SEX AFFECTS THE FEELING OF PAIN IN THE MICE, POSSIBLE INVOLVEMENT OF NITRIC OXIDE

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ABSTRACT

It has been shown that nitric oxide is a mediator with a major role in pain signaling at the level of dorsal root ganglion neurons of the spinal cord. The main objective of the present study was to elucidate the influence of sex on the effects of nitric oxide on pain mediation in mice. Painful stimuli such as heat induced by light beam focused on tail and hot plate chamber were applied. Animals were injected with either morphine (0.5, 5 and 50 mg/100g body weight) or L-NAME (0.1, 0.5 and 1 mg/100g body weight) intraperitonealy. Changes in tail flick latency and responses to the hot plate chamber were measured in different groups of mice. The tail flick latency was increased significantly in both male and female animals treated with morphine (control male (sec): 2.45 ± 0.16, male which received morphine 50 mg/100g body weight: 13.5 ± 0.6, control female: 3.4 ± 0.3, female which received morphine 50 mg/100g body weight: 13.8 ± 0.6; $P<0.001$ vs control in both cases). The response time to the hot plate chamber was also increased significantly by morphine pretreatment in both male and female mice. The tail flick latency and the response time to the hot plate chamber were significantly higher in the female mice (eg, the response time to the hot plate chamber (sec) in male: 7.3 ± 0.8, in female: 13.7 ± 1.6, $P<0.01$ vs female mice). Pretreatment with L-NAME at all concentrations caused a significant non-dose dependent increase in the response time to the hot plate chamber only in the male mice. These results may suggest that pain is mediated through different mediators in male and female mice and probable involves sex hormones. Furthermore, from the effect of L-NAME on pain sensation, it maybe suggested that L-arginine-nitric oxide pathway is more important in male in comparison with female in pain signaling.

Key words: Sex, Pain, Nitric oxide, Mouse.

INTRODUCTION

The exposure of skin and other organs to damaging or potentially-damaging, 'noxious' stimuli elicits intense unpleasant sensation of pain, an experience that is ultimately integrated in corticolimbic centers of the brain. Nitric oxide (NO) has been shown to participate in numerous physiological and pathophysiological processes (1). NO is produced within the central nervous system (CNS) from L-arginine by a constitutive (neuronal) form of NO synthase (nNOS), an enzyme which is localized in neurons of the CNS. The NO which is formed from L-arginine, activates soluble guanylate cyclase which causes an increase in cyclic GMP levels. Concerning the physiological roles of NO in the CNS, reports have emphasized on its participation in the perception of pain. A role of NO in nociceptive signaling was initially based on the localization of neuronal NOS in the superficial dorsal horn and intermediolateral cell column (2, 3), which led to the notion that NO regulate both autonomic tone and sensory transduction at the spinal cord level. However, there are some conflicting reports regarding the role of nitric oxide in pain sensation. For instance, some reports have shown that reduction of NO induces antinociception (4, 5) whereas recently, it has been shown that administration of NO by sodium nitroprusside infusion or nitroglycerine delivery has a beneficial effect on reduction of the pain in cancer patients (6). It is well known that gender affects the susceptibility to immune-mediated inflammatory diseases such as rheumatoid arthritis and multiple sclerosis, and women in comparison with men have higher risk for development of these diseases (7). In addition, the protective role of estrogen in the cardiovascular system has also been established (8, 9). However, so far little attention has been given to the influence of sex on processes which are involved in pain sensation. Thus, it was our interest to investigate any possible interaction of sex and the effects of L-NAME mediating in mice.

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MATERIAL AND METHODS

All experiments were performed in accordance with the Helsinki Declaration and IASP's guidelines for pain research in animals. Male and female albino Swiss mice (25-30g) were used in this study. Animals were housed (4-5) per cage in a temperature controlled room (22 ^{o}C) and exposed to a 12h light-dark cycle, and had free access to food and water. Both tail flick latency and the response time to the hot plate chamber were measured separately in different groups of male and female mice. These groups were mice treated with: saline (0.2 ml), morphine (0.5, 5 and 50 mg/100g) and mice treated with L-NAME (0.1, 0.5 and 1 mg/100g). All drugs were injected intraperitonealy in a volume of 0.2 ml. Hot plate and tail flick tests were conducted 15 min after injection of drugs (morphine and L-NAME).

Measurement of tail-flick latency and the response time to the hot plate chamber:

A tail-flick procedure similar to that described previously (10) was used to test the analgesia. Briefly, the noxious stimulation was produced by a beam of high intensity light focused on the lower third of the tail with a tail-flick apparatus (type AK72B). The animal was allowed to acclimatize to the environment of the apparatus at least 2 minute before exposure to a tail-flick. The cut-off was 10 sec and the operator was unaware of the specific treatment of animals of each group. In another sets of experiment, different groups of mice were placed on a hot plate chamber in order to test the analgesia. The hot plate apparatus consisted of an aluminum floor (35 x 35 cm), which was surrounded by clear Plexiglas walls (30 cm high) and was heated to 54 ^{o}C. The latency to display either hind paw licking or flinching/slapping was recorded (11). Animals were removed from the apparatus as soon as they showed any sign of response to stimuli.

Drugs

Morphine sulfate was provided by the narcotic drug control office at the Mashhad University of Medical Sciences, Iran. Indomethacin and N^{G}-nitro L-arginine methyl ester (L-NAME) were purchased from Sigma. All drugs were dissolved in distilled water.

Analysis of data

Results are expressed throughout as means ± S.E.M. Differences between groups have been compared using Unpaired Student *t* test and One Way ANOVA followed by Tukey-Kramer multiple comparison test. A *P* value less than 0.05 was considered statistically significant.

RESULTS

In the male mice received saline 0.2 ml, the tail flick latency was 2.5 ± 0.2, sec, which increased significantly (13.5 ± 0.6 sec) by injection of morphine in all concentrations (i.e.: 50 mg/100g, Figure 1-A). Pre-treatment of mice with L-NAME (0.1, 0.5 and 1 mg/100g) caused a non-significant (6.8 ± 1.8, sec, Figure 1-B) increase in the tail flick latency in comparison with the saline treated group. In male mice, the tail flick latency was significantly lower (3.4 ± 0.3 sec, Figure 1) than female mice. The response time to the hot plate chamber in male mice (7.3 ± 0.8, sec) increased significantly by either morphine or L-NAME pre-treatment in all concentrations (Figure 2). In female mice received saline 0.2 ml, the tail flick latency increased significantly (13.8 ± 0.6, sec, Figure 1-A) by injection of morphine at all concentrations (i.e.: 50 mg/100g). Pre-treatment of female mice with L-NAME in all three concentrations that were employed, caused a non-significant increase in the tail flick latency (Figure 1-B) in comparison with the saline treated group. Figure 2 shows that male mice (received saline 0.2 ml) could only tolerate the pain induced by a hot plate chamber (54^{o}C) for 7.3 ± 0.8 sec, which was significantly lower (13.7 ± 1.6, sec) than the response time to the hot plate chamber in female mice. Pre-treatment of female mice with L-NAME did not significantly modify the response time to the hot plate chamber (Figure 2). For instance injection of female mice with L-NAME (0.5 mg/100g) increased the response time to the hot plate chamber to 11.9 ± 1 sec, which was not quite significant in comparison with saline treated female mice (Figure 2-B).

DISCUSSION

The present investigation confirmed that in the male and female mice, morphine produced a significant antinociceptive effect as measured by the tail flick and hot plate tests. Female mice could tolerate pain for a longer time than male, which was statistically significant. The participation of nitric oxide in pain perception was also shown. Inhibition of nitric oxide synthesis by administration of the NOS inhibitor, N^{G}-nitro L-arginine methyl ester (L-NAME), resulted in diminished perception of pain as reflected by an increase in response time to the hot plate in the male but not in female mice. This effect of L-NAME in the male mice confirms the results of previous studies (4, 5), in which the pain reducing effect of L-NAME could be reversed by the administration of L-arginine (12). Hot plate test is an indicator of supraspinal analgesia whereas tail flick test is considered to be a measure of spinally

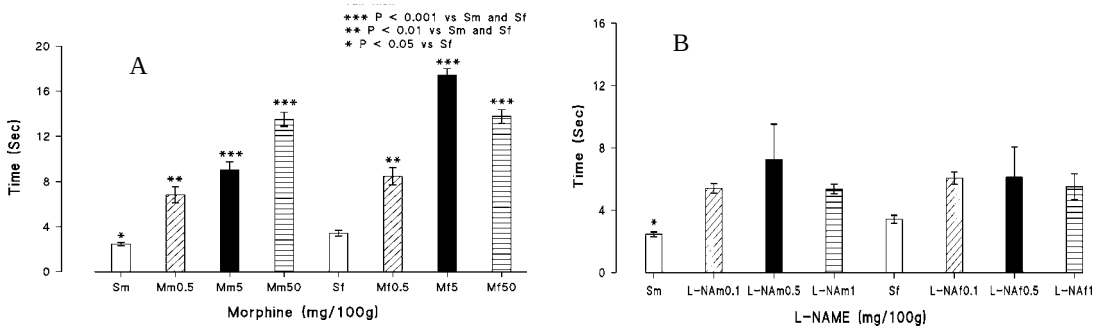

Figure 1. The tail flick latencies in male (Sm) and female (Sf) mice received saline (0.2 ml, i.p.), in comparison with : ***A:*** morphine group (Mm 0.5, Mm5, Mm50; Mf0.5, Mf5, Mf50: 05, 5 and 50 mg / 100g, i.p.) and ***B:*** L-NAME (L-NAm0.1, L-NAm0.5, L-NAm1; L-NAf0.1, L-NAf0.5, L-NAf1: 0.1, 0.5 and 1 mg / 100g, i.p.). Results of Tukey-Kramer multiple comparison test at each group showed a significant difference between mice (either male or female) receiving morphine in comparison to those of saline injected mice (*** P < 0.001 vs Sm and Sf). Unpaired Student t test showed a significant differences between male saline-treated to the female mice (* P < 0.05 vs Sf). The values are presented as mean ± S.E.M of 7 experiments.

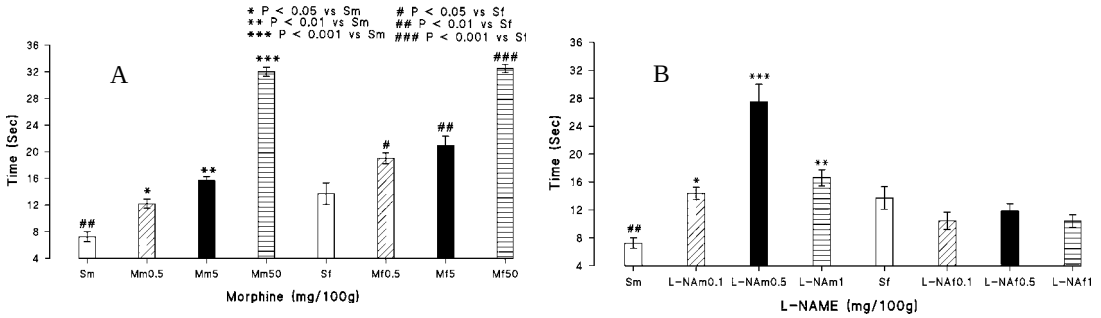

Figure 2. The time of jumping after placing animals on a hot plate chamber in male (Sm) and female (Sf) mice received saline (0.2 ml, i.p.), in comparison with ***A:*** morphine group (Mm0.5, Mm5, Mm50; Mf0.5, Mf5, Mf50: 05, 5 and 50 mg / 100g, i.p.) and ***B:*** L-NAME (L-NAm0.1, L-NAm0.5, L-NAm1; L-NAf0.1, L-NAf0.5, L-NAf1: 0.1, 0.5 and 1 mg / 100g, i.p.).
Results of Tukey-Kramer multiple comparison test at each group showed a significant difference between mice (either male or female) receiving morphine in comparison to those of saline injected mice (*** P < 0.001 vs Sm and ### P < 0.001 vs Sf). Unpaired Student t test showed a significant difference between male saline-treated to the female mice (## P < 0.01 vs Sf). Data are presented as mean ± S.E.M. of 7 experiments.

mediated antinociception (13). It has been suggested that the effect of nitric oxide for inhibition of antinociception is not opioid dependent because naloxone fails to reverse the antinociceptive effect of diminishing nitric oxide synthesis (14, 15).

To our knowledge, the result presented in this article is the first report which indicates that the involvement of nitric oxide pathway in mediation of acute pain in female mice is not as important as in male mice. It has been shown that the inflammatory pain induced by epinephrine injection in the rat is dependent on sex hormones and NOS inhibitors can antagonize the pain only in the male but not in female rats (16) which is in good agreement with our findings. However, at the present, it is not clear why the effects of sex hormones are more significant on the hot plate than tail flick. One possible explanation might be the number of nociceptors that are activated by different stimuli. However, further investigations are required to resolve this hypothesis. It has been reported that there are sex differences in many neurotransmitter systems. For example, sex differences exist in the regulation of central dopaminergic neurotransmission (17, 18). Therefore, as sex is a factor that influences a variety of neurotransmitter systems and different mediators are important in the response to the painful stimuli such as hot plate and tail flick tests in the mice. It has been shown that the levels of stable metabolites of NO, nitrite and nitrate, in the

rat brain show sex differences and, female rats in comparison with male rats have lower levels in the cortex and hippocampus (19). The response of male and female brain to neurotoxic insults such as ischemia has also been different and the severity was greater in males (20). In summary, the present data show that in male mice inhibition of NOS at the level of the brain but not at the spinal result in supraspinal analgesia. These results may suggest that sex steroid hormones such as estrogen have a role in the feeling of pain. It will be important to investigate any possible interaction between sex hormones and sensation of pain as well as the mechanism(s) in different experimental models.

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