Pharmacology, Biochemistry and Behavior 135 (2015) 60–63

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Pre-conditioned place preference treatment of chloral hydrate interrupts the rewarding effect of morphine☆

YongMei Sun [a,b,1], Wei Zong [a,1], MuRu Zhou [c], YuanYe Ma [a], JianHong Wang [a,*]

[a] Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences & Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, PR China
[b] Department of Molecular and Cellular Neurobiology, Center for Neurogenomics & Cognitive Research, Neuroscience Campus Amsterdam, VU University Amsterdam, De Boelelaan 1085, 1081 HV Amsterdam, Netherlands
[c] School of Pharmacy, Fu Dan University, Shanghai, PR China

ARTICLE INFO

Article history:
Received 17 July 2014
Received in revised form 13 May 2015
Accepted 15 May 2015
Available online 23 May 2015

Keywords:
Morphine
Chloral hydrate
Rewarding effect
Conditioned place preference

ABSTRACT

The medical use of morphine as a pain killer is hindered by its side effects including dependence and further addiction. As the prototypical μ receptor agonist, morphine's rewarding effect can be measured by conditioned place preference (CPP) paradigms in animals. Chloral hydrate is a clinical sedative. Using a morphine CPP paradigm that mainly contains somatosensory cues, we found that pre-CPP treatment in rats using chloral hydrate for 6 consecutive days could disrupt the establishment of CPP in a U shape. Chloral hydrate had no effect on the body weight of rats. Our results indicate that prior treatment with chloral hydrate can interrupt the rewarding effect of morphine.

1. Introduction

Morphine, a member of the opiate family, is parenterally administered as premedication for surgical procedures and for post-operative and chronic pain relief (Martell et al., 2007; Van Ree et al., 1999). However, as one of the abused drugs, chronic use of morphine leads to dependence, tolerance and addiction. Addiction is hypothesized as an aberrant learning and memory disorder, which alters the natural reward-related learning and memory pathways (Hyman et al., 2006; Torregrossa et al., 2011). Screening drugs, which could alleviate or eliminate the rewarding effect of morphine but promote its beneficial therapeutic effect, would be highly valued.

Previous studies imply that chloral hydrate (CH) might be such a drug. Firstly, CH is widely used clinically and experimentally to treat opioid-related diseases or symptoms. Clinically, chloral hydrate is a common pediatric sedative (Rutman, 2009; Schulte-Uentrop and Goepfert, 2010). In one retrospective study (Esmaeili et al., 2010), chloral hydrate was co-administered with clonidine to treat opiate withdrawal symptoms and neonatal narcotic abstinence syndrome encountered in neonates born to addicted mothers. Pre-clinically, chloral hydrate is a popular anesthetic (Silverman and Muir, 1993) in animals including laboratory animals. In morphine-dependent rats, preceding treatment using single chloral hydrate dose could decrease the withdrawal symptoms induced by naloxone (Streel et al., 2000). These clinical and behavioral results suggest that CH and morphine might have pharmacological interactions.

Morphine and chloral hydrate may have similar effects on the brain regions that are involved in opiate addiction. For instance, both chloral hydrate and morphine induced an increase of extracellular serotonin (5-HT) in the nucleus accumbens (Tao and Auerbach, 1994). Furthermore, the dose of morphine required to decrease spontaneous single unit activity in the globus pallidus was substantially less with chloral hydrate- than phenobarbital-anesthetized rats (Napier et al., 1983). In addition, chloral hydrate decreased dopamine overflow in the anterior striata (Hamilton et al., 1992). Dopamine is a well-known neurotransmitter in the process of reward learning and memory of addictive drugs.

The abovementioned studies suggest that morphine and chloral hydrate might share the same pathways in the central nervous system and chloral hydrate might also intervene with the rewarding effect of morphine.

To test this hypothesis, rats firstly received intraperitoneal injections at different doses of chloral hydrate (0 mg/kg, 50 mg/kg and 100 mg/kg) for 6 consecutive days, and then were used to establish morphine

Abbreviations: CH, chloral hydrate; CPP, conditioned place preference; 5-HT, 5-hydroxytryptamine; ANOVA, analysis of variance; LSD, least significant difference; NS, no significance; NMDA, N-methyl-D-aspartate; i.p, intraperitoneal; S.E.M., standard error of the mean.

☆ The authors declare no conflict of interests.

* Corresponding author.
E-mail address: wangjh@mail.kiz.ac.cn (J. Wang).

[1] These authors contributed equally to this study.

http://dx.doi.org/10.1016/j.pbb.2015.05.010
0091-3057/

conditioned place preference (CPP), which has been widely used to measure the rewarding effect of drugs (Bardo and Bevins, 2000; Tzschentke, 2007). In this study, we mainly used somatosensory cues, i.e., different textures of two conditioned chambers, as the conditioned stimuli.

2. Material and methods

2.1. Animals

Thirty-nine adult male Sprague Dawley (SD) rats weighing 180–220 g upon arrival were purchased from the Dashuo Biological Technology Company (Chengdu, China). Rats were housed with four to six per cage under conditions (a 12-h light/dark cycle with light on from 07:00 to 19:00, humidity 60% and temperature 23 ± 2 °C). Food and water were available *ad libitum*. Rats were handled twice per day during the two-week acclimation period. The experiments were conducted in accordance with the guidelines for the National Care and Use of Animals approved by the Chinese National Animal Research Authority.

2.2. Drugs

Morphine hydrochloride, 10 mg/ml per ampoule, was purchased from the Shenyang Number 1 Pharmaceutical Company (Northeast Pharmaceutical Group, Shenyang, China); and chloral hydrate (AR) from the Sino Chemical Reagent Company (Shanghai, China) was dissolved in sterile saline. Vehicle in this study was sterile saline.

2.3. Experimental design

Details are shown in Fig. 1. In experiment 1, sixteen rats (initially 8 in each group) were first used to establish morphine-induced CPP based on the modified protocol described in previous studies (Lin et al., 2011; Lin et al., 2010). In experiment 2, to test the effect of chloral hydrate on CPP, another 23 rats were randomly assigned to three groups. Before CPP conditioning, three groups were given intraperitoneal (i.p.) 0.9% saline (1 ml/kg) (n = 8), 50 mg/kg (n = 8) or 100 mg/kg (n = 7) chloral hydrate respectively at 9:00 for 6 days and then put back to their home cages. These rats were weighted daily and their body weight was used to measure the body weight variation.

2.4. CPP apparatus

The apparatus for CPP training and testing consisted of four identical three-chamber polyvinyl chloride (PVC) boxes, each separated by two removable guillotine doors. Two large black side chambers (30 cm long × 25 cm wide × 30 cm high) differed in floor texture (one with a grid plexiglas floor while the other had a rough PVC floor) and were connected by a white smaller box (11 cm long × 25 cm wide × 30 cm high with a white smooth PVC floor). The activity of the rats was recorded using a video camera mounted to the ceiling 1.5 m above the center of the CPP apparatus. Information regarding the rats' activity was transferred to a computer in a separate room for offline analysis. The rats were considered to have entered a side chamber when their heads and two front paws were inside the chamber. Rats that entered either of the side chambers less than four times in pre-conditioning phase were removed from the experiment (Meng et al., 2009). In experiment 1, one rat from each group was excluded. In experiment 2, one rat from 0 mg/kg group and 50 mg/kg group were excluded.

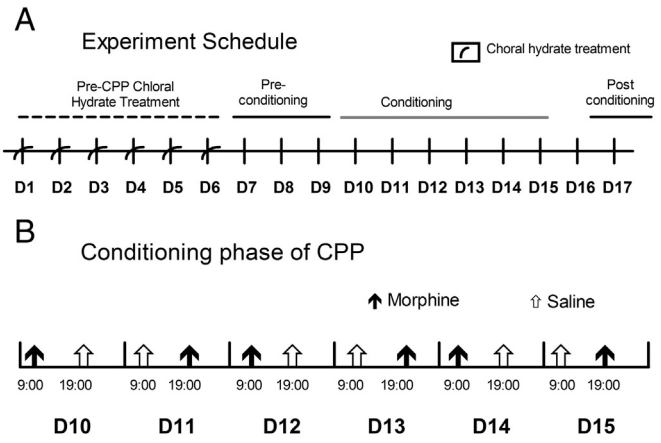

Fig. 1. Experiment design. The whole CPP procedure (A) consisted of 3 pre-conditioning days, 6 conditioning days (B) and one test day. For experiment 2, rats received chloral hydrate (50 mg/kg and 100 mg/kg) or saline treatment at 9:00 (A).

2.5. Behavioral procedure

The CPP conditioning procedure (see Fig. 1A) consisted of three periods: pre-conditioning (three days), conditioning (six days) and post-conditioning (one day).

The pre-conditioning phase started around 9:00 and consisted of three days. During this phase, the animals were put into the intermediate room with two arched doors open. They were allowed to explore the entire apparatus freely for 15 min. The videos of the third day were assessed and the time spent in the conditioning chambers was calculated as a pre-conditioning baseline.

The conditioning phase lasted for six days; two days comprised one unit. On the first day of each unit, the rats received morphine (10 mg/kg) at 9:00 and saline (1 ml/kg) at 19:00. On the second day, they were injected with saline at 9:00 and morphine at 19:00. After each injection, they were confined to the corresponding chambers (morphine with non-preferred chamber that was referred to as the drug-paired chamber; and saline with preferred chamber that was called the vehicle-paired chamber) for 45 min.

The post-conditioning test was carried out 38 h after the last conditioning. Rats were placed in the intermediate room with the doors opened and allowed free access to the conditioning chambers for 15 min.

2.6. Statistics

The CPP score represents the index of place preference of each rat, calculated by the following formula:

$$\text{CPP score} = \text{time in drug-paired chamber} / (\text{time in drug paired chamber} + \text{time in vehicle-paired chamber}).$$

Body weight variation during the chloral hydrate treatment period was measured as the following:

$$\text{Body weight variation} = (\text{body weight on day 6 (D6) - body weight on day 1 (D1)})/\text{body weight D1} * 100\%.$$

All behavioral data and body weight variation were presented as mean ± S.E.M. The establishment of CPP was analyzed by two-way ANOVA with drugs (morphine and saline) and trials (pre-conditioning and post-conditioning) as factors. In SPSS, two-tailed paired t-tests (pre-conditioning and post-conditioning as within subjects) and independent t-tests with drug (morphine and saline) as factors were then conducted to test the effect of morphine on the establishment of CPP and to decrease the family wise error, respectively, because there were only two trials and no post-hoc tests. In experiment 2, two-tailed paired t-tests were directly conducted to test chloral hydrates effects on place preference since the data did not meet the assumptions of mixed ANOVA. For body weight variation, a one-way ANOVA was