Effects of naltrexone and cross-tolerance to morphine in a learned helplessness paradigm

Abstract

Opiates have been implicated in learned helplessness (LH), a phenomenon known to be related to opiate stress-induced analgesia (SIA). In the present study, we investigated the role of opiates in the induction of LH and SIA under different conditions. Adult female Wistar rats were trained either by receiving 60 inescapable 1-mA footshocks (IS group, N = 114) or by confinement in the shock box (control or NS group, N = 92). The pain threshold of some of the animals was immediately evaluated in a tail-flick test while the rest were used 24 h later in a shuttle box experiment to examine their escape performance. The opiate antagonist naltrexone (0 or 8 mg/kg, ip) and the previous induction of cross-tolerance to morphine by the chronic administration of morphine (0 or 10 mg/kg, sc, for 13 days) were used to identify opiate involvement. Analysis of variance revealed that only animals in the IS group demonstrated antinociception and an escape deficit, both of which were resistant to the procedures applied before the training session. However, the escape deficit could be reversed if the treatments were given before the test session. We conclude that, under our conditions, induction of the LH deficit in escape performance is not opiate-mediated although its expression is opiate-modulated.

Introduction

Whether or not an organism can control aversive events has widespread behavioral and physiological consequences (1). Among these, exposure to unpredictable and uncontrollable shocks may impair subsequent learning responses to aversive stimuli. This effect is commonly referred to as “learned helplessness” (LH) (2,3) and represents an animal model that is useful for studying human depression (4). Uncontrollable shocks may also reduce responsiveness to noxious or aversive events from different sources (5). The antinociception thus obtained has been termed “stress-induced analgesia” (SIA) even though it has been recognized that stress itself is not a critical variable (6).

The similarity in the conditions required to produce opioid SIA and LH has been noted by several authors (5,7-10). Recent studies have even suggested a potential opioid basis for both of these phenomena (11). Thus, opiate antagonists can block the escape defi-
cit when administered either prior to the inescapable shock (IS) (11,12) or 24 h later, before the animals are tested for the acquisition of a shock-escape response (13-15). However, reversal is only obtained with high doses of naloxone (12,14,16).

The opiate or non-opiate nature of SIA has been shown to depend on the aversive stimuli studied (7) since different stimuli, or even the same stimulus under different conditions, may change the nature of the SIA response (5). In contrast, the extent to which experimental parameters may influence the opiate/non-opiate nature of LH has not been as fully investigated as that of SIA. Furthermore, although the reversibility of LH by opiate antagonists is a necessary condition for implicating opiate involvement, it is not sufficient (17) since opiate antagonists are not totally specific for the opiate receptor (18). Several other requirements that need to be fulfilled in order to establish opiate involvement have been suggested (17), the most important of these being cross-tolerance to morphine (19).

In the present study, we investigated the involvement of opiates in the production and expression of the above phenomena using naltrexone, an opiate antagonist, and cross-tolerance to morphine. Although our IS protocol differs in many aspects from those used by other authors, it induces similar LH effects (20) and long-term reinstated antinociception (21).

**Material and Methods**

Female Wistar rats (190-210 g) were used. The animals were housed individually in polypropylene cages (30 x 20 x 13 cm) with pine shavings as bedding, at a room temperature of 26 ± 2°C, on a 12-h light/dark cycle (lights on at 6:00 a.m.), and with free access to water and food.

After three days of adaptation to the housing conditions, the rats were randomly assigned to one of two groups and underwent a training session for the induction of LH (20). The first group received inescapable shocks (IS) while the other, which served as the control group, was simply confined in the shock box for the same period of time (NS). Twenty-four hours later, both groups were submitted to a test session involving an escape test. Another group of animals underwent the induction of LH, after which their pain threshold was determined by the tail-flick test applied immediately after the training session (21).

**Training and test sessions**

The rats were placed in a plexiglass box (25 x 25 x 30 cm) equipped with a grid floor consisting of stainless steel bars. The first group was subjected to a session of 60 scrambled, unsignalled, inescapable 1-mA footshocks (IS subgroup) delivered by a shock generator (Albarsch, Porto Alegre) connected to a microcomputer executing a software program (for details, see Ref. 22). This program controlled the shock on/off periods and was based on the escape latencies of 199 animals determined during a series of 60 footshocks delivered over 30 s in a shuttle box, as described below for the test sessions. The use of this program ensured that animals in the IS group received a footshock density “yoked” to an escapable shock group. Details of the yoked shock sessions and the program construction are provided elsewhere (22). The duration of the shocks varied from 0.58 to 18.18 s depending on the trial. Each trial was separated by an interval of 5-25 s. The second group was simply confined in the box for the same period of time (NS subgroup). Twenty-four hours after the end of the session, all of the animals underwent an escape test in a shuttle box (automatic shuttle box, Albarsch). The latter consisted of a two-way automated plexiglass box (50 x 25 x 30 cm) with a grid floor containing stainless steel bars. The box was divided into two equal chambers connected to each other.
via a small opening (6.0 x 7.5 cm) located 8 cm from the grid floor in the dividing wall (20). Thirty shocks were delivered continuously during each trial which lasted a maximum of 30 s. In each case, the rat was required to jump into the opposite chamber in order to turn off the shock. If no response was evoked, another shock was immediately delivered (random between-trial interval of 5-25 s). The time between the onset and the end of the shocks was considered to be the response latency or trial duration.

**Tail-flick test**

The rats were tested for their threshold responsiveness to radiant heat pain (23). The animals were placed on a tail-flick apparatus (Albarsch) which consisted of a 13 x 25 x 33 cm metal box that supported a 25 x 33 cm aluminum plate. This plate was divided by a single groove which passed through a small hole in the center of the plate. The hole served as the focus (through a condenser lens) for a Kondon 150-W projector lamp, thereby restricting the radiant heat to a defined region. The animal’s tail was placed in the groove and the hole positioned 2.5 cm rostral to the tip of the tail. Deflection of the tail activated a photocell located 6.5 cm above the hole and automatically terminated the test. The light intensity was adjusted so as to obtain a baseline tail-flick response within 6-9 s. A cut-off time of 20 s was used in order to reduce the possibility of tissue damage.

**Treatments**

Pharmacological treatments were applied to paired IS (experimental) and NS (control) groups to form drug-treated pairs. All drugs were prepared from pure salts (Sigma) and diluted in 0.9% (w/v) saline solution. Independent subgroups of rats received similar treatments before the training or test sessions. Naltrexone (8 mg/kg, ip) was given 60 min (7) before the training or test sessions as appropriate. Morphine (10 mg/kg, sc) was administered once daily for 13 days in the cross-tolerance (CT) experiments. Injections were stopped 24 h before the training or test sessions as appropriate. No injection of saline was used to substitute for the chronic morphine or saline injections after their suspension, in order to avoid any conditioned behavioral effect (19). As a control for the influence of drug treatment on the behavioral depression or antinociception induced by inescapable shocks, 0.9% (w/v) saline was administered either ip (acutely) or sc (chronically) to NS and IS rats before the training or test sessions, as appropriate.

**Statistical analysis**

The behavioral results are reported as the mean escape latency (± SEM) for blocks of five trials during each test session or as the mean tail-flick latency (± SD) for each test session. The results obtained for the escape test were analyzed using two-way analysis of variance in order to compare the IS vs NS groups for each drug treatment. Non-parametric Kruskal-Wallis analysis of variance followed by Dunn’s multiple comparisons test (24) was used to analyze the results obtained with the tail-flick test.

**Results**

Two-way analysis of variance indicated that only rats previously exposed to uncontrollable shocks showed an escape learning deficit characteristic of this model of behavioral depression when compared to non-shocked rats. This difference between the two groups of animals was significant (P≤0.001) for all treatments when they were applied before the training sessions (acute saline (F (1,14) = 17.6), naltrexone (F (1,24) = 15.9), chronic saline (F (1,14) = 16.1) and cross-tolerance to morphine (F (1,14) = 15.7) groups; Figures 1 and 2). No statistically
significant shock vs drug treatment interaction was observed. Independent of the previous treatment or shock exposure, all groups learned to jump in order to escape the shocks since throughout the trials there was a significantly higher mean escape latency at the beginning of the session than at the end. This relationship was observed with all treatments given before training ($P \leq 0.001$ for the acute saline ($F(5,70) = 16.0$), naltrexone-treated ($F(5,12) = 13.1$), chronic saline ($F(5,70) = 15.9$) and cross-tolerance to morphine ($F(5,70) = 17.0$) groups; Figures 1 and 2).

The escape deficit provoked by inescapable shocks in the control groups ($F(1,14) = 18.4$ for the acute and $F(1,14) = 19.2$ for the chronic saline treatments) was reversed by naltrexone (Figure 1) as well as by the cross-tolerance to morphine induced before the test session (Figure 2). No statistically significant shock vs drug treatment interaction was observed. Similar to that observed for the treatments given before the training session, treating the animals before the test significantly decreased the mean latencies from the first to the last block of trials ($P \leq 0.001$ each: $F(5,70) = 7.01$ for acute saline-treated, $F(5,70) = 6.9$ for naltrexone-treated, $F(5,70) = 9.4$ for chronic saline-treated and $F(5,90) = 16.6$ for the cross-tolerance to morphine groups), indicating that the animals retained their ability to perform the escape response.

Kruskal-Wallis analysis of variance ($KW = 39.8$, $P \leq 0.001$) followed by Dunn’s test revealed that only the IS group showed antinociception soon after the shocks ($P \leq 0.01$ for the acute saline and $P \leq 0.05$ for chronic saline groups). This antinociception was not affected by the manipulation of the opiate system ($P \leq 0.01$ for naltrexone and $P \leq 0.05$ for morphine CT; Table 1).

**Discussion**

The LH model of depression (also known as “interference effect”) is defined on the basis of a statistically significant difference between the escape latencies of inescapably shocked animals and those of a non-shocked...
Non-opiate-mediated learned helplessness group (control). Thus, the conclusion that an animal is (or is not) depressed under drug treatment requires that both the shocked and non-shocked groups are treated with the same drug of interest. This experimental design is routinely used in protocols involving drug administration (see for example, Ref. 25).

The present results indicate that exposure to inescapable shocks under the conditions used here produced an antinociceptive effect and substantial interference with the shuttle box escape learning 24 h later. These findings agree with those described in the pioneer work of Seligman and Maier (2) and also obtained in our laboratory using a different protocol (20,21).

It has been postulated that the SIA obtained soon after a series of escapable shocks dissipates rapidly (a phenomenon known as short-term analgesia) and does not lead to the behavioral depression typical of learned helplessness. Short-term SIA is unconditionable and cannot be induced by a brief re-exposure to inescapable shocks. Non-opiate mechanisms appear to be involved in this antinociception (7).

In contrast, opiate-mediated SIA is obtained after physically identical but inescapable shocks. This antinociception is long-lasting, produces LH (8,10) and can be conditioned as well as reinstated after a brief re-exposure to inescapable shocks (also known as long-term reinstated analgesia). Opiates are involved in this antinociception (7). The stress protocol used in our experiments allowed us to observe the development of long-term, conditionable (21) hypoalgesia which was not affected by the manipulation of the opiate system before the training session. Since the classification of SIA as opiate- or non-opiate-dependent is based on its susceptibility to opiate blockade (7), the SIA obtained in our study can be considered to be non-opiate.

Since the opiate nature of long-lasting antinociception has been extensively studied (5), it would be reasonable to suspect that the LH phenomenon that accompanies long-term SIA would also be opiate-dependent. This hypothesis was initially put forward by Drugan and Maier in 1983 (11) and later by others (12). The conclusions of both of these studies were based on the use of opiate antagonists given before the training session at

![Figure 2 - Cross-tolerance to morphine prior to the test session reverses learned helplessness. Data are reported as the mean escape latency (s) ± SEM for blocks of 5 trials for 5-15 rats in each group. The rats were made cross-tolerant (CT) to morphine or received saline (S) before the training or test session and were given either inescapable (IS) or no shocks (NS) in a shuttle box.](image)
doses as high as 14 mg of naltrexone/kg and 3 mg of naloxone/kg. Such doses, however, do not always produce complete blockade of LH (11).

In contrast, our results demonstrate that LH was not affected by any of the manipulations designed to reduce the functioning of the opiate system. However, the manifestation of LH induced under drug-free conditions could be blocked by these same manipulations when they were applied immediately before the test. A similar finding has been reported by others (13-15,26). The latter phenomenon is mediated principally by opiate receptors in the brain since centrally, but not peripherally, administered naltrexone is able to block the manifestation of the escape impairment as well as the increase in nociceptive threshold produced by prior exposure to drug-free inescapable shocks (26). The intracerebroventricular administration of naltrexone before the test reduces the manifestation of both the LH escape deficit (27) and the conditioned opioid antinociception (28). Our demonstration of an opiate involvement agrees with the well established fact that endogenous opiates participate in the manifestation of learned behavior by affecting retention and retrieval processes in a complex and profound manner (for a review, see 29). An effect on memory processes represents one of these pathways of interference.

Since opiate-mediated SIA is very sensitive to changes in the shock parameters, it would be reasonable to suspect that methodological differences could explain the discrepancy between our observations and the results of others (11,12). We and others (11,12) have used the shuttle box escape latency to estimate the LH escape deficit. However, apart from the already discussed possible non-specific effect resulting from the use of high doses of antagonists by these authors (11,12), we have employed female rats which received a smaller number of shocks. The authors of the above two studies used 80 shocks which were applied either as inescapable tailshocks (11) or as footshocks (12). In this respect, it has already been shown that the number of inescapable shocks is a crucial variable in the manifestation of opiate-/non-opiate-mediated SIA. Specifically, the transition from non-opiate SIA to opiate SIA occurs in the interval between 40 to 80 inescapable tailshocks (7). Sex is also recognized as being an important variable in the modulation of stress-induced opiate antinociception (30) and in the vulnerability of rats to the induction of depression (31), with females being more vulnerable than males. The estrous cycle strongly influences the inhibition of pain (32), there being a strong increase in opiate antinociception during diestrous. In addition, there are also differences between sexes in the response to the persistent pain induced by the formalin test (33). Such differences can be explained by variations in the cerebral β-endorphin concentrations for which the levels are higher in females, particularly in the periaqueductal gray matter (34), a region known to be very important in the organization of defensive

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Latency (s)</th>
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<tbody>
<tr>
<td>NS</td>
<td>Saline</td>
<td>6.7 ± 3.5</td>
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<tr>
<td>IS</td>
<td>Acute</td>
<td>19.2 ± 2.1*</td>
</tr>
<tr>
<td>NS</td>
<td>Saline</td>
<td>7.2 ± 5.4</td>
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<tr>
<td>IS</td>
<td>Chronic</td>
<td>17.1 ± 2.8*</td>
</tr>
<tr>
<td>NS</td>
<td>Naltrexone</td>
<td>9.3 ± 2.9</td>
</tr>
<tr>
<td>IS</td>
<td>Naltrexone</td>
<td>18.3 ± 2.5*</td>
</tr>
<tr>
<td>NS</td>
<td>Morphine CT</td>
<td>5.9 ± 4.5</td>
</tr>
<tr>
<td>IS</td>
<td>Morphine CT</td>
<td>12.0 ± 7.2*</td>
</tr>
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Table 1 - Antinociception is not blocked by naltrexone.

The numbers indicate the mean tail-flick latency (± SD in s) observed soon after the training session. IS indicates rats receiving inescapable shocks and NS, non-shocked rats. Prior to training, the rats received either 0.9% saline (acutely or chronically), or naltrexone (8 mg/kg) or were made cross-tolerant (CT) to morphine (10 mg/kg). *P<0.05 compared to the NS control group (Dunn’s test). N = 8 rats per subgroup.
behavior, including the escape reaction and endogenous pain modulation (35). As stated above, the use of female rats in our study may have had a profound effect on the outcome of the results, particularly since our rats were possibly more resistant to the reversal of depression and of the induced antinociception. In this regard, it is also possible that training may have resulted in the release of more β-endorphin than did the test sessions. Our results further suggest that activation by opiates is not absolutely necessary for the induction of LH in female rats. The learning of uncontrollability may activate a non-opiate system, thus leading to manifestation of the “interference effect” upon escape (2).

The results obtained above for non-opiate SIA associated with non-opiate LH reinforce the idea that the LH and SIA produced by inescapable shocks are positively related (36). However, this relationship does not mean that the two phenomena are necessarily coupled. Such a divergent association was originally suggested by MacLennan et al. (37) who observed that the selective blockade of SIA by hypophysectomy and dexamethasone administration did not affect the induction of the LH escape deficit.

We conclude that, under our experimental conditions, the induction of the deficit in escape performance that is characteristic of the LH model of depression is not opiate-mediated, although its expression is opiate-modulated.

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References


