Brain Research Bulletin 64 (2005) 417–424

BRAIN RESEARCH BULLETIN

www.elsevier.com/locate/brainresbull

The development of tolerance to locomotor effects of morphine and the effect of various opioid receptor antagonists in rats chronically treated with morphine

Júlia Timár[a,*], Zsuzsanna Gyarmati[a], Zsuzsanna Fürst[a,b]

[a] *Semmelweis University, Department of Pharmacology and Pharmacotherapy, Budapest, 1445, Nagyvárad tér 4, Hungary*
[b] *Hungarian Academy of Sciences-Semmelweis University, Neuropsychopharmacology Group, Budapest, 1445, Nagyvárad tér 4, Hungary*

Received 23 June 2004; received in revised form 10 September 2004; accepted 10 September 2004
Available online 26 October 2004

Abstract

Behavioural measures are considered to be highly sensitive indices of opioid withdrawal. Opioids, depending on dose and time protocols may induce both reduction and enhancement of locomotor activity and chronic opioid treatment results in tolerance and sensitisation to these effects. In the present study the locomotor activity as experimental model was used to assess the development of tolerance to subcutaneous morphine challenge at different time points following morphine withdrawal in rats exposed to gradually increasing subcutaneous doses of morphine for 11 days. Tolerance developed to the inhibitory action of morphine (10 mg/kg) was observed even 8 weeks after morphine withdrawal, while tolerance to its locomotor activity enhancing effect (3 mg/kg) was detected 18 h after withdrawal, but not 3 weeks later. In the other series of experiments the locomotor activity of animals exposed to chronic morphine treatment was tested 18 h after spontaneous or subcutaneously administrated opioid antagonists precipitated withdrawal. Spontaneous withdrawal resulted in a moderate decrease of locomotion. Both the non-selective antagonist naloxone in low, μ opioid-receptor selective doses and the δ opioid-receptor selective naltrindole induced marked reduction of locomotor activity. The results provide further evidence that both μ and δ opioid-receptors might be affected during chronic morphine treatment.

Keywords: Morphine-tolerance; Morphine-dependence; Naloxone; Naltrindole; Locomotor activity

1. Introduction

In spite of intensive research the mechanism of drug dependence is not clearly understood. The long-term behavioural changes observed in animals exposed to gradually increased doses of opioid drugs may help to know more about this phenomenon.

The different types of opioid-receptors affect the mesolimbic dopamine (DA) transmission that seems to play important role in their behavioural effects. μ and δ opioid-receptor agonists have been shown to stimulate, whereas κ opioid-receptor agonists to suppress the mesolimbic dopaminergic transmission [9]. The stimulatory effect of μ and δ opioid-receptor agonists is supposed to be exerted at the level of ventral tegmental area (VTA), the cell body region of the mesolimbic DA-ergic system. κ opioid-receptor agonists exert their inhibitory effect via presynaptic inhibition of DA nerve terminals in the nucleus accumbens (NAC) [7,12,28,34].

Morphine (MO) displays dual effect on the locomotor activity (LA), depending on the dose and on the time elapsed after the treatment. At the beginning of the observation period reduction of activity (sedation) is observed, followed by hyperactivity [3]. Both tolerance to these acute effects and sensitisation to the hyperactivity have been reported using various species of experimental animals and applying different treatment regimens and experimental methods [1,5,37].

* Corresponding author. Tel.: +36 12102930/6277; fax: +36 12104412.
E-mail address: timjul@pharma.sote.hu (J. Timár).

0361-9230/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.brainresbull.2004.09.005

Continuous exposure to MO results in development of tolerance to its analgesic and also to some behavioural effects, like sedation or reinforcement [18,27,38].

Administration of naloxone (NX), the μ-preferring, non selective opioid-receptor antagonist to rats pretreated with repeated doses of MO does not only precipitate the symptoms of physical dependence, but induces behavioural changes, too. Thus conditioned place aversion (CPA) induced by NX is much more pronounced in MO-pretreated animals [11,22] than in MO-naive ones [26]. A decrease in LA was also observed [25]. The majority of these experiments were done after short-term MO treatment or implantation of MO-containing pellet.

The aim of the present study was to investigate how the rats exposed to rapidly increasing s.c. doses of MO respond to an acute MO, a non-selective opioid-receptor antagonist NX and a selective δ opioid-receptor antagonist naltrindole (NTI) challenge. LA of animals was tested at different time points following withdrawal of MO administration. A higher dose of MO (10 mg/kg) was applied for studying the development of tolerance to the hypoactivity-inducing effect and a smaller one (3 mg/kg) was applied for studying the development of tolerance to the hyperactivity-inducing effect.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River, Budapest) weighing 140–160 g at the beginning of the experiments, housed at constant temperature (20–21 °C) and humidity (55 ± 5%) under a standard 12–12 h light/dark cycle (light on at 6.00 a.m.) were used. Water and food were available ad libitum, except during the tests. All the observations were made at comparable times of a day during the light period. Each animal was challenged only once, except in Experiments I and II, where the effect of MO as challenge drug was tested at various time points after withdrawal.

All experiments were performed according to the Semmelweis University guidelines on the use of experimental animals under the licence of the Ethical Committee.

2.2. Drugs and drug treatment protocols

Morphine HCl (MO), naloxone HCl (NX) and naltrindole HCl (NTI) (ICN Alkaloida, Tiszavasvári, Hungary) were dissolved in physiological saline and given subcutaneously (s.c.) generally in a volume of 0.1 ml/100 g bodyweight, but from the 8th day of MO-pretreatment in a volume of 0.2 ml/100 g.

Morphine tolerance/dependence was studied in rats treated chronically with MO according to Buckett's treatment schedule [6]. Gradually increasing doses of MO were given for 11 consecutive days in two daily portions, starting with a daily dose of 40 mg/kg and raised to as high as 345 mg/kg (see treatment schedule in Table 1). Control animals were given saline applying the same treatment schedule. Chronically treated animals were divided in 11 groups (20 in each), half of the animals in each group were given saline, the other half MO. During the treatment period both the MO- and saline-treated animals were placed individually in small (20 cm × 20 cm × 20 cm) wire mash cages.

Table 1
Treatment schedule of chronic MO administration (daily doses are given in divided doses twice daily, at 6-h intervals)

Day	Daily s.c. MO dose (mg/kg)
1	40.0
2	70.5
3	111.0
4	131.5
5	162.0
6	192.5
7	223.0
8	253.5
9	284.0
10	314.5
11	345.0

In Experiment I the locomotor activity decreasing effect of MO as challenge drug in a dose of 10 mg/kg was studied 18 h after the last dose (drug withdrawal) and then 3, 8 and 12 weeks later. In Experiment II the locomotor activity enhancing effect of MO as challenge drug in a dose of 3 mg/kg was studied first 18 h after drug withdrawal and then three weeks later. In Experiment III and IV the effect of saline and various doses of the opioid antagonists as challenge drugs were studied on LA 18 h after the last treatment. In all the cases the saline-pretreated animals (controls) were challenged with the same drug in the same dose as the MO-pretreated peers.

The challenge drugs were tested also in MO-naive animals and compared to acute saline-treated peers.

2.3. Measurement of locomotor activity (LA)

Horizontal LA (ambulation) was measured by an "Animal Activity Measurement System" (Research Institute of the Electrical Industry, Budapest, Hungary). The apparatus consists of two testing boxes (50 cm × 50 cm × 30 cm) each with a TV camera. The testing boxes are black-painted open field areas, set in an isolated dark room, illuminated by two standard laboratory lamps. The experimental animals are placed into the boxes individually and their movement is followed by the TV cameras. The time passed in LA during the whole observation period is recorded. All data are registered, processed and statistically analysed by a PC [29].

On the day of the observations the animals were transported to the testing room and thirty minutes later were treated with one of the challenge drugs. Ten minutes after the treatment they were placed into the testing boxes individually and the observation period started immediately without any habituation and lasted 20 min in all of the experiments except in Experiments I and II. In the Experiment I, when the