GENDER DEPENDENT DIFFERENCE IN ANALGESIC EFFECT AND TOLERANCE TO MORPHINE IN MICE

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Summary

There are controversial reports regarding the role of sex hormones in pain perception, analgesia ad tolerance. In the present study morphine tolerance and its analgesic effects in male and female mice was investigated.

Twenty mice were divided into male and female groups. Hot plate test was carried out as a base record, then the animals received 10 mg/kg (S.C.) morphine and analgesic effect was recorded every 15 min. Morphine (30 mg/kg; 3 times a day) was injected for 3 consecutive days to induce tolerance to analgesic effect of morphine and the analgesic effect of morphine was evaluated again.

The reaction latency times after injection of morphine (10mg/kg) was longer than base in both female and male groups while after tolerance, there was no difference between base time and reaction latency times. The reaction latency times after injection of morphine in male group were more than female group. There was no significant difference in reaction latency times in both male and female tolerated animals after tolerance to morphine.

Repeated treatment by morphine lead to tolerance in both sex. Analgesic effect of morphine in male mice was higher than female but tolerance to morphine was not sex dependent.

Keyword: Mice, Sex, Analgesia, Tolerance, Morphine.

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Introduction

Pain is defined as an unpleasant but beneficial sense which protects the organs or skin from damaging or potentially-damaging, noxious stimuli (1). It is now well known that the pain perception is affected by sociocultural, psychological and biological conditions (2). There is also good evidence that pain and analgesia is sex dependent(3,4,5,6,7)**.** The gender dependent difference in the prevalence of some chronic pain producing disorders (8,9) confirms this hypothesis. It has been also well documented that both male and female gonadal hormones such affect pain and analgesia (3,10,11,12,13).

The presence of estrogen and progestrone receptors in pain or analgesia related regions of the nervous system imply that these hormones have a role in pain perception or analgesia (14,15,16,17). The interaction of Sex hormones with neurotransmitters such as gama amino butyric acid (GABA), serotonin and calicitonin gene-related peptide (CGRP) may also contribute in the gender differences in pain perception and analgesia (18,19,20). Reduction in pain sensitivity after injection of testosterone in animal models (21) may imply that androgens have a tole role of in sex dependent difference of pain. Gender differences in opioid-induced analgesia have also been widely suggested (22,23,24). The results of animal studies imply that opioids have greater analgesic effect in males (7) while, the results of some studies indicate that opioids may exhibit more analgesia in females (25). Interaction sex hormones with other neurotransmittesrs such as GABA, acetylcholine , serotonin and dopamine has been widely documented (26,27,28,29). All of these neurotransmitters have some roles in antinociceptive properties of morphine and pain perception (30,31,32,33,34,35,36,37) . There are also controversial reports regarding the the gender difference in tolerance to analgesic effects of opioids(38,39,40,41,42). Therefore, the aim of the present study was to clarify the differences of morphine - induced antinociception between male and female rats**.**

Material and Methods

Animals and drugs

Twenty male and female mice (33±8 g) were used. All mice were housed in 10 per standard cages, at room temperature (22± 1°C) on a 12 h light/dark cycle. Food and water were available properly. Animal handling and all related procedures were in accordance with approved standards of animal caring. The morphine powder (TEMAD Ltd, Teheran, Iranand was dissolved in saline.

Nociceptive test

To assess nociceptive responses, hot plate method was used. In this method, the rats were placed on the hot plate with temperature setting controlled at 55±0.2 °C. Cut-off time was 60 seconds. Nociceptive response is defined as licking fore paws or moving hind paws. Time duration between placing the animals on hot plate and licking fore paws or moving hind paws was considered as reaction time. The hot plate test was performed as a base record 15 min before injection of morphine (10 mg/kg; s.c.)(43, 44) and consequently it was repeated 5 times, every15 minutes after injection.

Tolerance induction

Morphine tolerance (Tol) was induced in animals by injecting 30 mg/kg morphine (s.c.) 3 times/day for 3 consecutive days(45).

Experimental design

Male and femlae mice were divided into two groups: 1) male; 2) female. The hot plate test (55±0.2 °C; Cut-off 60 sec) was carried out as a base record 15 min before injection of morphine (10 mg/kg; s.c.) and consequently it was repeated every15 minutes after injection in the first day. Morphine tolerance was then induced in animals by injecting 30 mg/kg morphine (s.c.) 3 times/day for 3 days. In fifth day hotplate test was carried out again, as same as the day before tolerance induction.

Statistical analysis

All data were presented as mean ± S.E.M of reaction latency time. Statistical comparison of basal reaction time between groups was done with one-way analysis of variance (ANOVA) and post hoc tukey test. Repeated measure ANOVA followed by post hoc tukey test was used for comparison of reaction latency times after injection of morphine. Differences were considered statistically significant when $p<0.05$.

Results

Before tolerance, 60 and 75 min after morphine injection(10mg/kg) the reaction latency time was higher than base time in male group ($p<0.01$ and $p<0.001$, respectively) while, there was no significant difference between reaction latency times after morphine injection following tolerance induction. In female mice, the all reaction latency times measured after morphine injection were higher than base in the day before tolerance ($p<0.05$ to $p<0.001$, respectively). In fifth day, there was no significant difference between reaction latency times when compared to base time. The reaction latency times in non- tolerated male mice were higher than female however there was no significant difference between two groups after tolerance.

groups	base	15 min	30 min	45 min	60 min	75 min
male	13.35± 1.06	16.25± 1.86	22.3±2.56	22.75±2.75	31.45±5.5$^{**}_{*}$	26.22±3.37$^{*}_{*}$
female	8.69±1.02	19.37±3.72$^{**}_{*}$	14.83±0.72*	19.47±1.77$^{*}_{**}$	15.52±2.75*	17.36±2.76
male-Tol	16.35±1.02	19.73±3.72	20.26±2.29	20.34±2.28	17.47±1.81	17.36±2.76
female-Tol	13.55±0.74	13.12±1.16	16.55±1.15	14.77±0.82	14.65±1.2	14.3±1.93

Tab 1: Comparison of reaction latency times before (basal reaction latency time) and after injection of morphine (10mg/kg) in each group. Data are presented as mean ± SEM (n=10 in each group). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared to basal reaction latency time in each group.

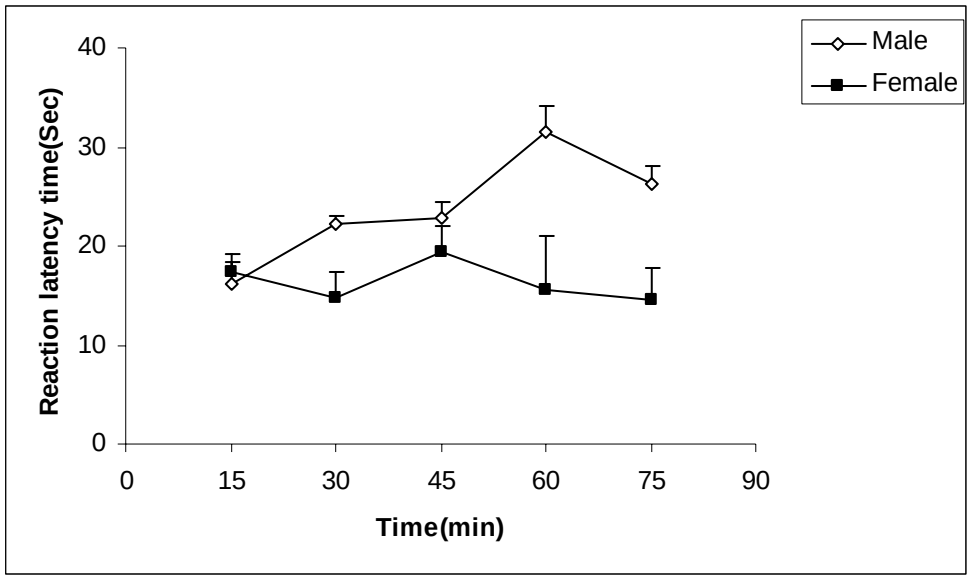

Fig.1: Comparison of reaction latency times following injection of morphine (10mg/kg) between male and female groups before tolerance . Data are presented as mean ± SEM (n=10 in each group).

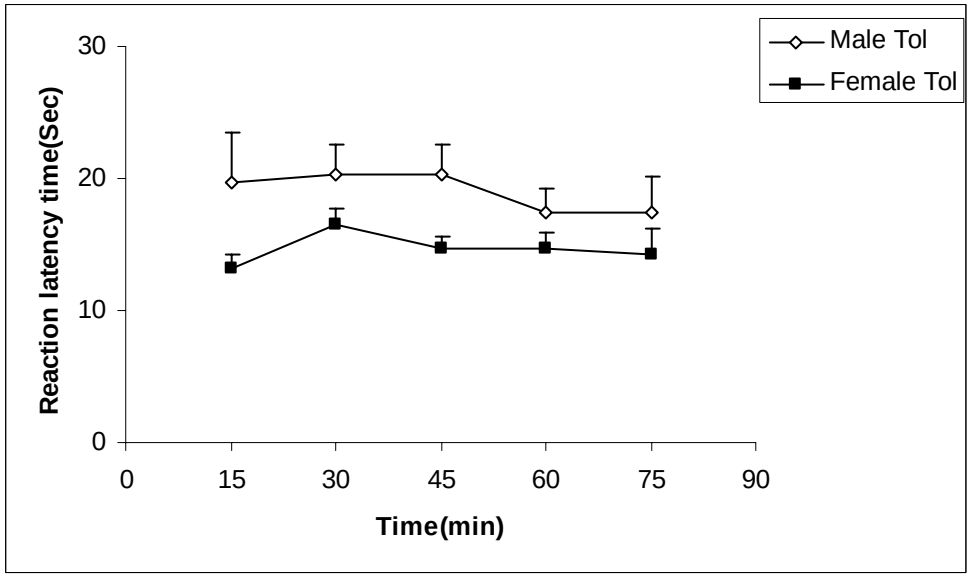

Fig.2: Comparison of reaction latency times following injection of morphine (10mg/kg) between male and female groups after tolerance. Data are presented as mean ± SEM (n=10 in each group).

Discussion

A gender dependent differences in pain perception and analgesia has been widely reported (3,4,5,46). There is evidence that female or male all female or male gonadal hormones have modulatory effects in pain and opioid antinociception(47,48,49). Therefore, we investigated the differences of morphine - induced antinociception and toleramce to morphine between male and female mice. Hot plate test used in the present study is a well known standard method for pain threshold evaluation after morphine or other analgesic drugs administration (50).

The results of present study showed that morphine has significantly more effects in male mice in comparison with female mice; reaction latency times in male mice were higher than females. It has been frequently reported that the potency of opioids to produce analgesis in male is greater than than femalels (47,51,52,38). It has been also suggested that testosterone increases the sensitivity of mu and kappa receptors to opioids (22). No significant sex difference in fentanyl and buprenorphine antinociceptive effects has been reported by Bartok and Craft (1997) (52). Furthermore, morphine has been shown to have twofold potency in male rhesus monkeys in comparison with ovariectomized females (53). It contrast to this findings, greater analgesic effect of pentazonic (kappa-receptor agonist) in female compared to male animals has been reported (54,55). Modulation of β-endorphin receptors in some areas of the brain by estradiol and progesterone and increased opioid receptor density in hypothalamus during proestrous phase, when estrogen levels are elevated, (56) confirms the role of sex hormones in analgesia (57). Estrogen also decreases the functional coupling of the μ-opioid and GABA receptors (58) which may affects the analgesic function of opioids. Co-increased levels of estrogen and proenkephalin gene expression has also been reported(59). It has also been shown that regulatory effects of estrogen on pre- proenkephalin mRNA is gender dependent in rats(60). All of these reports as well as the results of present study shows that analgesic effects of morphine is sex dependent. Gender dependent difference of many neurotransmitter systems such dopaminergic transmission has been reported (29,59.60). So it seemS that there is an interaction between sex hormones and opioids and neurotransmitter systems in the regulation of antinociceptive effects of morphine. It has been suggested that the discrepancy reported results due to analgesic effects of morphine my in part be due to kind of tested animal or temperature of hot plate test. Regarding to this hypothesis in the present study male and female mice was used and the temperature of hot plate was 55±0.2 °C. The results confirmed the results of our previous study when 52±0.2 °C was used to evaluate analgesic effect of morphine in rats(43,44). Repeated administration of opiates such as morphine is

accompanied by the development of tolerance. There are also controversial reports regarding gender difference in tolerance to morphine but there is any accepted view. (3,41,62, 63). The results of present study showed no significant difference in tolerance to morphine between male and female mice. The effect of estradiol on morphine tolerance has been widely reported(64,65). In the present study acute tolerance to analgesic effects of morphine was induced during three days. It has been reported the effect of estrous cycle is low when tolerance is induced acutely (64). Therefore, based on the results of present study it might be suggested that there is no difference between male and female in tolerance to morphine.

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