Pharmacology, Biochemistry and Behavior 99 (2011) 626–633

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

The response to naltrexone in ethanol-drinking rats depends on early environmental experiences

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ARTICLE INFO

Article history:
Received 23 March 2011
Received in revised form 20 May 2011
Accepted 4 June 2011
Available online 13 June 2011

Keywords:
Individual variability
Alcohol
Maternal separation
Ethanol intake
Voluntary drinking
Rearing environment

ABSTRACT

The opioid receptor antagonist naltrexone is currently used in the treatment of alcohol addiction. However, substantial individual differences have been reported for the efficacy of naltrexone. Genetic factors are known to contribute to these differences; however, little is known about the impact of early environmental influences. Based on previous findings that have suggested a link between ethanol, endogenous opioids and the early environment, it was hypothesised that early environmental factors affect naltrexone efficacy later in life. A population of Wistar rats was subjected to three different rearing conditions where the pups experienced a daily separation from the dam, for either 15 min or 360 min, or were just briefly handled. On postnatal day 26, the rats were given intermittent access to ethanol (5% and 20%) and water for six weeks before naltrexone (0.3 mg/kg or 3.0 mg/kg) or saline treatment using a randomised injection schedule with a one-week washout period between injections. Naltrexone reduced ethanol consumption, but there was high variability in the efficacy. In addition, there was an association between the rearing condition and the effectiveness of naltrexone. Naltrexone reduced ethanol intake in rats experiencing postnatal conditions that contrasted normal wildlife conditions, *i.e.*, prolonged absence or continuous presence of the dam, and naltrexone had no effect on the total ethanol consumption in rats reared under naturalistic conditions, *i.e.*, short absences of the dam. These rats reduced their intake of 5% ethanol but increased their preference for 20% ethanol. We conclude that rats with a history of early adversity responded well to naltrexone treatment, whereas rats reared in a social context similar to that found in nature did not benefit from treatment. The present study highlights the importance of not only considering genetics but also environmental factors when identifying individual responses to naltrexone.

1. Introduction

Alcohol use disorders (AUD) are a major health problem with a great need for individualised treatment strategies. Although pharmacotherapy is available today, it is evident that not all patients benefit from general treatment schedules (Spanagel and Kiefer, 2008). To achieve better pharmacotherapy of AUD, knowledge about how each individual responds to ethanol and drugs used for treatment is essential. Ethanol interferes with the function of a number of central transmitter systems, including acetylcholine, glutamate, GABA, monoamines and endogenous opioids (Soderpalm et al., 2009; Vengeliene et al., 2008). Individual differences in any of these networks may contribute to differences in the sensitivity to pharmacological agents and therapeutic outcome.

Opioid networks have attracted interest with regard to the aetiology of AUD and as a target for pharmacological therapy (Modesto-Lowe and Fritz, 2005; Nylander and Silberring, 1998; Oswald and Wand, 2004). Ethanol activates central opioids, although the exact mechanism has not been elucidated, and endogenous opioids have been implicated in addiction processes and in the neurobiological basis for AUD vulnerability (Barson et al., 2009; Gianoulakis, 2004; Trigo et al., 2010; Van Ree et al., 2000). The mu-opioid peptide receptor (MOPR) has been implicated in the actions of ethanol because genetic deletion or antagonism of the MOPR attenuates ethanol-induced mesolimbic dopamine release (Benjamin et al., 1993; Job et al., 2007). Opioid receptor antagonists reduce ethanol consumption in animal and human studies (O'Malley, 1996; Ulm et al., 1995; Volpicelli et al., 1995), and the MOPR receptor antagonist, naltrexone, is an FDA-approved medication for AUD treatment. These studies suggest that opioid antagonists act as anti-reward drugs; when they are administered prior to ethanol intake, the ethanol-induced reward is absent, which leads to a decrease in ethanol intake.

However, an increasing number of reports describe large individual variation in the effects of naltrexone in AUD patients (Kiefer et al., 2008; Spanagel and Kiefer, 2008), and the underlying mechanism has not been elucidated. Pharmacogenetic studies have revealed that genetic factors contribute to individual differences in the ability of naltrexone to reduce ethanol intake (Monterosso et al., 2001; Ray et al., 2010; Rubio et al.,

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doi:10.1016/j.pbb.2011.06.004

2005). For example, several reports have shown that MOPR polymorphisms affect the response to naltrexone (Mague and Blendy, 2010; Oslin et al., 2003). Little is known about the impact of environmental factors on the effects of opioid antagonists. Environmental experiences, especially early in life, may cause long-term changes in neuronal functioning and are known to affect the propensity for AUD (De Bellis, 2002; Langeland et al., 2004). Endogenous opioids represent putative targets for environmentally induced effects. Early in life, brain opioids play an important role in neuronal development and social behaviour (Nelson and Panksepp, 1998). Furthermore, the opioid networks develop and mature postnatally (Loughlin et al., 1985; Petrillo et al., 1987; Spain et al., 1985), and early experiences may evoke long-lasting changes in opioid function. To study the mediators and consequences of early environmental influence, maternal separation (MS) is commonly used. Maternal separation procedures, including the separation of the rat pups from the dam for short or prolonged periods during the first postnatal weeks, are used to assess the consequences of different environmental conditions (Holmes et al., 2005; Ladd et al., 2000; Pryce and Feldon, 2003). Previous studies using MS have shown that endogenous opioids are affected by early environmental factors as evidenced by long-lasting changes in the opioid networks (Gustafsson et al., 2008; Ploj et al., 2003) and an altered pain threshold (Kehoe and Blass, 1986; Pieretti et al., 1991). In addition, neurobiological alterations, caused by early environmental factors, affect the sensitivity to drugs that act on the opioid system. Long-term voluntary ethanol consumption can elicit different effects on opioid peptides and receptors, which is dependent on previous experiences, with enhanced ethanol-induced responses in rats exposed to prolonged MS (Gustafsson et al., 2007; Ploj et al., 2003). Furthermore, rats subjected to prolonged MS had an altered sensitivity to morphine compared to non-handled rats (Kalinichev et al., 2001a; Kehoe and Blass, 1986). Finally, when compared to non-handled rats, MS enhanced the effects of naltrexone on sucrose responses in male Long-Evans rats (Michaels and Holtzman, 2007).

Taken together, these previous studies suggest a link between ethanol, endogenous opioids and early environmental factors. However, it is not known whether the environment early in life also affects the efficacy of naltrexone in reducing ethanol consumption. Therefore, the present study was designed to test whether different environmental experiences early in life result in different responses to naltrexone in ethanol-drinking animals. If this hypothesis is true, environmental factors may contribute to the individual differences seen in treatment outcomes observed in the clinic. A population of Wistar rats with different rearing conditions was given free access to ethanol and water three days a week. After six weeks of drinking, naltrexone was administered prior to a 24 h session with free ethanol access during which its effects on ethanol consumption were assessed. Whether naltrexone differentially affected the consumption of low (5%) and high (20%) ethanol concentrations was also examined. The results provide novel evidence of the different effects of naltrexone and their dependence on rearing conditions.

2. Materials and methods

2.1. Animals

Time-mated outbred Wistar rats from Scanbur BK AB, Sweden, arrived at the animal facility on gestational days 15–16. Animals were then housed singly in standard cages (59×38×20 cm) with wood chip bedding as nesting material and were fed pellet food (R36 Labfor; Lactamin, Vadstena, Sweden) and water *ad libitum*. All animals were housed in temperature (21±1 °C) and humidity controlled (49±5%) animal cabinets in a room on a 12-h light–dark cycle with lights on at 08:00. All animal experiments were performed following an approved protocol in accordance with the Uppsala animal ethical committee and the Swedish Animal Protection Legislation.

2.2. Maternal separation

At birth, all pups from 15 time-mated Wistar rats were sexed, and the litters were arranged to minimise the use of biological littermates in the same experimental groups and culled into 10 pups (5–6 males, 4–5 females) per litter. The litters were separated from the dam for 0 min (MS0; $n=5$ litters), 15 min (MS15; $n=5$ litters) or 360 min (MS360; $n=5$ litters) daily from postnatal day (PND) 1–20. The separation procedure started daily at 10:15 and was performed during the light period. The separation was initiated by removing the dam from the nest to a macrolon cage (26×20×14 cm) containing wood chip bedding material, followed by removing the litter into a separate macrolon cage (26×20×14 cm) containing wood chip bedding material. In the MS15 and MS360 groups, the litters were moved to a heating cabinet (30±2 °C) in an adjacent room during the separation period. The dams in the MS360 groups were returned to their home cages during the separation procedure but were removed prior to the return of the litters. In the MS15 groups, the dams were kept in another cage during the separation, and the litters were returned to the home cages before the dams. In the MS0 groups, the litters were handled similarly to the MS15 and MS360 litters but then returned immediately to the home cage and were not separated from their mother more than 45 s. The rats were weaned on PND 21, and the 12-hr light–dark cycle was adjusted to lights off at 14:00. Thereafter, the male rats were housed three per cage with wood chip bedding material and a wooden house. The cages were changed once a week, and water access was given *ad libitum*. Thirty-four male rats, MS0 ($n=12$), MS15 ($n=11$) and MS360 ($n=11$), were used in the present study. The same person performed all separation sessions and care giving of the rats, and gloves were used during all animal contact. The rats were housed in the same room for the duration of the experiment.

2.3. Voluntary ethanol consumption

On PND 26, the male rats were singly housed in macrolon cages (42×26×18 cm) with wood chip bedding material and a wooden house in temperature (21±1 °C) and humidity controlled (49±5%) animal cabinets. Care was taken to place rats from the three experimental groups (MS0, MS15 and MS360) appropriately to avoid differences due to cabinet housing, and each cage remained in the same place during the length of the experiment, including the ethanol drinking and naltrexone injection periods. The animals were intermittently exposed to ethanol; a procedure that results in higher ethanol intake in rats compared to continuous access models (Simms et al., 2008; Sinclair and Senter, 1967; Wise, 1973). A modified version, previously described (Daoura et al., 2011), was used to examine the consumption of different concentrations of ethanol. At the beginning of the dark cycle, all rats were given free access to three bottles, 5% and 20% ethanol (v/v using 95% ethanol diluted in tap water) and tap water. Water was available at all times. The positions of the bottles (250-mL plastic bottles with ball-valve nipples obtained from Scanbur BK AB, Sollentuna, Sweden) were changed for each session to avoid place preference. Ethanol solutions were changed for each session, and water was changed every day. Body weights were measured before every session.

The rats had free access to ethanol three days a week (Monday, Wednesday, Friday) for nine weeks. During the 6th week of ethanol access, the animals were familiarised to the procedure used for measurement of naltrexone-induced effects. The ethanol consumption was measured at 30 min, 2 h and 24 h after the beginning of ethanol access.

2.4. Naltrexone treatment

During the 7th week of ethanol access, the naltrexone/saline injections started. The injections were performed once a week, on