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European Journal of Pharmacology 405 (2000) 103–112

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The role of atrial natriuretic peptide in alcohol withdrawal: a peripheral indicator and central modulator?

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Accepted 28 June 2000

Abstract

Changes in fluid and electrolyte homeostasis may accompany and are likely to modify the clinical symptoms of alcohol-withdrawal reactions. It was of obvious theoretical and practical interest therefore to investigate the changes in the secretion of hormones, which regulate the fluid and electrolyte homeostasis (atrial natriuretic peptide, aldosterone and plasma renin activity) during alcohol withdrawal in chronic alcoholic patients. In a phase of severe withdrawal, there were increased plasma renin activity and aldosterone levels observed. In a phase of partial recovery, on the other hand, the elevated plasma renin activity and aldosterone levels were back to the normal range. In 60% of the patients, delirium tremens was gradually developing during the observation period. In these patients, an elevated level of atrial natriuretic peptide was observed at the time of hospital admission, i.e. days before the actual onset of delirium tremens. It is concluded that the disturbed volume homeostasis and the consequently altered plasma atrial natriuretic peptide secretion might be associated with, and therefore used as an indicator of the onset of delirium tremens. To study the role of central nervous atrial natriuretic peptide, mice were rendered tolerant to and dependent on alcohol with an alcohol–liquid diet for 14 days. Five hours after withdrawal from alcohol, withdrawal hyperexcitability symptoms were analyzed. Intracerebroventricular (i.c.v.) injection of atrial natriuretic peptide attenuated, whereas that of an antiserum against atrial natriuretic peptide intensified the severity of handling-induced convulsions. *N*-methyl-D-aspartate induced behavioral seizures in a dose-dependent manner, whose effect was more intensive during the alcohol-withdrawal period than in alcohol-naive animals. I.c.v. injections of atrial natriuretic peptide dose-dependently inhibited, whereas that of antiserum against atrial natriuretic peptide potentiated the seizure-inducing effect of *N*-methyl-D-aspartate in alcohol-dependent mice. Although tentatively, it is concluded that peripheral secretion of atrial natriuretic peptide may be an indicator, whereas central nervous atrial natriuretic peptide a neuropeptide modulator of alcohol-withdrawal symptomatology.

Keywords: Alcohol withdrawal, human; Delirium tremens; Volume and fluid regulation; ANP (atrial natriuretic peptide) secretion; Withdrawal hyperexcitability, mouse; NMDA (*N*-methyl-D-aspartate) seizure; Glutamate

1. Introduction

The concept of neuropeptides implies that certain peptide hormones, which are released to the peripheral blood stream under physiological or pathological stimuli, may also be synthesized in and released from brain neurons. One of the pioneers, who elaborated on and experimentally proved the biological importance and widespread validity of the neuropeptide concept, is Professor David De Wied (De Wied, 1980, 1987, 1999, 2000) from the Rudolf Magnus Institute, University of Utrecht. Owing to this concept, neuropeptide research has become ever more sophisticated and focussed in the past two or three decades. It has been discovered that peripheral and central release of the same neuropeptides may occur to occasionally different stimuli and may also underlie different biological functions. Central release of neuropeptides (and that of centrally more active, but peripherally inactive, fragments of these neuronal peptides) has been shown to affect various adaptive functions of the central nervous system, including attention and motivation, learning and memory processes, addiction to morphine/heroin, cocaine or alcohol. The author of the present review has been working with Professor De Wied in a most creative, critical re-

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00545-8

search atmosphere since 1977/78 and for 14 subsequent shorter periods in the next two decades. Although the experiments described in the present review were not performed in collaboration with the Rudolf Magnus Institute, the long lasting, intensive and fruitful cooperation with David De Wied has deeply affected the author's research attitude and strategy, as have those of many other researchers from all over the world.

Alcohol is one of the most widely used recreational drugs, but also one of the most widely abused substances, causing vast economic, social and personal damage. Alcohol dependence is considered to be divisible into two categories. One of these is psychological dependence, in which the rewarding effects of alcohol play a primary role. The other is chemical dependence, in which adaptive changes in the brain initiate punishing effects on the withdrawal of alcohol, and suppression of these becomes the primary motive for using the drug (Littleton and Little, 1994).

Alcohol withdrawal in humans, which usually results from the abrupt cessation of regular alcohol intake in patients who had been dependent on alcohol, is characterized in its early stages by mild tremor, irritability, anxiety, slight disorientation and hallucinations (Liappas et al., 1983; Victor, 1992). Severe sympathetic hyperactivity (elevated blood pressure, tachycardia, fever) and global cerebral and cardiovascular dysfunctions (Berglund and Riserg, 1981) frequently accompany these symptoms. A certain proportion of withdrawal patients will also develop delirium tremens (severe tremor, autonomic hyperactivity, vivid hallucinations, total disorientation and loss of memory, etc.), a specific manifestation of the withdrawal syndrome. Patients with delirium tremens might get into critical, occasionally life-threatening conditions, the risk of which cannot be easily foreseen in the very first period of withdrawal reactions, since delirium typically begins 2–4 days after drinking is stopped. The exact pathophysiological mechanisms involved in the appearance of alcohol-withdrawal symptoms and particularly those related to the precipitation of delirium tremens await clarification (Morton et al., 1994).

Profound changes in volume and electrolyte homeostasis occur during alcohol withdrawal, especially during delirium tremens. These changes might also be associated with convulsions and tremulousness during delirium tremens. Moreover, disturbances of fluid and electrolyte homeostasis are also known to affect brain functions, as well as the functional activity of the cardiovascular system. Under extreme pathological conditions, rapid changes in serum electrolytes, mainly in serum sodium, may be directly involved in the development of severe brain damage associated with withdrawal reactions (e.g. central pontine myelinolysis). Hence, measurement of the hormonal regulation of these parameters — e.g. plasma renin activity, aldosterone and atrial natriuretic peptide — during alcohol withdrawal might be of clinical and theoretical importance in understanding pathophysiological mechanisms of delirium.

Natriuretic peptides, mainly produced in the heart (atrial natriuretic peptide and B-type natriuretic peptide: BNP), the brain (C-type natriuretic peptide: CNP) and the kidney (urodilatin), decrease blood pressure and increase salt excretion (for review: Espinger et al., 1995). In rats, intraperitoneal injections of alcohol increased the plasma content of atrial natriuretic peptide in a dose-dependent manner (Guillaume et al., 1994). Following chronic alcohol ingestion, however, B-type natriuretic peptide, rather than atrial natriuretic peptide was affected (Wigle et al., 1993a, 1993b). In humans, intake of alcohol inhibited the nocturnal increase of plasma atrial natriuretic peptide (Ekman et al., 1994). Acute consumption of low amounts of alcohol in humans induced an increase in the plasma content of atrial natriuretic peptide (Gianoulakis et al., 1997). Following high-dose alcohol intake, however, plasma immunoreactive atrial natriuretic peptide level significantly decreased (Leppaluoto et al., 1992).

Atrial natriuretic peptide and related peptides, as well as their receptors also exist in the central nervous system (for reviews: Geiger et al., 1991; Imura et al., 1992). Atrial natriuretic peptide and its smaller congeners are produced by and released from the brain to regulate the cardiovascular system, drinking behavior, and neurohormone release at the central level (Lee et al., 1995). The results indicate that atrial natriuretic peptide is capable of modulating the membrane excitability of rat neurons and suggest that the peptide acts as a neuromodulator/neurotransmitter within the central nervous system (Wong et al., 1986).

Based on these recent data, it was of interest to study the secretion of hormones related to the regulation of water and electrolyte homeostasis, and particularly that of atrial natriuretic peptide during alcohol withdrawal and in delirium tremens. Another relevant issue — which could only be studied in experimental animals and not in humans — is the possible modulatory role and the mechanism of action of central nervous atrial natriuretic peptide in alcohol withdrawal.

2. Regulatory hormones of fluid and electrolyte homeostasis in human alcohol withdrawal: atrial natriuretic peptide as a potential diagnostic indicator of delirium tremens

In a series of clinical studies, chronic alcoholic patients were investigated (Bezzegh et al., 1991; Kovács et al., 1992). These patients exhibited various symptoms of alcohol withdrawal (tremor, irritability, anxiety, profuse perspiration and tachycardia). Sixty percent of the patients gradually exhibited typical symptoms of delirium tremens (hallucinations, disorientation etc.), however, these characteristic symptoms of delirium tremens usually developed slowly, and were not yet present on the day of hospital

admission. Compared to age-matched controls, alcohol-withdrawal patients on day 1 of hospitalization, i.e. in a state of severe alcohol withdrawal, were characterized by increased plasma atrial natriuretic peptide, plasma renin activity and aldosterone values. The serum Na^+ level was also significantly decreased in these patients (Fig. 1). On day 10 of alcohol withdrawal, i.e. in a state of partial recovery from withdrawal, there was an almost complete normalization of laboratory parameters (serum Na^+, plasma renin activity and aldosterone), except for the atrial natriuretic peptide level, which remained high throughout the whole observation period (Fig. 1).

There was an interesting difference noticed between patients who eventually developed clinical symptoms of delirium tremens and those who underwent withdrawal, but did not exhibit delirium. In those patients who developed delirium, atrial natriuretic peptide levels on the day of hospital admission were almost 50% higher than in those alcoholic patients who did not have delirium later. The latter group of chronic alcoholic patients had normal atrial natriuretic peptide levels. It is particularly interesting to note that the plasma renin activity during this recovery phase from alcohol withdrawal was significantly lower in patients who developed delirium tremens during the observation period.

These results corroborate the notion that chronic alcoholic patients exhibit severe disturbances of the hormonal regulation of fluid and electrolyte homeostasis. In accordance with this suggestion, significantly elevated plasma renin activity and aldosterone values were observed at the time of hospital admission, together with a slightly (however statistically significantly) decreased serum sodium level. One might speculate that the primary event in the sequence of changes is the moderate decrease of serum sodium concentration, which is known to occur, even in a much more pronounced form, among alcohol-withdrawal patients (Messert et al., 1979; Laureno, 1983). The increased plasma renin activity and the elevated aldosterone secretion might reflect the compensatory mechanism of the organism, which serves to conserve Na^+, and which ultimately leads to a normalization of the serum Na^+ concentration during the recovery phase of alcohol withdrawal. This chain of events seems to be present in both groups of withdrawal patients, i.e. in those who do as well as in those who do not develop delirium tremens during the observation period. An important implication of these studies is that alcohol-withdrawal patients have a disturbed plasma renin activity and aldosterone secretion, which is reversible, and does not show correlation with the incidence of delirium tremens.

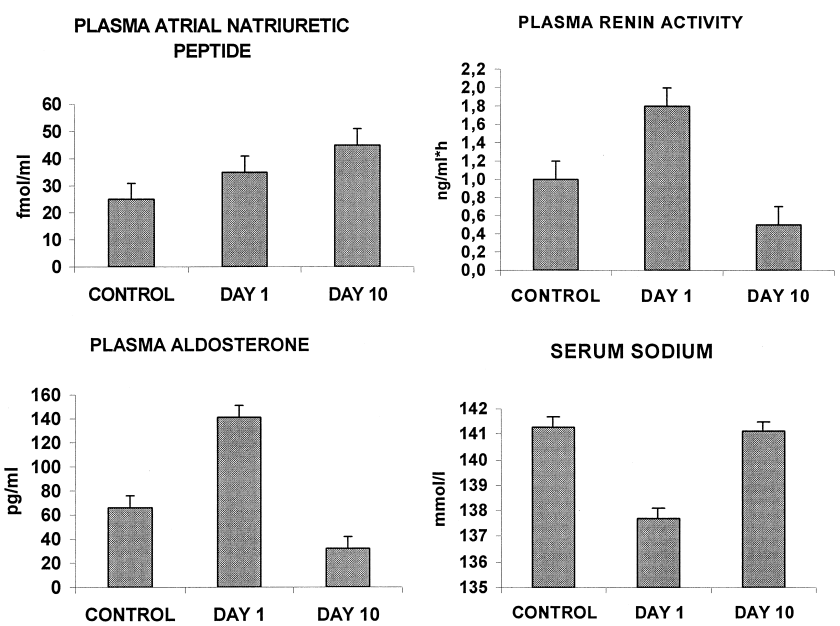

Fig. 1. Laboratory parameters during alcohol withdrawal in human patients. Thirty male chronic alcoholic patients were investigated. The mean age of the patients was 45 [26–64] years, with a mean duration of regular alcohol intake of minimally 5 years. Patients underwent the usual routine medical examinations (psychiatry, neurology and internal medicine). Patients with endogenous psychosis, organic central nervous diseases, other known metabolic or endocrine diseases were not included in the study. Those patients, who were found to consume alcohol during the investigation period of 10 days, were dropped out as well. For ethical considerations, liver biopsies were not performed. The patients received conventional therapy (high doses of minor tranquillants, polyvitamins). Age-matched hospital control values were taken as a basis for comparison. Blood was taken by venous puncture as part of the routine diagnostic procedure. Blood samples from day 1 and day 10 were not only assayed for routine purposes (electrolytes, white blood cells, sedimentation rate, liver enzymes [ASAT, ALAT, γ-GT, ALP], etc.: data not illustrated), but also for hormonal measurements. All hormones were measured with radioimmunoassay. For atrial natriuretic peptide, the diagnostic kit of Amersham (England) was used. Aldosterone was measured with the Aldosterone-Maia kit of Biodata (Italy). Plasma renin activity was measured with the Angiotensin I Test of Phadebas (Pharmacia, Sweden). Serum sodium was measured with an automated flame photometer (International Laboratory, IL-943, USA). Statistical evaluation of the data was performed with multiple analysis of variance (MANOVA) followed by post-hoc paired *t*-test.

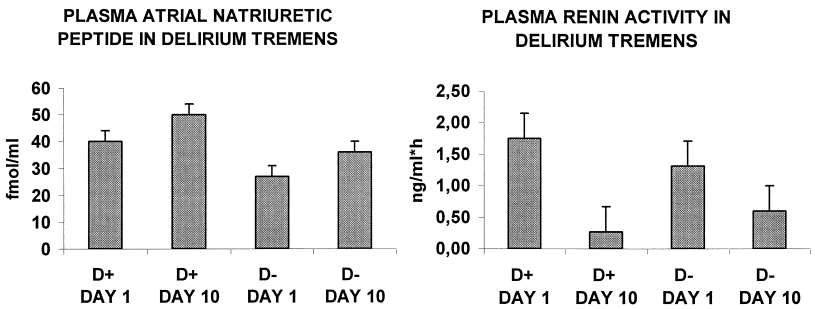

Fig. 2. Atrial natriuretic peptide and plasma renin activity in delirium tremens (DT + = delirium present, DT − = delirium absent).

A completely different picture seems to be present in the case of atrial natriuretic peptide, because the elevated level of atrial natriuretic peptide might be associated with the incidence of delirium tremens (Fig. 2). Alcohol-withdrawal patients, who did not develop delirium tremens, had a normal level of atrial natriuretic peptide. On the contrary, those withdrawal patients who developed delirium tremens had significantly (ca. 50%) elevated plasma atrial natriuretic peptide values, measured both in the early, severe, as well as during the recovery phase of withdrawal. It is not likely that the increased atrial natriuretic peptide would be directly related to changes in plasma renin activity, aldosterone, or serum Na^+. It is more plausible that the elevated atrial natriuretic peptide level reflects extracellular hypervolemia and the peptide is hypersecreted as a consequence of atrial stretch due to volume expansion (Tulassay et al., 1988). If so, one might argue that those patients, who, for any reason, have hypervolemia (and subsequently elevated plasma atrial natriuretic peptide levels) during the withdrawal reaction, would also have a significantly higher chance to develop delirium tremens. It is important to stress that in the early, severe phase of alcohol withdrawal, it was not possible to predict with conventionally available clinical methods, which patient of the total alcoholic population was going to develop delirium tremens.

It is a difficult question to answer, what the underlying reason might be, which ultimately results in putative volume expansion, in a higher atrial natriuretic peptide level and in an increased delirium risk in some of the patients. One reason might be the altered release of [Arg^8]vasopressin in the delirium group, which is known to affect the secretion of atrial natriuretic peptide (Needleman and Greenwald, 1986) and is supposedly related to withdrawal symptoms (Rigter and Crabbe, 1980; Szabó et al., 1987a,b). These hypotheses, however, need further experimental support.

Although tentative, our findings suggest the possibility that the increased level of atrial natriuretic peptide during the early phase of the alcohol-withdrawal syndrome is likely to be associated with the incidence of later delirium tremens. Thus, an elevated level of plasma atrial natriuretic peptide may also be used diagnostically, as an indicator to predict the threatening onset of delirium tremens.

3. Central nervous atrial natriuretic peptide and alcohol-withdrawal hyperexcitability in experimental mice

Animal models of the complete syndrome of alcoholism are difficult, if not impossible to achieve, but validated animal models exist for many of the different components of the syndrome. It is generally acknowledged that alcohol withdrawal is characterized by physiological hyperexcitability unmasked upon termination of alcohol administration. One of the withdrawal symptoms shared by humans and experimental animals is convulsion. Alcohol-withdrawal seizures have some characteristics of grand mal seizures, although there is no seizure focus, as in

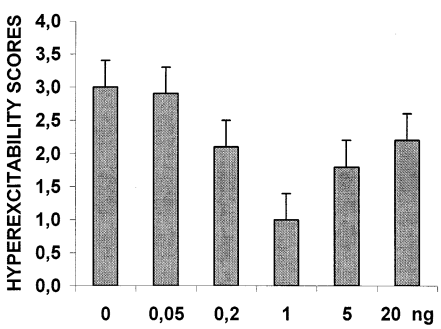

Fig. 3. Dose-dependent attenuation of withdrawal-related behavioral hyperexcitability following i.c.v. treatment with atrial natriuretic peptide. Male CFLP mice were used (Kovács, 1993). The animals received alcohol in the drinking water (5% v/v) for one week, then 7% for the second week. Seven days after the beginning of the experiments, mice were anesthetized with pentobarbital (Nembutal, 40 mg/kg) and equipped with a polyethylene cannula in the right lateral cerebral ventricle. Fourteen days after the start of the experiments, alcohol was removed and 5 h later handling-induced convulsions were scored: 0 = no convulsion; 1 = facial grimace (after 180° spin); 2 = tonic convulsion (after 180° spin); 3 = tonic-clonic convulsion (after 180° spin); 4 = tonic convulsion (when lifted by tail); 5 = tonic-clonic convulsion (when lifted by tail); 6 = severe tonic-clonic convulsions of long duration (when lifted by tail); 7 = severe tonic-clonic convulsions of long duration (before lifted by tail); 8 = severe tonic-clonic convulsions ending with death. Atrial natriuretic peptide (human, 1–28) was dissolved in physiological saline and injected into the lateral cerebral ventricle in a volume of 2 μl.

epilepsy. Freund (1969) noticed that alcohol-addicted mice displayed a sequence of characteristic behaviors following withdrawal from alcohol, starting with hyper-reactivity to external stimuli (acoustic, handling-induced, etc.), tremor and ending with recurring tonic-clonic convulsions and often death. Goldstein (1972) elaborated a sensitive scoring system to evaluate behavioral hyperexcitability in mice.

In a recent experiment by Kovács (1993), mice were rendered tolerant/dependent on alcohol, by replacing the drinking water with alcohol. When alcohol was removed, withdrawal-related central nervous hyperexcitability was studied, scoring handling-induced convulsions according to Goldstein (1972). Graded doses of atrial natriuretic peptide, injected into the lateral cerebral ventricle, resulted in an inverted U-shaped dose–response effect. Medium high doses of atrial natriuretic peptide significantly decreased handling-induced convulsion scores, whereas low and high doses were without effect. Thus, atrial natriuretic peptide attenuated central nervous hyperexcitability in alcohol-dependent mice during the withdrawal period (Fig. 3). Since atrial natriuretic peptide failed to affect this behavior in non-dependent control animals, a sedating effect of the peptide is not likely (Kovács, 1993). Anti-epileptic effects of atrial natriuretic peptide, independent of alcohol withdrawal, are also not likely, since Mazarati et al. (1993) found a late-onset pro-epileptic, rather than an anti-epileptic effect of natriuretic peptides in a picrotoxin-kindling model in rats.

Neutralization of endogenous neuropeptides in the brain by central injections of specific antisera was frequently used in various behavioral models to investigate the possible role of brain-born neuropeptides. In contrast to atrial natriuretic peptide, i.c.v. injection of an antiserum against atrial natriuretic peptide resulted in a more severe behavioral hyperexcitability (increased handling-induced convulsions) during alcohol withdrawal in mice (Fig. 4).

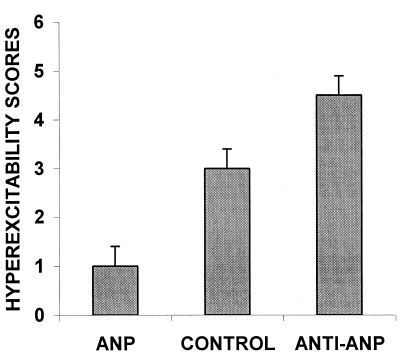

Fig. 4. Opposite effects of atrial natriuretic peptide and antiserum against atrial natriuretic peptide on behavioral hyperexcitability during alcohol withdrawal in mice. Antiserum against human atrial natriuretic peptide (anti-ANP) was produced in rabbits (Peptide Institute, Osaka, Japan) and dissolved in saline. A dilution of 1:20 was injected in a volume of 4 μl. The cross-reactivities for the antiserum were as follows: atrial natriuretic peptide (ANP, human, 1–28): 100%, atrial natriuretic peptide (human, 7–28): 100%, β-atrial natriuretic peptide (human, 1–28 dimer), atrial natriuretic peptide (rat, 1–28): 55%. No cross-reactivities were detected with oxytocin, [Arg^8]vasopressin, somatostatin, [Met^5]enkephalin, and β-endorphin. Atrial natriuretic peptide was injected in a dose of 1 ng. Treatments were given i.c.v. 4 h after alcohol withdrawal. Handling-induced convulsions were scored 1 h later.

Although the conclusion might be preliminary, the present data indicate that central administration of atrial natriuretic peptide attenuates, while the neutralization of endogenous atrial natriuretic peptide by central administration of an antiserum against atrial natriuretic peptide intensifies the symptoms of alcohol withdrawal. It is therefore hypothesized that endogenous atrial natriuretic peptide in the brain is involved in the control of the central nervous symptoms of alcohol withdrawal.

4. Central atrial natriuretic peptide and *N*-methyl-D-aspartate receptor interactions in alcohol withdrawal

Acute or chronic consumption of alcohol interferes differentially with transmission processes in the central nervous system, affecting many — if not all — of the known neurotransmitter systems. Selective pharmacological manipulations of some of these neurotransmitter systems have been shown to reduce alcohol intake and preference, as well as the severity of the alcohol-withdrawal syndrome in animal models, certain compounds having even been employed successfully in the clinic.

The role of the brain's major inhibitory amino acid transmitter system (γ-aminobutyric acid, GABA) has been widely studied over the past decade. There is a general consensus that acute alcohol treatment facilitates GABA-ergic transmission (by enhancing chloride conductance through the $GABA_1$ receptor). Conversely, the development of tolerance associated with chronic alcohol consumption leads to a reduced GABA-ergic function (Nevo and Hamon, 1995).

The major excitatory neurotransmitter in the central nervous system is L-glutamate, and one of the subtypes of L-glutamate receptors, the *N*-methyl-D-aspartate receptor, has been found to be quite sensitive to inhibition by low concentrations of alcohol (Michaelis and Michaelis, 1994). Recent evidence suggests that alcohol abuse produce diverse effects on the brain to a substantial degree by disrupting the function of glutamate. Alcohol, given acutely at concentrations associated with behavioral effects in humans, is a potent and selective inhibitor of the function of the *N*-methyl-D-aspartate subtype of glutamate receptors (Weight et al., 1991). It has been found that alcohol potently inhibits *N*-methyl-D-aspartate mediated synaptic currents in the basolateral amygdala, a brain region associated with actions of anxiolytic agents, such as alcohol (Calton et al., 1998). The effect of alcohol can be reversed by high concentrations of glycine, and non-equilibrium ligand binding studies in brain membrane preparations

suggest that alcohol may act by decreasing the frequency of ion channel opening (Littleton et al., 1991).

Chronic alcohol ingestion — leading to tolerance and physical dependence — results in up-regulation of the *N*-methyl-D-aspartate receptors in many brain areas (measured by ligand binding), so that abrupt withdrawal produces a hyperexcitable state that leads to seizures (Fig. 5), delirium tremens, and excitotoxic neuronal death (Tabakoff et al., 1991). This increase in receptors is clearly associated with alcohol-withdrawal seizures, which can be attenuated by *N*-methyl-D-aspartate receptor antagonists (Hoffman, 1995). This enhanced glutamatergic transmission probably results from a combination of increased *N*-methyl-D-aspartate receptor activation, decreased $GABA_1$ receptor activation and increased function of voltage-activated calcium channels (Lovinger, 1993). Therapeutic strategies aimed at correcting glutamatergic dysregulation in alcoholism have been studied by Hoffman and Tabakoff (1996) and Tsai and Coyle (1998), but still need to be further explored. In accordance with previous biochemical and behavioral findings (Lovinger, 1993), *N*-methyl-D-aspartate induced severe seizures in alcohol tolerant/dependent mice during a period of alcohol withdrawal. These results are fully in line with the observations that alcohol, given acutely, inhibits *N*-methyl-D-aspartate-activated current in brain cells, and also inhibits *N*-methyl-D-aspartate-stimulated Ca^{2+} uptake and cyclic GMP production (for summary: Weight et al., 1991). Alcohol presumably has a direct effect on the Ca^{2+} flux through voltage-operated calcium channel proteins (Littleton et al., 1991). Long-term modification of *N*-methyl-D-aspartate sites could possibly contribute to a receptor up-regulation. Indeed, chronic alcohol administration has been shown to increase the number of *N*-methyl-D-aspartate receptor-ion channel complexes in neuronal cells (Grant et al., 1990), which in turn, may be the underlying mechanism for an increased *N*-methyl-D-aspartate-induced seizure activity during alcohol withdrawal.

Based on these findings, it was of interest to investigate whether centrally administered exogenous atrial natriuretic peptide and the neutralization of endogenous atrial natriuretic peptide in the brain were able to modify the increased *N*-methyl-D-aspartate-induced seizure activity in mice during the alcohol-withdrawal period. In a recent experiment (Kovács, 2000), i.c.v. administration of atrial natriuretic peptide resulted in a dose-dependent attenuation of *N*-methyl-D-aspartate-induced seizures in alcohol-dependent mice. I.c.v. administration of an antiserum against atrial natriuretic peptide, presumably by neutralizing endogenous atrial natriuretic peptide, potentiated *N*-methyl-D-aspartate-induced seizure activity during alcohol withdrawal. The data therefore indicate that modulation of atrial natriuretic peptide levels in the central nervous system changes the severity of *N*-methyl-D-aspartate-induced behavioral seizures in alcohol tolerant/dependent mice during a period of alcohol withdrawal. This finding suggests that atrial natriuretic peptide might interfere with neuronal mechanisms related to the glutamate/*N*-methyl-D-aspartate receptor complex.

Neurochemical mechanisms of an interaction of acute atrial natriuretic peptide administration (or those of an acute neutralization of endogenous atrial natriuretic peptide by an antiserum against atrial natriuretic peptide) with a chronically developing process of *N*-methyl-D-aspartate hypersensitivity during alcohol tolerance/dependence remain to be studied. It is of interest, however, that neither

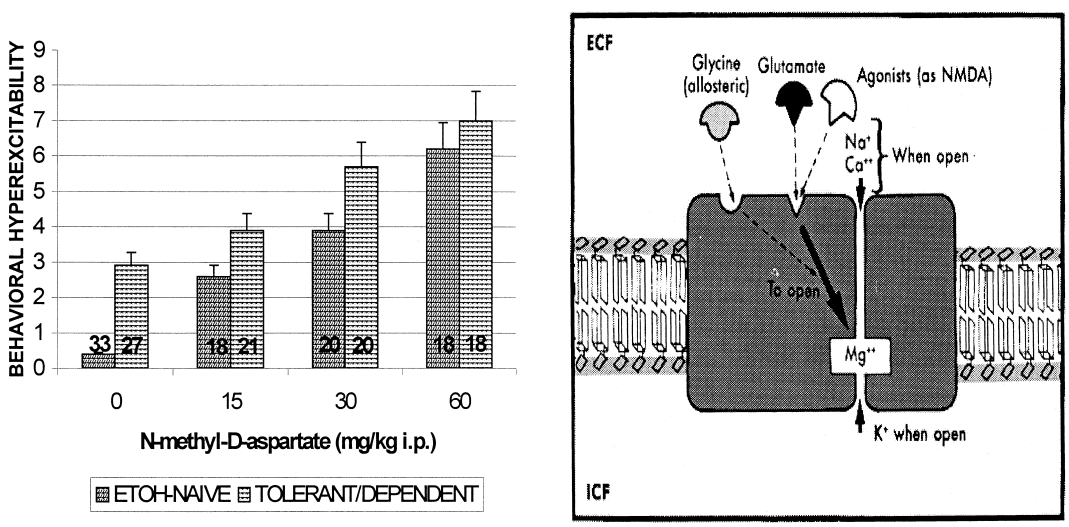

Fig. 5. *N*-methyl-D-aspartate, an agonist of glutamate binding sites, induces more severe seizures in alcohol-dependent, than in alcohol-naive mice. *N*-methyl-D-aspartate was dissolved in physiological saline and injected intraperitoneally (i.p.) in a volume of 0.1 ml/10 g body weight. *N*-methyl-D-aspartate administration resulted in behavioral seizures in a dose-dependent manner. This effect was present both in alcohol-naive ($P < 0.001$), as well as in alcohol-dependent mice ($P < 0.001$). The level of *N*-methyl-D-aspartate-induced seizures, however, was significantly ($P < 0.01$) more severe in the alcohol-dependent mice in the withdrawal period than in the alcohol-naive animals.

atrial natriuretic peptide, nor the investigated antiserum against atrial natriuretic peptide was able to modify *N*-methyl-D-aspartate-induced seizure activity in alcohol-naive animals (Kovács, 2000). These findings corroborate the hypothesis that the effect of atrial natriuretic peptide is more related to neurochemical and behavioral (e.g. seizure-inducing) properties of the hypersensitive glutamate/*N*-methyl-D-aspartate system of the alcohol tolerant/dependent organism.

Since atrial natriuretic peptide diminishes, and the antiserum treatment against atrial natriuretic peptide potentiates alcohol-withdrawal symptoms in alcohol tolerant/dependent mice, as well as the *N*-methyl-D-aspartate-induced seizure activity during alcohol withdrawal, one might speculate that atrial natriuretic peptide interferes with neurochemical mechanisms functionally 'distal' to the hyperexcitable *N*-methyl-D-aspartate-receptor complex. Further experiments are required to elucidate these mechanisms, especially since dopaminergic, cholinergic, beta-adrenergic (Bidzseranova et al., 1991a,b,c) and vasopressinergic (Shirakami et al., 1993) interactions of central atrial natriuretic peptide may be hypothesized.

5. Conclusions

Among the many factors that may influence physical dependence on, and withdrawal from alcohol are various neuropeptides. [Arg^8]vasopressin, administered exogenously, maintains alcohol tolerance in animals, once such tolerance has been established (for summary: Hoffman and Tabakoff, 1996). [Arg^8]vasopressin administration can also produce an increase in septal c-fos mRNA levels. An antisense oligonucleotide completely blocked the ability of [Arg^8]vasopressin to maintain alcohol tolerance, while a missense oligonucleotide was without effect. The antisense oligonucleotide also attenuated the increase in septal c-fos mRNA levels caused by [Arg^8]vasopressin (Szabó et al., 1996). Peripheral administration of oxytocin prevented the development of tolerance to alcohol in mice (Szabó et al., 1985, 1987b). Central administration of oxytocin was found to be at least 500 times more potent than peripheral injection at blocking the development of rapid tolerance to alcohol (Szabó et al., 1989), which lends support to the theory that oxytocin acts on central nervous system mechanisms to influence adaptive responses to drugs. Oxytocin-treated mice also displayed milder withdrawal convulsions in response to increasing dose of the peptide and the rate of lethality was also decreased (Szabó et al., 1987a). In human chronic alcoholic patients, plasma oxytocin levels were significantly elevated during the withdrawal period, suggesting an involvement of oxytocin in alcohol-induced neuropsychological deficits (Marchesi et al., 1997).

Neuropeptide Y is a hexatriacontapeptide amide that is now well characterized as a neuromodulator in the central nervous system. Recent studies suggest that exposure to chronic alcohol may affect neuropeptide Y-like immunoreactivity at the level of the hypothalamus. Differences in neuropeptide Y-like immunoreactivity in limbic areas and frontal cortex between alcohol-naive alcohol-preferring and non-preferring rats suggest that neuropeptide Y may also play a role in the development of alcohol preference either by modulating the 'tension-reduction' properties of alcohol, or by influencing consummatory behaviors (Ehlers et al., 1998). Central administration of neuropeptide Y in low concentrations has been shown to produce anxiolysis and suppression of locomotor activity, a behavioral profile not dissimilar to that of alcohol. These studies also demonstrate that neuropeptide Y and alcohol have a similar electrophysiological profile. In addition, the combined administration of neuropeptide Y and alcohol-produced additive effects (Ehlers et al., 1998).

In the central nervous system, cholecystokinin (CCK) is an important neurotransmitter that influences food intake, anxiety, nociception, and dopamine-related behavior. Okubo et al. (1999) examined the genetic variants of the cholecystokinin gene promoter region among healthy Japanese, and patients with alcohol-withdrawal syndrome (delirium tremens, hallucinations, and seizures). Patients with delirium tremens showed a significantly higher frequency of the cholecystokinin receptor allelic mutation in the promoter region. These data suggest that the cholecystokinin gene might be susceptible to delirium tremens caused by alcohol abuse. Crespi (1998) found that the endogenous tone of the cholecystokinin system is higher in alcohol-drinking rats than in water-preferring rats and that a treatment with a fragment of cholecystokinin (a cholecystokinin receptor agonist) selectively induced the water-preferring rats to drink alcohol. The data imply a CCK_1 receptor mechanism in the regulation of individual sensitivity towards alcohol. Thus, a potential therapeutic role for CCK_1 receptor antagonists in the treatment of alcohol abuse is proposed. In a recent experiment by Wilson et al. (1998) on mice and rats, on the other hand, CCK_2 receptor antagonists were effective in decreasing the majority of the anxiogenic effects of withdrawal from chronic alcohol treatment. Further studies by the same authors (Wilson and Little, 1998) suggest that changes in CCK_2 receptors may be involved in the later stages of the alcohol-withdrawal syndrome.

Corticotropin-releasing factor has also been implicated in alcohol withdrawal. It has been proposed that alcohol dependence may produce a prolonged dysregulation of the corticotropin-releasing factor system in the basal forebrain that may contribute to the increased motivational effect of alcohol withdrawal (Menzaghi et al. 1994; Koob et al. 1998).

Human and animal studies suggest that some of the reinforcing effects of alcohol may be mediated by the endogenous opioid system. In human studies, plasma levels of subjects genetically at high risk for excessive alcohol consumption showed lower basal activity of β-en-

dorphin and more pronounced release of β-endorphin in response to alcohol. In animal studies, the hypothalamus of mice bred for alcohol preference showed high basal activity of β-endorphin and more pronounced release of β-endorphin in response to alcohol than did alcohol non-preferring control mice. Increased opioidergic activity could mediate the rewarding effects of alcohol, reinforce the act of drinking, and increase alcohol consumption (Gianoulakis et al., 1996).

The concentration of [Met^5]enkephalin in the whole brain has also been implicated in the consumption of alcohol. The concentrations of [Met^5]enkephalin were significantly greater in withdrawal seizure-prone mice than in withdrawal seizure-resistant mice, whereas synthesis of [Met^5]enkephalin, as reflected by preproenkephalin mRNA levels, and transport out of the brain by peptide transport system-1, was not different. These results support a direct link between elevated concentrations of [Met^5]enkephalin in whole brain and proneness to withdrawal-induced seizures (Plotkin et al., 1998).

Taken together, these data strongly suggest that various neuropeptide systems in the brain may play a role in the symptomatology of the alcohol abstinence syndrome and raise the possibility that central nervous atrial natriuretic peptide may also be involved. Atrial natriuretic peptide and related peptides (brain natriuretic peptide, C-type natriuretic peptide), as well as their receptors, exist in the central nervous system (Geiger et al., 1991; Imura et al., 1992) and have been shown to modify various adaptive functions of the brain (Bidzseranova et al., 1991a,b,c; Mazarati et al., 1993). Atrial natriuretic peptide is released from the brain to regulate the cardiovascular system, drinking behavior, and neurohormone release at the central level (Lee et al., 1995). Recently, atrial natriuretic peptide has been shown to affect various adaptive behavioral processes in the central nervous system. Accordingly, extinction of an active avoidance learning (Bidzseranova et al., 1991a), electroconvulsive shock-induced amnesia (Bidzseranova et al., 1991b) and novelty-induced locomotor (open-field) activity (Bidzseranova et al. 1991c; Telegdy, 1994) were deeply affected in rats by i.c.v. administration of atrial natriuretic peptide, given in relatively high doses. Tolerance to and dependence on alcohol is a complex behavioral process also involving adaptive components. The results therefore indicate that atrial natriuretic peptide is capable of modulating neuronal membrane excitability and suggest that atrial natriuretic peptide may act as a neuromodulator/neurotransmitter within the central nervous system (Wong et al., 1986). In agreement with the central neuromodulator/neurotransmitter role of atrial natriuretic peptide is the major conclusion of the present animal studies indicating that this neuropeptide attenuated, whereas neutralization of atrial natriuretic peptide by central injections of a specific antiserum, intensified behavioral hyperexcitability during the withdrawal period in alcohol-dependent mice.

Repeated high, intoxicating doses of alcohol trigger neuroadaptive processes that lead to dependence and contribute to the manifestation of the abstinence syndrome upon withdrawal. An unbalance between inhibitory and excitatory neurotransmission is the most prominent neuroadaptive process induced by chronic alcohol consumption. Due to the diffuse glutamatergic innervation to all brain structures, the neuroadaptive alterations in excitatory neurotransmission can affect the function of most if not all of neurotransmitter systems. The adaptive changes, which cause the alcohol withdrawal syndrome, are not known for certain, but alterations in *N*-methyl-D-aspartate receptors have a definite claim. An increase in the activity of central nervous *N*-methyl-D-aspartate-receptors is clearly associated with alcohol withdrawal seizures. This enhanced glutamatergic transmission could be successfully inhibited by central atrial natriuretic peptide treatment and it was found to be more severely disturbed when central atrial natriuretic peptide was neutralized by antiserum against atrial natriuretic peptide.

These findings support the hypothesis that the central regulatory role of atrial natriuretic peptide in balancing the severity of alcohol withdrawal symptoms may be associated with glutamatergic neuronal mechanisms.

Peripheral atrial natriuretic peptide released from the heart lowers arterial blood pressure in association with vasodilatation and sympatholytic activity (Melo et al., 1999). Since alcohol induces a diuretic response and a decrease in atrial size (atrial distension), it was hypothesized that alcohol intake might be associated with a decrease in plasma atrial natriuretic peptide level. In humans, alcohol inhibited the nocturnal increase of plasma atrial natriuretic peptide (Ekman et al., 1994). Following high-dose alcohol intake, plasma atrial natriuretic peptide significantly decreased (Leppaluoto et al., 1992). However, plasma atrial natriuretic peptide concentration did not change in slightly volume-loaded subjects (Hynynen et al., 1992). An important observation of the present experiments is that elevated plasma level of peripheral atrial natriuretic peptide might be an early indicator of threatening delirium tremens in chronic alcoholic patients.

Most neuropeptides do not easily enter the brain from the periphery by diffusion across the blood–brain barrier. Although peripheral administration of various neuronal peptides has been demonstrated to affect adaptive behavioral processes, neuropeptides, when given through a peripheral route, had to be usually administered in doses of several orders of magnitude higher, to achieve reliable central nervous effects. It is a question, therefore, whether the altered levels of plasma atrial natriuretic peptide in alcohol withdrawal, a change in a non-pharmacological dimension, could have any impact on brain atrial natriuretic peptide levels. On the other hand, alcohol-withdrawal syndrome, and particularly delirium tremens is also known to damage the functional integrity of the blood–brain barrier and the barrier might be ‘leaking’ during delirium.

In conclusion, the exact mechanisms involved in the appearance of alcohol withdrawal symptoms and particularly those related to the precipitation of delirium tremens await further clarification. The proposal, that regular intake of alcohol causes cellular adaptation increasing cellular activity on withdrawal, is rather convincing. Global cerebral and cardiovascular dysfunctions are supposed to occur during alcohol withdrawal. The activity of central nervous neuronal pathways, operating with atrial natriuretic peptide as neuropeptide might be an integral part of the central nervous system, whereas peripheral secretion of cardiac atrial natriuretic peptide might be a component of such cardiovascular mechanisms. A better understanding of the mechanisms that support alcohol dependence in animals offers hope for the development of pharmacological interventions to block these mechanisms, an approach that is now being explored in humans. Ongoing research needs better communication between basic scientists and clinicians to establish research goals and to improve current models.

Acknowledgements

The skillful assistance of Katalin Kovács and László Németh is gratefully acknowledged. The experiments were supported by the research grant of OTKA (No.: T 023533).

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