Morphine induced changes in ethanol-and water-intake are attenuated by the 5-HT3/4 antagonist tropisetron (ICS 205-930)

Abstract The opiate agonist morphine has been shown to increase ethanol intake and mesolimbic dopamine (DA) levels. Conversely, the 5-HT3/4 antagonist tropisetron has been shown to decrease ethanol intake and morphine-induced increases in mesolimbic DA levels. This study was designed to test the effects of acutely administered tropisetron on morphine-induced changes in ethanol (6% v/v) and water intake in a two-bottle test procedure. Ten water restricted male rats were injected with combinations of morphine (0.0, 0.56, 1.0, 1.5, 10.0, and 17.0 mg/kg, SC) and tropisetron (0.0, 1.0, 10.0, and 17.0 mg/kg, SC) prior to test sessions. Morphine (1.0 and 1.5 mg/kg) significantly increased absolute (g/kg) and relative ethanol intake (ethanol/total fluid). Tropisetron alone did not affect ethanol or water intake. When tropisetron (10.0 and 17.0 mg/kg) was administered in combination with morphine (1.5 mg/kg), the increase in ethanol intake induced by morphine was attenuated. Tropisetron (1.0 mg/kg) reversed a decrease in ethanol intake induced by morphine (17.0 mg/kg). The two highest doses of tropisetron partially attenuated a significant decrease in water intake produced by morphine (17.0 mg/kg). These data suggest that opiate and 5-HT3 mechanisms could interact in the regulation of ethanol intake. However, the doses of tropisetron tested were high and, therefore, the potential involvement of 5-HT4 receptors or other neurotransmitter systems in regulating ethanol intake is discussed.

Key words Alcohol • Ethanol self-administration • Morphine • Tropisetron • Opiate • 5-HT3 antagonist • 5-HT4 • Rats

Introduction

Determining the neurotransmitter systems that are involved in regulating alcohol intake is the key to development of therapeutic interventions. Numerous compounds that act on neurotransmitter systems, including dopamine antagonists (Pfeffer and Samson 1986; Linsenmeier 1990), opioid antagonists (Froehlich et al. 1983; Schwarz-Stevens et al. 1992), benzodiazepines (Samson et al. 1989; Griffiths and Wolf 1990), Ca2+ channel blockers (Pucilowski et al. 1992), 5-HT reuptake inhibitors (Murphy et al. 1988; Haraguchi et al. 1990), and 5-HT3 antagonists (Fadda et al. 1991; Hodge et al. 1993b; Knapp and Pohorecky 1992), have been studied extensively for their ability to reduce voluntary alcohol intake. Conversely, under some experimental conditions, the opiate agonist morphine increases ethanol intake (Hubbell et al. 1986; Reid et al. 1991; Hodge et al. 1992a), suggesting that activation of the endogenous opioid system is involved in excessive ethanol consumption. Recent evidence indicates that systemic morphine injections increase the firing of ventral tegmental dopamine (DA) neurons (Matthews and German 1984) which increases release of DA in the terminals of the nucleus accumbens (Di Chiara and Imperato 1988; Carboni et al. 1989a). Further, ventral tegmental administration of morphine increases nucleus accumbens DA release (Leone et al. 1991). The dopamine stimulating effect of morphine is thought to be involved in some of the behavioral effects of opiates, including increased feeding (Jenck et al. 1986) and locomotion (Kalivas et al. 1983), and has been postulated to be involved in facilitation of ethanol intake (Hodge et al. 1992a; Schwarz-Stevens et al. 1992). Accordingly, local administration of the nonspecific DA agonist d-amphetamine in the nucleus accumbens increases intake by prolonging ethanol reinforced responding (Hodge et al. 1992b; Samson et al. 1993), whereas decreases in extracellular DA produced by nucleus...
accumbens administration of the D₂ antagonist raclopride (Samson et al. 1993), and ventral tegmental administration of the D₂/₃ agonist quinpirole (Hodge et al. 1993a), reduce ethanol reinforced responding.

Increases in nucleus accumbens DA levels produced by morphine (Carboni et al. 1989b; Imperato and Angelucci 1989) and ethanol (Wozniak et al. 1990) are attenuated by 5-HT₃ antagonists. The 5-HT₃ receptor is a ligand-gated, cation selective, ion channel that mediates depolarization and excitation (Wallis 1989). Antagonists of this receptor system, such as tropisetron (ICS 205-930) and ondansetron, have anxiolytic properties, and have been shown to inhibit diazepam, cocaine, and alcohol withdrawal (see Costall et al. 1990 for a review). 5-HT₃ antagonists reduce morphine-induced place conditioning (Carboni et al. 1989b; Higgins et al. 1992), morphine self-administration (Hui et al. 1993), and oral ethanol intake (Fadda et al. 1991; Knapp and Pohorecky 1992; Hodge et al. 1993b) without producing significant effects on other basal activities such as intake of water (Hodge et al. 1993b) or sucrose (Hui et al. 1993). 5-HT₃ antagonists may reduce ethanol intake by attenuating increases in mesolimbic DA levels (Fadda et al. 1991; Knapp and Pohorecky 1992), which may partly influence ethanol’s reinforcement function (Imperato and DiChiara 1986; Samson et al. 1992; Hodge et al. 1993b).

The present experiment was designed to test the potential interaction of opiate and 5-HT₃ neurotransmitter systems in the regulation of ethanol intake. If morphine-induced increases in ethanol consumption are due to increases in DA levels (Hodge et al. 1992a) and 5-HT₃ antagonists decrease ethanol intake by attenuating increases in DA levels (Fadda et al. 1991; Knapp and Pohorecky 1992), then antagonism of 5-HT₃ receptors should attenuate morphine-induced increases in ethanol intake. Specifically, the present study tested whether tropisetron, a highly selective 5-HT₃ antagonist, would alter morphine-induced changes in concurrent ethanol and water consumption.

Materials and methods

Animals

Male Long-Evans rats (n = 10, 60–90 days of age) were housed individually in standard stainless-steel hanging cages with food (Wayne Rodent Blox, Wayne Laboratories, Bartonville, Ill.) continuously available. Initial body weights (mean ± SEM) were 325 ± 14.5 g. The colony room was maintained on a 12 L:12 D light cycle (lights on at 0700 hours), and temperature and humidity were maintained within NIH guidelines (temperature: 18–26°C; humidity: 40–70%). All experimental sessions were conducted during the light portion of the cycle (1500–1600 hours). Access to fluids was limited to 1 h per day during experimental conditions, but food was always available. Rats were weighed and inspected daily for general health prior to each session.

Procedure

Daily sessions were conducted in standard stainless-steel hanging cages in a sound-attenuated room located adjacent to the animal colony. The front of each test cage was equipped with two plastic 100-ml graduated (1 ml graduations) drinking cylinders mounted with rubber-covered clamps to prevent bottle movement. Drinking tubes, containing stainless-steel balls to limit spillage, were fitted to the cylinders with rubber stoppers. The two drinking tubes were held 10 cm apart and 3.5 cm above the cage floor. A food hopper was centered between the drinking tubes.

Rats were given 1 week to adapt to individual housing conditions in the colony room with food and water available continuously. Following the initial adaptation period, the home cage water was replaced with ethanol (10% v/v) for 3 days (days 1–3). Subsequently, 1-h test sessions were conducted on 5 days per week (Monday–Friday), with water and an ethanol solution available concurrently as the only source of fluids. On weekends, water was available for 1 h as the only fluid in the home cages. During the first 15 days, ethanol concentration was increased from 2.0% (days 4–5), to 4.0% (days 6–7), to a final concentration of 6% (days 8–18) in a manner similar to that of Linseman (1987). Thereafter, drugs were administered on Tuesday and Friday, and the data from Thursdays were used as non-injection controls.

Prior to each session, rats were removed from their home cages and placed in individual holding chambers (20 × 12 × 15 cm) and moved to the sound attenuating room. On drug days, each rat received two drug injections. Various doses of tropisetron and morphine (alone and in combination with each other) were administered 30 min and 20 min prior to the session, respectively. Each morphine dose (0.0, 0.56, 1.0, 1.5, 10.0, and 17.0 mg/kg, SC) was tested alone in random order at the beginning of the experiment. Then, increasing doses of tropisetron (1.0, 10.0, and 17.0 mg/kg, SC) were tested in combination with randomly ordered morphine doses. Three dose combinations were not tested: 1.0 mg/kg tropisetron with 1.0 mg/kg morphine, and 17.0 mg/kg tropisetron with 1.0 and 10.0 mg/kg morphine.

Following injections, rats were returned to the holding chambers until the beginning of the session. At the start of each session, the rats were placed in two-bottle test cages and initial fluid levels were recorded to the nearest milliliter. Fluid levels were recorded at 15-min intervals during each 1-h session to assess patterns and rates of fluid consumption. Position of water and ethanol solutions was alternated daily to control for side preferences. The rats were returned to their home cages in the colony room at the end of each test session.

Drugs and solutions

The two solutions consumed orally during test sessions were tap water and ethanol (6% v/v) in tap water. Morphine sulphate (0.0, 0.56, 1.0, 1.5, 10.0, and 17.0 mg/kg) and tropisetron (0.0, 1.0, 10.0, and 17.0 mg/kg) were dissolved in 0.9% (w/v) saline and administered by subcutaneous (SC) injection in a constant volume of 1.0 ml/kg. Drug solutions were prepared immediately prior to injections.

Data analysis

Data are presented as mean ± SEM. The dependent variables were total ethanol (g/kg) and water (ml) consumption per session, relative ethanol intake [(ethanol intake/total water intake) × 100], and cumulative milliliters of ethanol and water consumed in 15-min intervals (four per session) to assess temporal patterns and rates of intake. Total fluid consumption was verified to be equal to the four measurements taken in each session. Data were analyzed according to repeated measures ANOVA and paired t-tests.