Available online at www.sciencedirect.com
SCIENCE DIRECT
Progress in Neuro-Psychopharmacology & Biological Psychiatry 30 (2006) 946–952
Progress In Neuro-Psychopharmacology & Biological Psychiatry
www.elsevier.com/locate/pnpbp

Tolerance to the antinociceptive effects of ethanol during ethanol withdrawal

Michael B. Gatch *

Department of Pharmacology and Neuroscience, University of North Texas Health Science Center at Fort Worth, 3500 Camp Bowie Blvd., Fort Worth, TX 76107-2699, United States

Available online 30 March 2006

Abstract

Prior research has indicated that tolerance develops to the antinociceptive effects of ethanol and continues even during withdrawal. Three potential pharmacological mechanisms for this tolerance are examined, using nitrendipine (L-type calcium channel blocker), theophylline (adenosine A_1/A_2 antagonist) and flumazenil (benzodiazepine antagonist). Rats received 10 days of exposure to an ethanol-containing liquid diet (6.5% w/v). A radiant heat tail-flick assay was used to assess hyperalgesia at 12 h after removal of the liquid diet, as well as tolerance to the effects of cumulative doses of ethanol (0.5–2 g/kg). Co-administration of flumazenil (10 mg/kg, i.p., b.i.d.), nitrendipine (5 mg/kg, i.p., b.i.d.) or theophylline (1 mg/kg, i.p., b.i.d.) with chronic ethanol prevented development of the hyperalgesia produced by ethanol withdrawal, but only theophylline reduced tolerance to the antinociceptive effects of ethanol administered during ethanol withdrawal. In contrast, when administered during ethanol withdrawal, theophylline (1–10 mg/kg) blocked the anti-hyperalgesic effects of ethanol during ethanol withdrawal, whereas nitrendipine (5–25 mg/kg) enabled ethanol to produce levels of antinociception comparable to non-dependent rats. These findings indicate that L-type calcium channels and adenosine receptors play important, but differing roles in the development of hyperalgesia during withdrawal, and to tolerance to the antinociceptive effects of ethanol.

Keywords: Adenosine receptors; Ethanol withdrawal; Hyperalgesia; L-type calcium channels; Rat

1. Introduction

Antinociception is seen during the first few days of chronic administration of ethanol, followed by the development of tolerance (Gatch, 1999; Gatch and Lal, 1999; Malec et al., 1987). During ethanol withdrawal, a significant degree of hyperalgesia is seen and the hyperalgesia can be reversed by low doses of ethanol administered during withdrawal (Gameiro et al., 2003; Gatch, 1999, 2002; Gatch and Lal, 1999; Gatch and Selvig, 2002; Rogers et al., 2004). The increase in sensitivity to stimulation produced by ethanol withdrawal is opposite to the response to other stressors, most of which produce small amounts of analgesia (Yamada and Nabeshima, 1995). Withdrawal from opioids and nicotine also produce hyperalgesia, which suggests that withdrawal-induced hyperalgesia is a completely separate phenomenon from stress-induced analgesia (Schmidt et al., 2001; Sweitzer et al., 2004).

If higher doses of ethanol are given during withdrawal, tail-flick latencies do not increase above baseline levels. The finding that ethanol can reverse the hyperalgesia seen during withdrawal, in spite of the tolerance to the antinociceptive effects of ethanol, suggests that the antinociceptive and hyperalgesic effects of ethanol might be mediated by different receptors. One alternative explanation is that metabolic factors might contribute to a lower blood ethanol concentration during withdrawal. However, when ethanol (2.0 g/kg) is administered in non-dependent rats or in rats undergoing ethanol withdrawal, there is no difference in the blood ethanol concentrations of the two groups (Jung et al., 1999, 2000), which suggests that pharmacokinetic factors are not involved. Another alternative is that ethanol is more potent at reversing ethanol-withdrawal-induced hyperalgesia than at producing analgesia, a phenomenon that has been reported with opioids (Negus et al., 1995).

When the benzodiazepine site antagonist flumazenil (10 mg/kg) is administered twice daily during exposure to the ethanol diet, the antinociceptive effects of ethanol are blocked and hyperalgesia does not develop during withdrawal (Gatch,

Abbreviations: GABA, gamma-amino butyric acid.

* Tel.: +1 817 735 2056; fax: +1 817 735 2091.
E-mail address: mgatch@hsc.unt.edu.

0278-5846/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2006.02.010

1999). However, the anti-hyperalgesic effects of ethanol are not reversed by administration of flumazenil (10–50 mg/kg) during withdrawal. This finding adds further support to the possibility of separate mechanisms, especially as flumazenil dose-dependently antagonizes the antinociceptive effects of ethanol in non-dependent rats (Gatch, 1999).

Because ethanol is far from being pharmacologically selective, there are a number of possibilities for other mechanisms. Chronic exposure to ethanol produces desensitization of receptor-stimulated cAMP production and adenosine transport, which produces tolerance to some effects of ethanol (Coe et al., 1996; Nagy et al., 1991; Sapru et al., 1994; Wannamaker and Nagy, 1995). Because chronic administration of the A1/A2 adenosine antagonist theophylline upregulates adenosine A_1 receptors (Szot et al., 1987), it is possible that chronic administration of theophylline can at least partially overcome the desensitization of adenosine receptors produced by ethanol. In support of this hypothesis, repeated administration of theophylline during exposure to the ethanol diet reduces withdrawal signs and prevents hyperalgesia during ethanol withdrawal (Gatch and Selvig, 2002).

Chronic administration of ethanol upregulates dihydropyridine-sensitive L-type calcium channels and dihydropyridine L-type calcium channel antagonists co-administered with chronic ethanol prevent the upregulation of binding sites for these compounds (Brennan et al., 1990; Whittington et al., 1991). It is then not surprising that calcium channel antagonists also block the development of tolerance to ethanol in hippocampal slices (Whittington and Little, 1991a,b) and in intact rats (Dolin and Little, 1989), and also reduce the severity of ethanol withdrawal signs and prevent the hyperalgesia seen during ethanol withdrawal (Gatch, 2002; Whittington et al., 1991).

The purpose of the present study was to examine potential mechanisms for the development of tolerance to the antinociceptive effects of ethanol. The present study characterizes the effects of flumazenil, nitrendipine (dihydropyridine L-type calcium channel antagonist) and theophylline (A_1/A_2 adenosine antagonist) on tolerance to the antinociceptive effects of ethanol during ethanol withdrawal. In the first set of experiments, compounds known to prevent the hyperalgesia during ethanol withdrawal were co-administered with the ethanol diet in the hope that they may also prevent the development of tolerance to the antinociceptive effects of ethanol. Flumazenil, nitrendipine and theophylline were chosen because of their effectiveness at blocking ethanol tolerance and withdrawal in both molecular (Brennan et al., 1990; Buck et al., 1991; Szot et al., 1987; Whittington et al., 1991) and behavioral studies (Gatch, 1999, 2002; Gatch and Selvig, 2002; Whittington et al., 1991). Doses and pretreatment times for this study were selected which produced maximal effects in the earlier studies. In the second set of experiments, nitrendipine and theophylline were administered during withdrawal to test whether they could reverse the tolerance to the antinociceptive effects of ethanol. Flumazenil given during withdrawal did not alter the antinociceptive effects of ethanol during withdrawal (Gatch, 1999) and so was not tested further in the present study.

2. Methods

2.1. Subjects

Male Long–Evans rats obtained from Harlan–Sprague (Indianapolis, IN) at 90 days of age were used in the experiments. Weights at the start of the experiment averaged 339 g. All rats were housed individually and maintained on a 12:12 light/dark cycle (lights on at 7:00 a.m.). All housing and procedures were in accordance with the guidelines of the Institute of Laboratory Animal Resources, National Research Council (Institute of Laboratory Animal Resources, 1996) and were approved by the University of North Texas Health Science Center Animal Care and Use Committee.

2.2. Drugs

Nitrendipine and theophylline were obtained from Research Biochemicals International (Natick, MA). Flumazenil was donated by Hoffmann-LaRoche (Nutley, NJ). Nitrendipine, theophylline and flumazenil were administered as a suspension in 3% carboxymethylcellulose. In dose–effect tests, ethanol was administered in a concentration of 15% (w/v). All injections were administered intraperitoneally.

2.3. Ethanol administration and withdrawal

During chronic ethanol/withdrawal experiments, rats received a nutritionally balanced liquid diet (100 ml) containing 6.5% (w/v) ethanol each morning at 8:00 AM for 10 days. On the last day of ethanol administration, the liquid diet was removed and the rats were gavaged with a dose of 3 g/kg ethanol in 10 ml of the liquid diet to standardize the starting time of ethanol withdrawal. Diet control animals were fed liquid diet with dextrin isocalorically substituted for ethanol (Lal et al., 1988) and were gavaged with 10ml of the dextrin diet on day 10.

2.4. Nociception assay

A radiant heat tail-flick assay was used to test changes in nociception as described previously (Gatch and Selvig, 2002). Two temperature settings were used, a low setting (0.5) at which rats ordinarily do not remove their tails to act as a control for increased agitation and motor activity during withdrawal, and a moderate setting (2.0) at which rats removed their tails in 6 to 9 s to test for the antinociceptive effects of ethanol. A maximum cutoff time of 20 s was used.

Cumulative dosing test sessions consisted of multiple cycles. At the beginning of each test session, baseline tail-flick latencies from both settings were determined. Subsequently, a dose of drug was administered at the beginning of each 30-min cycle. Fifteen minutes after each injection, tail-flick latencies were recorded from the low and high settings as described above. Each ethanol dose increased the cumulative amount by 0.5 g/kg. Cumulative dosing was used because in this and prior studies, cumulative doses of ethanol produced levels of antinociception,