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ScienceDirect
NeuroToxicology 28 (2007) 463–470
NeuroToxicology

The influence of ethanol intake and its withdrawal on the anticonvulsant effect of α-tocopherol in the penicillin-induced epileptiform activity in rats

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Received 18 September 2006; accepted 17 November 2006
Available online 24 November 2006

Abstract

Previous studies proposed the existence of a relationship between epilepsy and ethanol. Ethanol may have either proconvulsive or anticonvulsive effects on epileptic activity in different experimental epilepsy models. The influence of high dose ethanol intake and its withdrawal on the anticonvulsant effect of α-tocopherol was examined after intracortical injection of penicillin (500 units) to induce epileptiform activity. Thirty minutes after penicillin injection, the most effective dose of α-tocopherol (500 mg/kg) was administrated intramuscularly (i.m.). Ethanol-treated rats received a daily dose of 9.0 g/kg of 30% ethanol solution via an oesophageal probe for 15 days. All rats in the withdrawal group were anesthetized for induction of penicillin-induced epileptiform activity 28 h after the last ethanol administration. The epileptiform activity was verified by electrocorticographic (ECoG) recordings. Ethanol, in a dose of 9 g/kg, significantly decreased the mean frequency of penicillin-induced epileptiform ECoG activity without changing the amplitude. The mean frequency of ECoG activity was decreased in the 60 and 70 min period from penicillin injection in the ethanol-treated + α-tocopherol and ethanol withdrawal + α-tocopherol groups compared with the penicillin-injected (500 units, i.c.) group, respectively. α-Tocopherol was more effective in decreasing the mean frequency of epileptiform activity in the ethanol + α-tocopherol group than in other α-tocopherol administrated groups. Ethanol withdrawal caused an increase in frequency of epileptiform activity in the withdrawal + α-tocopherol group compared with other α-tocopherol administrated groups. α-Tocopherol did not affect the amplitude of epileptiform activity in any group. Possible mechanisms of ethanol influence on the neuroprotective actions of α-tocopherol are still a crucial issue associated with epilepsy.

Keywords: ECoG; Epileptiform activity; Ethanol; Penicillin; Rats; α-Tocopherol

1. Introduction

Alcohol (ethanol) is a drug that depresses the inhibitory mechanisms in the central nervous system (CNS) and its effect is dependent on the dose, tolerance and other factors (Gordon and Devinsky, 2001). The prolonged consumption of ethanol can cause neurological disorders such as dementia, neuronal degeneration, hallucinations, withdrawal convulsions, epileptic seizure, among others (Charness et al., 1989; Fischer, 2005; Becker et al., 2006). There are several studies showing a complex relationship between alcoholism and epilepsy (Hauser et al., 1988; Mello et al., 1990; Scorza et al., 2003; Fischer, 2005). Experimental studies on animal models of epilepsy revealed a depressant (Dember et al., 1953; Fischer, 2005) as well as an excitatory (Guerrero-Figueroa et al., 1970) effect of ethanol upon seizure.

Anticonvulsant properties of ethanol after acute and chronic applications have been previously found in various experimental models of epilepsy, including electrically- and chemically-induced seizures tests (McQuarrie and Fingl, 1958; Kokka et al., 1993; Zhuk et al., 2001). Fischer and Kittner (1998) reported that the administration of moderate doses of ethanol (0.5–1.5 g/kg, i.p.) reduced seizure severity in a dose dependent manner in the PTZ-kindling model. The anticonvulsant activity of ethanol was only slightly decreased by chronic ethanol treatment (5.0 g/kg, for 28 days) in the PTZ seizure threshold test in rats (Kokka et al., 1993). Furthermore, it was found that the ethanol administration induces behavioral

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0161-813X/$ – see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuro.2006.11.005

(frequency of seizures) and neuropathological changes during the chronic phase in the pilocarpine model of epilepsy (Scorza et al., 2003). They also noted that the ethanol withdrawal syndrome is a crucial event in the development of functional and neuropathological alterations associated with epilepsy (Scorza et al., 2003). During periods of ethanol withdrawal, the frequency of discrete 'brief spindle episodes', initiated, increased significantly, while the duration of this EEG activity remained unchanged (Veatch and Becker, 2002). On the other hand, intake of small to moderate quantities of ethanol appears not to increase seizure frequency in non-alcoholics patients (Hoppener et al., 1983). McQuarrie and Fingl (1958) did not find changes in the mean seizure threshold during the chronic administration of ethanol (2.0 g/kg, orally) in the low frequency electroshock seizure threshold test in mice. The subchronic administration of ethanol, in a high dose, also revealed neither a significant decrease of the MES seizure threshold nor a marked tolerance to the anticonvulsant activity tested 24 h after withdrawal in the MES threshold test (Fischer, 2005). In co-medication with valproate and carbamazepine, ethanol significantly increased the anticonvulsant effectiveness of both antiepileptic drugs without affecting the total plasma level of both antiepileptic drugs after single combination or following subchronic premedication of ethanol (2.0 g/kg, for 20 days) (Fischer, 2005). A widely used method for inducing epileptiform activity in rats is application of penicillin to the cerebral cortex (Holmes et al., 1987). Application of penicillin to the neocortex results in synchronous discharge of neurones, which bears an electrophysiological resemblance to human focal interictal epileptic discharges (Prince, 1972). There is lack of data showing the influence of ethanol intake and its withdrawal in the penicillin model of epilepsy.

However, there is also very limited experimental information about the influence of ethanol on the protective efficacy of α-tocopherol in epilepsy. This question seems to be a matter of special interest, since previous studies indicate that active oxygen free radical scavengers such as α-tocopherol prevent both epileptic discharges (Levy et al., 1992; Rauca et al., 2004; Ayyildiz et al., 2006a) and ethanol-induced toxicity (Nadiger et al., 1988; Nordmann et al., 1990; Agar et al., 2000). It is well known that α-tocopherol has the enormous advantage of a low toxicity, daily intakes of up to 1000 mg having been reported as not inducing side effects in normal humans (Diplock, 1995; Morinobu et al., 2002; Hathcock et al., 2005). In our previous studies, a high dose of α-tocopherol (500 mg/kg) was used to provide a maximal anticonvulsant effect in the penicillin-induced epileptiform activity (Ayyildiz et al., 2006a, in press). Therefore, the influence of subchronic treatment with ethanol, in a dose of 9 g/kg, intake and its withdrawal on the effects of α-tocopherol (500 mg/kg) was investigated in penicillin-induced epileptiform activity in rats.

2. Materials and methods

Adult male rats were obtained from University of Ondokuz Mayis Experimental Research Centre. The animal studies were conducted with governmental approval according to local guidelines for the care and use of laboratory animals. Fifty-six male Wistar rats (235–265 g) were housed individually on a 12-h light:12-h dark cycle (lights on at 07.00 h), at a temperature of 20 ± 2 °C and 50% humidity. Rats were assigned to the following experiments and groups: intracortical (i.c.) delivery of (1) 2.5 μl artificial cerebrospinal fluid [aCSF containing: NaCl, 124 mM; KCl, 5 mM; KH_2PO_4, 1.2 mM; $CaCl_2$, 2.4 mM; $MgSO_4$, 1.3 mM; $NaHCO_3$, 26 mM; glucose, 10 mM; HEPES, 10 mM; pH 7.4 when saturated with 95% O_2 and 5% CO_2] (i.c.); (2) 500 units penicillin (2.5 μl, i.c.); (3) 500 mg/kg α-tocopherol (i.m.); (4) 500 units penicillin (2.5 μl, i.c.) + 500 mg/kg α-tocopherol (i.m.); (5) ethanol-treated (9 g/kg, per day, for 15 days, intragastrically) + 500 units penicillin (2.5 μl, i.c.); (6) ethanol-treated (9 g/kg, per day, for 15 days, intragastrically) + 500 units penicillin (2.5 μl, i.c.) + 500 mg/kg α-tocopherol (i.m.); (7) ethanol withdrawal (9 g/kg, per day, for 15 days, intragastrically) + 500 units penicillin (2.5 μl, i.c.); (8) ethanol withdrawal (9 g/kg, per day, for 15 days, intragastrically) + 500 units penicillin (2.5 μl, i.c.) + 500 mg/kg α-tocopherol (i.m.). Each animal group was composed of seven rats.

2.1. Induction of epileptiform activity

The animals were anesthetized with urethane (1.25 g/kg, i.p.) and placed in a stereotaxic frame (Harvard Stereotaxic Instrument). Rectal temperature was maintained between 36.5 and 37.0 °C using a feedback controlled heating system (Harvard Apparatus Limited). A polyethylene cannula was introduced into the right femoral artery to monitor blood pressure, which was kept above 100 mmHg during the experiments (mean 118 ± 5 mmHg). All contact and incision points were infiltrated with procaine hydrochloride to minimize possible sources of pain.

The left cerebral cortex was exposed by craniotomy. The epileptic focus was produced by 500 units penicillin G potassium injection (2 mm posterior to bregma and 3 mm lateral to sagittal sutures, 1 mm beneath the brain surface by a Hamilton microsyringe type 701N; infusion rate 0.5 μl/min) (Ayyildiz et al., 2006b).

2.2. Drug and drug administration

Ethanol-treated and ''withdrawal'' rats received a daily dose of 9.0 g/kg of 30% ethanol solution via an oesophageal probe for 15 days. At the end of this period, ethanol administration was stopped and all rats were anesthetized for induction of epileptiform activity 3 and 28 h after the last administration of ethanol in the ethanol-treated and ''withdrawal'' groups, respectively. Ethanol intake was administered at approximately the same time (between 9.00 and 10.00 h) during the whole procedure. Blood ethanol concentrations were measured 90 min after ethanol administration in different groups ($n = 35$). Penicillin (500 units, 2.5 μl, i.c.) was injected to induce epileptiform activity after recording baseline activities within 10 min.