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Research report

Facilitation of avoidance behaviour in mice chronically treated with heroin or methadone

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Abstract

Although the repercussion of chronic treatment with large amounts of opioids on cognitive performance is a matter of concern, the effects of opioid drugs on passive avoidance learning have been scarcely studied. Here, we analyzed the effects of prolonged administration of heroin and methadone, as well as the impact of suffering repeated episodes of withdrawal on fear-motivated learning using the passive avoidance test. Mice received chronic treatment (39 days) with methadone (10 mg/kg/24 h), associated or not with repeated withdrawal episodes, or with heroin (5 mg/kg/12 h). Our results show that, regardless of the type of treatment received, all mice displayed similar basal thermal nociceptive thresholds during 25 days of treatment. In the hot plate test, both methadone and heroin induced antinociception 30 min after drug administration. The analgesic effect was absent when measured 4 h after heroin and 12 h after methadone. Pain behavioural responses elicited by growing intensities of electric shock, applied on day 28th of treatment, were similar in all groups of mice. Our results indicate that chronic opioid treatment had promnesic effects on passive avoidance behaviour in mice, unrelated to changes in the nociceptive state.

Keywords: *Leishmania donovani*; *Brugia malayi*; BmAFII; Hamsters; Co-infection; NO; LTT

1. Introduction

Opioid drugs, such as morphine, are the most powerful analgesics available in the clinic for the pharmacological treatment of acute and chronic pain [5]. Within the last few years, the mu-opioid agonist, methadone, has been increasingly used to manage neuropathic pain and cancer pain [26,35]. On the other hand, opioid drugs exhibit a strong addictive potential and, among them, heroin is widely abused. The maintenance programs for heroin addicts include the long-term treatment with opioid agonists such as methadone or, more recently, heroin [16,24].

A critical question is the extent to which chronic treatment with large amounts of opioids is associated with altered cognitive performance. In this regard, numerous reports indicate that chronic exposure to opioid drugs produces cognitive deficits in humans [13,14,19,20,31,37,52]. However, data from clinical studies are difficult to interpret in terms of cognitive dysfunction because, besides opioid consumption, there are numerous factors – including the presence of pain, neuropsychological status, premorbid medical and psychiatric problems, polydrug abuse, etc. – that may contribute to an impairment of cognitive capabilities [27,44].

In the experimental animal, nearly all studies have focused on the cognitive effects of morphine, the opioid most frequently used in the clinic. It has been reported that chronic morphine treatment impairs rodent learning in many experimental paradigms, including the radial arm maze, Y-maze choice escape, and Morris water maze [10,30,41,45]. Only a few reports indicated that methadone, either acutely or chronically administered [21,48], and chronic heroin [49] impair spatial learning in the Morris water maze.

Regarding fear-motivated avoidance learning, many studies have analyzed the effects of acute morphine in a variety of experimental paradigms, showing contradictory results. Some investigators have reported that morphine impaired passive avoidance responses in rodents [3,8,11,17,54], whereas, according to others, it facilitated memory retrieval [11,15,33,42,46,53]. The dose, time of administration or mice strain are among

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the factors that have been described to condition the improving/impairing effects of the opioid. It has been reported that low doses of morphine increased avoidance learning, whereas high doses exerted a negative effect [1]. When morphine was administered before training, memory retrieval was impaired [8], whereas post-trial administration facilitated learning of a passive avoidance task [33,46]. Opposite avoidance responses to morphine were described in mice from different strains, indicating a determinant role for the genetic make-up [11]. The intensity of the electric shock [50] as well as the sensitivity of the animal to the shock [18] are factors that have been also described as conditioning the effects of morphine on avoidance responses. Finally, concomitant effects of morphine on locomotor activity can distort its actual effects on avoidance learning, leading to contradictory results [2].

In contrast to morphine, the cognitive effects of other opioid drugs on passive avoidance learning have scarcely been or not studied at all. Moreover, the repercussion of long-term treatment with opioids on cognitive functions was not investigated so much in animals as in humans. Here, we have analyzed the effects of prolonged administration of heroin and methadone, as well as the impact of suffering repeated episodes of withdrawal on fear-motivated learning using the passive avoidance test. The possible influence of opioid-induced changes in nociceptive sensitivity on avoidance behaviour has also been evaluated.

2. Methods

2.1. Animals

Male C57BL/6 mice, 8-week old, were used. Mice were bred in our animal facility, housed three per cage in a room kept at 22 °C, and exposed for their whole lifespan to inverted 12-h light:12-h dark cycle (dark from 8:00 a.m. to 8:00 p.m.). Food and water were provided *ad libitum*. This study was approved by the Cantabria University Institutional Laboratory Animal Care and Use Committee and carried out in accordance with the Declaration of Helsinki and the European Communities Council Directive (86/609/EEC).

2.2. Drugs

Methadone and heroin (Dirección General de Estupefacientes, Ministerio de Sanidad y Consumo, Spain) were dissolved in saline at a concentration of 2 mg/ml and 1 mg/ml, respectively.

2.3. Experimental groups

Tables 1 and 2 summarize the timeline of the experimental design. For the evaluation of the behavioral effects induced by chronic methadone, mice received daily subcutaneous injections of methadone (10 mg/kg; $n = 24$) or saline (0.1 ml/mouse; $n = 24$) for 39 days. Behavioral repercussion of repeated withdrawal was assessed in a group of mice chronically treated with methadone and exposed to precipitated withdrawal every 6 days (6th, 12th, 18th, 24th, 30th and 36th days) by administering 1 mg/kg of the opioid antagonist naltrexone ($n = 24$). To evaluate the behavioral effects induced by heroin, mice received two daily subcutaneous injections of heroin (5 mg/kg; $n = 24$) or saline (0.1 ml/mouse; $n = 24$) for 39 days. Twelve mice from each experimental group were randomly selected to perform the nociceptive tests or the passive avoidance test.

2.4. Evaluation of nociception

Considering that the nociceptive sensitivity of the animals can influence the aversive response to the electric shock used for the avoidance test [18], 12 animals from each group of treatment were evaluated for nociception induced by thermal stimulus (hot plate) and by increasing intensities of electric shock. The experimental protocol is displayed in Table 1. The objective of this series was twofold: first to assure that the passive avoidance test was performed in the absence of opioid-induced antinociception, and second to evaluate the development of tolerance and/or changes in the nociceptive threshold during the chronic opioid treatments.

2.4.1. Hot plate

Each animal was placed in a metal cylinder, 15 cm high, immersed in a water bath at 52 °C. The stability of the plate temperature was assessed with an adhesive thermometer. The latency of jumping from the cylinder was used as a measure of pain sensitivity. The cut-off was set at 150 s. As shown in Table 1, mice were tested in the hot-plate every 3 days from days 1 to 25 of chronic treatment, before and 30 min after the corresponding methadone or heroin injection. On days 1 and 22, an additional test was performed 12 h after methadone and 4 h after heroin injections.

2.4.2. Electric shock

The same group of mice tested in the hot plate was submitted to a stimulus/response analysis by applying increasing intensities of electric shock through the floor of the dark box of the passive avoidance apparatus. The behavioural response was assessed on the 28th day of chronic drug treatment, 4 h after heroine or saline, and 12 h after methadone or saline injection. The behavioral response was quantified as follows: 0 = no response; 1 = temblor; 2 = jump less than 1 cm; 3 = jump more than 1 cm; 4 = vocalization. The animals used in this test were not tested on passive avoidance.

2.5. Evaluation of short- and long-term memory: passive avoidance test

In order to evaluate short- and long-term memory, 12 mice from each treatment group were submitted to the passive avoidance test, starting on the 29th day and finishing on the 39th day of treatment (Table 2). Mice performed the behavioral test during their activity (dark) phase, 10–12 h after being administered the corresponding dose of methadone or saline and 4–6 h after heroin or saline.

The apparatus (LE-870, Panlab, Barcelona, Spain) was a box with two compartments, a dark one and a light one. Four sessions were performed. During the first one (habituation session) mice were placed in the light box for 5 min and the latency to enter the dark compartment, the number of entries and the time spent in each compartment were registered. In a second session (the training session), carried out 24 h later, the mice were trained to associate an electric shock with the dark compartment by receiving an electric shock (0.8 mA) when they entered this compartment. Twenty-four hours (test session), and 7 days (retest session) later, mice were placed in the lit compartment and the latency to enter the dark compartment was registered and considered an index of short- (test) and long-term memory (retest).

2.6. Statistical analysis

Comparison of the means of two independent groups was performed by Student's *t*-test. The effects of one or several factors on a dependent variable were assessed by analysis of variance (ANOVA). The means of each experimental group were compared post hoc by the Bonferroni test. The analysis was performed by using SPSS for Windows, Version 11.0.

3. Results

3.1. Basal nociceptive responses in the hot plate test were similar for all groups of animals

As shown in Table 1, animals were submitted to the hot plate test every 3 days, from days 1 to 25 of chronic drug treatments. Data from selected representative days are