Neuropharmacology 118 (2017) 199–208

Contents lists available at ScienceDirect

Neuropharmacology

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

Hydrogen-rich saline attenuates anxiety-like behaviors in morphine-withdrawn mice

Di Wen [a], Peng Zhao [b], Rongji Hui [b], Jian Wang [b], Qianchao Shen [a], Miao Gong [c], Hongyan Guo [a], Bin Cong [a, **], Chunling Ma [a, *]

[a] Department of Forensic Medicine, Hebei Medical University, Hebei Key Laboratory of Forensic Medicine, Collaborative Innovation Center of Forensic Medical Molecular Identification, Hebei Province, Shijiazhuang 050017, PR China
[b] College of Basic Medicine, Hebei Medical University, Hebei Province, Shijiazhuang 050017, PR China
[c] Department of Histoembryology, Hebei Medical University, Hebei Province, Shijiazhuang 050017, PR China

ARTICLE INFO

Article history:
Received 10 December 2016
Received in revised form
24 March 2017
Accepted 25 March 2017
Available online 28 March 2017

Keywords:
Molecular hydrogen
Hydrogen-rich saline
Morphine withdrawal
Anxiety-like behavior
Hypothalamic-pituitary-adrenal axis

ABSTRACT

Hydrogen therapy is a new medical approach for a wide range of diseases. The effects of hydrogen on central nervous system-related diseases have recently become increasingly appreciated, but little is known about whether hydrogen affects the morphine withdrawal process. This study aims to investigate the potential effects of hydrogen-rich saline (HRS) administration on naloxone-precipitated withdrawal symptoms and morphine withdrawal-induced anxiety-like behaviors. Mice received gradually increasing doses (25–100 mg/kg, i.p.) of morphine over 3 days. In the naloxone-precipitated withdrawal procedure, the mice were treated with three HRS (20 μg/kg, i.p.) injections, and naloxone (1 mg/kg, i.p.) was given 30 min after HRS administration. Body weight, jumping behavior and wet-dog shakes were immediately assessed. In the spontaneous withdrawal procedure, the mice were treated with HRS (20 μg/kg, i.p.) every 8-h. Mice underwent naloxone-precipitated or spontaneous withdrawal were tested for anxiety-like behaviors in the elevated plus-maze (EPM) and light/dark box (L/D box) paradigm, respectively. In addition, the levels of plasma corticosterone were measured. We found that HRS administration significantly reduced body weight loss, jumping behavior and wet-dog shakes in mice underwent naloxone-precipitated withdrawal, and attenuated anxiety-like behaviors in the EPM and L/D box tests after naloxone-precipitated withdrawal or a 2-day spontaneous withdrawal period. Hypo-activity or motor impairment after HRS administration was not observed in the locomotion tests. Furthermore, HRS administration significantly decreased the levels of corticosterone in morphine-withdrawn mice. These are the first findings to indicate that hydrogen might ameliorate withdrawal symptoms and exert an anxiolytic-like effect in morphine-withdrawal mice.

1. Introduction

Opioids are used clinically for their analgesic effects on acute and chronic severe pain. Morphine is the most commonly used opioid in the clinic, but its application is limited due to severe side effects characterized by compulsive use and addiction. Addiction to opioids depends not only on their positive reinforcing effects, but also on avoidance of the negative, aversive consequences of withdrawal. Early withdrawal symptoms in opioids abusers, including diarrhea, yawning, dysphoria, irritability, loss of appetite, severe abdominal pain and nausea, emerge after drug abstinence (Heishman et al., 1989; Spanagel and Weiss, 1999). Furthermore, a number of psychological symptoms, such as bipolar disorder, hyperirritability, major depression and anxiety, gradually increase along with the intensity of the drug craving as the withdrawal time passes (Goeldner et al., 2011; Radke and Gewirtz, 2012). These negative emotional states are most commonly reported after compulsive and continued use and relapse followed by drug abstinence (Spanagel and Weiss, 1999).

Physical withdrawal symptoms in rodents are clearly manifested by an injection of the opioid receptor antagonist naloxone. Jumping behavior, body weight loss and wet-dog shakes have been considered hallmarks of naloxone-precipitated withdrawal

* Corresponding author.
** Corresponding author.
E-mail addresses: hbydbincong@126.com (B. Cong), chunlingma@126.com (C. Ma).

http://dx.doi.org/10.1016/j.neuropharm.2017.03.029
0028-3908/

symptoms in morphine-dependent mice (Linseman, 1977; Ritzmann, 1981). In addition, animal studies have shown that both spontaneous and naloxone-precipitated morphine withdrawal result in significant increases in anxiety-like behaviors in the elevated plus-maze (EPM) and light/dark box (L/D box) paradigm (Buckman et al., 2009; Miladi-Gorji et al., 2012). Hyperresponsive activity of the hypothalamic-pituitary-adrenal (HPA) axis, which induces an increase in corticosterone and cortisol secretion, was considered to be associated with morphine withdrawal (Gibson and Pollock, 1975). Stimulation of the HPA Axis and sympathetic nervous system by neural circuitry involving the amygdala, which regulates emotions such as anxiety and fear is thought to underlie anxiety-like behaviors (Papaleo et al., 2007). The increase in corticosterone secretion and blood cortisol levels is a quantitative index of the magnitude of changes in status of anxiety situation during withdrawal period (Kishioka et al., 1994; Ueno et al., 2011).

Molecular hydrogen is the lightest and most abundant chemical element in nature. Hydrogen selectively reduces cytotoxic oxygen radicals, and has recently become increasingly appreciated as a potential novel anti-oxidant in preventive and therapeutic applications (Takeuchi et al., 2015; Han et al., 2016; Zhao et al., 2016). Several methods are used to administer hydrogen, including inhalation of hydrogen gas (HG), drinking hydrogen-rich water (HRW) and injection with hydrogen-rich saline (HRS)(Kurokawa et al., 2015). Studies have reported that molecular hydrogen possesses the ability to modulate several pathophysiological processes in various animal models, including ischemia-reperfusion injury, stroke, neonatal hypoxia-ischemia, radiation-induced damage, traumatic brain injury, Alzheimer's disease and Parkinson's disease (Kajiyama et al., 2008; Nakao et al., 2010; Kang et al., 2011; Ohno et al., 2012; Han et al., 2015; Xue and Bian, 2015; Tian et al., 2016a). These functions of molecular hydrogen might be related to its efficient anti-oxidantive, anti-inflammatory, anti-apoptotic, and anti-allergy effects (Ohsawa et al., 2007; Tian et al., 2016a). A number of experimental studies found that reactive oxygen species (ROS) and reactive nitrogen species (RNS) are involved during morphine treatment or withdrawal (Desole et al., 1996; Pinelli et al., 2009; Skrabalova et al., 2013). The inhibition of morphine-induced oxidative stress in the brain attenuated the development of morphine tolerance and dependence, and the administration of inhibitors of oxidative stress may be an effective treatment of the opioid withdrawal syndrome (Sharma et al., 2007; Liu et al., 2012). We hypothesized that the molecular hydrogen, as an effective antioxidant, may be associated with the onset of withdrawal symptoms and critically involved in coping with adverse effects induced by morphine withdrawal. Notably, Zhang and colleagues have recently proposed that HRW treatment prevents chronic unpredictable mild stress-induced depressive-like behavior, indicating a potential role for HRW treatment in modulating stress-related disease (Zhang et al., 2016). Morphine withdrawal is a stressful event and induces anxiety-like behaviors in rodents. However, the potential effect of molecular hydrogen treatment on the increased anxiety-like behavior and stress hormones induced by morphine withdrawal needs to be clarified.

In the present study, we aim to investigate the potential effects of HRS on opioid withdrawal symptoms using a model of naloxone-precipitated withdrawal in morphine-dependent mice, and then to explore the effect of HRS on withdrawal-induced negative affective behaviors in naloxone-precipitated and spontaneous morphine-withdrawn mice using the EPM and L/D box test. When withdrawal symptoms and anxiety-like behaviors were inhibited by treatment with HRS during the morphine-withdrawal stage, we also measured the changes in corticosterone and cortisol in the blood, which are not only key mediators of the behavioral response to stressors but also hormones related to emotion.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice, initially weighing 20–25 g upon arrival, were purchased from the Beijing Vital River Laboratory Animal Technology Co. Ltd., China. The animals were housed in a climate-controlled environment. Constant temperature (21 ± 2 °C), humidity (approximately60%) and a 12-h light/dark cycle (lights off at 7:00 a.m.) were maintained. Food and water were available ad libitum. All experiments were conducted according to the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experimental procedures were approved by the Local Committee on Animal Care and Use and Protection of the Hebei Medical University.

2.2. Drugs

Morphine hydrochloride was obtained from Shenyang First Pharmaceutical Factory (Liaoning, China). Naloxone hydrochloride was purchased from Sigma-Aldrich (St. Lou- is, MO, USA). HRW was provided by Huoli Qingyuan Biotechnology Co. Ltd., Beijing, China and stored under atmospheric pressure at ambient temperature in an aluminum pot without dead volume. The drug concentrations were adjusted to an appropriate injection volume of 10 mL/kg body weight. HRW was dissolved in sodium chloride (0.45 g/50 mL) and then sterilized with a 0.22 μm filter for HRS preparation. A fresh solution was prepared for each day of experiments. The concentration of the HRS was maintained at approximately 2.0 mg/L in this study.

2.3. Induction of naloxone-precipitated and spontaneous withdrawal inmorphine- dependent mice

All of the mice were acclimated to the laboratory housing conditions for 7 days, and gently handled for 3 days before the experiment. To induce morphine dependence, mice were treated with escalating doses of morphine (25, 25, 50, 50, 50, 75, 75, 75, and 100 mg/kg) injected intraperitoneally every 8 h over a 3-day period according to a previous report (Wu et al., 2014). The last morphine injection (100 mg/kg) was given on day 4. After the induction of morphine dependence, naloxone-precipitated withdrawal and spontaneous withdrawal were conducted. Naloxone (1 mg kg^{-1}, i.p.) was administered 3.5 h after the last morphine injection to the mice for withdrawal precipitation. During the period of spontaneous withdrawal (day 4–8), saline injections (10 mL/kg, i.p.) were administered three times a day at 8-h intervals.

2.4. Behavioral tests

2.4.1. Assessment of naloxone-precipitated withdrawal symptoms

The animals underwent morphine withdrawal displayed characteristic withdrawal symptoms. Loss of body weight was calculated as the difference between the body weight determined immediately before saline or naloxone injection and a second determination made 30 min later. Jumping behavior was assessed by counting the number of jumps for 30 min immediately after the naloxone injection according to previous reports (Chen et al., 2011; Wu et al., 2014). Each mouse was placed in a blue opaque cylinder (32 cm height × 10 cm diameter) above a platform. The mouse remained in the cylinder for 30 min. Jumping behavior was recorded for 30 min and analyzed with the Animal Video Analysis System (JL Beh soft-tech Co. Ltd. Shanghai, China). Additionally, wet-