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PLASMA LEVELS OF BETA-ENDORPHIN, ADRENOCORTICOTROPIC HORMONE AND CORTISOL DURING EARLY AND LATE ALCOHOL WITHDRAWAL

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Abstract — Endogenous opioid peptides are thought to participate in the phenomena of alcohol tolerance and withdrawal. Since in the pituitary gland, beta-endorphin (β-EP) and adrenocorticotropic hormone (ACTH) are produced from the same precursor molecule, pro-opiomelanocortin, it may be expected that alterations in plasma ACTH and cortisol levels should parallel changes in plasma β-EP levels during alcohol withdrawal. The aim of the present study was to investigate the alterations of β-EP, ACTH and cortisol secretion patterns in alcohol-dependent patients with heavy intake in the early withdrawal period and, if any, whether these changes remained stable on long-term withdrawal. Twenty-two hospitalized male patients (mean age ± SD: 43.45 ± 9.22 years, mean daily amount of alcohol ± SD: 421.59 ± 116.57 g) who were diagnosed to have alcohol withdrawal and 20 age-matched healthy men (mean age ± SD: 38.35 ± 7.63 years) were included in the study. Morning and night levels of plasma β-EP, ACTH and cortisol were measured in the patients during the early (first week) and late (fourth week) withdrawal periods following alcohol cessation, and only once in the control subjects. It was found that both morning β-EP and morning ACTH levels were reduced during both early and late withdrawals, whereas cortisol levels were increased in early withdrawal and normalized towards the late withdrawal period. The finding that β-EP deficiency continued despite withdrawal symptoms subsiding in patients suggests that their β-EP deficiency is independent of the withdrawal syndrome and that reduced β-EP activity may be a trait contributing to alcohol craving.

INTRODUCTION

Endogenous opioid peptides are involved in a number of physiological and adaptive processes, such as analgesia, stress, reward, emotions, motivation, feeding behaviour, and temperature control. These peptides are found in many areas of the brain and may act as neurotransmitters or neuromodulators (Giuffre *et al*., 1988; Gianoulakis, 1993; Herz, 1997).

The major sites of beta-endorphin (β-EP) biosynthesis are the pituitary gland and the arcuate nucleus of the hypothalamus. Corticotropin-releasing factor (CRF) induces both hypothalamic and pituitary β-EP peptides. Since adrenocorticotropic hormone (ACTH) and β-EP share the same precursor molecule, pro-opiomelanocortin (POMC), and are co-released from the pituitary gland under various conditions, it may be expected that increases in plasma ACTH and cortisol levels should be observed in parallel to a β-EP increase (Herz, 1997).

It has long been thought that alcoholism and the endogenous opioid system are in some way related. However, the interactions between alcohol and endogenous opioids are still unclear. Endogenous opioids, in particular β-EP, play a key role in the rewarding (addictive) effects of ethanol (Herz, 1997). Some animal studies showed ethanol-induced alterations in biosynthesis, release, or receptor binding properties of various endogenous opioids in the central nervous system (Tabakoff and Hoffman, 1983). Other studies reported that β-EP concentrations in various brain regions were increased by acute alcohol administration (Schulz *et al*., 1980), and either decreased (Schulz *et al*., 1980; Seizinger *et al*., 1983; Aguirre *et al*., 1990) or unchanged (Brambilla *et al*., 1988) by chronic alcohol intake.

In addition to the idea that opioid receptors may be involved in some of the actions of ethanol, it is also thought that endogenous opioid peptides participate in the phenomena of alcohol tolerance and withdrawal (Hutchison *et al*., 1988; Vescovi *et al*., 1992). In alcoholic patients, the results of studies concerning β-EP levels during alcohol withdrawal have so far been inconsistent. Reduced (Aguirre *et al*., 1990; Vescovi *et al*., 1992; Marchesi *et al*., 1997; Inder *et al*., 1998) or normal (Brambilla *et al*., 1988) plasma levels of β-EP have been reported during the early (1–10 days) (Brambilla *et al*., 1988; Vescovi *et al*., 1992; Marchesi *et al*., 1997; Inder *et al*., 1998) and late (4–5 weeks) (Aguirre *et al*., 1990; Vescovi *et al*., 1992; Marchesi *et al*., 1997) withdrawal periods. Cerebrospinal fluid β-EP concentration was found to be decreased in early (Genazzani *et al*., 1982) and normal in late (Petrakis *et al*., 1999) abstinence (1–3 months) in alcohol-dependent individuals.

The aim of the present study was to investigate the alterations of β-EP, ACTH and cortisol secretion patterns in alcohol-dependent patients who were in early withdrawal and, if any, whether these changes remained stable during long-term withdrawal.

SUBJECTS AND METHODS

Subjects

Twenty-two hospitalized male patients (mean age ± SD: 43.45 ± 9.22, range 30–60 years) who were diagnosed with alcohol dependence and alcohol withdrawal according to the DSM-IV criteria (American Psychiatric Association, 1994) were included in the study (Table 1). The diagnoses were made by two psychiatrists (E.E. and S.S.) independently by using the DSM-IV criteria for alcohol dependence and alcohol withdrawal. No structured interview was used. The patients were in the state of withdrawal when they were admitted to hospital, but none had delirium. All patients had been alcohol dependent for more than 5 years (mean ± SD: 19.7 ± 8.3 years)

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Table 1. Some clinical variables of the patients and controls

Clinical variables	Patients (n = 22)	Controls (n = 20)
Age (years)	43.4 ± 9.2	38.3 ± 7.6
Age at the onset of alcoholism (years)	21.40 ± 4.0	Nil
Duration of dependence (years)	19.7 ± 8.3	Nil
Amount of alcohol consumed (g/day)	421.6 ± 116.6	Nil
Diazepam dose given (mg/day)	33.4 ± 12.9	Nil

Values are means ± SD.

with an alcohol intake of >175 g/day (421.6 ± 116.6 g/day). Only subjects with normal levels of transaminases were included in the study. All patients were cigarette smokers. Thirteen of them had a positive family history of alcoholism. Those who had major physical illnesses, including hepatic or endocrine disorder, substance abuse other than alcohol, or a history of primary mood disorder, were excluded from the study. Twenty age-matched healthy men who did not have a history of alcohol dependence, drug dependence, psychiatric or neurological illnesses served as control subjects (mean age ± SD: 38.4 ± 7.6, range 30–55 years). In order to control the smoking status, which has been reported to have the ability to affect β-EP and cortisol secretions (Rasmussen, 1998; del Arbol *et al*., 2000), the control subjects were selected from smoking individuals, as were the patients.

Demographic and clinical data were collected after the patients had returned to sobriety. Severity of anxiety and depressive symptoms were assessed with the Clinical Anxiety Scale (CAS) (Snaith *et al*., 1982) and the 21-item Hamilton Rating Scale for Depression (HRSD) (Hamilton, 1960), respectively. The patients whose depression scores in HRSD were ≥10 from the first or fourth week evaluations were excluded from the study.

During the first 2 weeks of withdrawal, the patients were given diazepam (mean dose ± SD: 33.4 ± 12.9 mg/day) and polyvitamins, and the patients were drug-free from 2 weeks after admission onwards.

This study was carried out in accordance with the Helsinki Declaration of the World Medical Association, and was approved by the local Ethics Committee. All subjects gave their written informed consents after full understanding of the study.

Procedures

Morning and night levels of plasma β-EP, ACTH, and cortisol were measured in the patients during the early (first week or 7 days after alcohol cessation) and late (fourth week or 28 days after alcohol cessation) withdrawal periods following alcohol cessation, whereas they were not intoxicated with alcohol, but only once in the control subjects. The patients remained in the hospital throughout the study while the control subjects were held in the hospital solely on the day the blood samples were taken. Blood samples were collected with an indwelling catheter inserted into an antecubital vein at 07.00 after an overnight fast, and at 23.00. We chose these time points to evaluate the circadian rhythms of hormones because 07.00 is a time point generally accepted to herald the ascending limb of the daily circadian secretion curve of these hormones, whereas 23.00 is the time point of the lowest levels of the hormones (Buydens-Branchey and Branchey, 1992; Marchesi *et al*., 1997). The same individual performed all venepunctures. Venous blood was drawn into an ice-cold vacutainer plastic tube containing a mixture of EDTA and aprotinin, and centrifuged immediately in cold conditions. Separated plasma was frozen within 10 min after collection and stored at –70°C until analysed. Plasma β-EP, ACTH and cortisol levels were determined in duplicate by using standard radioimmunoassay kits (Nichols Institute, USA for β-EP, CIS Bio International, France for ACTH, and Amerlex, UK for cortisol). For β-EP, the lowest sensitivity limit was 14 pg/ml with a cross-reactivity of 16% with human β-lipotropin, the inter- and intra-assay coefficients of variation (CV) were 4.1% for a 213 pg/ml concentration and 9.0% for a 190 pg/ml concentration, respectively. For ACTH, the lowest sensitivity limit was 2 pg/ml, the inter- and intra-assay CV were 2.9% for a 59 pg/ml concentration and 4.8% for a 203 pg/ml concentration, respectively. For cortisol, the inter- and intra-assay CV were 7.9% and 7.0%, respectively, the lowest sensitivity limit of the method was 0.1 μg/dl and the normal range was 5.9–26.1 μg/dl.

Statistical analysis

Comparison of the ages of the patients and controls was performed by using the unpaired *t*-test. For statistical analysis of each hormone, two-way analysis of variance followed by post-hoc Bonferroni's test was used with three alcohol-related groups (normal subjects, alcoholic subjects in the first week of withdrawal and alcoholic subjects in the fourth week of withdrawal) as one of the independent variables and the time of day blood samples were taken (morning or night) as the other independent variable. Whenever a significant *F*-ratio was found, the means were compared using *t*-tests. Statistical comparisons of the values of hormonal variables in the first and the fourth weeks of the patients were carried out with the paired *t*-test. To compare the hormonal values of the patients with those of controls, the two-tailed independent *t*-test was used. Comparisons of the hormonal values of the alcoholic patients with and without a family history of alcoholism were also carried out by the two-tailed independent *t*-test. Non-parametric Spearman's correlation test was used to investigate the relationships between plasma β-EP, ACTH and cortisol levels and clinical variables (age, age at the onset of alcoholism, duration of alcoholism, amount of daily alcohol consumed, benzodiazepine doses, CAS and HRSD scores).

RESULTS

The ages of the patients and control subjects (Table 1) were not significantly different from each other ($t = 1.95$, $P > 0.05$). Table 2 presents the endocrine indices of the groups studied. β-EP values in both the first and fourth weeks of the patients were significantly lower than those of the controls ($F = 11.40$, $P < 0.001$). There was a significant interaction on β-EP values between the groups and the time of day ($F = 4.17$, $P < 0.05$). When the morning and night values were analysed separately, both at the end of the first and fourth weeks of withdrawal, morning plasma values of β-EP were significantly lower in the patients than in the control subjects ($t = 3.20$, $P < 0.01$;

Table 2. Plasma beta-endorphin (β-EP), adrenocorticotropic hormone (ACTH) and cortisol values of the patients who were in the early and late withdrawal periods and of controls

Hormonal variables	Patients		Control subjects (n = 22)
	Early withdrawal (day 7) (n = 20)	Late withdrawal (day 28) (n = 22)	
Morning β-EP (pg/ml)	36.7[a] ± 20.7	32.5[b] ± 14.9	56.0 ± 18.3
Night β-EP (pg/ml)	14.5[c] ± 7.1	18.9 ± 11.2	23.3 ± 8.2
Morning ACTH (pg/ml)	18.2[d] ± 9.4	15.3[e] ± 9.6	35.5 ± 19.7
Night ACTH (pg/ml)	15.2 ± 10.1	13.5 ± 7.5	13.7 ± 10.6
Morning cortisol (μg/dl)	28.3[f] ± 9.2	15.9 ± 7.0	19.5 ± 3.3
Night cortisol (μg/dl)	13.3[f] ± 11.9	7.7 ± 7.0	4.6 ± 2.1

Values are mean ± SD.
[a–e]Significantly lower than that of the control group: [a]t = 3.20, $P < 0.01$; [b]t = 4.41, $P < 0.001$; [c]t = 3.71, $P = 0.001$; [d]t = 3.55, $P = 0.001$; [e]t = 4.06, $P < 0.001$.
[f]Significantly higher than those in the late withdrawal period and in the control group: F = 18.05, $P < 0.001$ (ANOVA).

t = 4.41, $P < 0.001$, respectively). Night β-EP values were significantly lower in the patients compared to controls only in the first week, but not in the fourth week (t = 3.71, $P = 0.001$; t = 1.41, $P > 0.05$, respectively).

Morning ACTH values were significantly lower in the first and fourth weeks of the patients, compared with those of the controls (F = 6.15, $P < 0.005$). Again, a significant interaction was observed between the groups and the time of day (F = 0.48, $P < 0.05$). Morning ACTH values were significantly lower in the patients than in the controls in both the first and fourth weeks (t = 3.55, $P = 0.001$; t = 4.06, $P < 0.001$, respectively), whereas night ACTH values were not different from each other (t = 0.46, $P > 0.05$; t = 0.05, $P > 0.05$, respectively).

In the first week, cortisol values were significantly higher in the patients than in the controls (F = 18.05, $P < 0.001$), but not different in the fourth week. Cortisol values in the first week were also significantly higher than those in the fourth week in the alcoholic patients. There was no significant interaction between the time of day and groups in terms of cortisol values (F = 2.46, $P > 0.05$).

In the patients, positive correlations were observed between HRSD scores and the amount of alcohol consumed (r = 0.55, $P < 0.05$), and between night ACTH values and β-EP values (r = 0.42, $P < 0.05$) in the first week, and between HRSD scores and morning cortisol values in the fourth week of the withdrawal (r = 0.48, $P < 0.05$). There were no significant differences in any hormonal variables between the patients with and without a family history of alcoholism in the first or fourth weeks of the withdrawal.

DISCUSSION

Findings of previous studies concerning β-EP levels during withdrawal in alcoholic patients have been inconsistent. The finding in the present study that β-EP levels in the early phase (first week) of the withdrawal were reduced in the alcohol-dependent patients is consistent with most of the previous studies (Aguirre *et al*., 1990; Vescovi *et al*., 1992; Marchesi *et al*., 1997; Inder *et al*., 1998), but not with some others, which reported normal values of β-EP in this period (Brambilla *et al*., 1988). Concerning β-EP levels in the late withdrawal (fourth week), the finding of continued lower levels of β-EP in the fourth week agrees with some previous reports (Aguirre *et al*., 1990; Marchesi *et al*., 1997), but not with that of Vescovi *et al*. (1992), who reported a restoration of plasma β-EP concentrations after 5 weeks of alcohol cessation. These inconsistencies may be due to different patient-selection criteria, variations in the severity of alcohol dependence or methodological differences. Since β-EP deficiency continued despite withdrawal symptoms subsiding in our patients, it may be suggested that their β-EP deficiency is independent of the withdrawal syndrome, and that reduced β-EP activity may contribute to alcohol craving. Nevertheless, we consider that a 4-week duration after alcohol cessation may not be sufficient for normalization of plasma β-EP levels in severe alcohol dependence, as we did not investigate them after a longer term of abstinence.

β-EP deficiency observed during alcohol withdrawal in the alcoholic patients in this study and in some previous studies may be explained in different ways. Firstly, it might originate from a genetic defect (Topel, 1988; Gianoulakis *et al*., 1996; Wand *et al*., 1998, 1999; Froehlich *et al*., 2000). One of the hypotheses proposed for the implication of the endogenous opioid system in alcoholism is the 'opioid deficiency' or 'opioid compensation' hypothesis, which suggests that high-risk subjects, those from families with alcoholism history, have inherited a deficiency in the basal activity of the endogenous opioid system. Since ethanol enhances the activity of the opioid system (Schulz *et al*., 1980; Li *et al*., 1996), high-risk subjects consume high quantities of alcohol to compensate for this deficiency (Gianoulakis *et al*., 1996). The finding of the present study of the continuation of β-EP deficit in late withdrawal may support this idea. This hypothesis has also been supported by studies reporting an increased responsiveness of the endogenous opioid system to alcohol in humans and animals in high-risk groups for alcoholism (Froehlich *et al*., 1990; De Waele *et al*., 1992; Gianoulakis *et al*., 1992, 1996). It has also been reported that the pituitary β-EP system of the high-risk subjects is more sensitive to ethanol than that of low-risk subjects, and this is associated with the lower basal plasma β-EP levels of the former (Gianoulakis *et al*., 1996). Moreover, it has been suggested that high-risk subjects have altered hypothalamic opioid activity, compared with low-risk subjects (Wand *et al*., 1998, 1999), and that β-EP response to alcohol might represent

a biomarker of genetic risk for alcoholism (Froehlich *et al*., 2000).

Another explanation of the finding of reduced β-EP levels during the withdrawal period in alcoholics may be the fact that chronic alcohol intake causes a decrease in β-EP level with time (Schulz *et al*., 1980; Seizinger *et al*., 1983; Aguirre *et al*., 1990). Therefore, we might have found the β-EP level to be low as a result of the effects of chronic alcohol consumption on the endogenous opioid system. A competitive or direct effect of alcohol on the synthesis of POMC (Dave *et al*., 1986) or increased degradation of β-EP due to liver enzymatic induction by chronic alcohol misuse (Brambilla *et al*., 1988) could account for this decrease.

The simultaneous decrease in plasma β-EP and ACTH levels and the positive correlation between decreased night β-EP and ACTH levels in the early withdrawal period in our patients support the hypothesis that alcohol leads to the reduced synthesis of their precursor molecule POMC at the pituitary level. In support of the relationship between alcohol and POMC-related peptides, it has been reported that long-term exposure to ethanol not only decreases POMC mRNA levels in the anterior pituitary due to alterations in POMC gene transcription, but also CRF-binding at corticotropic cells (Dave *et al*., 1986), and it has also been reported that pre-treatment of cultured corticotropic cells with ethanol resulted in decreased CRF-stimulated ACTH secretion (Rivier *et al*., 1984). Accordingly, it has been reported that alcoholic patients show blunted ACTH and cortisol responses following intravenous CRF during active alcohol consumption (Wand and Dobs, 1991) or after short- and long-term abstention from alcohol (Heuser *et al*., 1988; von Bardeleben *et al*., 1989; Ehrenreich *et al*., 1997), indicating a persistent impairment of CRF function in chronic alcoholics. Decreases in β-EP and ACTH, and blunted responses to CRF in chronic alcoholics may be due to long-term dysregulation of CRF-R_1 receptors (Ehrenreich *et al*., 1997), reduction in adenylyl cyclase activity in the anterior pituitary caused by chronic alcohol misuse (Dave *et al*., 1986; Wand and Dobs, 1991), or interference of alcohol in pathways controlling ACTH and β-EP secretions from the hypothalamic–pituitary unit (Vescovi *et al*., 1997).

In our study, the findings that cortisol levels were higher during the first week following alcohol cessation, and decreased to approach the control values with the course of time, are consistent with the results of most previous studies (von Bardeleben *et al*., 1989; Buydens-Branchey and Branchey, 1992; Heinz *et al*., 1995; Adinoff *et al*., 1991, 1996). However, they are not in agreement with Marchesi *et al*.'s (1997) study, which reported increased cortisol levels both after 7 and 28 days of abstinence. Although it was proposed that increased cortisol levels of the patients might be the reason for the reduced ACTH and β-EP concentrations in this report (Marchesi *et al*., 1997), our findings do not support this idea as ACTH levels were low, whereas cortisol levels were normal, in the fourth week of withdrawal. It has been suggested that the neurotoxic effect of alcohol itself, additional to stress-induced arousal of hypothalamic–pituitary–adrenal axis, could be responsible for the hypercortisolaemia which occurs during the withdrawal period (Adinoff *et al*., 1991). It appears that acute withdrawal from alcohol is associated with hypercortisolaemia that is parallel to an increase in sympathetic nervous system activity (Ehrenreich *et al*., 1997). One can consider that the adrenal glands become hyper-responsive to ACTH, because of the direct effects of alcohol on the adrenal cortex and/or alcohol-induced chronic ACTH insufficiency due to defective synthesis of the precursor molecule in alcoholic patients. Thus, minimal elevations in ACTH during stressful events, such as alcohol withdrawal, may cause hyper-secretion of cortisol from the adrenals, and, as withdrawal symptoms subside and the stress levels decrease, cortisol may return to normal levels while ACTH levels remain lower. The finding of some previous studies that CRF-stimulated ACTH response does not normalize and continues to be blunted in chronic alcoholic patients, although hypercortisolaemia returned to normal levels within several weeks after the cessation of alcohol (von Bardeleben *et al*., 1989; Ehrenreich *et al*., 1997), also supports the idea that a cortisol-suppressing effect cannot account for the lower ACTH levels.

Benzodiazepines can also reduce β-EP and ACTH levels (Marchesi *et al*., 1997), but this cannot explain the reduced levels of β-EP and ACTH after 4 weeks of abstinence, since benzodiazepines were administered just for 2 weeks.

In conclusion, we observed a deficient β-EP secretion associated with reduced ACTH concentration which persisted even after the subsidence of the withdrawal symptoms. This deficiency may be the result of chronic alcohol consumption, or may contribute to its development. Future studies with measurements during longer-term abstinence will reveal more accurately the relationship between the pathophysiology of alcoholism and endogenous opioid activity.

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