

COMPARISON OF ANTINOCICEPTIVE EFFECTS OF MORPHINE BETWEEN MALE AND FEMALE RATS

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Summary

The role of gonadal hormones on pain perception have been widely investigated. In the present study the differences of morphine - induced antinociception between male and female rats was investigated. Twenty rats were divided into two groups: 1) female 2) male. All animals were tested on the hot plate test (52 ± 0.2 °C; Cut-off 80 sec) for evaluating the antinociceptive effects of morphine. The hot plate test was performed as a base record 15 min before injection of morphine (10 mg/kg; s.c.) and consequently it was repeated every 15 minutes after injection of morphine. There were no significant differences in baseline latencies among two groups. Reaction time after injection of morphine in male group was higher than female group ($P < 0.01$). It is concluded that sex hormones such as testosterone and estrogen have a role in pain perception and analgesia.

Keywords: Male, Female , Morphine, Antinociception.

Running title: sex dependent effects of morphine analgesia

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Introduction

Pain is an unpleasant sensation which is elicited by exposure of skin and other organs to damaging or potentially-damaging, noxious stimuli (1). Several factors such as sociocultural, psychological and biological conditions affect pain perception (2). There are now strong documents for sex differences in pain and analgesia (3,4,5,6,7). Migraine, fibromyalgia and temporomandibular joint disorder which are accompanied with chronic pain, have a higher prevalence in female in comparison with male (8,9). These differences have been attributed to modulatory effects of gonadal hormones such as estradiol and testosterone on pain or analgesia (3,10,11,12,13). The presence of estrogen receptors in areas of the brain that are related to pain, imply that sex hormones have a role in pain perception or analgesia (14).

High localization of estrogen receptors in periaqueductal gray (PAG) confirms that estrogens influence the function of descending analgesia pathways (15,16). Progesterone receptors are also present in the ventrolateral medulla, the reticular formation and the nucleus of the solitary tract thus, progesterone probably has a role in pain processing (17). The interaction of estrogen with neurotransmitters such as gamma amino butyric acid (GABA), serotonin and calcitonin gene-related peptide (CGRP) may also contribute in the sex differences in pain modulation (18,19,20). Reduction in pain sensitivity after injection of testosterone in animal models has been also reported (21). Therefore the role of androgens in sex dependent difference of pain should not be ignored. Sex differences in opioid antinociception have also been widely reported (22,23,24). Animal studies suggest greater opioid analgesia for males (7) while some of human studies indicate that opioids may exhibit more analgesia in females (25). Interaction sex hormones with other neurotransmitters 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). 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

In the present study, 20 male and female wistar rats (200±20 gr) were used. All rats were housed in 4–6 per standard cages, at room temperature (22± 1 °C) on a 12 h light/dark cycle. Food and water was available properly.

Animal handling and all related procedures were in accordance with approved standards of animal caring. Morphine sulfate (TEMAD Ltd, Teheran,Iran) was dissolved in saline solution.

Nociceptive test

To assess nociceptive responses, hot plate method was used. In hot plate method, rats were placed on the hot plate with temperature setting controlled at 52 ± 0.2 °C, Cut-off time was 80 seconds. Nociceptive response was 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.) (38) and consequently it was repeated 5 times , every 15 minutes after injection.

Experimental design

Twenty rats were divided into two groups: 1) female; 2) male. The animals of All animals were tested on the hot plate test for evaluating the antinociceptive effects of morphine.

Statistical analysis

All data were presented as mean \pm S.E.M of reaction 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 times after injection of morphine. Differences were considered statistically significant when $p < 0.05$.

Results

The results indicate several facts: 1-The basal reaction time in female and male was 30.150 ± 1.815 , 26.848 ± 2.811 sec respectively; there were no significant differences between groups (Fig 1). 2- 30 and 45 min after injection of morphine, the reaction times in male group were 50.778 ± 6.751 and 55.333 ± 8.184 sec respectively, and were significantly higher than basal reaction time (26.848 ± 2.811 sec)($p < 0.05$, $p < 0.01$; tab 1). 3- In female group, 45 and 60 min after injection of morphine, the reaction times were 52.080 ± 4.64 and 52.5 ± 6.57 sec respectively, and were significantly higher than basal reaction time (30.15 ± 1.81 sec)($p < 0.001$; tab 1).

Tab 1: Comparison of reaction times before (basal reaction 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 time in each group.**

groups	Base	15 min	30 min	45 min	60 min	75 min
male	26.84± 9.81	48.25± 9.71	50.77±6.75 *	55.33±8.18 **	41.13±6.87	41.73±10.15
female	30.15±1.81	29.37±3.28	37.02±3.37	52.08±4.64 ***	52.5±6.57 ***	31.32±4.24

4- 15 and 30 min after injection of morphine the reaction times in male group was higher than female group ($P < 0.01$; fig 2).

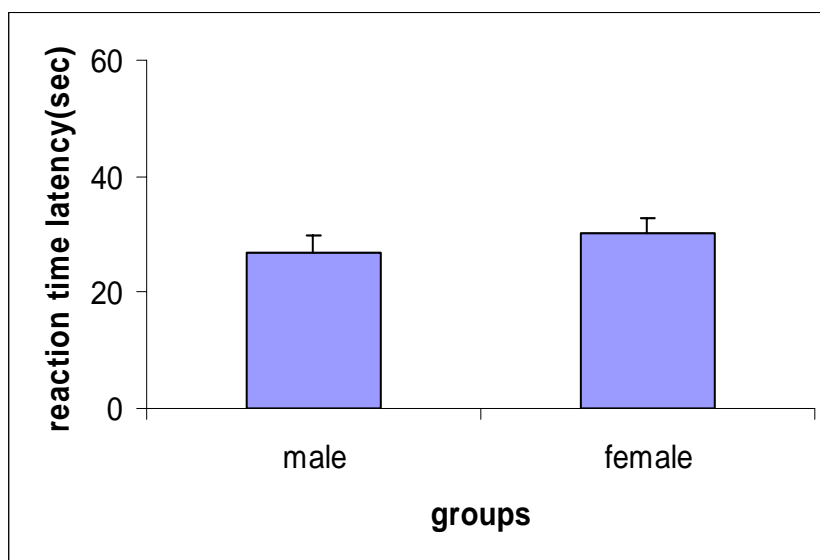


Fig 1: Comparison of basal reaction time between female and male groups. Data are presented as mean ± SEM (n=10 in each group).

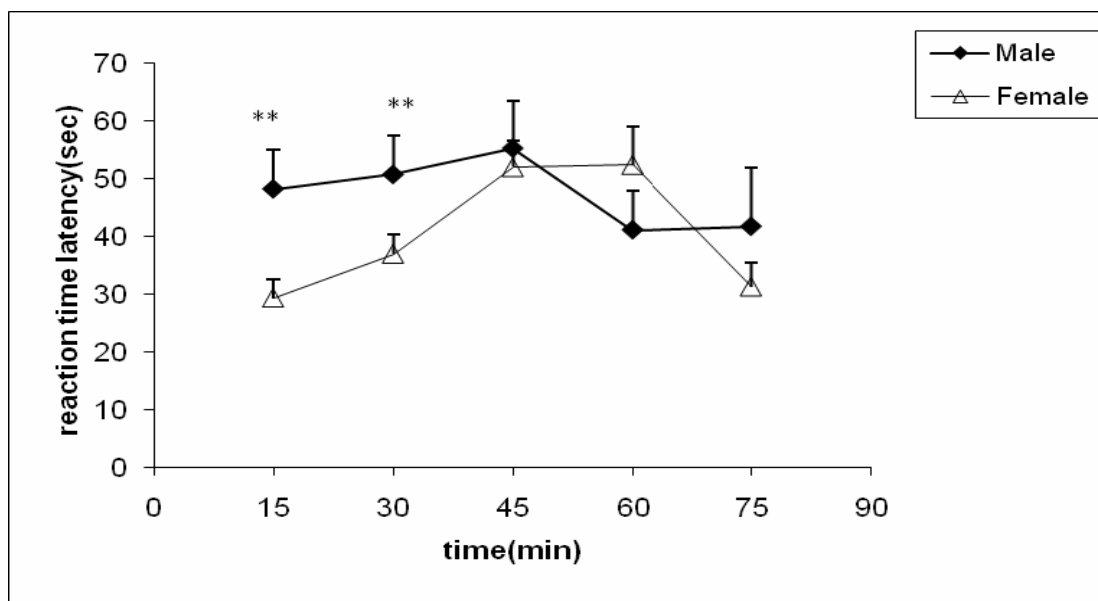


Fig. 2: Comparison of reaction times after injection of morphine (10mg/kg) between male and female groups. Data are presented as mean \pm SEM (n=10 in each group). ** $P < 0.01$ compared to female group.

Discussion

Animal and human studies confirm that there is a sex dependent differences in pain and analgesia (3,4,5,39). It has been hypothesized that sex differences in opioid antinociception and pain perception are a consequence of actions of female or male gonadal hormones(40,41,42). For this reason, we was investigated the differences of morphine - induced antinociception between male and female rats. 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 (43).

The results showed no significant differences in baseline hotplate latencies among male and female rats. It may be concluded that there is no difference in nociception threshold between male and female rats. This result is in agreed with the study of Negus et al (2004) who reported no difference between female and male animals in sensitivity to thermal stimuli(44). However, in another study, Negus and Mello (1999) Showed that, baseline sensitivity to thermal stimuli was similar in male and ovariectomized monkeys (45).

Tall et al (2004) showed that paw withdrawal latency in response to mechanical stimulus is greater in female compared to male rats (46). In contrast, more sensitivity of female animals to thermal stimuli has also been reported (47). All of these findings have shown that sex hormones may have a role in pain perception however, the reports are sometimes controversial.

The results of present study also showed that morphine has significantly more effects in male rats in comparison with female rats; reaction time latencies in male rats were higher than females. Several studies have demonstrated that opioids are generally more potent in producing an antinociception in male than female animals (40,47,48,49). This effect may be due to increased sensitivity to mu and kappa antinociception by testosterone (22).

No significant sex difference in fentanyl and buprenorphine antinociceptive effects has been reported by Bartok and Craft (1997) (50). However, other findings have shown that, morphine has been approximately twofold more potent in male rhesus monkeys than in ovariectomized females (45). Therefore testosterone may not be the only determinant factor. In contrast, it has been demonstrated that pentazonic (kappa-receptor agonist) has produced significantly greater analgesia in female than male animals (51,52).

Decreased β -endorphin receptors in hypothalamic, thalamic and midbrain areas by estradiol and progesterone have been reported (53). Estrogen also decreases the functional coupling of the μ -opioid and GABA receptors and suppresses μ -opioid and GABA-mediated hyperpolarization of hypothalamic arcuate neurons (54). High opioid receptor density in hypothalamus during proestrous phase, when estrogen levels are elevated, (55) may affect the analgesic function of opioids. The association of high levels of estrogen with increased levels of proenkephalin gene expression has also been reported (56). It has also been shown that estrogen has different regulatory effects in male vs female rats on hypothalamic pre-proenkephalin mRNA (57). All of these reports as well as the results of present study shows that analgesic effects of morphine is sex dependent. On the other hand, it has been suggested that sex differences present in many neurotransmitter systems such dopaminergic transmission (29,58). So these data confirm that there are potential levels of interaction between sex hormones and opioids and neurotransmitter systems in the regulation of antinociceptive effects of morphine.

Acknowledgments

Authors would like to thank the Vice Presidency of Research of Mashhad University of Medical Sciences, for financial supports.

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