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Gender Related Differences in Antinociceptive Properties of Morphine after Gonadectomy in Male and Female Rats

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Abstract: In this study gonadectomy was undertaken to evaluate gender-differences in antinociceptive effects of morphine. Ninety NMRI adult male and female rats in three groups of intact, sham-operated and gonadectomized were tested by Hot-plate assay. The latency time was recorded three times before and every 15 min until 2 h after morphine (7 and 12 mg kg^{-1} s.c.). Same procedure was repeated in days 7, 21 and 35 after surgical operation. The data were expressed as latency time and maximum possible effect (%MPE).There was not any significant gender differences in base line latency time in intact and also in sham operated rats at days 7, 21 and 35, while in gonadectomized rats the base line latency time of male were decreased significantly at 21st and 35th day compared to female rats. The analgesic response to 12 mg kg^{-1} s.c. of morphine was significantly higher in intact male rats while in day 35th after gonadectomy it was decreased significantly compared to females. By 7 mg kg^{-1} s.c. of morphine %MPE of males was decreased only in 35th day. In sham operated and all other groups no gender differences was observed in %MPE. So gonadectomy decreased pain threshold sensation especially in male rats. The analgesic response to morphine decreased in both sexes but in male rats it was more pronounced, especially by higher doses of morphine (12 mg kg^{-1} s.c.). This phenomenon may be described by different density and affinity of opioid receptors which their function depends on morphine dosage and/or sex hormone level.

Key words: Sex, antinociception, morphine, gonadectomy, rat

INTRODUCTION

There are gender-related differences in the acute pharmacological response to psychoactive drugs and in the development of tolerance, dependence and perhaps drug addiction[1,2]. Gender-differences have been found in term of opioid antinociception induced by stress[3,4]. It has been suggested that hormones could be involved in sex-differences effects of morphine. Nock *et al*.[5] reported that there are sex differences in the plasma level of physiologically active corticosterone by chronic exposure to morphine, which might foster drug-seeking behavior. While others have found that morphine interacts reciprocally with other hormones such as LH[5,6] or might be related to testosterone[7] and/or corticosterone[8,9].

In some other studies, the role of sexual hormones has been emphasized in gender-differences in tolerance, dependence and antinociceptive effect of morphine[3,4,8]. It has been shown that testosterone sensitized post-partum rats as well as ovariectomized rats to morphine[8] and reversed the deficits in Continuous Cold Water Swim (CCWS) and/or Intermittent Cold Water Swim (ICWS) analgesia in gonadectomized rats[10]. However, estradiol and progesterone decreased ß-endorphin receptors in hypothalamic, thalamic and midbrain areas[11]. Additionally it was demonstrated that sex hormones modulate pattern and content of opioid receptors in sexually dimorphic medial preoptic area[13,14] in terms of density and distribution of opiate receptors[14] the subsequent decrease of receptor density may be related to receptor turnover[15] or gene expression[16,17].

In previous studies, it has been observed that males were generally more sensitive to analgesic effect of morphine than females, but when gonadectomy was occurred to explain the modality of these differences, the results were controversial[8,18-20]. So this study was performed to evaluate the gender differences to the various doses of morphine during different periods after gonadectomy in male and female rats.

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MATERIALS AND METHODS

Subjects: Ninety NMRI rats (45 males and 45 females) weighing 250-270 g were used. All animals had 60-80 days of ages to ensure adulthood state. They were housed three per cage at a room controlled temperature (22±2°C) and a 12-h light/dark cycle (light on 07:00 h) with free access to standard rat breeding diet and tap water.

Nociceptive testing: All animals were tested on the hot-plate test for evaluating the antinociceptive properties of morphine. The heated surface was maintained at 55±0.5°C to yield baseline reaction times up to 7 sec. The outranked were excluded from analysis. The baseline reaction time as a threshold to nociceptive stimulus is the mean of three measurements before drug treatment.

The animals were gently placed on the plate and the time required for paw licking or jumping out of the cylinder which occurs rarely was taken as hot plate latency time. The latency time more than 20 sec was considered as a cut-off point to prevent any damage to animals.

The test was performed in three groups of male and females rats: intact, sham-operated and gonadectomized three times before administration of morphine and every 15 min until 120 min after injecting the drug subcutaneously (s.c.) at the doses of 7 and 12 mg kg^{-1} of morphine sulfate (Temad Co., Iran).The same procedure was used to record the latency time on 7, 21 and 35 days after gonadectomy and/or sham-operation.

To evaluate the sensitivity of animals to nociceptive stimulus, we consider the individual latency time before drug treatment as the pain threshold sensation.

To assess antinociceptive effect, the mean value of %MPE of each rat in any hot-plate test, was indicated as maximum possible effect (%MPE), defined as follows:

$$\%MPE = \frac{\text{Actual response time (sec)-baseline (sec)}}{\text{Cut-off time (sec)-baseline (sec)}} \times 100$$

Gonadectomy: To determine whether gender-related differences changes in lack sex of steroids, male or female rats, were castrated or ovariectomized, respectively. Under anesthesia, by sodium thiopental (60 mg kg^{-1}, i.p) the testes or ovaries were removed. Prior to experimentation, all animals were allowed to recover from surgery for 7 days. In sham-operated group rats were treated in the same way as for gonadectomized but without removal of gonads.

Data analysis: The results obtained are expressed as mean±standard error of at least 6 rats in each group. The reaction time or %MPE between males and females were compared by Student's paired t-test. On the other hand, data in each groups (Intact, sham-operated, gonadectomized: male or female) were subjected to the One-way ANOVA followed by post-hoc analysis, as needed. The level of $p<0.05$ was considered to represent a significant difference.

RESULTS

Pain threshold sensation: The results of the study showed that there was not any significant difference in hot-plate latency time before drug treatment between male (5.19±0.22 sec) and female (5.22±0.22 sec) in intact rats. Also the latency time in intact rats was not significantly different from sham-operated rats at days 7, 21 and 35 after surgical operation in both sexes (Fig. 1). Additionally, the results have been shown that there were no significant differences in baseline reaction time among the gonadectomized groups (7th, 21st and 35th days after gonadectomy) of each sex. The comparison of baseline reaction time between males and females showed no significant differences in intact animals and also in all three sham-operated groups (7th, 21th and 35th days after surgical operation). In gonadectomized rats, after 7 days baseline reaction time is still similar between males and females while after 21 days (male; 3.84±0.20 sec, female; 3.27±0.14 sec) and also 35 days (male; 3.53±0.17 sec female; 4.29±0.25 sec) that of male's were significantly less than female's (Fig. 1).

Gender differences in antinociceptive effect of morphine: In these experiments, male and female rats were received 7 and 12 mg kg^{-1} morphine (s.c.) in both gonadectomized and sham-operated groups in 7th, 21st and 35th days. The reaction times were recorded by hot-plate as described in material and methods for evaluating the antinociceptive effects of morphine as %MPE.

After injection of 7 mg kg^{-1} morphine (s.c), there were no significant differences of %MPE between sexes in intact animals and also after 7th and 21st day of gonadectomy. but in 35th day after gonadectomy, %MPE in male rats (4.85±3.19) was decreased significantly compared to female rats (24.02±6.88) ($p<0.05$) (Fig. 2)

The comparison of %MPE of 7 mg kg^{-1} morphine (s.c.) during the days of gonadectomy showed a significant decrement of %MPE in male rats while in female rats %MPE didn't change during the same period after gonadectomy (Fig. 2).

In sham-operated animals which received the same dose of 7 mg kg^{-1}, no gender difference of %MPE was observed in 7th, 21st and 35th day. Additionally %MPE

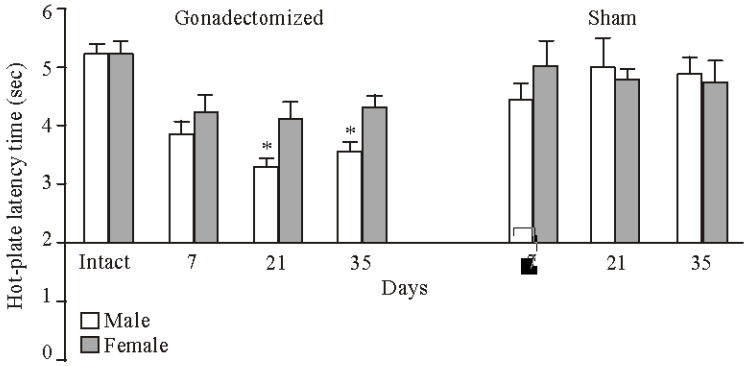

Fig. 1: Reaction time to thermal stimuli in hot-plate apparatus in three groups of male and female rats (intact, sham-operated and gonadectomized). * Significantly different as compared to females ($p<0.05$)

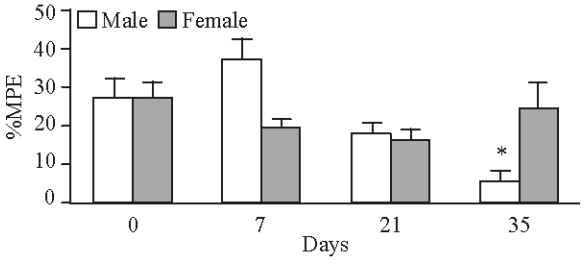

Fig. 2: Maximum possible effect (%MPE) after morphine (12 mg kg^{-1} s.c) in intact and 7, 21 and 35 days after gonadectomy in male and female rats. *Significantly different as compared to females ($p<0.05$)

Table 1: %MPE in sham-operated groups (7th, 21st and 35th day) after injection of 7 and 12 mg kg^{-1} morphine (s.c) in male and female rats

Morphine mg kg^{-1}	7th day	21st day	35th day
7	19.74±9.00	22.95±10.02	29.87±0.93
	15.53±2.53	**36.18±8.01**	**46.09±27.04**
12	86.01±13.98	64.39±20.57	54.58±17.34
	52.00±13.19	**79.26±12.01**	**65.99±14.58**

The data are expressed by Mean±SEM. The data in the first line represents %MPE in male and second line represents (bold lines) that in female rats

didn't change during days of operation in males neither in females sham-operated (Table 1).

After injection of high dose of morphine (12 mg kg^{-1} s.c), intact male rats showed significantly higher %MPE (77.9±9.3) compared to females (47.68±8.71). After gonadectomy %MPE decreased in both sexes, however no gender difference was observed in day 7 and/or 21 while in 35th day %MPE of male rats (15.4±1.2) decreased significantly compared to females (34.6±6.1) (Fig. 2).

In sham-operated animals which received the dose of 12 mg kg^{-1}, no gender difference of %MPE was observed in 7th, 21st and 35th day. Additionally %MPE didn't change during days of operation in males neither in females sham-operated (Table 1).

DISCUSSION

The results of this study showed that prior to morphine administration, there was no significant gender differences in the latency of response to thermal stimuli in intact and sham-operated animals at days 7, 21 and 35 after operation. However, the latency response to the thermal stimuli in castrated rats was significantly decreased at 21 and 35 days after gonadectomy as compared to ovariectomized rats.

Forman *et al.*[20] reported that both male and female rats become hyperalgesic within one week of gonadectomy. Also this is in contrast with the report of Ali *et al.*[22] that there was no significant differences between intact, sham-operated or gonadectomized rats in the latency response to thermal stimuli 7 to 10 days after gonadectomy. These differences may be ascribed to differences in the analgesic test used, to differences in animal strain or other unknown confounding factors[20,22].

Forman *et al.*[21] proposed that gonadal steroids increase the reaction time to thermal stimuli, but testosterone treatment did not completely reverse the effect of castration on antinociception, probably indicating the involvement of other factors. Also some investigators found that castration decreases male rat brain opioid receptor content[10,18,24], while others have found no evidence of this changes[23].

The analgesic response to morphine (7 mg kg^{-1}, s.c) was not significantly different between intact male and female rats, However, male rats showed significantly higher analgesic response to higher doses of morphine (12 mg kg^{-1}, s.c.), than females ($p<0.05$). which is in agreement with some other studies that males are more

sensitive to the antinociceptive properties of morphine than females in the different assays, I. e. Hot plate, tail-flick and abdominal constriction tests[3,10 18,23]. In accordance to the results of this study, it was shown that gonadectomy reduced antinociceptive effect of morphine in both sexes, probably via interaction with central opioid and gonadal steroids receptors[10,18]. Morphine subsensitivity in castrated rats may be attributed to lack of testosterone[8]. Since testosterone reversed the deficits in CCWS (Continuous Cold Water Swim) and ICWS (Intermittent Cold Water Swim) in both castrated and ovariectomized rats[10]. While estradiol administration as a sex hormone in gonadectomized rats decreased significantly β endorphin in Hypothalamus/ thalamus/midbrain axis[11].

Although the analgesic response to morphine (7 and 12 mg kg^{-1}, s.c.) between gonadectomized male and female rats were not significantly different at 7 and 21 days after gonadectomy, It was seen that there was a significant decrease of %MPE in castrated rats as compared to ovariectomized rats and their respective controls only at 35 days after gonadectomy in hot plate test. Ali *et al.* also reported that the increase in the reaction time, after morphine (5 mg kg^{-1}) treatment, was significantly higher in ovariectomized than in castrated rats 7 to 10 days after gonadectomy[22].

The precise mechanism(s) in the change of antinociceptive effect of morphine in long term gonadectomy in male rats is not completely understood, since the testosterone treatment did not completely reverse the effect of castration on antinociception, probably indicating the involvement of other factors[21,22]. Some investigators proposed that gonadectomy may change the kinetics of morphine, but Cicero *et al.*[23] showed that there were no differences between males and females in the serum morphine levels attained at the time which maximal differences in the antinociceptive activity of morphine.

In agreement with other reports, we sustain that the neuronal development of opiate receptors, as the structural and sexual dimorphisms of brain regions, depends on sex hormones. These organizational effects occurred in early postnatal or prenatal period[12,25-27]. A reasonable hypothesis is the existence of different density and/or subtype of morphine antinociceptive receptors, of their affinity and function could depend on morphine dosage and/or sex hormone levels.

In conclusion, present experiments demonstrate pronounced gender related differences in the antinociceptive effects of morphine and also our results suggest that the acute effects of steroids play little role in gender related differences observed. The mechanisms underlying these gender differences are unknown and remain to be established.

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