Elevation of blood β-carboline alkaloids in essential tremor

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Abstract

Background—β-Carboline alkaloids are normal body constituents but are also potent tremor-producing chemicals that are naturally present in the food chain.

Objective—To explore the hypothesis that high concentrations of β-carboline alkaloids are associated with essential tremor (ET).

Methods—One hundred cases and 100 controls were frequency matched on age, sex, and ethnicity. Blood concentrations of harmane and harmine were quantified by high-performance liquid chromatography, blinded to clinical information.

Results—The mean log blood concentration of harmane was higher in cases than controls (0.72 ± 0.53 vs 0.51 ± 0.64 g^{-10}/mL; $p = 0.01$). A nonparametric test on nontransformed data (median harmane = 5.21 g^{-10}/mL in cases and 2.28 g^{-10}/mL in controls) confirmed this difference ($p = 0.005$). The mean log blood concentration of harmine was 0.20 ± 0.48 g^{-10}/mL in cases and 0.10 ± 0.65 g^{-10}/mL in controls ($p = 0.20$). Log harmane concentrations were stratified based on the median value; 62% of cases vs 39% of controls had a high log harmane concentration ($p = 0.001$). Mean log harmane concentration was similar in the cases with (0.74 ± 0.58 g^{-10}/mL) and without (0.71 ± 0.50 g^{-10}/mL) an affected relative ($p = 0.83$).

Conclusions—Blood concentrations of harmane were measured in ET cases compared with controls. Concentrations were elevated in cases with and without a family history of ET.

Essential tremor (ET) affects 1 to 6% of the population.[1,2] The pathogenesis is poorly understood, but there may be cell damage or death in the cerebellum.[3] Although genetic susceptibility is important,[4] nongenetic factors may contribute to the etiology[5]; specific environmental factors, however, have not been identified.

The β-carboline alkaloids, including harmine and harmane, are a group of highly tremorogenic chemicals. Laboratory animals injected with large doses of these chemicals acutely exhibit an action tremor that resembles ET.[6] Human volunteers acutely exposed to

large doses display a reversible coarse tremor.[7,8] The effect of low-level, chronic exposure to β-carboline alkaloids is unknown.

Sources both exogenous and endogenous to the body exist. β-Carboline alkaloids are naturally present in small concentrations in the food chain, in commonly eaten plant-derived foods (wheat, rice, corn, barley), beverages (wine, beer, whisky, and sake), and inhaled substances (tobacco),[9,10] although their concentrations have been quantified in few food items. Also, these chemicals are normal body constituents and are produced in vivo by the cyclization of indole-alkylamines with aldehydes, occurring in most tissues in animals and humans.[11,12] Under physiologic conditions, the magnitude of exogenous vs endogenous sources has not been studied. Many β-carboline alkaloids are highly lipophilic,[13,14] and brain concentrations in rats are as much as 55 times higher than those of the plasma.[11] The specificity of individual β-carboline alkaloids for different brain receptors suggests that they probably have normal biological functions.[12] To explore the hypothesis that high concentrations of β-carboline alkaloids are associated with ET, blood concentrations of harmane and harmine, which are among the most tremorogenic and most well-studied β-carbolines, were measured in ET cases and control subjects.

Methods

Participants

Recruitment began in 1999. The 100 ET cases were initially ascertained from a community survey and then from a tertiary referral center. As described previously,[15] community cases had been participants in a population-based familial aggregation study of ET in the Washington Heights–Inwood community in northern Manhattan, NY (1995 to 1999). Seventeen were alive and had contact information when recruitment began for the current study. These were frequency matched by 5-year age strata, sex, and ethnicity (white, African American, Hispanic) to 17 control subjects from the same community-based study. An additional 83 ET cases were recruited between 2000 and 2002. All 83 were patients seen at the Neurologic Institute of New York, Columbia–Presbyterian Medical Center. They were identified from a computerized database listing names and diagnoses of all patients billed within the last 3 years supplemented by a computerized database at the Center for Parkinson's Disease and Other Movement Disorders, Columbia–Presbyterian Medical Center, which listed names and diagnoses of patients seen within the last 10 years. All patients had received a diagnosis of ET from their treating neurologist at the institute. ET patients were selected, alphabetically, for enrollment. Office records were reviewed, and patients with diagnoses or physical signs of dystonia, PD, or spinocerebellar ataxia were excluded. From 2000 to 2002, 83 control cases were identified from the New York metropolitan area using random digit dialing. These control cases were frequency matched to Columbia–Presbyterian Medical Center cases on 5-year age strata, sex, and ethnicity.

Clinical evaluation

A trained tester evaluated all subjects in person. The tester was trained for 1 month by a neurologist to administer clinical questionnaires and to perform a videotaped examination. Most evaluations were home visits and therefore were performed in the late morning or early

afternoon, making fasting levels impractical. Data suggest that plasma concentrations of harmane do not change significantly after meals.[16] In one study,[16] human subjects ingested food or ethanol, and plasma harmane concentrations were measured hourly for 8 hours. The concentration remained stable. The same investigators also demonstrated that variability in concentration was minimal over a longer (3-week) period.[16]

The tester collected demographic, clinical, and family history information. Data on current diet were collected using a Willett Semi-Quantitative Food-Frequency Questionnaire. This 20-minute food-frequency questionnaire[17] included questions on frequency of current consumption of 61 foods and on the use of vitamins and mineral supplements. Food frequency data may be used to compute mean daily caloric, protein, carbohydrate, fat, and ethanol intake. The questionnaire has shown good reliability and validity related to recent nutrient intake.[17] Subjects were considered current ethanol users if they drank at least one alcoholic beverage (beer, wine, other) per month. Current smoking status was assessed in each subject. Medical comorbidity was assessed with the Cumulative Illness Rating Scale, in which the subject reported the presence or absence of illness within 14 bodily systems (cardiac; vascular; respiratory; ear, nose and throat; upper gastrointestinal; lower gastrointestinal; hepatic; renal; genitourinary; musculoskeletal; hypertension; neurologic [other than ET]; endocrine; malignancy).[18] The names of all current medications were recorded separately and then also as 19 classes of medications (e.g., neuroleptic medications, cardiac medications, anticancer medications, narcotics, medications for ET, selective serotonin reuptake inhibitors, oral hypoglycemic agents).

Weight and height were assessed using a balance scale designed for field surveys (Scale-Tronix 5600; White Plains, NY) and a movable anthropometer (GPM Martin Type; Pfister, Carlstadt, NJ). Body mass index was calculated as weight in kilograms divided by the square of height in meters.

The tester videotaped a tremor examination in all 200 subjects,[15,19] and each of 12 videotaped action tremor items was rated by the neurologist on a scale from 0 to 3, resulting in a total tremor score (range = 0 to 36 [maximum]). The neurologist also noted the presence or absence of head tremor on the videotaped examination. The diagnosis of ET was confirmed by the neurologist using published diagnostic criteria (moderate amplitude tremor during three activities or a head tremor).[15,19] None of the cases or control subjects had PD or dystonia.

Blood harmane and harmine concentrations

During the clinical evaluation, phlebotomy was performed. Blood concentrations of harmane and harmine were measured, blinded to any clinical information including age, sex, and diagnosis. Samples were run in batches of 10, containing an approximately equal number (4 to 6) of cases and control specimens. A novel high-performance liquid chromatography (HPLC) method for quantifying harmane and harmine in blood has been reported previously.[20] One volume (9 to 12 mL) of whole blood was mixed with a half-volume (5 to 6 mL) of 1 mol/L NaOH. Following vortexing for 30 seconds, the samples were placed on a horizontal rotator and shaken at room temperature for 30 minutes. An aliquot (15 mL) of the extraction solution consisting of ethyl acetate and methyl-*t*-butyl ether (2:98, vol/vol) was

added to the tube. The tube was then vigorously shaken by hand for 1 to 2 minutes, followed by shaking on a horizontal rotor at room temperature for 45 minutes. After centrifugation at 3,000 g for 10 minutes, the upper organic phase was separated. The extraction procedure was repeated two additional times. The organic phase was combined and evaporated under nitrogen to dryness. The samples were reconstructed in 0.25 mL of methanol. After centrifugation at 3,000 g for 10 minutes, the supernatant was transferred to autosampler vials with sealed caps for HPLC analysis.

A Perkin–Elmer model LC-250 binary liquid chromatographic system (Foster City, CA) equipped with an LC-600 autosampler and an LS-40 fluorescence detector was used for separation and quantitation. Separation was accomplished using an ion-interaction, reversed-phase Econosphere C_{18} column (ODS2; 5 μm, 250 × 4.6 mm) attached to a Spherisorb guard column (ODS2; 5 μm, 10 × 4.6 mm). Both analytical and guard columns were purchased from Alltech (Deerfield, IL). An isocratic mobile phase consisted of 17.5 mmol/L of potassium phosphate buffer (pH 6.5; equal molar concentration of both monobasic and dibasic potassium salts) and methanol (30:70, vol/vol). A 50-μL aliquot of sample extracts was injected and the separation performed at room temperature at a flow rate of 1 mL/min. The detector was set at an excitation wavelength of 300 nm and an emission wavelength of 435 nm. A Macintosh computer equipped with Mac Integrator II (Rainin; Apple, New York, NY) was used to collect and analyze the data. The identity of harmane and harmine on high-performance liquid chromatographs previously has been clarified.[14,20] The intraday precision, measured as a coefficient of variation at 25 ng/mL, was 6.7% for harmane and 3.4% for harmine. The interday precision was 7.3% for harmane and 5.4% for harmine.[20]

Statistical analyses

The empirical distributions of both harmane and harmine were positively skewed. Using a one-sample Kolmogorov–Smirnov test, we tested whether harmane and harmine concentrations were normally distributed. Rejection of the null hypothesis (i.e., $p < 0.05$) is consistent with a nonnormal distribution. Neither harmane nor harmine was normally distributed (Kolmogorov–Smirnov test, $z = 4.23$ and 5.31, both $p < 0.001$). Therefore, harmane and harmine concentrations were logarithmically transformed and case-control differences compared using Student's t-tests. In confirmatory analyses, a nonparametric (Mann–Whitney U) test also was performed on harmane and harmine data that were not logarithmically transformed. In some analyses, log blood harmane and log blood harmine concentrations were stratified, based on the median values for the entire sample (n = 200), into those with high vs low values. Also, concentration quartiles were determined. χ^2 tests were used to analyze proportions and Student's t-test to examine group differences in continuous variables. Pearson (r) correlation coefficients were used to assess correlations between continuous variables. When comparing the proportion of cases vs controls who reported illness in each of the 14 bodily systems and when comparing the proportion of cases and controls who were taking a particular class of medication, Bonferroni corrections were used. To assess the null hypothesis that blood harmane concentration was not a predictor of diagnostic group (ET vs control), data were assessed using a logistic regression analysis with diagnostic group as the outcome and blood harmane concentration and age as

independent variables. This resulted in odds ratios (OR) with 95% CI. Statistical analyses were performed in SPSS (version 9.0; Chicago, IL).

Our sample size calculation necessitated 100 cases and 100 control subjects. This sample size would provide 80% power to detect a difference between cases and controls such that 60% of cases vs 40% of controls would have blood harmane or harmine concentrations above the median values for these chemicals.

Results

There were 100 ET cases and 100 controls (mean age = 68.4 ± 15.4 vs 64.7 ± 13.5 years; $t = 1.80$, $p = 0.07$). Sex was identical (57% women in each group) and ethnicity nearly identical (80 vs 81% white; $\chi^2 = 0.03$, $p = 0.86$). Three cases and two controls were current cigarette smokers. Mean ± SD pack-years did not differ between these three cases and two controls (10.3 ± 9.5 vs 15.0 ± 7.1 pack-years; $t = 0.58$, $p = 0.60$). Cases and controls did not differ in terms of the proportion who reported illness in each of the 14 bodily systems on the Cumulative Illness Rating Scale or, with the exception of medications for ET, in terms of the proportion who reported currently taking each of the different classes of medication. Cases had a higher mean total tremor score than controls (18.9 ± 8.1 vs 4.6 ± 3.0; $t = 16.6$, $p < 0.001$). Mean disease duration (cases) was 21.3 ± 19.3 years. Thirty-four cases were taking a medication to treat ET.

Using our control sample, we examined potential confounding variables. Age was marginally correlated with log blood harmane ($r = 0.18$, $p = 0.08$) and log blood harmine ($r = 0.16$, $p = 0.11$) concentrations. There was no difference between sexes in mean log blood harmane concentration. Mean log blood harmine concentration was −0.04 ± 0.61 g^{-10}/mL in men and 0.21 ± 0.66 g^{-10}/mL in women ($t = 1.92$, $p = 0.06$). There were no ethnic differences in mean log blood harmane (for whites = 0.53 ± 0.65 g^{-10}/mL vs nonwhites = 0.43 ± 0.64 g^{-10}/mL; $t = 0.58$, $p = 0.56$) or mean log blood harmine (for whites = 0.08 ± 0.63 g^{-10}/mL vs nonwhites = 0.17 ± 0.73 g^{-10}/mL; $t = 0.50$, $p = 0.62$) concentrations. There was no correlation between body mass index and log blood harmane or log blood harmine concentrations. In separate analyses for cases and controls, the mean log blood harmine and mean log blood harmane concentrations did not differ between current ethanol users and nonusers.

Log blood harmane concentration was correlated with log blood harmine concentration ($r = 0.28$, $p < 0.001$). The mean log blood harmane concentration was higher in cases than in controls (0.72 ± 0.53 vs 0.51 ± 0.64 g^{-10}/mL; $t = 2.56$, $p = 0.01$). A nonparametric test (Mann–Whitney U) on nontransformed data (median harmane = 5.21 g^{-10}/mL in cases and 2.28 g^{-10}/mL in controls) confirmed this difference ($z = 2.78$, $p = 0.005$). There was no difference in mean log blood harmane concentration in the cases who were taking a medication to treat ET vs those who were not (0.76 ± 0.59 vs 0.70 ± 0.51 g^{-10}/mL; $t = 0.52$, $p = 0.60$).

The mean log blood harmine concentration was 0.20 ± 0.48 g^{-10}/mL in cases and 0.10 ± 0.65 g^{-10}/mL in controls ($t = 1.29$, $p = 0.20$). A nonparametric test (Mann–Whitney U) on

nontransformed data (median harmine = 1.60 g^{-10}/mL in cases and 0.65 g^{-10}/mL in controls) provided similar results ($z = 1.32$, $p = 0.19$). Mean log blood harmine concentration did not differ in the cases who were taking a medication to treat ET vs those who were not (0.09 ± 0.55 vs 0.25 ± 0.44 g^{-10}/mL; $t = 1.56$, $p = 0.12$).

Strata were created based on the median log blood harmane (0.59 g^{-10}/mL) and median log blood harmine (0.18 g^{-10}/mL) concentrations. Sixty-two percent of cases vs 39% of controls had a high log blood harmane concentration ($\chi^2 = 10.58$, $p = 0.001$), and 56% of cases vs 44% of controls had a high log blood harmine concentration ($\chi^2 = 2.88$, $p = 0.09$). The log blood harmane and log blood harmine concentrations were further divided into quartiles. The proportion in each log blood harmane quartile differed in cases and controls ($\chi^2 = 12.31$, $p = 0.006$; table 1). The proportion in each log blood harmine quartile did not differ ($\chi^2 = 4.67$, $p = 0.20$; table 2).

In a logistic regression analysis, log blood harmane concentration (OR = 1.80, 95% CI = 1.10 to 2.93, $p = 0.02$) was associated with the outcome (diagnosis of ET vs normal), but age was not (OR = 1.02, 95% CI = 1.00 to 1.04, $p = 0.12$). In a logistic regression analysis, log blood harmane quartile (OR = 1.32, 95% CI = 1.02 to 1.69, $p = 0.03$) was associated with the outcome (diagnosis), but age was not (OR = 1.02, 95% CI = 1.00 to 1.04, $p = 0.13$).

Among ET cases, the correlation (r) between log blood harmane concentration and total tremor score was 0.16 ($p = 0.10$). There was no correlation between total tremor score and log blood harmine concentration ($r = -0.08$, $p = 0.43$). Disease duration was not correlated with either log blood harmane or log blood harmine concentration ($r = 0.03$, $p = 0.76$, and $r = -0.13$, $p = 0.24$). We stratified the ET cases by family history, and there was no difference in mean log blood harmane concentration in those with (n = 42; 0.74 ± 0.58 g^{-10}/mL) vs those without (n = 58; 0.71 ± 0.50 g^{-10}/mL; $t = 0.22$, $p = 0.83$) an affected relative. Results were similar for mean log blood harmine concentration (0.16 ± 0.54 vs 0.23 ± 0.44 g^{-10}/mL; $t = 0.72$, $p = 0.47$).

There were no differences between cases and controls in reported mean daily calories ($1,444.0 \pm 418.9$ vs 1420.4 ± 523.3 kcal; $t = 0.28$, $p = 0.78$) or mean daily grams of protein (67.1 ± 23.2 vs 64.0 ± 20.2 g; $t = 0.83$, $p = 0.41$), carbohydrate (182.0 ± 66.2 vs 178.2 ± 85.8 g; $t = 0.28$ $p = 0.78$), fat (27.1 ± 11.6 vs 27.2 ± 13.8 g; $t = 0.02$, $p = 0.98$), or ethanol (8.1 ± 12.9 vs 7.4 ± 10.1 g; $t = 0.35$, $p = 0.72$).

Discussion

Low doses of β-carboline alkaloids are present in food (e.g., 2×10^{-3} mg of harmane in one 8-ounce glass of wine).[9,10] β-Carboline alkaloids are normal body constituents as well.[11,12]

We demonstrated an increased blood concentration of harmane in ET cases. Concentrations of harmine and harmane were correlated. Harmane may be metabolized to form harmine.[14] Although concentrations of harmine were elevated in cases compared with controls, the difference was not significant. This consistency of direction, but difference in strength of association, suggests that the same process may have been operating more strongly for harmane than for harmine.

The normal physiologic level of harmane in whole blood or the relationship in humans between whole blood, urine, and CSF or CNS concentrations has not been studied. In rats given an intraperitoneal injection of norharmane, a related compound, brain concentrations were several fold higher than plasma concentrations and were proportional to the intraperitoneal dose.[11] Many β-carboline alkaloids are highly lipophilic, so that third-compartment sequestration in the brain is likely to occur.

This study did not provide an explanation for the case-control difference. Possibilities include increased exogenous (dietary) exposure or increased endogenous production or impaired metabolism in ET cases. Increased dietary exposure is difficult to assess because harmane and harmine concentrations have not been quantified in most food items. In one study, plasma concentrations were minimally variable over a 3-week period, suggesting that alterations in plasma β-carboline concentrations reflect long-standing metabolic alterations (i.e., increased formation or reduced metabolism).[16] Little is known about the normal endogenous production or the metabolism of these compounds in humans.

Some evidence has linked ET with an enhanced expression of brain catecholamines. In one study,[21] a high brain level of norepinephrine was observed in three ET cases compared with three controls. This observation is consistent with the efficacy of adrenergic β-receptor-blocking agents in treating ET.[22] Whereas peripheral β-adrenergic receptors probably mediate the effects of β-adrenergic-blocking agents, central mechanisms may be involved as well.[23,24] Norepinephrine is metabolized primarily by monoamine oxidase type A.[25] Several of the β-carbolines have been shown to inhibit monoamine oxidase,[26,27] particularly type A.[28,29]

We did not assess fasting levels of harmane and cannot exclude the possibility that the difference we observed may reflect a difference between cases and controls in dietary intake of harmane rather than an endogenous difference in the metabolism of harmane. Also, we did not assess liver function, variability in the cytochrome P450 system, or renal function to see whether these factors, which could influence the metabolism of harmane or harmine, differed in cases and controls.

Acknowledgments

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Table 1

Log blood harmane concentration by quartile

Quartile	ET cases, n = 100	Controls, n = 100
1 (lowest)	23	32
2	15	
3	34	
4 (highest)	28	22

$\chi^2 = 12.31$, $p = 0.006$.

ET = essential tremor.

Table 2

Log blood harmine concentration by quartile

Quartile	ET cases, n = 100	Controls, n = 100
1 (lowest)	12	21
2	32	35
3	30	20
4 (highest)	26	24

$\chi^2 = 4.67$, $p = 0.20$.

ET = essential tremor.