- and within-subjects. Standard transformations were tried and the square root of cpm best met the assumptions for this ANOVA based

**RESULTS AND DISCUSSION**

Figure 1 shows that 5-HT uptake, measured at 1000 nM [5-HT], was greater in EOA than LOA or controls (P = 0.016 and P = 0.012, respectively). To our knowledge, a comparison of platelet 5-HT uptake has never been made between EOA and LOA and this finding supports the hypothesis that 5-HT function may vary between these two types of alcoholics. Previous studies have shown that platelet 5-HT uptake is higher in abstinent alcoholics than control subjects (Daoust et al., 1991; Rausch et al., 1991; Ernouf et al., 1993). These previous studies, however, did not directly compare EOA and LOA and, therefore, further study might distinguish these two subtypes.

Our results show that neither LOA nor EOA + LOA differed from controls, the latter of which conflicts with previous studies (Daoust et al., 1991; Rausch et al., 1991; Ernouf et al., 1993). Several differences exist between our study and these previous studies, however, related to the concentration(s) of \([^{3}H]5\text{-HT}\) used, distribution of LOA and EOA among the alcoholics in the study, and the measurement of active vs total (active and passive) serotonin uptake. It is interesting to note that Rausch et al. (1991) reported that males with a FHP (alcoholic father) had a higher \(V_{\text{max}}\) for platelet 5-HT uptake, than those with a FHN. FHP is more likely in EOA (Cloninger et al., 1988).

The higher platelet 5-HT uptake that we observed in EOA vs LOA and controls, was apparently not due to acute effects of the presence of ethanol, i.e. not a state, but a trait, effect. To test this possibility, we performed a correlational analysis of days of abstinence vs 5-HT uptake at 1000 nM of \([^{3}H]5\text{-HT}\). The \(r^2\) value for the regression was 0.08 and the \(P\) value was 0.24 (not statistically significant), which suggests that platelet 5-HT uptake did not decrease during abstinence. Arranz et al. (1999) reported that platelet \([^{3}H]\)paroxetine binding decreased
with 2 weeks of abstinence. The reason for the difference between our finding and theirs is not clear at present, although it is possible that uptake and binding do not co-vary (Doust et al., 1991).

We did not observe any correlation between age and platelet 5-HT uptake among our control subjects ($r^2 = 0.003$). Several previous studies have addressed the relationship between age and platelet 5-HT transporter. Increasing age correlated in a statistically significant fashion with decreased imipramine (Langer et al., 1980; Marazziti et al., 1987; Halbreich et al., 1991) or paroxetine (Nakai et al., 1994; Sigurdh et al., 1999) binding to human platelet membranes in some studies. However, the results of other studies were in disagreement. For example, Marazziti et al. (1999) reported that increasing age correlated with decreased $V_{\text{max}}$ for platelet 5-HT uptake, but not with $B_{\text{max}}$ for paroxetine binding. Thus, there may be other age-related factors required for uptake that do not affect binding to the SERT. Marazziti et al. (1998) showed that neither the maximum binding capacity nor the dissociation constant ($K_d$) of paroxetine for platelet SERT was significantly different in aged vs young subjects. Finally, Andersson et al. (1992) found no age-related changes in binding capacity ($B_{\text{max}}$) or binding affinity ($K_d$) of paroxetine to human SERT sites in the cortex of cingulate gyrus and the amygdala of post-mortem human subjects.

Caution is required when extrapolating results from platelet studies to brain, because the regulation of the transporter may be different in the two tissues (Pletscher, 1988; Wirz-Justice, 1988). Nevertheless, the transporter in brain, platelets, and lymphocytes has identical amino acid sequences and pharmacological sensitivities (Lesch et al., 1993; Villiger et al., 1994). It is tempting, therefore, to speculate that enhanced 5-HT uptake in the brain might result in reduced serotonin tone, thereby contributing to the impulsive and violent behaviours associated with early-onset alcoholism. This notion is supported by the work of Fils-Aime et al. (1996), in which they showed that EOA had lower cerebrospinal fluid 5-HIAA concentrations, suggesting reduced serotonin turnover, i.e. a decrease in serotonin function or tone. Those results are in agreement with ours that elevated 5-HT uptake in brain, as observed by us in platelets, would probably result in reduced turnover in brain, as observed by Fils-Aime et al. (1996).

The role of serotonin function related to alcoholism is not yet clear. Heinz et al. (1998) reported reduced central SERT density in the brainstem of alcoholics. Arranz et al. (1999) reported an increased SERT density in platelets of alcoholics that was reversed with abstinence for 14 days, suggesting that the elevation was a state, not a trait, variable. For our study, platelet 5-HT uptake in alcoholics did not correlate with days of abstinence; the longest period of abstinence was 13 days. Finally, Greenberg et al. (1999) reported that the long and short polymorphic forms of the 5-HT transporter gene resulted in different platelet 5-HT uptake, but no difference in the density of paroxetine binding sites. Additional study is required to clarify these apparently inconsistent results.

Finally, our results indicate that 5-HT uptake differed between EOA and LOA when tested at 1000 nM 5-HT. Bunin and Wightman (1999) estimated the intraneuronal concentration of 5-HT to be about 50 nM, a concentration thought to be optimal for receptor and transporter activation. However, in the synapse immediately after stimulated release, it is likely that the concentration reaches 6 mM according to their estimates. Differences of 5-HT uptake at this higher concentration of 5-HT, i.e. well above the $K_m$ of 5-HT for the transporter, could have a significant effect on the synaptic availability of 5-HT and therefore on behaviour.

Acknowledgement — This study was funded by a grant from NIAAA.

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