ELSEVIER

Alcohol 43 (2009) 119–126

ALCOHOL

Potency of naltrexone to reduce ethanol self-administration in rats is greater for subcutaneous versus intraperitoneal injection

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Received 15 September 2008; received in revised form 13 November 2008; accepted 19 November 2008

Abstract

The opioid antagonist naltrexone (NTX) is used to treat alcohol dependence and may reduce alcohol consumption by selectively blocking opioid receptors. In rat experiments, discrepancy exists across studies regarding the potency of NTX to reduce ethanol consumption. One cause of this discrepancy may be the use of different routes of NTX administration (e.g., intraperitoneal vs. subcutaneous). The purpose of this study was to directly compare the effects of intraperitoneal and subcutaneous injections of NTX on ethanol self-administration. Rats pressed a lever for a sweetened ethanol solution (10% wt/vol in 0.1% saccharin) during 20 min daily sessions. One group received intraperitoneal injections of 1, 3, 10, and 30 mg/kg NTX before the sessions. Another group received subcutaneous injections of 0.03, 0.1, 0.3, and 1 mg/kg NTX before the sessions. The group that received subcutaneous NTX was also tested with a single intraperitoneal injection of 0.3 mg/kg NTX. Naltrexone significantly reduced ethanol self-administration, and NTX was more potent when administered via subcutaneous injection versus intraperitoneal injection. Ethanol intake (g/kg) was significantly reduced after subcutaneous injection of NTX 0.1 mg/kg and higher. In contrast, ethanol intake was significantly reduced after intraperitoneal injection of NTX 3 mg/kg and higher. A comparison of the NTX ED_{50} values showed that subcutaneous NTX was approximately 30-fold more potent than intraperitoneal NTX. For the subcutaneous 0.3 mg/kg NTX dose, a detailed bin analysis showed that responding during the first 2 min after injection was similar to that during the first 2 min after a saline injection while responding after NTX decreased in subsequent bins. These findings suggest that researchers should carefully consider the route of NTX administration when discussing potency and selectivity of NTX's effects on ethanol-related behaviors in rats. These findings further support the notion that NTX acts by terminating responding early rather than reducing the initial responding.

Keywords: Ethanol; Naltrexone; Naloxone; Intraperitoneal; Subcutaneous

Introduction

The opioid antagonist naltrexone (NTX) effectively reduced alcohol craving and relapse rates in early clinical trials with alcohol-dependent patients (O'Malley et al., 1992; Volpicelli et al., 1992). Since then, multicenter clinical trials and meta-analyses have confirmed that alcohol-dependent individuals experience some benefit from treatment with NTX (Anton et al., 2006; Pettinati et al., 2006). Other opioid antagonists, such as nalmefene, may produce benefits as well (Drobes et al., 2004; Mason et al., 1999). Opioid antagonists are hypothesized to reduce alcohol consumption by blocking the rewarding effects associated with an alcohol-stimulated increase in endogenous opioid activity (for reviews see Gianoulakis, 2001; Modesto-Lowe and Fritz, 2005).

The specific opioid receptor subtype that mediates the effects of ethanol and the ability of opioid antagonists to reduce ethanol consumption continue to be explored. The evidence in the literature is complicated and points to the involvement of multiple opioid receptor subtypes. For example, μ-selective antagonists such as CTOP, naloxonazine, and β-funaltrexamine (β-FNA) reduced ethanol consumption in rat studies (Hyytia and Kiianmaa, 2001; Krishnan-Sarin et al., 1998; Mhatre and Holloway, 2003; Stromberg et al., 1998). In addition, δ-selective antagonists such as ICI 174,864, naltrindole, and naltriben reduced ethanol intake in some studies (Froehlich et al., 1991; Krishnan-Sarin et al., 1995a,b). Even the κ-opioid receptor may be involved in ethanol consumption. Walker and Koob (2008) suggested that the κ receptor plays a role in the aversive properties of alcohol dependence, as they showed that nor-binaltorphimine decreases ethanol consumption only during ethanol withdrawal. Thus, ethanol consumption may be modulated by one or more opioid receptor subtypes.

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0741-8329/09/$ – see front matter
doi: 10.1016/j.alcohol.2008.11.003

NTX, along with naloxone, is more commonly used in animal experiments and is typically considered a ''nonselective'' antagonist. NTX and naloxone have the ability to bind to all three opioid receptors but do so at different concentrations. In vitro experiments using guinea pig ileum, mouse vas deferens, rat brain, or monkey brain show that NTX and naloxone preferentially bind to the μ receptor at low concentrations and then bind to δ or κ receptors at higher concentrations (Childers et al., 1979; Emmerson et al., 1994; Paterson et al., 1984; Takemori and Portoghese, 1984). This binding profile has been applied as rationale for the effects of systemically injected NTX or naloxone on ethanol self-administration in rodents. For example, Mhatre and Holloway (2003) indicated that low doses of NTX (<1 mg/kg) occupy μ receptors, whereas doses up to 20 mg/kg occupy μ- and δ receptors. They indicate that doses greater than 20 mg/kg additionally occupy κ receptors. However, the potency of NTX or naloxone to reduce ethanol consumption varies dramatically according to the literature. For instance, some studies show that NTX doses as low as 0.1 mg/kg reduced ethanol consumption or ethanol-reinforced responding (Holter and Spanagel, 1999; Hyytia and Kiianmaa, 2001; June et al., 1998), whereas other studies show that acute injections as high as 3 or 30 mg/kg failed to significantly reduce ethanol-reinforced responding (Bienkowski et al., 1999; Williams, 2007). The observed potency differences may be influenced by many experimental variables, such as the self-administration paradigm (operant responding vs. free access), strain of rat (outbred vs. ethanol-preferring rats), or route of administration (intraperitoneal vs. subcutaneous injection).

In the present study, we used a self-administration model with an ethanol solution (10% wt/vol) that was sweetened with 0.1% saccharin. Although the addition of saccharin may confound our ability to draw conclusions about manipulations that are specific to ethanol alone, the sweetened ethanol solution has many benefits for researchers using the self-administration model. First, ethanol intakes (g/kg) are maintained at a higher level in outbred rats compared to solutions containing ethanol alone. The average intakes in the present experiments were close to 1 g/kg during a 20 min period in an outbred strain of rats that had free access to food and water in the home cage. Although the added sweetener enhances the palatability of the ethanol solution, consumption is likely maintained by the pharmacological properties of ethanol as demonstrated by a behavioral economic analysis (Heyman, 1997; Heyman et al., 1999). A second benefit of using a sweetened alcohol solution is the reduced training time by avoiding the time spent ''fading out'' the sweetener. A third benefit is that the model has face validity compared to the human condition. Humans consume ethanol in beer, wine, or mixed drinks, which allows palatability to play a role in alcohol consumption and, therefore, may influence effects of pharmacotherapy.

The purpose of this study was to examine the route of administration by directly comparing the effects of intraperitoneal and subcutaneous injections of NTX on ethanol self-administration in outbred rats. Although these experiments are not highly novel, they do fill a gap in the literature. The ''common knowledge'' that route of administration influences potency is not clearly supported by published empirical evidence. The literature demonstrates a lack of a standard for antagonist route of administration. Many articles make comparisons of the effective antagonist doses across studies without mentioning the differing type of injection.

Materials and methods

Animals and housing

Male Long-Evans rats weighing 126–150 g (Charles River, Wilmington, MA) upon arrival to the laboratory were individually housed and maintained in a temperature- and humidity-controlled room with a 12-h light/dark cycle with lights on at 9:00 a.m. Operant experiments were conducted six days per week during the light cycle between 10:00 a.m. and 2:00 p.m. The rats had free access to food and water in their home cages except during initial training for the lever-press response. The experimental protocol was approved by Institutional Animal Care and Use Committee, and the animals were treated in accordance with the *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (National Research Council, 2003).

Apparatus

Experiments were conducted in standard operant chambers (Med Associates, St. Albans, VT) housed in melamine sound-attenuated cubicles. Each chamber (30.5 × 24.2 × 29.2 cm) contained a rolled-edge standard lever approximately 7 cm from the grid floor on the right wall near the rear of the chamber. A single-cup receptacle, placed on the center of the right wall approximately 3 cm from the grid floor, was fitted to receive food pellets from a modular dispenser and fluid deliveries from a syringe pump via 18-gauge stainless steel tubing connected to the receptacle. A white stimulus light was located above the cup receptacle. The house light was on the center of the left wall near the top of the chamber. Operant chambers were controlled with programs written in Med-PC MedState Notation version IV (Med Associates).

Training

Training for the lever-press response started after food depriving the rats for 24 h. Rats began lever pressing for 45-mg food pellets (Bio-Serv #F0021, Frenchtown, NJ) during shaping sessions that lasted 10–20 min and were conducted twice per day for up to 4 days. When the rats acquired the lever-press response, 20% sucrose was substituted for the food pellets for a single 20-min session