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Atrial natriuretic peptide modulates *N*-methyl-D-aspartate-induced hyperexcitability in ethanol-dependent mice

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Abstract

The role of central nervous atrial natriuretic peptide was investigated for behavioral hyperexcitability in alcohol-dependent mice. Mice were made tolerant to and dependent on ethanol with an ethanol–liquid diet for 14 days. Five hours after withdrawal from ethanol, withdrawal symptoms were analyzed by scoring handling-induced convulsions. *N*-methyl-D-aspartate (NMDA) induced behavioral seizures in a dose-dependent manner, an effect which was more intensive during the ethanol withdrawal period than in alcohol-naive animals. Intracerebroventricular (i.c.v.) injections of α-atrial natriuretic peptide (atrial natriuretic peptide) dose-dependently inhibited, whereas injection of an antiserum against atrial natriuretic peptide potentiated, the seizure-inducing effect of NMDA in ethanol-dependent mice. The main conclusion is that central nervous atrial natriuretic peptide plays a modulatory role in behavioral hyperexcitability during alcohol withdrawal.

Keywords: Ethanol tolerance; Ethanol dependence; Withdrawal; NMDA (*N*-methyl-D-aspartate); ANP (atrial natriuretic peptide); Antiserum against atrial natriuretic peptide

1. Introduction

Although alcoholism is one of the most common psychiatric diagnoses, understanding of its pathophysiology remains poor. 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 (NMDA) receptor, has been found to be quite sensitive to inhibition by low concentrations of ethanol (Michaelis and Michaelis, 1994). Recent evidence suggests that ethanol abuse produces its diverse effects on the brain to a substantial degree by disrupting the function of glutamate.

Ethanol, given acutely at concentrations associated with behavioral effects in humans, is a potent and selective inhibitor of the function of the NMDA subtype of glutamate receptors (Weight et al., 1991). It has been found that ethanol potently inhibits NMDA-mediated synaptic currents in the basolateral amygdala, a brain region associated with actions of anxiolytic agents, such as ethanol (Calton et al., 1998). The effect of ethanol can be reversed by high concentrations of glycine, and non-equilibrium ligand binding studies with brain membrane preparations suggest that ethanol may act by decreasing the frequency of ion channel opening (Littleton et al., 1991).

Chronic ethanol ingestion — leading to tolerance and physical dependence — results in upregulation of the NMDA receptors in many brain areas (measured by ligand binding), so that abrupt withdrawal produces a hyperexcitable state that leads to seizures, delirium tremens, and excitotoxic neuronal death (Tabakoff et al., 1991). This increase in receptors is clearly associated with ethanol withdrawal seizures, which can be attenuated by NMDA receptor antagonists (Hoffman, 1995). Ethanol's inhibition of the NMDA receptor in the fetal brain is likely to contribute to the central nervous system manifestations of fetal alcohol syndrome. This enhanced glutamatergic transmission probably results from a combination of increased NMDA receptor activation, decreased $GABA_A$ receptor activation and increased function of voltage-activated calcium channels (Lovinger, 1993). Therapeutic strategies

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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.

Natriuretic peptides, mainly produced in the heart (atrial and B-type natriuretic peptide, BNP), brain (C-type atrial natriuretic peptide) and kidney (urodilatin) decrease blood pressure and increase salt excretion (for review, see Espinger et al., 1995). All of the peptides and their receptors exist in the central nervous system (for reviews, see Geiger et al., 1991; Imura et al., 1992). Atrial natriuretic peptide and its smaller congeners are produced by and released from the brain, and regulate the cardiovascular system, drinking behavior, and neurohormone release at the central level (Lee et al., 1995).

In rats, intraperitoneal injections of ethanol increase the plasma content of atrial natriuretic peptide in a dose-dependent manner (Guillaume et al., 1994). Following chronic ethanol ingestion, however, BNP is affected rather than atrial natriuretic peptide (Wigle et al., 1993a,b). In humans, intake of ethanol inhibits the nocturnal increase of plasma atrial natriuretic peptide (Ekman et al., 1994). Acute consumption of low amounts of ethanol in humans induces an increase in the plasma content of atrial natriuretic peptide (Gianoulakis et al., 1997). Following high-dose ethanol intake, on the other hand, the level of plasma-immunoreactive atrial natriuretic peptide level decreases significantly (Leppaluoto et al., 1992). During ethanol withdrawal in human patients, a fair correlation was found between the plasma atrial natriuretic peptide levels and the onset of delirium tremens (Bezzegh et al., 1991; Kovács et al., 1992).

Recent experimental research on alcohol withdrawal suggests that central nervous atrial natriuretic peptide may participate in the neural mechanisms which regulate the onset and severity of ethanol withdrawal symptoms (Kovács, 1993). Based on the above reports, it was of interest to investigate whether central nervous administration of atrial natriuretic peptide and the neutralization of endogenous atrial natriuretic peptide by intracerebroventricular (i.c.v.) injections of an antiserum against atrial natriuretic peptide would be able to modify the seizure-inducing effect of NMDA in the ethanol-dependent organism during the alcohol withdrawal period.

2. Materials and methods

2.1. Experimental animals and alcohol withdrawal

Male CFLP mice (Gödöllö, Hungary), weighing 35 ± 5 g, were used in the experiments (Kovács, 1993). The animals received ethanol in the drinking water (5% [v/v] for 1 week, then 7% for the second week). Seven days after the beginning of the experiments, the 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 initiation of the experiments, ethanol was removed and behavioral hyperexcitability due to alcohol withdrawal was measured by testing handling-induced convulsions according to Goldstein (1972): 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 being lifted by tail); 8 — severe tonic–clonic convulsions ending with death.

2.2. Pharmacological treatments

The schedule of pharmacological treatments is summarized in Table 1. NMDA (Sigma) was dissolved in physiological saline and injected intraperitoneally (i.p.) in a volume of 0.1 ml/10 g body weight. Doses of 15, 30 or 60 mg/kg were administered. Convulsions were tested 3,

Table 1
Summary of treatment schedules

Treatment schedule	Ethanol-naive	Ethanol-tolerant/dependent
For 14 days continuously	Animals drinking water	Animals with ethanol in the drinking water 5% v/v, 7 days and then 7% v/v, 7 days
Day 7	–	Implantation of i.c.v. cannula
Day 15	0 min	Ethanol replaced by drinking water
	240 min	i.c.v. treatment: atrial natriuretic peptide, antiserum against atrial natriuretic peptide or vehicle
	270 min	i.p. treatment: NMDA or vehicle
	273 min	Behavioral hyperexcitability tested
	300 min[a]	Behavioral hyperexcitability tested[a]
	330 min	Behavioral hyperexcitability tested
	390 min	Behavioral hyperexcitability tested

[a] Only these results presented.

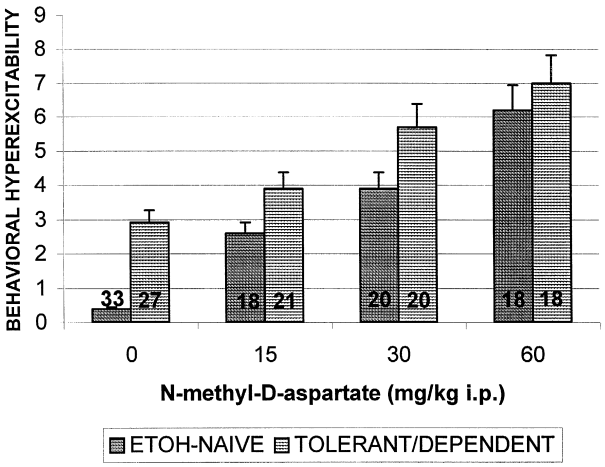

Fig. 1. NMDA-induced seizures in ethanol-naive and ethanol-tolerant/dependent mice. Behavioral hyperexcitability: arbitrary units (mean ± SD) of seizure scores. The numbers in the bars represent the number of experimental animals. ETOH-naive: ethanol-naive animals.

30, 60 and 120 min after the i.p. injection of NMDA. There were no differences noticed, after any treatment combination, in the time course of convulsions. Therefore, only results for the maximal convulsion intensity, measured 30 min after the injection of NMDA, are shown.

Atrial natriuretic peptide (human, 1–28) was dissolved in physiological saline and injected in a volume of 2 μl. Antiserum against human atrial natriuretic peptide was produced in rabbits (Peptide Institute, Osaka, Japan) and dissolved in saline. Dilutions of 1:20 1:10 and 1:5 were injected in a volume of 4 μl. The cross-reactivities for the antiserum were as follows: atrial natriuretic peptide (human, 1–28): 100%; atrial natriuretic peptide (human, 7–28): 100%; β-atrial natriuretic peptide (human, 1–28 dimers), atrial natriuretic peptide (rat, 1–28): 55%. No cross-reactivities were detected with oxytocin, [Arg^8]-vasopressin, somatostatin, [Met^5]enkephalin or β-endorphin. Control animals received saline (2 μl) or normal rabbit serum (4 μl of 1:5 dilution). No differences were noticed between the two control groups.

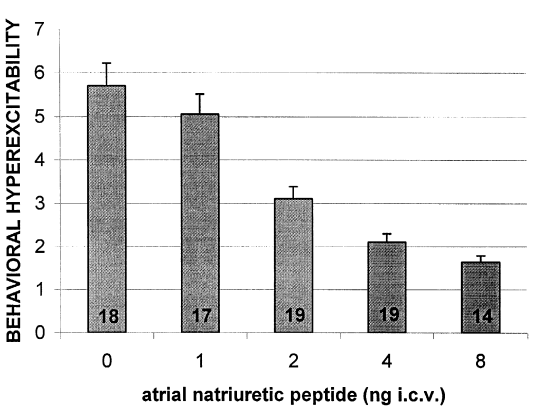

Fig. 2. The effect of atrial natriuretic peptide on NMDA-induced seizures in ethanol-tolerant/dependent mice (for symbols, see Fig. 1).

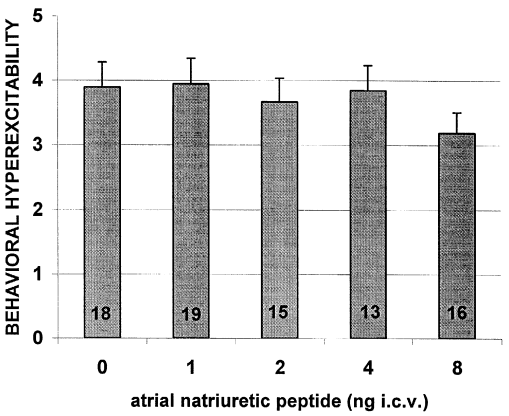

Fig. 3. The effect of atrial natriuretic peptide on NMDA-induced seizures in ethanol-naive mice (for symbols, see Fig. 1).

2.3. Statistical analysis

Results were analyzed with a non-parametric analysis of variance (Kruskal–Wallis test). A probability level of less than 5% was accepted as indicating significant differences.

3. Results

NMDA, an agonist ligand of one type of L-glutamate receptor, resulted in behavioral seizures in mice. The seizure-inducing effect of NMDA was dose-dependent, i.e. increasing doses of the receptor agonist resulted in more severe convulsions (Chi-square = 109.0, $df = 3$, $P < 0.001$). This effect was present in both ethanol-naive (Chi-square = 64.2, $df = 3$, $P < 0.001$) and ethanol-dependent mice (Chi-square = 49.8, $df = 3$, $P < 0.001$). The NMDA-induced seizures, however, were significantly (Chi-square = 5.1, $df = 1$, $P < 0.05$) more severe in the ethanol-dependent mice in the withdrawal period, than in ethanol-naive animals (Fig. 1). Based on these dose–response studies, a higher — although submaximal — dose of NMDA was used to investigate the inhibitory effect of

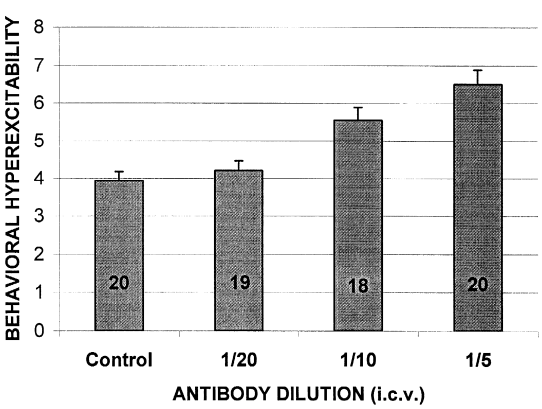

Fig. 4. The effect of antiserum against atrial natriuretic peptide on NMDA-induced seizures in ethanol-tolerant/dependent mice (for symbols, see Fig. 1).

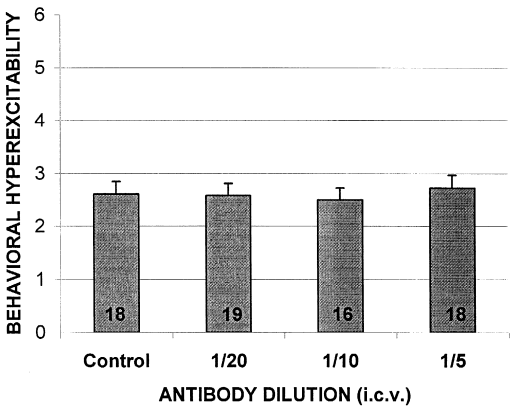

Fig. 5. The effect of antiserum against atrial natriuretic peptide on NMDA-induced seizures in ethanol-naive mice (for symbols, see Fig. 1).

atrial natriuretic peptide, whereas the smallest effective convulsive dose of the receptor agonist was administered to analyze the potentiating effect of the antiserum against atrial natriuretic peptide.

Accordingly, a test dose of 30 mg/kg NMDA was subsequently used to investigate the interaction of atrial natriuretic peptide with NMDA-induced seizures. In ethanol-dependent mice, i.c.v. administration of atrial natriuretic peptide resulted in a dose-dependent attenuation of NMDA-induced seizures (Chi-square = 66.6, $df = 4$, $P < 0.001$, Fig. 2).

The less intensive seizure activity of alcohol-naive mice, on the other hand, was not affected by i.c.v. administration of atrial natriuretic peptide (Chi-square = 2.7, $df = 4$, $P > 0.05$, Fig. 3).

A lower (15 mg/kg) dose of NMDA was administered to study the effect of the antiserum against atrial natriuretic peptide on NMDA-induced seizures. Graded dilutions of the antiserum (1:20, 1:10 and 1:5 dilutions) resulted in a dose-dependent potentiation of NMDA-induced seizures in ethanol-dependent mice (Chi-square = 38.4, $df = 3$, $P < 0.001$, Fig. 4).

In ethanol-naive animals, on the other hand, antiserum against atrial natriuretic peptide failed to modify the seizure-inducing effect of NMDA (Chi-square = 0.6, $df = 3$, $P > 0.05$, Fig. 5).

4. Discussion

In accordance with previous biochemical and behavioral findings (Lovinger, 1993), NMDA induced more severe seizures in ethanol-tolerant/dependent mice during a period of ethanol withdrawal than in ethanol-naive animals. These results are fully in line with observations that ethanol, given acutely, inhibits the NMDA-activated current in brain cells and also inhibits the NMDA-stimulated Ca^{2+} uptake and cyclic GMP production (for summary, see Weight et al., 1991). Ethanol presumably has a direct effect on the Ca^{2+} flux through voltage-operated calcium channel proteins (Littleton et al., 1991). Long-term modification of NMDA sites could possibly contribute to receptor upregulation. Indeed, chronic ethanol administration has been shown to result in an increase in the number of NMDA receptor–ion channel complexes in neuronal cells (Grant et al., 1990), which in turn may be the underlying mechanism for the increased NMDA-induced seizure activity during ethanol withdrawal.

Recently, atrial natriuretic peptide has been shown to affect various adaptive behavioral processes in the central nervous system. Accordingly, extinction of 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, although the peptide was given in relatively high doses. Tolerance to and dependence on ethanol is a complex behavioral process also involving adaptive components. The i.c.v. administration of atrial natriuretic peptide has been shown to attenuate ethanol withdrawal symptoms in tolerant/dependent mice (Kovács, 1993).

Presumably, both exogenously administered atrial natriuretic peptide and endogenous atrial natriuretic peptide have various effects on adaptive neuronal processes. In keeping with this hypothesis, i.c.v. administration of an antiserum against atrial natriuretic peptide, by neutralizing endogenous atrial natriuretic peptide, affects ethanol tolerance in mice (Kovács, 1993) and various types of motivated behavioral reactions in rats (Telegdy, 1994). The effect of antiserum against atrial natriuretic peptide is always the opposite of that of atrial natriuretic peptide itself.

The mode of action of central nervous atrial natriuretic peptide on ethanol tolerance/dependence is unknown. The present data indicate that modulation of atrial natriuretic peptide levels in the central nervous system changes the severity of NMDA-induced behavioral seizures in ethanol-tolerant/dependent mice during a period of ethanol withdrawal. These data suggest that atrial natriuretic peptide might interfere with neuronal mechanisms related to the glutamate/NMDA receptor complex.

The neurochemical mechanisms of the interaction between acute atrial natriuretic peptide administration (or acute neutralization of endogenous atrial natriuretic peptide by its antiserum) and the chronic development of NMDA hypersensitivity during ethanol tolerance/dependence remain to be studied. It is of interest, however, that neither atrial natriuretic peptide nor its antiserum modified NMDA-induced seizure activity in alcohol-naive animals. These findings support the hypothesis that the effect of atrial natriuretic peptide is closely related to neurochemical and behavioral (e.g. seizure-inducing) properties of the hypersensitive glutamate/NMDA system in the ethanol-tolerant/dependent organism.

Atrial natriuretic peptide diminishes, and the antiserum against atrial natriuretic peptide potentiates the ethanol withdrawal symptoms in ethanol-tolerant/dependent mice (Kovács, 1993), as well the NMDA-induced seizure activity during ethanol withdrawal (present data). Therefore, one might speculate that atrial natriuretic peptide interferes with neurochemical mechanisms functionally ''distal'' to the hyperexcitable NMDA–receptor complex. Further experiments are required to elucidate these mechanisms, especially since the existence of dopaminergic, cholinergic, beta-adrenergic (Bidzseranova et al., 1991b) and vasopressinergic (Shirakami et al., 1993) interactions of the central atrial natriuretic peptide may be hypothesized.

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