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BLOOD HARMANE (1-METHYL-9H-PYRIDO[3,4-*B*]INDOLE) CONCENTRATION IN ESSENTIAL TREMOR CASES IN SPAIN

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Abstract

Background—Environmental correlates for essential tremor (ET) are largely unexplored. The search for such environmental factors has involved the study of a number of neurotoxins. Harmane (1-methyl-9H-pyrido[3,4-*b*]indole) is a potent tremor-producing toxin. In two prior case-control studies in New York, we demonstrated that blood harmane concentration was elevated in ET patients vs. controls, and especially in familial ET cases. These findings, however, have been derived from a study of cases ascertained through a single tertiary referral center in New York.

Objective—Our objective was to determine whether blood harmane concentrations are elevated in familial and sporadic ET cases, ascertained from central Spain, compared to controls without ET.

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5. Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

Methods—Blood harmane concentrations were quantified by a well-established high performance liquid chromatography method.

Results—The median harmane concentrations were: 2.09 g^{-10}/ml (138 controls), 2.41 g^{-10}/ml (68 sporadic ET), and 2.90 g^{-10}/ml (62 familial ET). In an unadjusted logistic regression analysis, log blood harmane concentration was not significantly associated with diagnosis (familial ET vs. control): odds ratio = 1.56, p = 0.26. In a logistic regression analysis that adjusted for evaluation start time, which was an important confounding variable, the odds ratio increased to 2.35, p = 0.049.

Conclusions—Blood harmane levels were slightly elevated in a group of familial ET cases compared to a group of controls in Spain. These data seem to further extend our observations from New York to a second cohort of ET cases in Spain. This neurotoxin continues to be a source of interest for future confirmatory research.

Keywords

essential tremor; epidemiology; β-carboline alkaloid; harmane; toxin; environmental risk factors

1. Introduction

Essential tremor (ET) is one of the most commonly-encountered neurological diseases (Louis and Ferreira, 2010). Although its prevalence is highest among the elderly, ET may begin along the full age spectrum, from childhood through advanced age (Louis and Ferreira, 2010; Louis et al., 2001; Louis et al., 2009). Genetic factors clearly play a role in disease etiology (Tan and Schapira, 2008; Merner et al., 2012); non-genetic (environmental) factors have also been posited to play a role, either alone or through their interactions with genetic factors (Salemi et al., 1998; Jiménez-Jiménez et al., 2007; Louis, 2008). Environmental factors are thought to play a role in several other predominantly late life neurological diseases, including Parkinson's disease (PD) and Alzheimer's disease. Yet the environmental correlates for ET are largely unexplored.

The search for environmental factors in ET has involved the exploration of a number of neurotoxins, and especially the β-carboline alkaloids (Louis, 2008). β-carboline alkaloids are a group of neurotoxins that produce tremor. Laboratory animals injected with high doses acutely exhibit action tremor that shares clinical features with ET (Fuentes and Longo, 1971; Zetler et al., 1972). Human volunteers exposed to high doses display a coarse, reversible action tremor (Lewin, 1928).

Harmane (1-methyl-9H-pyrido[3,4-*b*]indole) is a potent tremor-producing β-carboline alkaloid; subcutaneously administered harmane produces tremor in laboratory animals (Zetler et al., 1972). Harmane is also very lipid soluble (Zetler et al., 1972), and broadly distributed within the rat brain in experimental settings (Moncrieff, 1989; Anderson et al., 2006). Harmane is produced endogenously, but it is among the most highly-concentrated β-carboline alkaloids in the human diet, especially in meats but also in many plant-derived foods; exogenous exposure is thought to be the main source of bodily exposure to harmane (Pfau and Skog, 2004).

In 2000, we hypothesized that this neurotoxin could play a role in the etiology of ET, and in 2002, demonstrated that blood harmane concentration was elevated in an initial sample of 100 ET patients compared with 100 controls enrolled in New York (Louis et al., 2002). Between 2002 and 2007, we assembled a new sample of 150 ET cases and 135 controls, again in New York; blood harmane concentrations were similarly elevated in those cases vs.

controls (Louis et al., 2008a). In the second study, the concentrations were most elevated in familial ET cases (Louis et al., 2008a).

Yet, our findings have been derived from a study of ET cases ascertained through a single tertiary referral center in New York. We asked the unique question whether, if one were to sample a group of ET cases on a different continent, would a similar pattern of elevated blood harmane be found? The aim of this case-control study, conducted between 2010 and 2011, was to determine whether blood harmane concentrations are elevated in ET cases (especially familial ET) ascertained from central Spain compared to of their counterparts without ET. Given prior results in New York (Louis et al., 2008a), we had a particular *a priori* interest in familial ET cases.

2. Methods

2.1 Participants

ET cases were recruited either from a population-based study in central Spain (Neurological Disorders in Central Spain, NEDICES) or two general neurology clinics in central Spain (Arévalo Health Center, Arévalo, Ávila, Spain, and Villaverde-El Cruce Health Center, Madrid, Spain) that were selected because of pre-existing research collaborations with the NEDICES investigators. Controls were recruited either from NEDICES, spouses of patients who came to the neurological clinics for reasons other than tremor (e.g., headache, dizziness), or from a geriatric social center in Madrid dedicated to promotion of cultural activities. Enrollment at clinics and social centers was consecutive, beginning in April 2010.

NEDICES is a longitudinal, population-based survey of the prevalence, incidence, and determinants of major age-associated conditions of the elderly (Morales et al., 2004; Vega et al., 2010). The study population was composed of elderly subjects, ≥65 years old, living in three communities in central Spain (Las Margaritas, Lista and Arévalo). Detailed accounts of the study population and sampling methods have been published (Morales et al., 2004; Vega et al., 2010). Between 2010 and 2011, all surviving ET cases were contacted and enrolled, as was an age-matched control (i.e., a participant in this population-based sample who did not have a neurological diagnosis). The Columbia University Internal Review Board and University Hospital “12 de Octubre” Ethics Board approved of all study procedures; written informed consent was obtained upon enrollment.

All diagnoses of ET were assigned by Spanish physicians (J.B.L, S.M.G., J.P.R., S.V., all neurologists or geriatricians) using NEDICES criteria (see full criteria in Louis et al. 2008b); all ET cases then underwent a detailed videotaped neurological examination. Each videotape was reviewed by a senior neurologist specializing in movement disorders (E.D.L.) who re-assessed ET diagnosis in each case using Washington Heights-Inwood Genetic Study of Essential Tremor diagnostic criteria (WHIGET, moderate or greater amplitude kinetic tremor during three or more activities or a head tremor, in the absence of PD) (Louis et al., 1998). Of the 138 ET cases who were videotaped, 130 (94.2%) met WHIGET criteria; two (1.4%) of 138 had dystonic tremor, one (0.7%) of 138 had ET and early PD, and 5 (3.6%) of 138 had action tremor that was not present during a minimal of three activities.

2.2 Clinical Evaluation

All cases and controls were evaluated in person by one of the Spanish physicians who administered clinical questionnaires and performed a videotaped examination. Study restraints required that most examinations take place in the early afternoons; therefore, fasting blood concentrations was impractical, but data were carefully collected as to the time/details of any food consumption during the day of testing.

The physician collected demographic, clinical and family history information using a structured questionnaire. ET cases were classified as having a family history of tremor if they reported at least one relative with tremor. Current smoking status was assessed in each subject. Medical comorbidity was assessed using the Cumulative Illness Rating Scale (CIRS, range = 0 – 42 [maximal co-morbidity]) (Linn et al., 1968). Weight and height were assessed, and body mass index (weight/height^2) was calculated and expressed as kg/m^2.

2.3 Blood Harmane Concentrations

As part of the clinical evaluation, phlebotomy was performed. Blood concentrations of harmane were measured blinded to all demographic, clinical and diagnostic information. Harmane concentrations in blood were quantified by a well-established high performance liquid chromatography method at Purdue University used in our previous studies (Zheng et al., 2000; Louis et al., 2002; Louis et al., 2008a).

2.4 Sample Size

In pre-study sample size calculations, which were based on pilot data from 10 ET cases and 10 controls in Spain (2008), enrollment of 150 ET cases and 150 controls would provide 93.2% power to detect as little as a 10% difference in blood harmane concentrations between cases and controls; enrollment of 130 ET cases and 130 controls would provide 89.5% power to detect as little as a 10% difference between cases and controls (assuming two-sided tests and alpha = 0.05).

2.5 Statistical Analyses

Statistical analyses were performed in SPSS (Version 19.0). Chi-square tests (X^2) were used to analyze proportions, and analysis of variance (ANOVA) and Student's t tests were used to examine group differences in continuous variables. Pearson (r) correlation coefficients were used to assess correlations between continuous variables.

Using a one-sample Kolmogorov-Smirnov test, we tested whether harmane concentration was normally distributed and it was not (Kolmogorov-Smirnov test, $z = 3.74$, $p < 0.001$). Therefore, harmane concentrations were logarithmically transformed; the log-transformed blood harmane values were normally distributed (Kolmogorov-Smirnov test, $z = 0.81$, $p = 0.53$).

Our previous studies have indicated that familial ET cases have the highest blood harmane concentrations (Louis et al., 2008a). To assess the null hypothesis that blood harmane concentration was not a predictor of diagnostic group (familial ET vs. control), we used logistic regression analysis to obtain odds ratios (OR) with 95% confidence intervals (CI). As in prior studies (Louis et al., 2002; Louis et al., 2008a), we considered a number of potential confounders (age in years, sex, race, years of education, body mass index, CIRS, current cigarette smoker, "yes" to the question "have you had something to eat today", and start time of the evaluation), and included these in the adjusted logistic regression analyses if they were associated with diagnosis and blood harmane concentration.

3. Results

There were 130 ET cases (62 familial ET and 68 sporadic ET) and 138 controls (Table 1). The three groups were similar in terms of age, sex, race, education, marital status, body mass index, smoking (Table 1). The groups differed in three respects: (1) while all controls had eaten something on the day of evaluation, a small percentage of cases (esp. familial cases) had not, (2) the evaluation start time was on average 1.5 –2 hours later in the control group, and thus, took place on average after rather than before the typical 2 – 3 pm mid-day

meal (lunch) in Madrid, (3) the CIRS was on average low, but was 1 point lower in controls than either case group.

Using our control sample, we examined the correlates of log blood harmane concentration. Log blood harmane concentration was positively correlated with evaluation start time (Pearson's r = 0.27, p = 0.006, i.e., later start time was associated with higher blood harmane concentration). It was not associated with CIRS score (Pearson's r = 0.06, p = 0.50). All controls had eaten something on the day of evaluation, so that it was not possible to assess the correlates of this variable within controls. There were no other relevant associations between log blood harmane concentration and clinical or demographic variables (e.g., gender, age, smoker).

The median harmane concentrations and mean log blood harmane concentrations are shown (Table 2); the highest median and mean values were in familial ET cases and the lowest were in controls (Table 2). The differences did not reach statistical significance (Table 2).

The potential association between familial ET and elevated blood harmane concentration was furthered considered. To further assess the role of confounding, logistic regression analyses were performed. First, in an unadjusted logistic regression analysis, log blood harmane concentration was not significantly associated with the outcome (familial ET vs. control) (OR = 1.56, 95% CI = 0.72 – 3.36, p = 0.26). Evaluation start time was a significant potential confounder because it was associated both with diagnosis and with log blood harmane concentration. In a logistic regression analysis that adjusted for evaluation start time, the OR increased to 2.35, 95% CI = 1.002 – 5.53, p = 0.049, indicating that accounting for this factor indeed enhanced the association. In that model, evaluation start time was also associated with the outcome (p = 0.001). Inclusion of other variables in the adjusted model (age, sex, education, body mass index, smoking, pack years, gender, medications) did not change the results.

There was no association between log blood harmane concentration and age of tremor onset in either familial ET cases (Pearson's r = −0.15, p = 0.27) or sporadic ET cases (Pearson's r = −0.13, p = 0.33).

Analyses in which we stratified ET cases and controls by source (NEDICES [20 familial ET, 20 sporadic ET, 13 controls] vs. non-NEDICES [42 familial ET, 48 sporadic ET, 125 controls]) did not change the results. Also, including source (NEDICES vs. non-NEDICES) in the final model that also adjusted for evaluation start time, the OR increased to 2.65, 95% CI = 1.10 – 6.40, p = 0.03, indicating that accounting for this factor only enhanced the association.

4. Discussion

Blood harmane levels were slightly elevated in a group of familial ET cases compared to a group of controls in Spain. These data seem to further extend our observations from New York to a second cohort of ET cases in Spain. This neurotoxin continues to be a source of interest for future confirmatory research.

β-carboline alkaloids such as harmane are a reasonable choice for investigation in the search for possible toxic environmental causes of ET. These toxins are structurally similar to MPTP, a neurotoxin which serves as one of the main animal models for PD (Langston et al., 1984). The administration of β-carboline alkaloids to a broad range of laboratory animals, including mice, cats, and monkeys, produces an action tremor that shares several clinical and drug-response features with ET (Fuentes and Longo, 1971; Du et al., 1997). β-carboline alkaloid administration is the main animal model for ET, and new pharmacotherapies are

being tested using exposed animals (Handforth and Krahl 2001; Martin and Handforth 2006).

In our earlier study, we demonstrated that blood harmane concentrations seemed to be highest among ET cases with a family history of ET (Louis et al., 2008a) These results were observed now in Spain. The higher concentration in familial ET cases suggests that the mechanism for this elevated concentration may be at least partly genetic and/or metabolic (i.e., possibly some combination of an inherited tendency for decreased metabolism in the setting of increased exposure). ET itself is a highly familial disorder although no genes have yet been identified.

The median harmane concentration we now observe in Spanish controls (2.09 g^{-10}/ml) is similar to that which we observed in controls in our 2008 study in New York (1.82 g^{-10}/ml) (Louis et al., 2008a). The same may be said for familial ET cases (2.90 g^{-10}/ml now vs. 3.39 g^{-10}/ml in our 2008 study) (Louis et al., 2008a). The median value for familial ET in Spain, though, was somewhat lower than reported in our initial 2002 study in New York (Louis et al., 2002). Differences in dietary factors could account for cohort differences.

This study had limitations. First, we did not assess fasting blood harmane concentrations; fasting concentrations are less likely to reflect recent food consumption. Study restraints required that most examinations take place in the afternoons; therefore, fasting blood concentration was impractical, but data were carefully collected regarding the timing of our evaluation and whether the study subject had fasted or not. Of interest is that the evaluation start time was later in controls than cases (on average, the start time was 1 – 2 hours after the mid-day meal in controls and before that meal in cases), and evaluation start time was correlated with blood harmane concentration, likely reflecting consumption of food at the mid-day meal. Therefore, evaluation start time was an important confounder to consider. Indeed, consideration of possible confounding effects of this factor in our models only served to increase the magnitude of the association between log blood harmane concentration and familial ET diagnosis. Second, ET cases were classified as having a family history of tremor if they reported at least one relative with tremor; prior research has shown that there is a tendency on the part of ET probands to both over-report and under-reported tremor in affected relatives; thus, the questionable validity of these reports likely had the effect of biasing our results towards the null hypothesis. Third, we did not assess liver function, variability in the cytochrome P450 system, or renal function to see whether factors that might influence the metabolism of harmane differed between cases and controls. This study also had several strengths. This is the first study outside of the United States, extending the results of earlier studies in New York. Blood harmane levels were measured in the same laboratory using identical analytic methods in all subjects; this is the same method used in our earlier studies. All blood harmane determinations were performed blinded to clinical information. In the analyses, we were able to assess the potential confounding effects of multiple relevant covariates.

In summary, genetic and environmental factors are likely to play a role in the etiology of ET, either alone or synergistically. The contribution of environmental factors to disease etiology has been examined in numerous epidemiological studies of PD as well as Alzheimer's disease and amyotrophic lateral sclerosis (Gorell et al., 1997; Gorell et al., 1998; Racette et al., 2001; Dick, 2006; Baldereschi et al., 2007; Morahan et al., 2007; Shcherbatykh and Carpenter, 2007). Yet the study of toxins in ET lags far behind. Our studies of harmane hold some promise; the environmental determinants for this tremor should continue to be explored.

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HIGHLIGHTS

- Environmental correlates for essential tremor (ET) are largely unexplored.
- Harmane (1-methyl-9H-pyrido[3,4-*b*]indole) is a potent tremor-producing toxin.
- Blood harmane levels were quantified by high performance liquid chromatography.
- Blood harmane levels were elevated in familial ET cases vs. controls in Spain.
- These data extend observations from New York to a second cohort of cases in Spain.

Table 1

Characteristics of 268 study subjects

Characteristic	Familial ET (N = 62)	Sporadic ET (N = 68)	Controls (N = 138)	Significance
Age in years	71.0 ± 13.2	71.0 ± 10.7	69.3 ± 11.1	ANOVA F = 0.72, p = 0.49
Age of tremor onset in years	47.4 ± 24.6	52.5 ± 21.7	Not applicable	t = 1.14, p = 0.26
Female sex	37 (59.7)	35 (51.5)	79 (57.2)	$X^2_{df=2}$ = 0.98, p = 0.61
White race	62 (100)	68 (100)	138 (100)	$X^2_{df=2}$ = 0.00, p = 1.00
Years of education	7.9 ± 5.0	7.5 ± 5.4	8.7 ± 4.7	ANOVA F = 1.36, p = 0.26
Currently married	42 (67.7)	46 (67.6)	99 (71.7)	$X^2_{df=2}$ = 0.52, p = 0.77
Body mass index in kg/m^2	27.8 ± 4.0	28.4 ± 5.0	27.9 ± 4.9	ANOVA F = 0.31, p = 0.73
Current cigarette smoker	9 (14.5)	5 (7.4)	15 (11.5)	$X^2_{df=2}$ = 1.71, p = 0.42
Number of cigarettes smoked on the day of the evaluation	0.4 ± 1.5	0.2 ± 1.3	0.4 ± 1.6	ANOVA F = 0.46, p = 0.63
Prescribed ET medication	23 (37.1)	26 (38.2)	Not applicable	$X^2_{df=1}$ = 0.02, p = 0.89
Answered “yes” to the question “have you had something to eat today”.	59 (95.2)	67 (98.5)	138 (100)	$X^2_{df=2}$ = 6.81, p = 0.03
Evaluation start time	2:25 pm	2:19 pm	3:57 pm	ANOVA F = 9.90, p < 0.001
Cumulative Illness Rating Scale (CIRS) score	4.6 ± 3.5	4.8 ± 3.6	3.4 ± 2.7	ANOVA F = 5.93, p = 0.003

Values are mean ± standard deviation, or numbers (percentages).

ANOVA = analysis of variance.

Table 2

Log blood harmane concentration by group

Group	Median harmane concentration in g^{-10}/ml	Mean ± S.D. log blood harmane concentration in g^{-10}/ml
Controls	2.09	0.33 ± 0.39
Sporadic ET	2.41	0.35 ± 0.51
Familial ET	2.90	0.40 ± 0.42
	Kruskal-Wallis test$_{df = 2}$ = 1.45, p = 0.48[1]	ANOVA $F_{df = 2}$ = 0.56, p = 0.58[2]

[1]For comparison of familial ET vs. controls, Mann Whitney z = 1.26, p = 0.21.

[2]For comparison of familial ET vs. controls, t = 1.13, p = 0.26.

In an unadjusted logistic regression analysis, log blood harmane concentration was not significantly associated with the outcome (familial ET vs. control) (OR = 1.56, 95% CI = 0.72 –3.36, p = 0.26). In a logistic regression analysis that adjusted for evaluation start time, the OR increased to 2.35, 95% CI = 1.002 – 5.53, p = 0.049. Also, including source (NEDICES vs. non-NEDICES) in the final model that also adjusted for evaluation start time, the OR increased to 2.65, 95% CI = 1.10 – 6.40, p = 0.03, indicating that accounting for this factor only enhanced the association.