CLINICAL ASPECTS

Impact of Tryptophan Metabolism on the Vulnerability to Alcohol-Related Blackouts and Violent Impulsive Behaviours

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(Received 3 October 2008; first review notified 9 December 2008; in revised form 12 June 2009 and 20 July 2009; accepted 24 July 2009)

Abstract — Aims: We examined (1) the association of SLC6A4 genotypes and alcohol dependence (AD) in a sample of alcoholics; (2) the validity of lifetime occurrence of blacked-out violent impulsive behaviour (BOVIB) during binge drinking bouts as a criterion for subtyping AD patients and (3) a mechanistic hypothesis for BOVIB involving tryptophan-2,3-dioxygenase (TDO) activity. Methods: Three common polymorphisms of the SLC6A4 gene (5-HTTLPR, A/G SNP of LPR region and VNTR in intron 2) were genotyped. An oral tryptophan (Trp) load (OTL) was administered to a sample of patients seeking help for AD. BOVIB history and psychological status were screened by BOVIB-Q, depression (BDI), anxiety (BAI, STAI) and personality (TCI) questionnaires. During the 7 h following Trp load, serum kynurenine (Kyn) and Trp were monitored. Results: BOVIB+ patients showed significantly higher scores on depression, anxiety and character scales but no significant association was found between SLC6A4 polymorphisms and BOVIB. Patients with a history of BOVIB (BOVIB+ subgroup) differed from those exempt from such episodes (BOVIB− subgroup) for TDO activity response to OTL assessed by the Kyn/Trp ratio (P = 0.043) and the slope of concentration increase ratio (SCIR) of serum Kyn (P = 0.043).

Conclusions: Put together, these findings support the validity of the BOVIB criterion to differentiate a sub-group of vulnerable AD subjects and suggest that OTL may help to concurrently define a specific endophenotype.

INTRODUCTION

Shifting from moderate non-harmful consumption of alcoholic drinks to deleterious alcohol dependence (AD) is highly specific to the human beings and is accounted for by complex interactions of environmental and genetic factors (Stoltenberg and Burmeister, 2000). As a whole, gene-environment studies on AD remain inconclusive, mainly due to the phenotypic heterogeneity of the population samples used in clinical research, as pointed out by many authors. Attempts have therefore been made to specify more homogeneous subtypes of alcoholics. From a clinical perspective, the level of impulsivity has commonly been used as the most consistent proposition of alcoholism typology in order to differentiate subgroups of alcohol-dependent subjects (Cloninger, 1987; Babor et al., 1992). In addition, the memory impairments that may black-out some episodes of impulsive and violent behaviours under alcohol intoxication (Perry et al., 2006) have been found to be of importance in discriminating heavy drinkers who actually bear one of the characterized diagnoses of alcohol use disorder (Hasin et al., 1997). From a neurobiological perspective, central serotonin (5-hydroxytryptamine, 5-HT) neurotransmission has been shown to modulate both alcohol consumption and impulsivity. Some variations in 5-HT neurotransmission may thus contribute to a risk of AD, especially the forms of AD associated with a high level of impulsivity (Sellers et al., 1992; Kranzler and Anton, 1994; LeMarquand et al., 1994; Zhou et al., 1994). As the extracellular concentration of 5-HT is regulated by the activity of the 5-HT transporter (5-HTTP) (Fabre et al., 2000), the gene SLC6A4 encoding this protein represents an important potential candidate gene for AD risk. The role of a polymorphism in the promoter region of SLC6A4 (5-HTTLPR) with demonstrated biochemical effects has been extensively investigated in diverse neuropsychiatric phenotypes in more than 300 studies (Glatt and Freimer, 2002). Using a meta-analysis approach, Feinn et al. found evidence for an association of the short (s) allele of the 5-HTTLPR with AD, but the overall effect size estimated by odd ratios was found weak (Feinn et al., 2005). However, association of the long (L) allele with AD has also been reported (Kweon et al., 2005), as well as an absence of association between the 5-HTTLPR and AD (Kranzler et al., 2002).

As expected in a complex condition like AD, the disagreement in the association between AD and 5-HTTLPR likely reflects the impossibility for a single genetic determinant to explain the whole of the risk. In addition to genotypic variations of 5-HTT functional capacity, central serotonergic neurotransmission is also highly dependent on the availability of serotonin precursor, the essential amino acid tryptophan (Trp). Acute ethanol consumption enhances the liver enzymatic activity of tryptophan-2,3-dioxygenase (TDO) (Buydens-Branchey et al., 1988; Banò et al., 1996), which catalyses the initial rate-limiting reaction of an oxidative metabolic pathway, resulting in an increase in L-kynurenine (Kyn) production at the expense of Trp availability (Badawy et al., 1995). In other words, the alcohol-related increase of TDO activity can induce a central depletion of the serotonin content by siphoning its metabolic precursor Trp. As the gene TDO2 encoding the enzyme TDO is inducible by steroids (Knox and Auerbach, 1955) and possibly by alcohol (Badawy, 2003), and has been shown to bear mutations associated with some psychiatric diseases (Comings et al., 1996; Nabi et al., 2004), variations in the individual response to alcohol may be linked to TDO2 polymorphisms. Besides, the metabolite Kyn readily crosses the blood brain barrier (BBB) and penetrates into the central nervous system (CNS), where it is metabolized by astrocytes of the glial tissue mainly into neuroactive metabolites (Swartz et al., 1990;
Some of those kynurenine derivates may potentiate the antagonistic action of alcohol on glutamatergic receptors and play a significant role in alcohol-induced amnesia (White and Best, 2000).

In the first part of our study, we examined the association of SLC6A4 genotypes and AD in a sample of alcoholics seeking help for AD from a French addiction clinic. In addition, we investigated whether the lifetime occurrence of alcohol-induced impulsive violent episode with amnesia constitutes a valuable criterion for subtyping AD patients and could reduce the inconsistencies reported in previous association studies. In the second part of the study, we tested this hypothesis by exploring TDO activity through an oral Trp load test and examined the relationship of serum Trp and Kyn kinetics with the anamnestic report of episodes of blacked-out violent impulsive behaviours.

MATERIALS AND METHODS

Patients
In the first part of the study, 31 alcohol-dependent patients seeking help from the addiction clinic of the University Hospital of Lille were included in the study that was approved by the ethic committee board of the Northern France region. The 31 alcohol-dependent subjects received a set of psychometric assessments including a questionnaire designed to document lifetime history of blacked-out violent impulsive behaviours (BOVIB). This questionnaire, named BOVIB-Q (Fig. 1), was used for patient subtyping, as follows: the AD patients who fulfilled the criteria of at least one BOVIB episode in their lifetime were classified BOVIB+ and those who did not, BOVIB−. The age of the 31 alcohol-dependent participants ranged between 26 and 57 years (mean age: 40 ± 8 years). The 17 patients’ BOVIB+ were aged between 26 and 56 years (mean age: 39.0 ± 7 years) and the 14 patients’ BOVIB−, 31 and 57 years (mean age: 43 ± 7 years). The difference of age was not statistically significant between the 17 BOVIB+ and 14 BOVIB− patients (P = 0.127).

In the second part of the study, the initial nine recruited BOVIB+ male patients and the initial nine recruited BOVIB− male patients were selected to participate in the biochemical challenge study, consisting of an oral tryptophan load (OTL) test. By Day 10 following admission for alcohol withdrawal, the patients were screened for present and past psychiatric Axis I diagnoses by a senior psychiatrist and received physical examinations, including electrocardiogram, blood and urine tests. These tests ensured that all patients (i) fulfilled inclusion criteria, i.e. alcohol physical dependence attested by an initial increase in the Cushman score during the first days of admission, age between 18 and 60 years and a willingness to participate and (ii) presented no non-inclusion criteria, i.e. any severe medical or psychiatric condition, HIV, HBV (other than isolated anti HBs) or HCV antibodies, stagnation or worsening of altered liver function if present on admission, recent consumption of psychoactive drugs (positive urine test), corticoid or psychotropic medications (other than systematic diazepam 10 days decreasing dose protocol used for alleviating alcohol withdrawal symptoms).

Psychometric measurements
For the purpose of our study, a specific questionnaire BOVIB-Q was designed to assess BOVIB. Self-report measures of nicotine dependence were collected by the Fagerstrom Tolerance Questionnaire. The Beck Depression and Beck Anxiety Inventories (BDI and BAI, respectively; Beck et al., 1961, 1988) were used as screening tools during the initial interview and as state-dependent (during the previous week) measures of depression and anxiety. Anxiety evaluation was completed by Spielberger’s State-Trait Anxiety Inventory (STAI-S, STAI-T). Cloninger’s Temperament Character Inventory (TCI) was used to assess personality characteristics, as some of them have been correlated with serotonergic functioning. TCI raw-score analysis measures several personality dimensions, i.e. novelty seeking (NS), harm avoidance (HA), reward dependence (RD), persistence (P), self-directedness (SD), cooperativeness (C) and self-transcendence (ST). With the view of bridging the gap...
between this dimensional approach of personality and psychopathology, the concept of ‘pathological personality’ has been defined by the authors and supported by other works (Bayon et al., 1996). Owing to the predictive power of low scoring at (C) and (SD) subscales for the risk of psychopathology (Svrakic et al., 1993), the algebraic sum of (C) and (SD) scores provides a simple and reliable indicator of the subjects’ protection against psychopathology. Conversely, withdrawing this ‘protection’ indicator from the sum of maximum scores results in a ‘risk’ predictor named ‘Pathological Personality Index’ (PPI) calculated as follows: PPI = 86 – (C + SD). In the last part of the study, i.e. the OTL test, self-report measures of nicotine dependence, mood state and biochemical measures of Tryptophan metabolism were related to TDO activity as either sources of confounding state-dependent interferences or additional expressions of TDO variability.

**SLC6A4 genotyping**

Genomic DNA from each of the 31 alcohol-dependent patients included in the study was extracted from venous blood collected in EDTA containers using the Nucleon BACC3 kit (Amersham-Biosciences, Saclay, France) according to the manufacturer’s instructions. Genotyping assays were carried out blindly without knowledge of the diagnostic status. Additionally, 49 genomic DNAs from a cohort of unrelated non-AD volunteers were genotyped and used as a control population. All the participants were French Caucasian and gave written informed consent.

We genotyped three common polymorphisms of the *SLC6A4* gene, i.e. the serotonin transporter-linked polymorphic region (5-HTTLPR), a single nucleotide polymorphism A/G (rs25531) in the long (L) allele of the LPR region and a variable number of tandem repeat in intron 2 (VNTR-2). Two distinct PCRs were carried out in a final volume of 25 μL of Tris–HCl buffer (pH 8.4) containing 50 mM KCl, 0.2 mM of each dNTP, 0.4 μM of each primer, 200 ng of genomic DNA, 0.625U of *Taq* DNA polymerase (Invitrogen, Cergy-Pontoise, France) and an optimized MgCl₂ concentration of 1 mM and 1.5 mM for the 5-HTTLPR and the VNTR-2 amplifications, respectively. Both primer sets used for amplification of the regions of interest in the *SLC6A4* gene have been previously described (Kaiser et al., 2002). After an initial 2-min denaturation step at 94°C, 35 cycles of 1-min denaturation step at 94°C, 1-min annealing step at an optimized temperature (64°C for 5-HTTLPR and 56°C for VNTR-2) and 1-min extension step at 72°C were carried out and followed by a 7-min final extension at 72°C. The 5-HTTLPR A/G polymorphism was subsequently detected by *MspI* endonuclease digestion as follows: 10 μL of the corresponding PCR fragment were digested overnight at 37°C with 10 U of *MspI* enzyme (Ozyme, Saint Quentin en Yvelines, France) in a final volume of 20 μL. The digested products were separated and visualized on an ethidium bromide-stained 8% acrylamide gel, the Lₐ allele is characterized by three fragments of 340, 127 and 62 bp, whereas the Lₐ allele is characterized by four fragments of 174, 166, 127 and 62 bp. An ethidium bromide-stained 2% agarose gel was run to separate the VNTR-2 amplicons and to identify the various VNTR with 9, 10 or 12 repeats.

**Tryptophan load**

Between Day 13 and Day 15 of withdrawal, all patients were referred to the Research Centre of the University Hospital, where they stayed in a quiet room from 07.30 to 16.30 under close supervision throughout the Trp challenge, with systematic record of any adverse or unexpected events. All patients were smokers and were periodically allowed to go out to smoke in the yard adjacent to the Research Centre. They received a standard light snack (apples and bread) at 12.00 and 16.00 and were free to drink water. At 09.00, dietary tryptophan 300 mg capsules were administered (50 mg/kg). After bladder emptying on arrival (urines kept for toxicological analysis), subsequent urines were collected from 08.00 to 16.00, and placed in a bottle with hydrochloric acid for 5-hydroxyindoleacetic acid (5HIAA) determination. An intravenous catheter was placed on forearm at 08.00 for all blood collections. Samples for kynurenine and tryptophan quantification were collected at 08.00, 09.30, 10.00, 11.00, 12.00 and 16.00 and were centrifuged at 3000 g for 5 min after standing for 1 h at room temperature. The obtained sera were stored at −80°C until analysis. Samples for serum and platelet serotonin determinations were collected at 08.00 (the latter was drawn in the EDTA anticoagulant). Concerning dietary Trp load performed in AD patients, only one reference was found in the literature (Friedman et al., 1988). The authors reported using a standard 2 g dietary Trp dose whereas we opted for an adaptation to body weight (50 mg/kg), as suggested by other authors (Costa et al., 1979; Truscott and Elderfield, 1995; Martinsone et al., 1996). In Friedman’s protocol, only two blood samples were drawn at 2 h and 4 h following Trp load, which is insufficient to determine accurate kinetics parameters (e.g. peak time). We also added early intermediate samples (at 30, 60 and 180 min) and a late one (at 7 h post-ingestion).

**Chemicals and serum compounds’ determination.** Tryptophan and kynurenine were quantified according to a rapid and specific HPLC protocol, as published previously (Vignau et al., 2004). Shortly, serum samples were precipitated with perchloric acid and centrifuged for 10 min at 64 g. Injection of the clear supernatant into the HPLC system allowed simultaneous quantification of tryptophan and kynurenine by fluorescence and UV detection, respectively. HPLC with coulometric electro-chemical detection—monitored by an internal standard—was used for quantification of serum and platelet serotonin (after sera precipitation and platelet concentrates lysis, respectively).

Apart from safety monitoring, no specific psychological assessment was performed during the tryptophan challenge. Contrary to research designs using Trp intravenously to test acute cognitive and mood changes within laboratory setting (Brummet et al., 2008), no relevant behavioural effect was expected from the oral administration of Trp in the present study. The administration of a single Trp dose is known to induce only inconsistent, mild and non-specific behavioural effect (Dougherty et al., 2007).

**STATISTICS**

Concerning allelic variations of *SLC6A4*, inter-group (controls × patients, controls × BOVIB+, controls × BOVIB− and BOVIB+ × BOVIB−) allele and genotype frequencies were compared with χ² tests and deviation from the Hardy–Weinberg equilibrium was assessed according to Mendell and
Simon using the Internet tool of the Institute for Human Genetics, Technical University Munich (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

For the analysis of the tryptophan load test, the independent variable was the history of BOVIB. Two indicators of TDO activity were extracted from the Kyn and Trp serum concentrations at −60 min, 30 min, 60 min, 120 min, 180 min and 420 min time points, and both were used as the main dependent variables. The first one consists of the kynurenine:tryptophan ratio (K:T) calculated at every time point (six values per subject). The second indicator of TDO activity is more complex and takes into account the kinetics of Kyn production. It is based on the calculation of the speed (slope) of concentration increase ratio (SCIR). Inter-group BOVIB x BOVIB+ comparisons for the six K:T values and the SCIR index, together with the various psychometric assessments, serum and urine biochemical levels, were performed using Student’s two-tailed t-tests, if applicable, or an alternative Mann–Whitney U-test, if the t-test not applicable.

RESULTS

Case control genetic study

Alcohol-dependent clinical subtyping. The data elicited by the BOVIB-Q are presented in Table 1. Around 90% of the reported BOVIB occurred after many drinks. Of the 26 subjects who experienced BOVIB, 19 (62% of total sample) reported at least one external intervention: 5 experienced the four types of external intervention (physician, emergency services, fire brigade and police), 4 required three types, 1 two types, 9 only one type. Nine of the fourteen BOVIB− subjects reported violent impulsive behaviour insufficient to classify them into BOVIB+ subgroup either because the disrupted behaviour never required external intervention (seven subjects) and/or because they were not covered by memory black-out (five subjects). Results of psychological assessment of AD patients are presented in Tables 2 and 3. Compared to BOVIB− subjects, BOVIB+ subjects scored higher at BDI (t = 3.31, P = 0.002), STAI-S (t = 5.85, P = 0.001) and STAI-T (t = 2.79, P < 0.009) self-rating scales. Concurrently, BOVIB+ subjects showed lower raw scores at the two TCI scales or subscales highly predictive of psychopathology, i.e. Responsibility (SD1) subscale (t = 2.81, P = 0.009), Cooperation scale (C) (t = 2.17, P = 0.040) and the Social Acceptance (C1) (t = 2.66, P = 0.013) and Compassion (C4) (t = 3.07, P = 0.005) subscales. Logically, BOVIB+ scored higher at PPI (t = 2.53, P = 0.018), a composite score calculated from C and SD scales. However, no difference was found between BOVIB+ and BOVIB− for the four TCI scales assessing Temperament (Novelty Seeking, Harm Avoidance, Reward Dependence and Persistence).

SLC6A4 genotypes. Concerning the triallelic (A/G) 5-HTTLPR polymorphism, inter-group comparisons (Controls × AD, Controls × BOVIB+, Controls × BOVIB− and BOVIB+ × BOVIB−) of the respective distributions of genotype and allele frequencies did not show any significant differences (data not shown).

Similarly, when Lg and S alleles were grouped together, as they both represent low expressing alleles of SLC6A4, there were still no significant differences either in allele frequency or in genotype distribution (data not shown). For the VNTR-2 of SLC6A4, inter-group comparisons (Controls × AD, Controls × BOVIB+, Controls × BOVIB− and BOVIB+ × BOVIB−) of the respective distributions of genotype and allele frequencies did not show any significant differences (data not shown).
Oral tryptophan load

Within the group of AD patients who participated in the OTL test, the 9 BOVIB+ subjects aged between 34 and 56 years (mean age: 39.0 ± 7 years) and the 9 BOVIB− subjects between 31 and 57 years (mean age: 42 ± 8 years). The age difference between the BOVIB+ and the BOVIB− patients was not statistically significant (P = 0.354). The psychometric and genetic characteristics of these two subgroups of AD patients did not differ from those of the respective groups to which they belonged, i.e. the BOVIB+ and BOVIB− groups. Due to smaller sample sizes, less statistical significance in data analysis was elicited compared to BOVIB− and BOVIB+ total samples, but the same trends were observed for higher psychopathology level in BOVIB+ subjects versus BOVIB− subjects (data not shown).

In contrast, patients’ baseline biological profile reveals no statistical difference between BOVIB− and BOVIB+ subgroups concerning serum concentrations of Trp (respectively 47.3 μmol/L ± 8.5 versus 42.3 ± 14.9), Kyn (221.5 μmol/L ± 0.36 versus 191.1 ± 0.50), 5-HT (94.4 μg/L ± 21.0 versus 88.3 ± 47.4) and platelet 5-HT concentrations (469.2 ng/10^9 platelets ± 41.0 versus 418.0 ± 245.5).

As expected, a substantial increase of serum Trp was recorded in patients, following the ingestion of dietary tryptophan capsules, with a concentration peak reaching 800% on average, at 2 h (Fig. 2). Serum Kyn levels followed a similar and delayed pattern, increasing by an average of 700%, with a 1-h lag compared to the Trp response (Fig. 3). Mann and Whitney U-tests showed a significant group effect for the last measure of K:T at 7 h (Fig. 4) and for the mean SCIR (Fig. 5). In BOVIB− and BOVIB+ subgroups, K:T at 7 h were respectively 8.28% ± 2.42 versus 13.04 ± 6.06 (Mann–Whitney U-test P = 0.0469), and mean SCIR values, 267 ± 116 versus 423 ± 206 (Mann–Whitney U-test P = 0.0305). The area under the concentration–time curves (AUC) for tryptophan and kynurenine were calculated for each subject. The inter-group comparison of mean AUC values showed no significant difference between BOVIB− and BOVIB+ samples (data not shown).

DISCUSSION

Our pilot study points towards several reasons for considering the co-occurrence of episodes of alcohol-induced black-outs with violent impulsive behaviours (BOVIB) in the lifetime of AD subjects. In the absence of a validated tool, we designed a new short questionnaire (BOVIB-Q) that we tested on a sample of 31 inpatients consecutively recruited among the population referred to our clinic. The original design of the BOVIB-Q questionnaire relies on the conjunction of two different effects commonly observed after alcohol use. The first one is the
occurrence of an acute and severe disruption in the behaviour of the individual with externalized manifestations of impulsivity and violence. A large body of evidence collected both in real-life environments and in experimental settings has confirmed the intuitive assumption that alcohol consumption, especially heavy or binge drinking, is a determining causal factor of violent impulsive behaviours—VIB (Gustafson, 1985, 1989; Murdoch et al., 1990; Lau and Pihl, 1994; Hoaken and Stewart, 2003; Richardson and Budd, 2003; Daeppen et al., 2005; Miller et al., 2007). For the BOVIB-, factual data attested by the necessity of an external intervention of various emergency services were privileged information in order to minimize the subjectivity of the retrospective appreciation of the violence. Our study confirms the high frequency of VIB in the population seeking help for alcohol use from specialized clinics (84% of the total sample of AD patients, 62% if the criterion of external intervention is taken into account).

This confirms that Type II or ‘male-limited’ subtype (characterized by no maternal alcohol problems and biological fathers with a history of criminal activity and an early onset of alcohol abuse) is over-represented in treatment-seeker alcoholics (Weisner, 1993; Grant, 1996). The second alcohol-induced effect screened by BOVIB-Q relates to memory impairment. Typically, alcohol-induced episodes of amnesia alter the person’s ability to form new memories during the intoxication, with no effect on the memories formed previously (Goodwin, 1995). Partial amnesia or complete black-outs have been reported in all forms of alcohol binge drinking, both in clinical and general populations (e.g. college students). In these studies, the most frequent behavioural manifestations of the blackout episode are at-risk behaviours including some impulsiveness and the exposition to various potential sources of danger (White et al., 2002). The BOVIB-Q includes only one item, addressing the en bloc versus fragmentary variety of memory impairment, because the former has been pointed as the most characteristic and consistent form of alcohol-induced blackouts by previous studies (Goodwin et al., 1969; White et al., 2004). In the psychiatric literature, the combination of both alcohol-related VIB and blackouts in alcohol-dependant (Batabyal and Yeh, 2007) clinical samples is well documented (Branchey et al., 1985; Roy et al., 1986) and is known in the traditional French psychiatric nosography as ‘Complicated Intoxication’. Surprisingly, despite apparent consistency, this clinical sequence (VIB secondarily covered by memory blackout), highly imputable to the binge drinking pattern of alcohol use, has not received official recognition as a specific discrete diagnostic category. In our study, around 90% of the BOVIBs were related to binge drinking—defined as the consumption of five or more alcoholic drinks on one occasion for a man or four or more drinks on a single occasion for a woman (National Institute on Alcohol Abuse and Alcoholism, Winter 2004)—excluding the diagnosis of idiosyncratic intoxication. Furthermore, AD patients fulfilling the BOVIB criterion revealed to present a high level of psychopathology as attested by the higher scores on BDI, STAI-S,
STAI-T and TCI-PPI scales. More sophisticated analyses (multivariate) requiring larger sample populations would be useful to explore the relationship between lifetime history of BOVIB and psychopathology. From present data, we can assume that the relatively poor social and individual maturity pointed out by the TCI Character subscale, including the propensity to blame others and to withdraw from personal responsibility, may be a source of overestimation of the allegation of BOVIB. Actually, many forensic medicine and legal papers often question the validity of the claim of amnesia in court, especially in the case of criminal violent acts committed under alcoholic intoxication (van Oorsouw et al., 2004).

Convincing evidence supports the notion that the complicated forms of alcoholism (combining co-morbid psychiatric condition or dissociative traits) are associated with serotonin transporter (5-HTT) gene (SLC6A4) polymorphisms (Sandet et al., 1998; Hallikainen et al., 1999; Feinn et al., 2005). Since HPA’s response to stress is being modulated by serotonin (Oroszi and Goldman, 2004), some authors have suggested, on the grounds of data from nonhuman primate models, that SLC6A4 geno-type may influence the consequences of early stressful events and pave the way for later alcohol preference at adolescence (Bennett et al., 2002; Barta et al., 2004). This speculation is corroborated by another (non-significant) trend in the BOVIB+ population to display lower platelet serotonin levels, previously shown to be linked to alcoholism (Pivac et al., 2004), especially when depressed mood accompanies the early phase of withdrawal (Bailly et al., 1993). Nevertheless, effect of SLC6A4 polymorphisms remains low, with odds ratio around 1.18 (Feinn et al., 2005) and, together with the weak effective of our samples, may explain why we found no significant association between AD patients (total sample or BOVIB+ subgroup) and non-AD controls.

This study is the first to demonstrate that alcohol-dependent subjects differ in their enzymatic TDO activity, according to clinical characteristics. TDO activity in response to the oral tryptophan load (OTL) actually uncovered a more persistent elevation of the kynurenine–tryptophan ratio (K:T) and a more abrupt initial increase of the kynurenine concentration (SCIR) in AD patients who report BOVIB episodes in their lifetime than those who did not report such episodes. Although these converging results point towards a correlation between BOVIB occurrence and enhanced TDO activity, several methodological and mechanistic issues need to be clarified.

The first methodological issue in our study was the use of a non-validated tool to form the subgroups on which the biochemical comparisons were subsequently performed. As we discussed above, a positive diagnosis of BOVIB (derived from the BOVIB-Q) consistently correlated with higher scores on several quantitative psychological assessments (BDI, STAI, TCI-226). Nevertheless, considered one by one, none of these quantitative assessments correlated with any of the biochemical variables of OTL, when systematic linear regression tests were performed on the total sample of the 18 participants (data not shown). The unique study exploring Trp levels along with the history of alcohol-induced blackouts (Branchey et al., 1985) was based on the baseline Trp serum concentration. This indicates the necessity of a conjunction of several clinical factors to correlate significantly with the biochemical markers. Another potential source of Type 1 error in the correlations found between BOVIB diagnosis and the substrate-metabolite measures of TDO activity is an inter-individual variation in the dietary Trp digestive absorption, prior to reaching liver TDO. This could have jeopardized the slope of initial Kyn increase upon which the SCIR variable has been calculated. Similarly, a large dispersion of Trp and Kyn values is observed as soon as within the 30 min post-OTL. At 7 h, the mean Kyn concentrations are equivalent in both groups and the difference observed in K:T seems to be attributable to larger differences in Trp concentrations. In the ratio calculation, both Kyn (numerator) and Trp (denominator) mean levels were slightly higher in BOVIB+ subgroup, excluding a simple artificial amplification of two minor opposite differences. The examination of the patients’ individual K:T and the means obtained from these individual values support the assumption that there are substantial inter-individual variations in the intensity of TDO activity. For other reasons discussed further, differences in the severity of alcohol withdrawal are susceptible to represent additional confounding factor acting at the level of TDO enzymatic activity. Kynurenine synthesis is influenced by alcohol withdrawal in the form of an induction of TDO, attested by an increase of the kynurenine urine level in humans (Buydens-Branchey et al., 1988) and by an augmentation of TDO activity and mRNA expression in animal models (Bano et al., 1996). However, the authors showed that ethanol impacted TDO activity during the early phase of withdrawal, and, in the study, the two-week delay before OTL makes it unlikely for these effects to challenge significantly our results. Furthermore, there were neither biological nor clinical inter-group differences in the intensity of withdrawal symptoms monitored on the ward, which could explain those observed in serum kynurenine kinetics. Another Type 1 error that may have altered the measurements of TDO activity is represented by the potential confounding effect generated by carbohydrates intake during the course of the trial. In order to minimize this bias, a standardized light snack was systematically dispensed to all participants. We chose not to exclude carbohydrate intake since protracted, i.e.7-h added to overnight, fasting would have biased our results in an even worse manner, by triggering metabolic processes (neoglucogenesis) with potentially considerable individual differences due to the heterogeneous nutritional status of chronic alcoholic subjects.

The postulated mechanism linking alcohol consumption, TDO activity and BOVIB occurrence involves the following three aspects: (i) induction of liver TDO by alcohol binge drinking, producing two central effects, i.e. (ii) a depletion of central serotonergic function contributing to VIB through the siphoning of 5-HT precursor Trp and (iii) an excess of Kyn neuroactive metabolites contributing to blackouts by a potentiation of alcohol on neuroinhibitory transmission.

The first mechanistic issue is the parallel that can be made between the effects of alcohol on TDO activity under ‘ecologic’ conditions (binge drinking) and the effect of OTL on TDO in the experimental setting. The question arises to what extent the conclusions derived from the latter can be transferred to the former. Due to a low affinity for its substrate, TDO activity is physiologically low under basal conditions (e.g. after a fasting night) and is triggered by the afflux of dietary Trp by an important post-translational regulation process that can be explored by OTL (Minatogawa et al., 1991). TDO being a haem-containing enzyme present as an inactive form with its prosthetic group in the haem-ferric form, the afflux of Trp results in the reduction of TDO to the haem-ferrous active form (heme-Fe2+),...
capable of oxidizing Trp efficaciously (Batabyal and Yeh, 2007). By contrast, alcohol binges may act through a transcriptional mechanism mediated by glucocorticoids. Apart from moderate doses of alcohol, producing an inconstant rise in TDO activity independent of endocrine mechanisms (Badawy et al., 1995), the consumption of large amounts of ethanol is followed by a dramatic increase in glucocorticoid secretion and TDO activity, in both animal models (Gjerde et al., 1985; Morland et al., 1985) and AD human subjects (Kutscher et al., 2002). TDO2 is one of the first glucocorticoid responsive genes ever described in enzyme physiology (Knox and Auerbach, 1955) and since these early works, the stimulating effect of glucocorticoids on TDO has been confirmed (Danesch et al., 1983; Sadler et al., 1984; Nakamura et al., 1987) and a glucocorticoid responsive element (GRE) sequence has been described in the promoter region of TDO2 (Comings et al., 1995). The two different regulation processes underlying the substrate and endocrine induction of TDO may have a synergistic action, as a transcriptional stimulation by glucocorticoids is responsible for an increase in the enzymatic potential (larger stock of inactive form of the enzyme) unapparent under basal conditions and unmasked by the post-translational challenge represented by OTL. Variations in either genetic TDO2 inducibility or level of glucocorticoid secretion may explain differential vulnerability to glucocorticoid-mediated alcohol-induced BOVIB. One of the mechanisms to explain the interindividual variability of TDO inducibility would be polymorphisms on the TDO2 gene, in particular polymorphisms affecting consensus sequences involved in TDO2 expression regulation. Screening of the TDO2 promoter region would then be of particular interest to explore the potential genetic origin of the variability of TDO induction. Another mechanism to explain the variations in the vulnerability to BOVIB would be a variation in the response of the hypothalamic pituitary adrenal (HPA) axis to alcohol. An indirect cause of the over-stimulation of the HPA axis could be a depression of the mood, on account of the extensive evidence linking the two phenomena (Joyce and Paykel, 1989). In the study, the observed high scores at the Beck Depression Inventory related to BOVIB diagnosis give support to this mechanistic hypothesis.

In conclusion, provided BOVIB-Q basic reliability is confirmed by further research, the study encouragingly supports its concurrent validity in spite of the weak sample size. BOVIB diagnosis appears to be linked to both psychological and biochemical specific profiles and may represent a valuable criterion to better define homogenous subtypes of AD subjects. In particular, the preliminary results of the OTL study show a significant difference in the TDO response between the subgroups of AD patients defined by BOVIB diagnosis. Larger samples are required to confirm these conclusions. The analysis of SLC6A4 polymorphism was not able to confirm or invalidate the association with BOVIB diagnosis. However, our results suggest a potentially important contribution of genetic variations of the gene encoding TDO, especially the regulation regions of the promoter, the analysis of which has already been initiated in our lab. Finally, two mechanistic issues remain unresolved and need further exploration, namely the respective contributions to VIB of the depleting effect of alcohol on central serotonin and that of increased Kyn metabolites to memory impairments characterizing BOVIB episodes.

Acknowledgements — This study was supported by the Université Lille Nord de France, the Centre Hospitalier et Universitaire de Lille and the Conseil Régional Nord-Pas de Calais. This work is part of a research program of the Pôle Interdisciplinaire sur les Conduites Addictives (PIRCAAd). We extend our gratitude to Professor Christian Libersa, Centre d’Investigations Cliniques, CHU Lille, for his great help. Marion Soichot is co-funded by the CHU Lille and the Conseil Régional Nord-Pas de Calais. We are grateful to Dr. S. W. Ellis for the English revision of the manuscript.

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