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Prevalence of High Serotonin Uptake in Lymphocytes of Abstinent Alcoholics*

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ABSTRACT. An impairment in serotonergic neurotransmission may be associated with alcoholism. We recently identified a high-affinity serotonin transporter (5-HTT) in human peripheral blood lymphocytes (PBLs). Moreover, molecular analysis of RNA samples of human lymphocytes using reverse transcription, coupled with polymerase chain reaction, enabled us to confirm the expression of a 5-HTT identical to the one reported in neuronal tissues, as evidenced by hybridization and sequence analysis. In this investigation, we measured the serotonin (5-HT) uptake in PBLs of recovering alcoholics (N = 10) with long-term abstinence (2–10 years) and non-alcoholic controls (N = 10). 5-HT uptake was measured by incubating 1×10^7 cells of PBLs with [^{3}H]5-HT (3–1000 nM; sp. act. 23 Ci/mmol) for 10 min at 37°. The results of this preliminary study revealed that abstinent alcoholics had significantly ($P < 0.01$) increased uptake of 5-HT (43.6 ± 5.70 pmol/10^7 cells) as compared with controls (23.33 ± 2.50 pmol/10^7 cells). An enhanced uptake of 5-HT in PBLs of abstinent alcoholics agrees with previously reported observations of increased 5-HT uptake in brain and platelets of former alcoholics and their descendants. This suggested that a serotonergic mechanism may be linked to the heredity of alcoholism. *Copyright © 1996 Elsevier Science Inc.*, BIOCHEM PHARMACOL 53;1:53–57, 1997.

KEY WORDS. alcoholism; serotonin; human lymphocyte; serotonin uptake activity; impulsive violence; lymphocyte 5-HT transporter cDNA

Studies in recent years have demonstrated that an impairment in 5-HT¶ central neurotransmission may be involved in alcohol drinking behavior [1–3]. Reduced levels of 5-HT, as well as its metabolite, 5-HIAA, have been found in the brain serotonergic neurons of alcohol-preferring (P) as compared with a non-preferring line of rats (NP) [4–6]. There was a significant reduction in alcohol consumption by P rats as a result of either increased 5-HT synthesis or the administration of 5-HT receptor agonists [7–10]. Consistent with these observations are the studies of the relationship between 5-HT uptake and alcohol ingestion. An increased 5-HT uptake has been found in striatal synaptosomes of P rats. The administration of selective 5-HT reuptake inhibitors (SSRIs) has been shown consistently to reduce alcohol intake in laboratory animals [11, 12] and human heavy drinkers [13–15]. Thus, it appears that conditions that increase the activity of serotonergic neurons tend to decrease alcohol consumption.

We recently identified the presence of a high-affinity transport mechanism for 5-HT in peripheral blood lymphocytes of human subjects [16]. This transporter bears a close resemblance to the one found in neuronal tissues [17]. This presented us with an opportunity to utilize lymphocytes as a readily accessible human cellular preparation for investigating the influence of alcoholism on 5-HT uptake. To minimize the effect of chronic alcohol exposure, 5-HT uptake activity was measured in lymphocyte preparations isolated from the blood of abstinent alcoholics. Also, an attempt was made to determine whether an association exists between the period of abstinence from alcohol and 5-HT uptake activity.

MATERIALS AND METHODS

Subjects

The abstinent alcoholics evaluated in this study consisted of seven males and three females between the ages of 20 and 60 years (40 ± 18). Of these alcoholics, eight were white and two were black. Prior to their sobriety, these alcoholics met the DSM-III-R criteria for alcohol dependence [18] and had had previous in-patient detoxification for alcoholism. These subjects were members of Alcoholics Anonymous, and the average length of their abstinence was 5.8 years (1–10 years). Subjects with major psychiatric disorders, neurologic or medical illnesses, or individuals who

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¶ *Abbreviations:* 5-HT, serotonin; 5-HIAA, 5-hydroxyindole acetic acid; CSF, cerebrospinal fluid.

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were taking medications known or suspected of affecting 5-HT uptake activity were excluded from the study.

Control Group

This group consisted of seven male and three female adults that were age, race, and sex matched with the recovering alcoholics evaluated in this study. They were members of the staff of Emory University School of Medicine. These individuals had no history of alcoholism or drug addiction, had no physical or psychiatric illness, were on no medications, and had no history of any chronic medical problems. Informed consent, approved by the Human Investigations Committee of Emory University, was obtained from all subjects prior to enrollment into the study. Blood samples were taken in the morning after an overnight fast.

Sample Collection

Blood samples for the determination of 5-HT uptake were drawn from a peripheral vein into an evacuated tube containing heparin as an anticoagulant.

5-HT Uptake Assay

Isolation of lymphocytes and measurement of 5-HT uptake activity was carried out according to the procedure described by Faraj *et al.* [16]. Briefly, suspended lymphocytes (1×10^7 cells) in Hanks' buffer and [^{3}H]5-HT (sp. act. 23.7 Ci/mmol; 3–1000 nM) were incubated for 10 min at 37°. Lymphocytes were isolated by centrifugation, washed repeatedly with buffer, and sonicated, and the radioactivity in the samples was counted.

Statistical Analysis

Correlation analyses were done using Pearson products moment estimates of linear association. Comparisons of 5-HT uptake activity in lymphocytes of abstinent alcoholics and controls were done using the Kruskal–Wallis test. Statistical significance was set at 0.05 levels.

RESULTS

Patient Profile

The abstinent alcoholics described in this study bear some resemblance to type II alcoholics [19]. These alcoholics were more likely to consume alcohol at an earlier age, to have an earlier age of onset of illness and entry into treatment, and to experience more social consequences and legal problems in association with their alcoholism. Furthermore, a greater percentage of subjects had a family history of alcoholism.

Serotonin Uptake

5-HT uptake activity was determined in lymphocytes isolated from the blood of abstinent alcoholics and controls. Abstinent alcoholics had significantly ($P < 0.01$) higher uptake of [^{3}H]5-HT in their lymphocytes (43.6 ± 5.70 pmol/10^7 cells) compared with controls (23.33 ± 2.50 pmol/10^7 cells) (Table 1 and Fig. 1). Kinetic analysis, as computed by regression analysis from Lineweaver–Burk plots, revealed a substantially higher ($P < 0.01$) maximal velocity (V_{max}) for the uptake of [^{3}H]5-HT in lymphocytes of abstinent alcoholics (V_{max} = 564 ± 70 pmol/10^7 cells) as compared with that of controls (V_{max} = 166 ± 30 pmol/10^7 cells). Similar changes in K_m values were observed (Table 1). In Table 2 are shown [^{3}H]5-HT uptake levels observed among these former alcoholics according to their length of abstinence from alcohol. The data showed that the uptake of [^{3}H]5-HT was not modified by the period of abstinence.

DISCUSSION

In view of the observation that the rate of 5-HT uptake may represent a biological trait closely associated with alcoholism [11–15], we investigated whether lymphocytes of abstinent alcoholics transport 5-HT differently from that of non-alcoholics. The results of this preliminary study showed that abstinent alcoholics had a significant increase of 5-HT uptake in their lymphocytes as compared with controls. Kinetic analysis revealed that the change in 5-HT uptake activity in abstinent alcoholics was due to a change in maximal velocity (V_{max}) with a corresponding change in the affinity constant (K_m) of the transporter toward 5-HT.

TABLE 1. Lymphocyte [^{3}H]5-HT uptake in recovering alcoholics and controls

Group	N	Lymphocyte* [^{3}H]5-HT uptake (pmol/10^7 cells)	Kinetics	
			V_{max} (pmol/10^7 cells)	K_m (nM)
Controls	10	23.33 ± 2.50	166 ± 30	547 ± 75
Recovering alcoholics	10	43.6 ± 5.70†	564 ± 70†	1068 ± 120‡

Values are means ± SD.

* The concentration of [^{3}H]5-HT used to measure lymphocyte uptake was 110 nM.

† Significantly different from controls ($P < 0.01$).

‡ Significantly different from controls ($P < 0.05$).

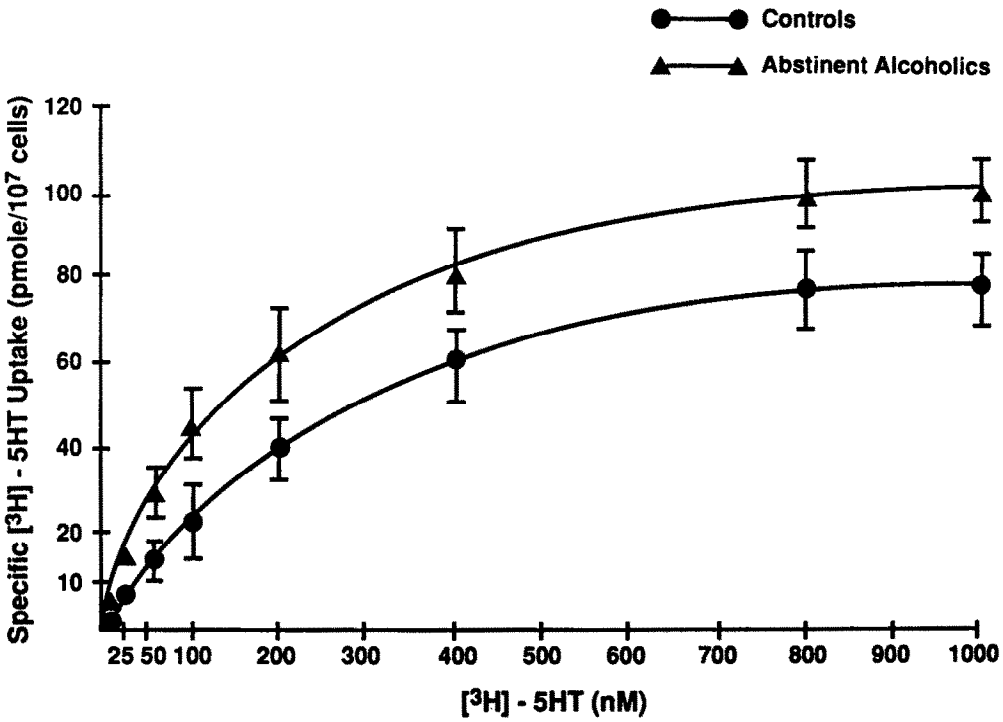

FIG. 1. Uptake observed in the presence of various concentrations of [^{3}H]5-HT (25–1000 nM) in lymphocytes isolated from the blood of controls (●—●) and abstinent alcoholics (▲—▲). Each point (mean ± SD) represents the average of six experiments.

These observations are in accordance with those of Boismare *et al.* [20] and Ernouf *et al.* [21], who showed that former alcoholics and their descendants had elevated 5-HT uptake in their blood platelets. No apparent difference in 5-HT uptake was noted in lymphocytes of alcoholics with varying lengths of abstinence. This suggested to us that the observed increase in 5-HT uptake in lymphocytes of abstinent alcoholics may be indicative of a heritable trait and not the long-lasting effect of chronic alcohol abuse. In support of this thesis, Rausch *et al.* [22] measured 5-HT uptake in blood platelets of young men who are potentially vulnerable to develop alcoholism on the basis of family history. They showed that individuals with a family history positive (FHP) had a significantly higher mean V_{max} for platelet 5-HT uptake as compared with those of family history negative (FHN) serving as controls.

The increased V_{max} of 5-HT uptake in lymphocytes of abstinent alcoholics is consistent with animal studies in which a higher capacity of 5-HT uptake was found in the brain of selectively bred alcohol-prone rodents [3]. A higher capacity for 5-HT uptake suggests an increased ability to sequester extracellular 5-HT from access to 5-HT receptors [23]. This, presumably, reduces the activation of serotonergic neurons. This argues in favor of the hypothesis that central nervous system 5-HT neurotransmission may be involved in the regulation of ethanol intake [24, 25]. However, the present data cannot confirm whether differences in 5-HT uptake in lymphocytes of abstinent alcoholics are related to a central serotonergic mechanism of hereditary risk to develop alcoholism.

TABLE 2. Kinetics of 5-HT uptake in lymphocytes of recovering alcoholics with abstinent time

Abstinent time (years)	N	Kinetic parameters* V_{max} (pmol/10^7 cells)	Kinetic parameters* K_m (nM)
1–2.9	4	41	1110
3–4.9	3	45	1050
5–10	3	42	950

* Means.

The application of molecular biology techniques should prove pivotal to efforts aimed at identifying whether a serotonergic mechanism underlies the development of alcoholism. Pharmacological properties of 5-HT uptake in lymphocytes have been characterized and shown to be similar to that in brain [16]. Using reverse transcription coupled with polymerase chain reaction (PCR) amplification and direct referencing, we have isolated and identified the cDNA encoding the primary structure of a 5-HT uptake site in lymphocytes from human blood and have shown it to be identical to that of human brain 5-HT transporter [26, 27].

The elucidation of the primary structure of 5-HT transporter in lymphocytes and the demonstration of its identity with 5-HT transporter in brain may have important implications. Human lymphocytes, a readily accessible, nucle-

ated cell type, could conceivably be used as a model of brain 5-HT transporter to study the regulation and expression of this gene in alcoholics and attain insight into the possible association between altered 5-HT transporter function and vulnerability for alcoholism.

What are the possible consequences of decreased serotonin neurotransmission in alcoholics? Impairment of 5-HT neurotransmission among alcoholics may precipitate behavioral traits that could have adverse psychosocial implications. Considerable preclinical and clinical evidence links impulsive aggressive behaviors to reduced brain 5-HT function [28, 29]. Individual vulnerability may render certain groups of alcoholics to be more at risk to exhibit violent behavior while intoxicated. One such group may be type II alcoholics [19, 30], where early onset of alcohol-related problems and criminality are the central features. Interestingly, Buydens–Branchey *et al.* [31] demonstrated the existence of an association between aggressive behavior and low plasma tryptophan/neutral amino acid ratio among individuals who had early onset of alcohol-seeking behavior.

Linnoila *et al.* [32] and Virkkunen and Linnoila [33] demonstrated that habitually violent criminals with a DSM-R III diagnosis of alcohol dependence had a low CSF concentration of 5-HIAA. In a follow-up and family history investigation, it was shown that low CSF 5-HIAA was predictive of recidivist violent criminality under the influence of alcohol. Furthermore, sons of an alcoholic father, who have been convicted of violent crimes, had significantly low CSF levels of 5-HIAA [34]. Early onset of impulsive violence among alcoholics, therefore, may represent physical and behavioral manifestations of an underlying defect in 5-HT function. Recognition and treatment of such individuals with serotonergic medications may reduce alcohol consumption and prevent aggressive outbursts.

In closing, the discovery of a naturally occurring structural variant of the 5-HT transporter gene in peripheral blood lymphocytes may provide us with an opportunity to elucidate the origin of complex behavioral traits with brain 5-HT function.

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