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Short communication

α-Atrial natriuretic peptide attenuates ethanol withdrawal symptoms

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Mice were rendered 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. Intracerebroventricular (i.c.v.) injection of α-atrial natriuretic peptide (α-ANP) attenuated, whereas that of an antiserum against α-ANP (anti-ANP) intensified the severity of handling-induced convulsions.

Ethanol withdrawal; α-ANP (α-atrial natriuretic peptide); Anti-ANP serum

1. Introduction

The alcohol withdrawal syndrome, which results from abrupt cessation of drinking in humans and experimental animals who are alcohol-dependent, encompasses a highly variable range of symptoms which can be measured objectively (see Litten and Allen, 1991). Among other symptoms, profound changes in volume and electrolyte homeostasis occur during alcohol withdrawal (Liappas et al., 1987) and these changes might also be associated with convulsions and tremulousness. α-Atrial natriuretic peptide (α-ANP) is known to exert a major influence(s) on volume homeostasis (Lang et al., 1985; Iitake et al., 1986; Cantin and Genest, 1987). Plasma concentrations of α-ANP have been shown to change during ethanol withdrawal in human patients (Bezzegh et al., 1991; Kovács et al., 1992) and in mice (manuscript in preparation), and a correlation was found between the plasma α-ANP levels and the onset of delirium tremens (Kovács et al., 1992). It was of interest, therefore, to study the role of central α-ANP in ethanol withdrawal.

2. Materials and methods

Male CFLP mice (Gödöllö, Hungary) weighing 35 ± 5 g were used (for reference see Szabó et al., 1987; Kovács, 1991). The animals received ethanol 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, ethanol was removed and 5 h later handling-induced convulsions were scored according to Goldstein (1972): 0 = no convulsion; 1 = facial grimace (after 180 degree spin); 2 = tonic convulsion (after 180 degree spin); 3 = tonic-clonic convulsion (after 180 degree 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.

α-ANP (human, 1–28) was dissolved in physiological saline and injected in a volume of 2 μl. Antiserum against human α-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: ANP (human, 1–28): 100%, ANP (human, 7–28): 100%, β-ANP (human, 1–28 dimer), ANP (rat, 1–28): 55%. No cross-reactivities were detected with oxytocin, [Arg^8]vasopressin, somatostatin, [Met^5]enkephalin, and β-endorphin. Control animals received saline (2 μl) or normal rabbit serum (4 μl of 1:20 dilution). No differences were noticed between the two control groups. Data are expressed as the median (lower and upper quartile) of the scores. Results were analyzed with a non-parametric test for location (Z-test, Statistical Graphical Company, USA).

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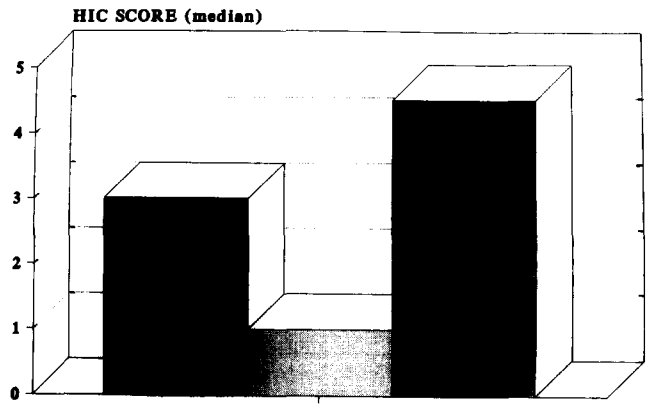

Fig. 1. Opposite effect of α-ANP and anti-ANP on ethanol withdrawal in mice. Treatments were given i.c.v. 4 h after ethanol withdrawal. Handling-induced convulsions were scored 1 h later. Columns indicate medians (first column: ethanol-dependent control, n = 45; second column: 1 ng α-ANP treatment, n = 15; third column: anti-ANP antiserum, n = 33).

3. Results

The dose-dependent effect of ANP on handling-induced convulsion scores is shown in table 1. Graded doses of ANP resulted in an inverted U-shaped dose-response effect. The smallest dose (0.05 ng) did not affect handling-induced convulsions. Medium to high doses of 0.2–1 ng (i.c.v.) significantly decreased handling-induced convulsion scores, whereas higher doses of 5 or 20 ng were again without effect. (Graded doses of α-ANP were also tested in non-dependent control animals: handling-induced convulsion scores in these animals were consistently below score 1. Graded doses of α-ANP (0.05–20 ng i.c.v.) failed to affect handling-induced convulsions in non-dependent control animals (data not illustrated).)

The time course of handling-induced convulsions in the ethanol-dependent control animals and in mice treated with 1 ng of α-ANP (i.c.v.) is shown in table 2. α-ANP inhibited the gradual development of handling-induced convulsions.

In contrast to the i.c.v. injections of α-ANP (1 ng), anti-ANP (1 : 20) resulted in an increased (Z = 0.0005) handling-induced convulsion score in ethanol-dependent mice (fig. 1).

TABLE 1

Dose-related effect of α-ANP on handling-induced convulsions in ethanol-dependent mice.

Dose (ng)	n	Handling-induced convulsions		
		Median	%	Z
0.00	15	3 (2–4) [a]	100	–
0.05	10	3 (2–3)	100	NS
0.20	10	2 (2–2)	66	0.01
1.00	14	1 (1–3)	33	0.004
5.00	15	2 (1–3)	66	NS
20.00	16	2 (1–3)	66	NS

[a] Lower and upper quartile.

TABLE 2

The time course of handling-induced convulsions in ethanol-dependent mice.

Time after withdrawal (h)	Handling-induced convulsions in ethanol-dependent animals		
	Control (n = 15)	α-ANP (n = 14)	Significance (Z)
0	1 (0–1) [a]	1 (1–1)	NS
1	2 (1–2)	1 (1–1)	0.0008
2	2 (1–2)	1 (1–1)	0.001
3	2 (2–3)	1 (1–1)	0.02
4	3 (2–3)	1 (1–2)	0.0005
5	3 (2–4)	1 (1–3)	0.004

[a] Median and lower and upper quartile.

4. Discussion

Recent research on alcohol withdrawal suggests that various central nervous system mechanisms (e.g. noradrenergic neurotransmission, GABA/benzodiazepine receptor complex, calcium channels, etc.) — including neuronal peptides — may play complementary roles in the onset and severity of withdrawal symptoms (for summary Litten and Allen, 1991). The present data indicate that central administration of α-ANP attenuates, while the neutralization of endogenous α-ANP by central administration of anti-ANP intensifies, handling-induced convulsions in ethanol withdrawal. Since α-ANP failed to affect this behavior in non-dependent control animals, a sedating effect of the peptide is not likely. α-ANP inhibited the gradual development of handling-induced convulsions. It is not likely, therefore, that the inhibitory effect of α-ANP on handling-induced convulsions is related to a peptide-induced shift in seizure activity in ethanol-dependent mice. It is therefore hypothesized that endogenous α-ANP in the brain is involved in the control of the central nervous symptoms of ethanol withdrawal. Further research is needed to elucidate the biological significance and mechanisms of action of α-ANP in ethanol withdrawal.

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