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Morphine-induced analgesia subsequent to formalin injection in female Wistar rat

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ABSTRACT

Background and Objectives: Previous studies have shown the antinociceptive effect of morphine in animal models, but the specific anti-pain consequence of the abuse drug in female animals is unclear. The present research showed the morphine antinociception in female Wistar rats using formalin test.

Materials and Methods: Subjects were 40 female Wistar rats purchased from Pasteur Institute of Iran. They were treated intraperitoneally (i.p.) with morphine (3-12 mg/kg) 10 min before of the formalin test. In order to induce the chemical pain, the rats received intraplantar injection of formalin (2.5% / 50 µL) and given the test. The control rats received saline solution (1 mL/kg, i.p.) instead of the morphine. The nociceptive response was divided into two phases: phase acute (0-15 min) and phase chronic (15-60 min). The animals were graded by a 4-point scale each 15 sec continuously throughout the test.

Results: This study revealed the morphine-induced antinociception at the early as well as the late phases of the formalin test. Moreover, the response to the lower doses of morphine in the early phase was considerable in comparison to the late phase.

Conclusion: The data indicate a definite morphine antinociception role in female Wistar rats. The female rats may exhibit less anti-pain effect of morphine in the late phase of the chemical pain unless if receiving the effective doses of the drug.

1. Introduction

Researchers have revealed the antinociceptive effect of morphine in animal models. In view of gender-dependent effect, several studies have also reported sex-related differences to morphine antinociception in the rats (1-3). Lot of the studies has been mainly focused on painful stimuli to show the role of genital hormones in the sex differences to the morphine antinociception (4-6).

Some of them have suggested a role for the genital hormones in the morphine response, (7-9) and the others have postulated the involvement of the neuronal modulators in the proposed effect of the abused drug (10). Considering the above-mentioned matter, the possible mechanisms which cause the sex-dependent differences in the morphine effects are included the sex hormones, and disparities in morphine pharmacokinetic.

Although the previous achievements suggest that there are sexually difference mechanisms governing on morphine effects, but, relatively few studies have studied morphine anti-inflammatory pain effect in female Wistar rats. Therefore, the reason of the present study was to show particularly morphine antinociception result in female Wistar rats by the formalin test.

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2. Materials and Methods

2.1. Animals

40 female Wistar rats (weighing: 200-250 g) were used in this research.

All animals were housed in specific animal room as virgin under 21 ± 3°C in a 12 h light /dark cycle with food and water available *ad libitum*. Each animal was used only once. All trials in this study were performed based on ethical guidelines of animal welfare and approved by the local Ethics Committee.

2.2. Drugs

Morphine sulfate (TEMAD Co, Tehran, Iran) was liquefied fresh in saline solution.

2.3. Experimental Procedure

Rats were given morphine (3-12 mg/kg) intraperitoneally (i.p.) 10 min before of the formalin injection. The rats then under restrained were subcutaneously (s.c.) injected with 50 µL of 2.5% formalin into the plantar surface of right hind paw and underwent the test. The control vehicle group received the saline (1 mL/kg) instead of the opioid drug.

For testing, the animals were placed in a 30×30×30 cm^3 Plexiglas box with a mirror below the surface at a 45° angle to allow a clear view of the animal' paws. Calculating of the drug effect was done by a 4-point scale grading recorded each 15 sec continuously throughout the test (60 min). The score was measured for each 5-min time block by determining the scale in each using the following behavioral weighted nociceptive counting: 0) the injected paw is not favored; 1) the injected paw has little or no weight placed on it; 2) the injected paw is elevated and is not in contact with any surface; and 3) the injected paw is licked, bitten or shaken (11). Scores are expressed during the initial (acute) and the late (chronic) phases. The first phase includes five min and the second one lasts from 15 min to 60 min.

2.4. Statistical analysis

All data were analyzed by one-way ANOVA, followed by Tukey's *post-hoc* comparison test. $P<0.05$ was statistically significant.

3. Results

3.1. Morphine antinociception effect in female Wistar rats

Fig. 1 shows the morphine anti-pain effect compared to the saline solution in female Wistar rats. As shown there was statistically significant differences in both phases of formalin test in animals.

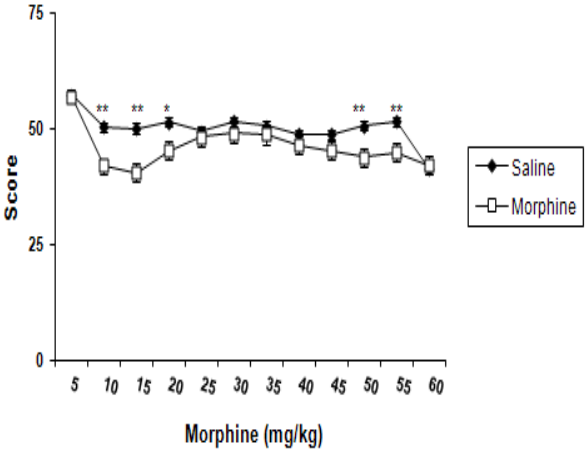

Fig 1. Figure shows the response to morphine or saline in the rat formalin test. Data are calculated as the scores of nociceptive behavior in female Wistar rats by formalin injection (50 µL at 2.5%) 10 min after receiving morphine or saline. Each animal was observed for 60 min after formalin injection. Each point is the mean±SEM of the accumulative time of nociceptive behavior/first or /later phase. The comparison was done between *p<0.05, **p<0.01 indicate the differences vs. vehicle based on Tukey's *post hoc*.

After injection of different concentrations of the drug, the differences were observed in the analgesic behavioral response to the morphine in the animals (Fig 2).

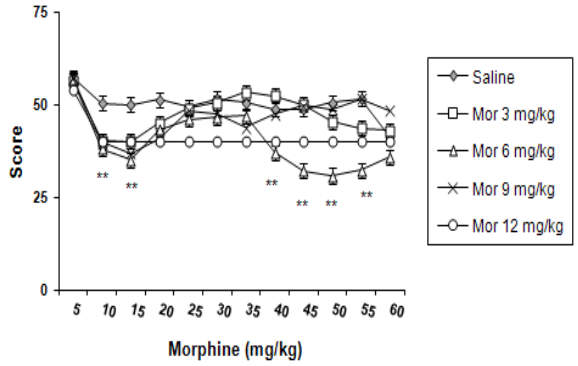

Fig 2. This figure shows the response to different concentration of morphine or saline in the rat formalin test. Data are calculated as the scores of nociceptive behavior in female Wistar rats by formalin injection (50 µL at 2.5%) 10 min after receiving morphine (3-12 mg/kg, i.p.) or saline (1 mL/kg). Each animal was observed for 60 min after formalin injection. Each point is the mean±SEM of the accumulative time of nociceptive behavior/first or /later phase. The comparison was done between *p<0.05, **p<0.01 indicate the differences vs. vehicle based on Tukey's *post hoc*.

As Fig. 3 shows the analgesic result of morphine in female rats was induced both at the early as well as the late phases of the formalin test. Moreover, the response to the lower doses of the opioid drug, morphine, in the early phase was considerable in comparison to the late phase ($F_{4,35} = 7.181$, $p < 0.001$).

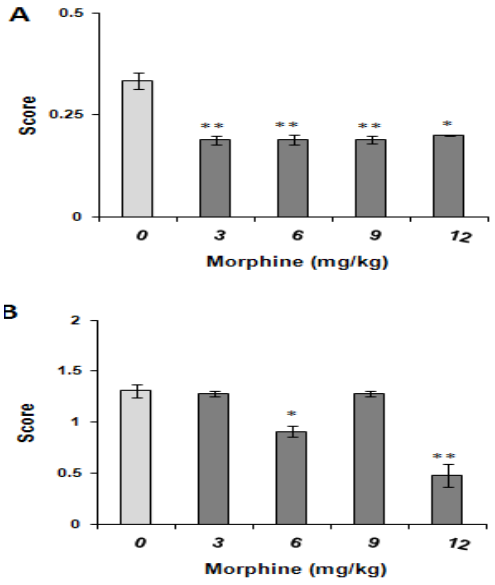

Fig 3. Figure shows the response to morphine doses or saline in the rat formalin test. Data are calculated as the scores of nociceptive behavior in female Wistar rats by formalin injection (50 µL at 2.5%) 10 min after receiving morphine (3-12 mg/kg, i.p.) or saline (1 mL/kg). Each animal was observed for 60 min after formalin injection. Each point is the mean±SEM of the accumulative time of nociceptive behavior/first (acute A) or /later (chronic B) phase. The comparison was done between *p<0.05, **p<0.01 indicate the differences vs. vehicle based on Tukey's *post hoc*.

4. Discussion

This research aimed to study the morphine antinociception in female Wistar rats by the formalin test. The results showed the morphine anti-pain effect in the female rats in respect to the control group in the formalin test.

To discuss the data, accordingly, morphine antinociception has been previously evidenced in other strain of rat on the tail flick assay (12). However, the drug antinociception as has been mentioned in the previous article was greater in male rather than in female Sprague-Dawley rats as been demonstrated by the researchers. The different results have been explained by involving the estrous cycle in the females to develop the response to the morphine (12).

It appears that the female cycle has a distinct role in the response to the drug. The female sex hormones most likely have impact on the morphine antinociception. For example, the sex-related control to the morphine-tolerance has been documented previously in the rats (1).

According to the present finding the morphine analgesic effect was developed at all concentration of the drug in the early phase. Although non-equivalently to the ours, to deal with our achievements, the authors have reported that female Sprague-Dawley rats had more tolerate latencies to the noxious stimuli (13,14).

We may include the female Wistar rats' hormones in the presented significantly more nociceptive responses in the acute phase. In contrast, it has been reported that female mice show lower pain-related responses in models of persistent nociceptive stimulation such as the formalin and the writhing tests (15,16).

In addition, differences were observed in morphine antinociceptive dose effect in the chronic phase in the female Wistar rats, but, there are not consistent studies to clearly interpret the findings.

The findings of the present study demonstrated no marked response to the moderate concentration of the drug in the late phase though the responses were no differently significant at lower or higher doses in the female rats. It is possible that the doses powers of the morphine as

well as the strain of the rats are involved in the results.

In conclusion the present data indicate a definite morphine antinociception role in female Wistar rats. The female rats may exhibit less anti-pain effect of morphine in the late phase of the chemical pain unless if receiving the effective doses of the drug.

Acknowledgments

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