PHARMACOLOGY AND CELL METABOLISM

Alteration of Glutamate/GABA Balance During Acute Alcohol Withdrawal in Emergency Department: A Prospective Analysis

G. Brousse1,2,3,*, B. Arnaud1, F. Vorspan4, D. Richard5,6, A. Dissard1, M. Dubois1, D. Pic1, J. Geneste1, L. Xavier1, N. Authier2,5,6, V. Sapin2,7, P.-M. Llorca2,3,8, I. De Chazeron3,8, R. Minet-Quinard2,7 and J. Schmidt1,2

1CHU Clermont Ferrand, Urgences Adultes, 28 Place Henri Dunant BP 69, 63003 Clermont-Ferrand Cedex 01, France, 2Université Clermont 1, UFR Médecine, Place Henri Dunant, Clermont-Ferrand F-63001, France, 3Université Clermont 1, UFR Médecine, EA 3845, Clermont-Ferrand F63001, France, 4Inserm U705, UMR CNRS 8206, Neuropsychopharmacologie des Addictions, Université Paris Diderot, Hôpital Fernand Widal Assistance Publique des Hôpitaux de Paris, 200 rue du Fg Saint-Denis, 75 475 Paris Cedex 10, France, 5CHU Clermont-Ferrand, Service de Pharmacie, Hôpital G. Montpied, F-63003 Clermont-Ferrand, France, 6INSERM 1107, Neurodol, Université d’Avergne, BP38, 63001 Clermont-Ferrand, France, 7Department of Biochemistry and Molecular Biology, Laboratoire de biochimie, CHU Gabriel Montpied, Rue Montalembert, BP69, 63003 Clermont Ferrand CD1, France and 8CHU Clermont Ferrand, Service Psychiatrie de l’adulte CMP B rue Montalembert 63003 Clermont-Ferrand Cedex 01, France
*Corresponding author. Tel.: +33-04-73-754-785; Fax: +33-04-73-754-781; E-mail: gbrousse@chu-clermontferrand.fr

(Received 1 December 2011; first review notified 13 January 2012; in revised form 4 June 2012; accepted 12 June 2012)

Abstract — Aims: Animal studies suggest that in alcohol withdrawal the balance of neurotransmitters gamma aminobutyric acid (GABA) and glutamate is altered. To test this in humans, we aimed to measure plasma levels of glutamate, GABA and glutamate/GABA ratio in alcoholics patients presenting with complicated AWS with the same values in non-alcohol abuser-dependent controls and to determine prognostic factors for severe withdrawal. Methods: 88 patients admitted to the emergency room for acute alcohol intoxication (DSM-IV) were prospectively included. Measurements of GABA and glutamate were performed on admission (Time 1, T1) and after 12 ± 2 h (T2). The experimental group (EG) was composed of 23 patients who presented at T2 with a severe AWS. The control group (CG) consisted of healthy subjects paired with the EG (gender and age). Logistic regression was performed in order to compare associated clinical and biological variables that could predict severe withdrawal. Results: The concentration of GABA in the EG at T1 was significantly lower than that in the CG. The concentration of glutamate in the EG at T1 was significantly higher than that in the CG. The glutamate/GABA ratio in the EG at T1 was significantly higher than the ratio in the CG. With a multivariate logistic regression model, glutamate level at admission remained the only criterion identified as a predictor of AWS at 12 h. Conclusion: Decreased synthesis of GABA and increased synthesis of glutamate might be related to withdrawal symptoms experienced on brutal cessation of chronic alcohol intake.

INTRODUCTION

About 50% of alcohol-dependent patients develop clinically relevant symptoms of withdrawal (Schuckit, 2009). Clinical features of alcohol withdrawal syndrome (AWS), which follow the cessation of regular high-dose alcohol ingestion as soon as the blood level decreases significantly, are quite common in emergency rooms (Etherington, 1996a,b; Hall and Zador, 1997; Holbrook et al., 1999a). AWS occurs in 8% of hospitalized patients in general hospitals and between 10 and 15% of patients during a programmed detoxification (Foy and Kay, 1995; Monte et al., 2009).

Commonly, AWS is divided into three sets of symptoms (Hall and Zador, 1997): The first consists of symptoms of autonomic hyperactivity, which appear within hours of the last drink and usually peak within 24 h. The most common features are trembling, sweating, nausea, vomiting, anxiety and agitation. The second set of symptoms concerns neuronal excitation and includes epileptiform seizures and global confusion, usually occurring within 24–48 h of abstinence. The third set of symptoms comprises delirium tremens or alcohol withdrawal delirium (AWD) with auditory and visual hallucinations, confusion and disorientation, clouding of consciousness, impaired attention and pronounced autonomic hyperactivity (Knot et al., 1981; Kosten and O’Connor, 2003; Mayo-Smith et al., 2004). AWD would occur in 5–20% of alcohol-dependent individuals who present in inner city hospitals for detoxification (Ferguson et al., 1996). If AWD is untreated, death may occur from respiratory or cardiovascular collapse (Cushman, 1987). Treatment today consists of good supportive care, including intravenous fluids, thiamine, correction of electrolyte disorders and benzodiazepines therapy delivered according to a symptom-triggered approach (Holbrook et al., 1999b). The aim of the treatment is to prevent fatal progression and to relieve uncomfortable symptoms (Holbrook et al., 1999b; Kosten and O’Connor, 2003; Mayo-Smith et al., 2004). Indeed, during alcohol withdrawal, the symptoms that are perceived negatively by the patients play an important role in the continuation of alcohol dependence, alcohol craving and relapse (Koob, 2003, Heilig et al., 2010).

AWS in emergency rooms ranges from uncomplicated withdrawal to delirium tremens (Etherington, 1996b). Diagnosing acute alcohol-related conditions is challenging for emergency-room physicians who have to treat discomfort and to prevent complications of AWS, particularly a problematic evolution to AWD (Etherington, 1996b). Several factors have been associated with severe withdrawal. These include the number of days since the last drink (Ferguson et al., 1996), patients referred from emergency room to detoxification unit (Mennecier et al., 2008), age (Liskow et al., 1989), prior complicated alcohol withdrawal, medical serious co-morbidity, elevated blood pressure (Ferguson et al., 1996; Fiellin et al., 2002) and amino transferase level (Mennecier et al., 2008).

Most of the symptoms observed during the withdrawal period seem to be related to the adaptive changes in central inhibitory and excitatory systems that result from continuous...
alcohol intake (Tsai, 1998; Koob, 2003; Esel, 2006; Vengeliene et al., 2008). A down-regulation of gamma-aminobutyric acid (GABA) central inhibition confirmed in animal (Faingold et al., 2000; Cagetti et al., 2003) and human (Gomez et al., 2012) studies appears commonly as one of the main causes of insufficient central inhibition during alcohol withdrawal, which leads to the symptoms of hyperexcitation and to relapse (Dahchour and De Witte, 2003a; De Witte et al., 2003; De Witte, 2004). Otherwise, raised extracellular glutamate levels during alcohol withdrawal have been observed in animals (Rossetti and Carboni, 1995). Thus, Dahchour et al. (1998) reported an augmentation of the extra cellular level of glutamate by 300% in animal models during acute withdrawal. The increase in glutamatergic activity could be responsible for many significant symptoms of withdrawal such as hyperexcitation, anxiety and epileptic seizures. This increased activity can be also responsible for the neurodegeneration that develops during withdrawal periods (Kretschmer et al., 2002; Nagy et al., 2003).

Few human studies have partially confirmed dysregulation in glutamate/GABA ratio during withdrawal syndrome. Recently, Hermann et al. (2012) have measured brain glutamate levels during detoxification in 47 alcohol-dependent patients and in 57 healthy control subjects, and found significantly increased glutamate levels during acute withdrawal in prefrontocortical regions. Monitoring for increase in intracerebral glutamate concentration and decrease in GABA concentration could have diagnostic and predictive value. However, in clinical conditions (emergency department), it is impractical to employ the invasive instrumentation required to determine directly central nervous system glutamate or GABA levels. To that end, some researchers have quantified systemic glutamate and GABA concentration (most often in plasma) as an indirect measure of brain glutamate efflux. The first study in emergency room was carried out by Aliyev et al. (1994). They studied plasma levels of glutamate and GABA in 20 male patients following alcohol withdrawal and in 20 normal controls. The levels of glutamate were higher and the value of GABA was lower in the patients than in the controls. The correlation between the plasma level of excitatory amino acid and the rating of subjective discomfort was positive. Otherwise, in 2002, Aliyev and Aliyev (2002) reported comparisons of plasma levels of GABA and glutamate in 106 men. The subjects for this study were 46 patients hospitalized for delirium tremens, 20 patients with an AWS, 20 alcohol-dependent patients and 20 non-alcoholic controls. In this analysis, subjects with delirium had significantly lower serum values for GABA and higher values for glutamate. Moreover, Tsai et al. (1998) have published data showing an augmentation of glutamate in cerebrospinal fluid (CSF) and a diminution of GABA in alcohol-dependent patients compared with non-alcoholics 1 week and 1 month after cessation of ethanol ingestion. All these studies confirm a possible plasma dysregulation of glutamate/GABA balance during withdrawal, reflecting brain dysregulation.

To our knowledge, there are no data on prospective measures of plasma concentration of GABA and glutamate during the first hours of alcohol cessation and the physiopathology of AWS remains poorly understood, particularly during this acute phase. We wanted to focus on these changes occurring during the withdrawal syndrome, particularly concerning inhibitory and excitatory systems, which interact with each other and are purported to play an important role in the development of AWS. We have hypothesized that alterations to glutamate and GABA plasma levels are present during the first hours of the acute phase of AWS and that this dysregulation could be a prognostic factor for severe withdrawal. To confirm this, we have prospectively assessed plasma levels of glutamate, GABA and glutamate/GABA balance in alcoholic patients presenting with AWS in the emergency department.

MATERIALS AND METHODS

Subjects

Eighty-eight adult patients hospitalized for alcohol acute intoxication in the emergency room of the Centre Hospitalier Universitaire of Clermont-Ferrand (France), between 1 February and 30 June 2008, were included in the study. The experimental protocol was approved by the Committee for the Protection of Individuals (Comité de Protection des Personnes, CPP, opinion of November 2007, ID RCB: 2007-A00920-53). The inclusion criterion was diagnosis of alcohol acute intoxication according to the DSM-IV criteria [(303.00), American Psychiatric Association, 1994]]. The exclusion criterion was the patients’ refusal to participate in the study (<5%) or the presence of serious somatic disability (<2%). Among the 88 participants, 79 were presented with AWS [diagnosed on the basis of DSM-IV, (291.8)]. Among this 79 participants, an experimental group (EG, n = 23) was constituted with patients presenting with severe AWS according to the Cushman score in the first 12 ± 2 h after admission (score ≥28). The control group (CG) was matched by age and gender to the EG. The CG consisted of healthy subjects. The inclusion criteria for the CG concerned the negative screening for abuse and dependence according to the CAGE Questionnaire (Ewing, 1984) and the MINI (Lecrubier et al., 1997).

Measures of AWS and alcohol problems

AWS was assessed by the French adaptation of the Cushman clinical score (Cushman et al., 1985; Cushman and Sowers, 1989; Mennever et al., 2008), which has been validated for this purpose (Société Française d’Alcoologie, 2006). This scale, which is classically used in emergency departments for assessing the severity of the withdrawal syndrome, takes into consideration pulse, systolic blood pressure, respiratory rate, tremor, sweating, agitation and sensorial disorders (Table 1) (Mennever et al., 2008).

A withdrawal syndrome with a Cushman score of ≥8 is considered severe and that with a Cushman score of ≥12 and/or the presence of criticized hallucinations (Mennever et al., 2008) is considered complicated. The MINI is a semi-structured questionnaire used for diagnostic purposes (Lecrubier et al., 1997). We used the ‘Alcohol abuse/Alcohol dependence’ module of this questionnaire to identify the type of alcohol misuse in our sample. This module and the CAGE questionnaire were used to verify the absence of alcohol problems into the CG. The CAGE is a short questionnaire developed to detect life-time alcohol dependence with a threshold score of >1 (Ewing, 1984).
Glutamate/GABA balance during acute alcohol withdrawal

Table 1. Cushman score

<table>
<thead>
<tr>
<th>Score</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulse*</td>
<td>≤80</td>
<td>81–100</td>
<td>101–120</td>
<td>&gt;120</td>
</tr>
<tr>
<td>Systolic blood* (mmHg)</td>
<td>≤135</td>
<td>136–145</td>
<td>146–155</td>
<td>&gt;155</td>
</tr>
<tr>
<td>Respiratory rate* (cycles per minute)</td>
<td>≤16</td>
<td>16–25</td>
<td>26–35</td>
<td>&gt;35</td>
</tr>
<tr>
<td>Tumor</td>
<td>0</td>
<td>Extended hand</td>
<td>Entire upper limb</td>
<td>Affecting whole body</td>
</tr>
<tr>
<td>Perspiration*</td>
<td>0</td>
<td>Palms</td>
<td>Palms and forehead</td>
<td>Affecting whole body</td>
</tr>
<tr>
<td>Agitation</td>
<td>0</td>
<td>Discrete</td>
<td>Generalized/controllable</td>
<td>Generalized/uncontrollable</td>
</tr>
<tr>
<td>Sensorial disorders</td>
<td>0</td>
<td>Retreat from noise or light, pruritis</td>
<td>Hallucinations (criticized)</td>
<td>Hallucinations (non-criticized)</td>
</tr>
</tbody>
</table>

*aCriteria if corporal temperature <38°C.
*bCriteria between 31 and 50 years old; if age >50 add 10 mmHg.

Biochemical measures

Blood alcohol levels were measured by means of the automated alcohol dehydrogenase enzyme method (Modular, Roche®, Meylan, France), which was a routine part of the examination for these patients. Data on the following biological variables were also recorded: mean corpuscular volume (MCV), serum levels of aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transferase (GGT) and carbohydrate deficient transferring (CDT). In parallel, a sample of blood was taken to measure the plasma concentration of GABA and glutamate. Separated amino analysis with a specific procedure was necessary to analyse for the two acids. Concerning GABA, each sample was transferred quickly on ice before centrifugation. Then the serum was stored at 80°C before analysis. A specific protocol of extraction for GABA was practised: a solid-phase extraction with cation-exchange polymeric groups was used (OASIS® MCX, Waters), and a specific derivation by silylation (MTBSTFA/ACN, 35/15, v/v) at 70°C for 30 min. Quantification of GABA was undertaken with an internal standard (6-aminocaproic acid, 6-ACA) and different calibration standards. The analytical protocol used gas chromatography (HP 6890, Agilent) coupled with a mass spectrometry detector (HP 5973, Agilent). Measurement of the area under the curves for specific fragments for quantification and qualification of the GABA (m/z = 274, 258, 316) and its internal standard (6-ACA, m/z = 302, 344, 170) allows a highly specific and sensitive quantitative analysis. Concerning glutamate, the blood sample was centrifuged (+ 4°C, 4500g, 10 min) and the plasma was deproteinized with sulfosalicylic acid (50 mg/ml). The supernatant was stored at −80°C before the plasma amino analysis which was performed by ion exchange chromatography with an amino acid autoanalysers (JLC-500/V AminoTac; JEOL). The results of our participation in ERNDIM (the European Quality Control Scheme) are characterized using descriptive methods. The normality of dependent variables was assessed by skewness and kurtosis statistics (the chosen criterion was that the dividend of the coefficient and the standard error did not exceed ± 2.0). Analysis of variance F-test was used to conduct between-and within-group comparisons. Because EG and CG were paired, plasma concentrations of EG (T1 and T2) and of CG were defined in the same within-subject factor (three levels).

Statistical analysis

The SPSS software (SPSS Inc., Chicago, IL, version 15.0) was used for statistical analyses. Numerical data were expressed as frequency and proportions (%). Measured data were expressed as means with standard deviations. First, data were characterized using descriptive methods. The normality of dependent variables was assessed by skewness and kurtosis statistics (the chosen criterion was that the dividend of the coefficient and the standard error did not exceed ± 2.0). Analysis of variance F-test was used to conduct between-and within-group comparisons. Because EG and CG were paired, plasma concentrations of EG (T1 and T2) and of CG were defined in the same within-subject factor (three levels). Simple contrast type was chosen to compare the mean of each level with the mean of a specified level (in General Linear Model repeated measures procedure).

In order to identify prognostic factors of severe withdrawal syndrome, logistic regression (backward stepwise) was used to examine a multivariate prediction model that included all potentially useful variables for discriminating patients who progressed with severe AWS at T2 from patients with non-severe AWS at T2. Because these predictive variables are unknown, stepwise procedure was selected in this exploratory research context (Agresti and Finlay, 1997). Then, backward elimination rather than forward inclusion was chosen as the method of stepwise regression. Indeed, backward stepwise elimination has the advantage of keeping in the equation variables that increase the predictive validity of another variable, or a set of variables (i.e. a suppressor effect). With backward elimination, because all variables are already in the model, there is less risk of failing to find a relationship when one exists (Menard, 2001).

All hypotheses were tested by using a two-sided test and a significance level of α = 0.05.

RESULTS

Among the 88 patients included in our study, 79 were diagnosed as presenting with AWS [19 females (24%), 60 males...
A history of treatment for alcohol-related disorders was present in 85% of these participants and 45% had a history of AWS. All were alcohol dependent. They were predominantly excess consumers of wine. In this sample, serum levels of AST, ALT, GGT and CDT were disturbed (Table 2).

Among these 79 AWS cases, 23 had a Cushman score superior or equal to 8 at T2 (severe or complicated AWS). This experimental sample was composed by 22 males and 1 female (mean age: 45.04 years, SD: 8.51). All these patients had a history of receiving treatment for alcohol-related disorders and 73% had a history of AWS. The body mass index of the EG was into the normal World Health Organization range (M: 23.54; SD: 6.01) and did not differ from the BMI of the CG (M: 24.52; SD: 6.01).

The total CAGE mean score of the EG was 3.43 (± 0.59). This score was greater than that of the CG (0.04 ± 0.21), F(1,44) = 676.000, P < 0.001, which highlighted the lack of a problem of alcohol in this population (≤1). The total Cushman mean score at T1 (6.43 ± 2.97) was lower than at T2 (8.78 ± 2.11), F(1,22) = 17.970, P < 0.001.

The mean plasma concentration of GABA at T1 (8.17 µmol/l ± 2.95) and at T2 (8.05 µmol/l ± 2.65) were not significantly different, F(1,21) = 0.153, P > 0.05. By contrast, the mean plasma GABA concentration for the CG (27.66 µmol/l ± 3.12) was greater than the mean plasma GABA concentration at T1 (8.17 µmol/l ± 2.95), F(1,21) = 368.362, P < 0.001, and at T2 (8.05 µmol/l ± 2.65), F(1,21) = 406.787, P < 0.001 (Fig. 1).

The mean plasma concentration of glutamate in T1 (88.06 µmol/l, SD: 59.92) was higher than at T2 (69.78 µmol/l ± 44.91), F(1,22) = 9.790, P < 0.01, and higher than in the CG (48.42 µmol/l ± 15.59), F(1,22) = 10.527, P = 0.004. In the same way, the mean plasma concentration of glutamate at T2 (69.78 µmol/l ± 44.91) was greater than in the CG (48.42 µmol/l ± 15.59), F(1,22) = 46.637, P < 0.001 (Fig. 2).

Concerning logistic regression analysis, 10 variables were examined. Seven were the perturbed variables documented in the literature as associated with withdrawal (gender, age, personal antecedents of withdrawal syndrome, serum levels of AST and ALT, serum levels of GGT and alcohol blood level at admission). There were variables supporting our hypothesis of acute glutamate/GABA dysregulation [mean plasma concentrations of GABA and glutamate at admission (T1), and ‘glutamate/GABA ratio’ at T1]. Because of strong co-linearity with the mean plasma concentration of glutamate (T1), the variables ‘GGT’, ‘MCV’ and ‘glutamate/GABA ratio’ were not included in the analysis. The other variables were gender (male vs. female), age (≤45 vs. >45), personal antecedents of withdrawal syndrome (yes vs. no), serum levels of AST and ALT (normal vs. elevated), serum levels of GGT (normal vs. elevated), alcohol blood level at admission (≤3 g/l vs. >3 g/l), and mean corpuscular volume (MCV) (normal vs. abnormal).

Table 2. Biological parameters of the 79 alcohol-dependent patients with AWS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal value</th>
<th>Mean</th>
<th>SD</th>
<th>Mini</th>
<th>Maxi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol blood level T1 (g/l)</td>
<td>0</td>
<td>2.81</td>
<td>1.44</td>
<td>0.8</td>
<td>5.11</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>80–100</td>
<td>95.68</td>
<td>6.82</td>
<td>80.3</td>
<td>119</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>10–40</td>
<td>101.6</td>
<td>58.4</td>
<td>20</td>
<td>419</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>10–50</td>
<td>63.3</td>
<td>63.5</td>
<td>8</td>
<td>460</td>
</tr>
<tr>
<td>GGT (IU/l)</td>
<td>7–32</td>
<td>284.9</td>
<td>451.2</td>
<td>10</td>
<td>2271</td>
</tr>
<tr>
<td>CDT (%)</td>
<td></td>
<td>&lt;1.7</td>
<td>5.2</td>
<td>0.3</td>
<td>25.8</td>
</tr>
</tbody>
</table>

MCV, mean corpuscular volume; AST, aspartate amino transferase; ALT, alanine amino transferase; GGT, gamma glutamyl transferase; CDT, carbohydrate deficient transfering.
ratio’ were excluded from the regression model in accordance with classical procedure (Menard, 2001) \( (r = 0.843, P < 0.01, r = 0.347, P < 0.01\) and \( r = 0.728, P < 0.01\), respectively). Likewise, serum levels of ALT, which were highly correlated with the serum level of AST \( (r = 0.783, P < 0.01)\), were excluded from the regression analysis. Despite a significant positive correlation between glutamate level and the serum levels of AST \( (r = 0.362, P < 0.01)\), ‘AST’ (which reflects hepatic enzyme activity) was kept in the regression analysis to evaluate the involvement of liver status in predicting severe AWS. In addition, because CDT is a marker of recent alcohol intake, it does not constitute a relevant biochemical variable for our predictive model. The logistical regression was performed using the data resulting from the 79 participants who presented with AWS. Among the seven remaining variables, only the mean plasma concentration of glutamate at T1 is a significant predictor of AWS at T2, \( \chi^2 = 6.356, P = 0.012 \). The model’s statistical results yield a satisfactory, but not optimal, response predictability \( (\chi^2 = 12.156; df = 4; P = 0.014; \text{Nagelkerke } R^2 = 0.254) \).

**DISCUSSION**

We have shown in this prospective study the dysregulation of glutamate/GABA plasma ratio during acute withdrawal syndrome. Hyper-glutamatnergic activity (>200% compared with non-alcoholics) in this human study could confirm the results of animal studies that showed a similar increase in the brain [00% for Dahchour et al. (1998)]. It is also compatible with early clinical manifestations of withdrawal, particularly those such as hyper-excitation and anxiety. Glutamate plasma levels seem to decrease during the acute period, unlike hypo-GABA activity, which seems stable during the first 12 h of withdrawal. These results are consistent with other human studies (Aliyev et al., 1994; Aliyev and Aliyev, 2002). Moreover, we have emphasized the precocity of this dysregulation.

In our CG, the mean plasma concentration of glutamate is in the normal range for plasma concentration (Hediger and Welbourne, 1999; Rainesalo et al., 2004). In our EG, the mean plasma concentration of glutamate is lower than the serum levels found by Aliyev et al. (1994) for 20 male patients following AWS \((160 + 3 \mu\text{mol/l})\). Concerning GABA plasma levels, our measurements for the control and EGs are not in line with previous published works, which are themselves heterogeneous. Thus, Petty et al. (1997) reported GABA plasma levels in alcoholic males of about 0.100 \( \mu\text{mol/l} \) and Aliyev and Aliyev (2002) found about 3.5 \( \mu\text{mol/l} \) for patients with AWS, while in our study, levels of about 25 \( \mu\text{mol/l} \) were reported. However, the coefficient of variation intra-assay was around 11% in our study while it was reported to be around 6.4% by Petty et al. (1997), testifying to the quality of the two analyses. We can assume that these discrepancies are linked with differences in methodology between studies rather than with techniques and measurement equipment used. Indeed, the time of collection of plasma for measurement of amino acids was not the same between these studies. In our work, plasma was collected a few minutes after admission while it was collected later in other studies. In addition there is an important variability in the GABA plasma levels reported in specialized articles (Rainesalo et al., 2004; Vaiva et al., 2006; Küçükibrahimolu et al., 2009), testifying to the instability of this amino acid and the necessity of a rapid analytical procedure like the one we used. As regards techniques and equipment, various techniques have been used to determine plasma amino acid concentrations. For Reynolds et al. (2002), it may also account for the varying conclusions reached by different research groups.

The most important question posed by this study concerns the difficulty in correlating plasma and brain glutamate and GABA levels. Several data support the following hypothesis: First of all, elevated plasma and brain glutamate levels have been reported for many pathophysiological conditions associated with neurologic injury (including stroke, motor neuron disease, olivopontocerebellar atrophy and preterm birth asphyxia) (Castillo et al., 1997; Babu et al., 1998). In the same way, variation of glutamate and GABA plasma levels have been reported to be prognostic factors or biomarkers during psychiatric disorders (Vaiva et al., 2006; Küçükibrahimolu et al., 2009) or human epilepsy (Rainesalo et al., 2004). Then animal data support the hypothesis of a correlation between the time course changes of glutamate and GABA in plasma and of those of CSF (Apud et al., 1981; Küçükibrahimoglu et al., 2009). In this way, glutamate and GABA plasma dysregulation could reflect brain activity, which presents similar changes during withdrawal (Aliyev et al., 1994; Tsai et al., 1998; Hermann et al., 2012).

In our study, hyperglutamatnergic activity appears as a prognostic factor in the evolution of withdrawal; that is, the higher the plasma concentration of glutamate in the acute phase, the greater the risk of serious immediate withdrawal. These results could support the hypothesis of the neurotoxicity of elevated glutamate concentration. Tsai et al. (1998) have suggested that augmentation of excitatory neurotransmission in CSF during withdrawal may lead to enhanced oxidative stress (Tsai et al., 1998). This augmentation could promote acute neurological damage (Delirium, Gayet Wernicke) or chronic sensitization of the brain as a kindling phenomenon (Gass and Olive, 2008).

In our study, GABA was not a prognostic factor for complicated withdrawal. Adinoff et al. (1995) have measured GABA in both plasma and CSF from 14 male alcohol-dependent patients at day one of acute alcohol withdrawal and 21 days after inpatient treatment. They did not find significant correlations between the indices of alcohol withdrawal and plasma or CSF GABA levels, suggesting that the involvement of other neurobiological factors should be investigated. Adinoff et al. (1995) also suggested that there would be major variation of GABA during the acute withdrawal phase but they did not assess variation of GABA during the first hours. It was thus impossible to compare their findings with our results that show stability of GABA during the first 12 h (Adinoff et al., 1995). In the same way, according to Roy et al. (1990), CSF GABA was not associated with the antecedents of severe AWS. According to Petty et al. (1997), GABA could be an indicator of brain sensitization and relapse: Thus, they followed 49 alcohol-dependent patients for up to 18 months of continuous abstinence following inpatient treatment. Alcohol-dependent patients with low-plasma GABA had significantly better outcomes than patients with GABA plasma values in the normal control range.
Knowledge of the dysregulation phenomenon of glutamate/GABA balance during the acute phase of withdrawal, with a prognostic role for glutamatergic hyperactivity, can help to supplement the classical acute therapeutic response to the withdrawal syndrome in the emergency room. Indeed, the classical approaches consist of GABA reinforcement (Hall and Zador, 1997; Mayo-Smith et al., 2004) by benzodiazepines, but concerns about their abuse potential drive the search for new treatments. It could be interesting to prevent rapid hyper-glutamatergic activity during the acute phase of withdrawal by using antiglutamatergic treatment (Carroll, 2008). Acamprosate seems to be a protective agent during hyper-glutamatergic syndrome occurring during alcohol withdrawal (Dahchour and De Witte, 2003b) by reducing calcium-related neurotoxicity (Al Qatari et al., 2001, Koob et al., 2002). This protective effect appears in animal models when acamprosate is chronically and preventively administered before withdrawal but an acute administration during AWS could not stem the massif influx of glutamate (Mann et al., 2008). High doses could be tested, especially since this product has low toxicity and seems to reduce central brain glutamate activity during withdrawal in human studies (Umhau et al., 2010). Moreover, for a significant number of patients, chronic evolution of alcohol dependence is marked by alternation of consumption and withdrawal periods. For them, long-term treatment by acamprosate could prevent irritable discharges of hyper-glutamatergic activity and protect the brain from long-term damage (Kiefer and Mann, 2010). Other new antiglutamatergic therapeutic approaches using drugs such as topiramate, memantine or lamotrigine (Krupitsky et al., 2007; Amato et al., 2011) could be investigated.

Our study has other limitations. Firstly, the sample was of a limited size, especially the severe withdrawal group, although other studies published in this area have similar sample sizes. These include the only three prospective studies assessing predictive factors of delirium tremens and severe withdrawal (Thiercelin et al., 2012). Secondly, metabolism and plasma levels of glutamate and GABA (derived from glutamate, Buddhala et al., 2009) are classically determined and influenced by liver status and, to a lesser extent, by dietary intake (Hediger and Welbourne, 1999). In this study, the experimental and CGs did not differ in nutritional status. We found an absence of correlation between body mass index of the EG and levels of glutamate and liver enzymes, but significant positive correlation between glutamate levels and AST and GGT. These correlations could implicate liver status in the glutamate plasma level. However, results of backward logistic regression highlight the role of glutamate in predicting risk of AWS when hepatic variables are excluded from the equation (Table 3). Thirdly, the two selected time points for the study (at admission and 12 ± 2 h later) only reflect the acute phase and are insufficient for exhaustive evaluation of the kinetics of amino acid plasma level evolution. Fourthly, we have not studied exhaustively all the potential factors that could be implicated in the occurrence of severe withdrawal. In fact, we have focused our attention on glutamate and GABA dysregulation and found that glutamate plasma concentration could predict the gravity of AWS. It is true that the model’s predictive capacity is not high but consistent with previous work (Aliyev et al., 1994; Aliyev and Aliyev, 2002). A larger study should investigate exhaustively the implication of other amino acids such as homocysteine and taurine (Bleich et al., 2000a; Bleich et al., 2000b; Olive, 2002; Bayerlein et al., 2005; Hillemacher et al., 2007). Otherwise, meta-analysis should be done in order to determine definitively the implication of clinical and biological factors. Genetic factors should also be studied (Van Munster et al., 2007). Further work is required, but without doubt, therapeutic brain protection should be instigated as early as possible during alcohol cessation therapy in alcoholic patients.

### CONCLUSION

In this study, we have confirmed a change in the glutamate/ GABA balance in alcohol-dependent subjects during withdrawal. The decreased synthesis of GABA and increased synthesis of glutamate might be related to chronic alcohol intake and may be made apparent by brutal cessation. Withdrawal symptoms are directly caused by this acute dis-equilibrium. It seems that hyper-glutamatergic activity produces an oxidative stress which induces acute brain symptoms such as seizures and may lead to chronic alterations, especially since withdrawals are iterative and there is no therapeutic protection. Alteration of GABA could

---

Table 3. Logistic regression analysis (backward) of selected variables to distinguish patients with severe AWS at 12 ± 2 h from patients with non-severe AWS

<table>
<thead>
<tr>
<th>Step</th>
<th>Variable</th>
<th>B</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>Sig.</th>
<th>Exp(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(a)</td>
<td>Gender</td>
<td>1.668</td>
<td>0.989</td>
<td>2.848</td>
<td>1</td>
<td>0.091</td>
<td>5.304</td>
</tr>
<tr>
<td></td>
<td>Age</td>
<td>0.088</td>
<td>0.041</td>
<td>4.625</td>
<td>1</td>
<td>0.032</td>
<td>1.092</td>
</tr>
<tr>
<td></td>
<td>PAWS</td>
<td>0.959</td>
<td>0.758</td>
<td>1.603</td>
<td>1</td>
<td>0.206</td>
<td>2.610</td>
</tr>
<tr>
<td></td>
<td>Alcohol admission</td>
<td>-0.532</td>
<td>0.229</td>
<td>2.105</td>
<td>1</td>
<td>0.147</td>
<td>0.717</td>
</tr>
<tr>
<td></td>
<td>TI AST</td>
<td>0.003</td>
<td>0.004</td>
<td>0.617</td>
<td>1</td>
<td>0.432</td>
<td>1.003</td>
</tr>
<tr>
<td></td>
<td>TI GABA</td>
<td>-0.089</td>
<td>0.108</td>
<td>0.685</td>
<td>1</td>
<td>0.408</td>
<td>0.914</td>
</tr>
<tr>
<td></td>
<td>TI GLUT</td>
<td>0.020</td>
<td>0.008</td>
<td>5.931</td>
<td>1</td>
<td>0.015</td>
<td>1.020</td>
</tr>
<tr>
<td></td>
<td>Constant</td>
<td>-7.614</td>
<td>3.058</td>
<td>6.198</td>
<td>1</td>
<td>0.013</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Variables included in the model: gender, age; Personal Antecedents of Withdrawal Syndrome (PAWS), serum levels of AST, alcohol blood level at admission, mean plasma concentrations of GABA and glutamate (GLUT) at admission (T1).

Intercept and covariates: -2Log = 62.791; R² (Nagelkerke) = 0.254; Max rescaled R² = 0.173; χ² = 12.516; df = 4; P = 0.014; *P < 0.05.
strengthen this phenomenon. The implication of withdrawal symptoms in craving and relapse must be taken into account. AWS is ‘a bad old companion’ to patients and clinicians. It is surprising that it is still so poorly understood and treated as difficult.

Funding — This study was supported by the University Hospital of Clermont-Ferrand and financed by a grant from the French Society of Emergency Medicine.

REFERENCES


Kretschmer BD, Schmidt WJ, Kostrzewska RM et al. (2002) Amino acids in neurobiology: neuroprotective and neurotoxic aspects of


