Physiology & Behavior 105 (2012) 195–202

Contents lists available at ScienceDirect

Physiology & Behavior

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

Anxiety profile in morphine-dependent and withdrawn rats: Effect of voluntary exercise

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ARTICLE INFO

Article history:
Received 8 April 2011
Received in revised form 6 August 2011
Accepted 9 August 2011

Keywords:
Anxiety
Elevated plus-maze
Light/dark box
Morphine dependence
Voluntary exercise
Withdrawal

ABSTRACT

Withdrawal from chronic opiates is associated with an increase in anxiogenic-like behaviours, but the anxiety profile in the morphine-dependent animals is not clear. Thus, one of the aims of the present study was to examine whether morphine-dependent rats would increase the expression of anxiogenic-like behaviours in novel and stressful conditions. Additionally, recent studies have shown that voluntary exercise can reduce anxiety levels in rodents. Therefore, another aim of this study was to examine the effect of voluntary exercise on the anxiety profile in both morphine-dependent animals and animals experiencing withdrawal. Rats were injected with bi-daily doses (10 mg/kg, at 12 h intervals) of morphine over a period of 10 days in which they were also allowed voluntary exercise. Following these injections, anxiety-like behaviours were tested in the elevated plus-maze (EPM) model and the light/dark (L/D) box. We found reductions in time spent in, and entries into, the EPM open arms and reductions in time spent in the lit side of the L/D box for both sedentary morphine-dependent and withdrawn rats as compared to the sedentary control groups. The exercising morphine-dependent and withdrawn rats exhibited an increase in EPM open arm time and entries and L/D box lit side time as compared with the sedentary control groups. We conclude that voluntary exercise decreases the severity of the anxiogenic-like behaviours in both morphine-dependent and withdrawn rats. Thus, voluntary exercise could be a potential natural method to ameliorate some of the deleterious behavioural consequences of opiate abuse.

1. Introduction

A higher prevalence of mood disorders, such as anxiety, major depression, and bipolar disorder, has been clinically demonstrated among substance abusers [1,2]. These negative emotional states may contribute to escalation of compulsive use, persisting use, and relapse following abstinence [1,3,4]. Stressful situations or anxiety are potent stimulator of drug cravings and drug-seeking behaviours in heroin addicts [5,6]. Thus, reversing or preventing of the anxiety-like behaviours induced by drugs of abuse might be useful in the treatment of relapse after periods of abstinence.

Animal studies have shown that both spontaneous and antagonist-precipitated morphine withdrawal result in significant increases in anxiety-like behaviours in the elevated plus-maze (EPM) and defensive probe-burying paradigm [7–9]. Systemic or central administration of the μ-opioid receptor agonist morphine induces anxiolytic-like effects in the EPM, which are reversed by the opioid receptor antagonist naloxone [10,11]. Similarly, κ-opioid receptor agonists have strong anxiolytic effects in the EPM, which are also antagonised by naloxone [12]. A recent study showed that microinjection of morphine into the ventral hippocampus or the nucleus accumbens induces an anxiolytic response, which is prevented by naloxone pre-treatment [13]. These findings indicate that acute morphine has anxiolytic-like actions in the EPM, but the underlying mechanisms are not well known.

Recent studies have shown that exercise is associated with a reduction in anxiety in humans [14] and rodents [15,16]. Some evidence indicates that wheel running might be useful as a natural substitute for drug reward and help in the reduction of drug abuse and the treatment of addicts [17,18]. In fact, voluntary wheel running in rodents is associated with a number of adaptive behavioural and physiological effects including improved cognitive function in rats [19], a reduction in stress-associated behaviours [15,16], enhanced neurogenesis and angiogenesis, and an increase in neurotrophic factors such as IGF-I and BDNF [20]. However, the effects of exercise on chronic morphine-induced anxiety-like behaviours are unknown.

In the present study, we tested the specific hypotheses that voluntary exercise can diminish the severity of naloxone-precipitated

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doi:10.1016/j.physbeh.2011.08.010

withdrawal responses, and reduce anxious behaviours in morphine-dependent and withdrawn rats.

2. Materials and methods

2.1. Animals and drugs

Adult male Wistar rats (220 ± 10 g) were obtained from the breeding colony of the Semnan University of Medical Sciences (Semnan, Iran). All rats were individually housed in cages for a 12 h light/dark cycle at 22–24 °C and had ad libitum access to food and water. All experimental procedures were conducted in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. Additionally, care was taken to use the minimum number of animals in each experiment. A new set of rats was used for each experiment.

Morphine sulphate (Temad Company, Iran) and naloxone hydrochloride (Sigma-Alderich, GmbH, Germany) were dissolved in physiological saline. Morphine sulphate was injected subcutaneously at a dose of 10 mg/kg. Naloxone hydrochloride was injected intraperitoneally at a dose of 0.4 mg/kg. All injections were made in a volume of 0.1 ml/100 g body weight.

2.2. Induction of morphine dependence

The rats were chronically treated with subcutaneous injections of morphine sulphate (10 mg/kg). These injections were given twice per day at 12 h intervals (06:00 and 18:00 h) for 10 days in the presence or absence of exercise (see below), as described previously [21]. Control rats were treated similarly, except that normal saline was used.

2.3. Withdrawal rating scale

Immediately after naloxone injection, rats were taken to a quiet, isolated room with moderate illumination, and their behaviour was monitored for 30 min by a rater blinded to the rats' treatment. Withdrawal signs were recorded and scored according to a modified version of the Gellet–Holtzman scale [22,23]. Briefly, on the Gellert and Holtzman scale graded signs with the exception of weight loss are assigned a weighting factor 1 to 4 based on frequency of appearance, and checked signs receive values of 2–3 depending upon the particular withdrawal sign noted, but regardless of frequency of appearance. Body weights were recorded immediately before and 60 min after naloxone injection, and the percentage of body weight changes was calculated. The weighting factor for weight was 1.0 for each 1.0% loss above the weight lost by control rats. Graded signs including jumps, wet dog shakes, and abdominal contractions were counted as the number of events occurring during the total test time. Checked signs including diarrhoea, ptosis, erection or genital grooming, teeth chattering, writhing, and irritability were counted as positive if the sign occurred at any time during the observation period. After completion of the observation session, the overall withdrawal severity was calculated by summing the proper weighting factor of somatic signs [22,23].

2.4. Exercise paradigm

Each of the exercising rats was given access to a cage that was equipped with a running wheel (diameter = 34.5 cm, width = 9.5 cm, Novidan. Tab, Iran) that was freely rotated against a resistance of 100 g. Each wheel was equipped with a magnetic switch that was connected to a counter located outside of the animal house that monitored the revolutions per hour. The number of revolutions for each wheel was recorded every day at 6 a.m. The sedentary rats were confined to similar cages with no access to a wheel. The exercising groups were allowed to exercise during the development of dependence on morphine, which took 10 days before the start of the EPM and the LD box experiments. Food and water consumption were checked daily, and animals were weighed on both the 1st and 10th days of exercise.

2.5. Anxiety measurement

2.5.1. The EPM

The EPM was a wooden plus-maze with two open arms (50 × 10 cm, with a ledge of 5 mm) and two closed arms (50 × 10 × 40 cm). The arms radiated from a central platform (10 × 10 cm). The apparatus was placed at a height of 70 cm from the floor. As rats have an innate fear of elevated open places, they entered the open arms less frequently and stayed in the open arms for less time compared to the closed arms when allowed to freely explore the maze [24,25].

To start the test, the rats were individually placed in the centre of the maze facing an open arm. Next, they were allowed 5 min of free exploration. The following variables were measured during each 5 min test: (1) time spent in open and closed arms as a percentage of the total time spent exploring both the open and closed arms; (2) the number of entries into the open and closed arms as percentage of the total number of entries into both open and closed arms. Percent time spent in, and entries into, the open arms were used as measure of anxiety [24,25]. In addition, the total number of arm entries was used as relative index of general activity [26]. The apparatus was cleaned after each trial with water.

2.5.2. The L/D box

Rodents prefer to spend most of their time in the dark. Rats that are more anxious will spend more time in the dark half of the chamber, whereas rats that are less anxious will spend approximately equal time exploring the light and dark compartments of the box. The L/D paradigm in rodents has been validated previously as a measure of anxiety in rats using agents that are known to have either anxiolytic or anxiogenic effects in humans [27].

The L/D box consisted of a Plexiglas box divided into two compartments of the same size (30 × 30 × 30 cm). The illuminated chamber was made from transparent plastic, illuminated by a 100 W desk lamp located above the box and connected through an 8 × 8 cm guillotine door to the dark compartment. The dark compartment was black and opaque. Each rat was placed in the illuminated compartment and observed for 5 min. Time spent in the lit compartment, the number of transitions between the light and dark compartments, and the number of rearings in the lit compartment were recorded. Significant decreases in any of these variables were interpreted as anxiety enhancement in this test [27]. The apparatus was cleaned after each trial with water.

2.6. Locomotor activity measurement

Spontaneous locomotor activity of each animal was measured using an automated activity monitor system (TSE infraMot, TSE, Bad Homburg, Germany), as described elsewhere [28]. Locomotor activity of each rat was measured for 5 min. Only one animal was placed in each activity chamber per measurement time.

2.7. Statistical analysis

The data from anxiety, exercise and locomotor activity testes expressed as the mean ± standard error of the mean (S.E.M.). These data were analysed using one-way or two-way analyses of variance (ANOVA), with repeated measures as required. Post-hoc analyses included Tukey's, and Student's *t* test. Graded somatic signs of opiate withdrawal showed normal distribution. Thus, they were analysed by Student's *t*-test, and expressed as the mean ± S.E.M. Checked