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ORIGINAL ARTICLE

Study of the effects of Maternal Psychological and physical stress on Morphine-induced Tolerance in F2 NMRI Generation Mice

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ABSTRACT
Maternal stress can influence brain development in the second generation. In the present study, the effects of maternal psychological and physical stress on the morphine-induced tolerance have been investigated in second generation (F2) of mice. Female pregnant NMRI mice (25±2 g) were placed in two stress and control group. Stressed group divided to two physical and psychological subgroups. Pregnant animals were received stress in stress box (60 min/day for 15 days). After growth,F2 generation offspring received acute administration of morphine (1, 3 and 6 mg/kg) for evaluation of morphine-induced analgesia. Seconds group of animals received chronic administration of morphine (50mg/kg, twice daily; 3 days), for evaluation of morphine-induced tolerance. In addition, the interaction effects of sulpiride (25mg/kg), dexterometorphan (20mg/kg) and L-NAME (10mg/kg) and chronic administration of morphine examined on the morphine-induced tolerance. Our data showed that both psychological and physical stress did not change pain-threshold. Moreover, acute administration of morphine causes analgesia in the both 3 and 6 mg/kg doses in all groups (Fig.1).However, the effective dose of morphine for analgesia was 3 mg/kg in control group, but in the both stressed groups were6 mg/kg. Moreover, we revealed that the physical and psychological stress during pregnancy produces permanent changes in glutamatergic and nitric oxide pathways and not dopaminergic in the F2 generations. It could be concluded that physical and psychological stress could produce changes in different parts of central nervous system in F2 generation.
Key words: *Psychological stress, physical stress, morphine, pain, mouse*

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INTRODUCTION

Addiction is one of the most intense mental illnesses that accompanied with many complications including medical, political, legal and social problems [1, 2]. It seems that genetic factors, events of fetal life and also environmental factors have critical contribution in the incidence of addiction[3]. One of the most important environmental factors in the occurrence of addiction is the stress. Studies have shown that the stress has two aspects: psychological and physical [4]. Some types of stress can induce secretion of corticosterone, which they have a stronger psychological effect, such as exposure to the novel environment or an environment that is uncontrolled. Other types of stress can increase secretion of epinephrine, that they have a stronger physical component. It seems that due to increased concentrations of the corticosterone hormone in both groups (psychological and physical stress), a similar effect have seen in both groups. However, because of less secretion of epinephrine during psychological stress, effects of corticosterone hormone is more severe in psychological stress [5, 6].Preclinical studies showed that physical stressors such as footstock, restraint stress, and or psychological stressors such as noise pollution can be a reason of drug relapse[2]. Several evidences showed that prenatal stress can lead to strong disorders in offspring brain development, such as deficits in memory and learning, sex atypical

behaviour. Also, prenatal stress can induce various behavioral disorders, such a heightened emotionality, prolonged freezing, reduced movement and exploratory behaviours in novel environments. These disturbances seems to be mediated by the hypothalamic–pituitary–adrenal axis (HPA axis) in offspring's that changedcorticosteron secretion in response to stress[7]. Cross-sectional studies of animals and humans proposed that large quantities glucocorticoids (GCs) cause atrophy of dendritic processes, prevention of adult neurogenesis and eventually cases neuronal death [8].Also stress can be effect on pain threshold[9]. Opioids are one of the best pharmaceutical groups that reduce pain [10]. However, the major problem of long-term administration of opioids is the tolerance and dependency to them. Although mechanisms of physiological addiction, psychological dependence and tolerance to morphine have been studied but there is still a lot of unclear points. Several neurotransmitter systems are involve in this phenomenon, including serotonin, dopamine, nitric oxide (NO) and glutamate [11].In recent years, the role of NO is considered on the synaptic dopamine in the nigrostriatal pathway and other nucleus accumbens mesolimbic system. NO can increase the concentrations of synaptic dopamine by increasing release or decreasing of dopamine reuptake. This effect in increased extracellular dopamine levels have also been reported by opioids and other abused drugs[12].Some studies showed that application of NO inhibitors also reduced the induction of opioids-induced tolerance.Also, many studies illuminated that the role of the bio amine systems in appearance of physical and psychological effects of opioids [13]. One of the main directions in the development of tolerance and dependence to opioids is the neuronal dopaminergic pathways [11]. The results of previous studies demonstrated that D_2 dopamine receptors played an important role on brain rewarding and physical and psychological dependence[14, 15]. Stimulation of this receptor decreased the activity of Adenylatecyclase and also induced the dependency and tolerance in the cellular and molecular level[11].Glutamate is also one of the most important neurotransmitters in the mammalian central nervous system. It also demonstrated that NMDA receptors involved in the process of synaptic plasticity associated with opioids[11].By activating this receptors, the calcium entry into the cell increased and this leads to activation of many calcium-dependent of secondary messengers as well as production of NO[12]. Today, the exact mechanisms of these three important pathways on opiods-induced tolerance are far from clear. Therefore, due to the lack of knowledge about the effects of maternal physical and psychological stress on opiods-induced tolerance in the F2 generation, the aim of the present study was to investigate the effects of maternal physical and psychological stress on analgesic effects of morphine and morphine-induced tolerance in F2 generation of mice. Moreover, the effects of dextromethorphan (as a NMDA receptor antagonist), sulpiride (as a dopamine D_2 receptor antagonist) and L-NAME (as an inhibitor of nitric oxide synthesis) on morphine-induced tolerance were studied in F2 generation of mice.

MATERIALS AND METHODS

Animals

Male and female NMRI mice (Pasture Institute, Tehran, Iran) weighing (25±2 g), were used. 10 animals were kept per cage under standard conditions: 12/12-h light–dark cycle with free *ad libitum* access to food and waterand constant temperature (21±3°C),except during the experiments (stress induction). All Experiments were done in accordance with standard ethical guidelines and approved by the local ethical committee (The Baqiyatallah (a.s.) University of Medical Committee on the Use and Care of Animals, 81/021, July 10, 2002).

Drugs

Morphine sulphate (Temad, Iran), Dextromethorphane (Sigma, U.S), L-NAME (RBI, U. S), Sulpiride (Sigma, U. S) was dissolved in physiological saline (0.9%) (except Sulpiride that dissolved in acetic acid). Solutions were freshly prepared on the days of experimentation (0.10 ml/10g). The morphine was used subcutaneous and Dextromethorphane, Sulpiride and L-NAME were used intra-peritoneal.

Induction of stress

First female and male animals (ratio 2:1) house in one cage and after 24h to ensure pregnancy (if sperm or vaginal plug were found), this was considered day zero of gestation.Then the mice were separated the stress and control groups. The stress groups based on completely randomized program was transferred to lab every day and was exposed stress tests for an hour by the stress apparatus (Communication Box). The control group was transferred to the testing room and kept therefore an hour. This was continuing for 15 days. Then natal (pups) animals were left to raise their kids. After maturation of first generation of animals, mature female of physical stress groups by a ratio of 2to1paired with mature male in the same group with the physical stress and mature female of mental stress group stress with mature males of same group, to obtain the second generation. After maturation of the second generation and reach of infantsweighing20-25g, our tests were performed on the second generation.

Stress induction system feature and electro-shock instruments

Communication box consisted of 9 distinct compartments (16×16 cm), made by Plexiglas, with 8 holes (2 mm in diameter) in their contact sides allowing reception of visual, olfactory and auditory cues. The floor of the compartment was equipped with stainless steel rods (4 mm in diameter) placed 1.3 cm apart. Intensity and duration of the induced electro and induction time was determined randomly by the computer. Thus, 5 animals in this area were subjected to electric shock that was a physical stress everydayaccidentally.4 other sections in which the floor was made of plexiglass, animals only see 5 other animals, and this means that a severe psychological stress. In this study the electro- shock (Stimulator) were setting for auto time (the voltage of 45 mV and a frequency 10 Hz and 10 seconds), to be able to for 60 minutes, and each minute only primary 10 seconds imposed the physical stress to the pregnant mice and after10seconds and before the next minute, it was not a shock to the animal.

Evaluation of analgesic tolerance to response the second generation

In this phase of study, to evaluate of the analgesic effects of morphine in control and both stressed groups, animals were transferred to animal's room of School of Public Health in Baqiyatallah (a.s.) University.To eliminate the stress effect of moving and getting used to the new environment, all experiments were performed a week after the transfer.

Tail-flick test

We used a standardized tail flick test to evaluate mice threshold responsiveness to radiant heat pain [16]. The radiant heat source intensity was adjusted in 7. The animal's tail was placed in the groove and the hole positioned 2.5 cm rostral to the tip of the tail. Deflection of the tail activated a photocell located 6.5 cm above the hole and automatically terminated the test. A cut-off time of 10 s was used in order to reduce the possibility of tissue damage. The analgesia nociception index (ANI) derived by heart rate (HR) variability was equal with:% Analgesia Index= Test A Latency – Control Latency cutoff (10 sec) – Control Latency

Step 1: evaluation of the effectiveness of acute administration of morphine and morphine dose determination in response to stress and control groups

In order to determine of the dose responses to morphine 24 male laboratory mice as the control group, 24 mice for physical stress and 24 mice for psychological stress of the generation were randomly selected and each were divided in 4subgroup. So that there were6 mice in each second subgroups. In all, the stress and control groups, the first, second, third and fourth subgroups were received normal saline, 1 mg/kg, 3 mg/kg and 6 mg/kg morphine respectively. In all subgroups after 30 minutes the delay time in pulling the tail were recorded by the Tail Flick device.

Step 2: evaluation of the development of tolerance to the analgesic effects of morphine in the stress and control groups

Same of first step the animals of the subgroups three consecutive days, morning and evening, were received the drug and then 30 minutes later, 50 mg/kg morphine (to induce tolerance) the fourth day (the test day) received 6 mg/kg morphine. 30 minutes after administration of morphine, the delay time in pulling the tail were recorded by the Tail Flick device. The animals of all subgroups three consecutive days in two times, 30 minute before administration of morphine, the animals were received saline, 20 mg/kg dextromethorphan, 10 mg/kg L-NAME and 25 mg/kg sulpiride, respectively.

Statistical analysis

All experimental results were expressed as mean±SEM. The data were analyzed by one-way ANOVA, followed by Tukey post-test. A significant difference was defined as a p value <0.05.

RESULTS

Morphine-inducedanalgesia in F2 generation

The animals which they had a history of psychological stress in two generations ago and also the animals that had a history of physical stress in two generations ago were compared with the control animals that don't had a history of stress. The results revealed that acute administration of morphine causes analgesia in the both 3 and 6 mg/kg doses in all groups. However, the morphine-induced analgesia in psychological stress group was more than physical stress group. Moreover, in the control group, the morphine-induced analgesia at dose of 3 mg/kg was more than dose of 6 mg/kg. However, in the both stress groups, the morphine-induced analgesia at dose of 6 mg/kg was more than dose of 3 mg/kg (Figure 1).

Effects of dopamine, glutamate and nitric oxide inhibitors on themorphine-induced tolerance in the F2 generation

Our results showed that morphine administration for several days could be induced tolerance to analgesic effect in all groups. Additionally, current results revealed that the administration of dextromethorphan significantly reduced morphine-induced tolerance in control group as compared with

saline group. However, dextromethorphan could not change morphine-induced tolerance in two stressed groups. Moreover, application of L-NAME significantly decreased morphine-induced tolerance only in physical stressed group. Also, administration of sulpiride could not change morphine-induced tolerance in all groups. Furthermore, the administration of dextromethorphan had greater effect on pain threshold as compared with the L-NAME and sulpiride effects in the control group, but in the both stressed groups did not show any effect. Also, the L-NAME decreased the morphine-induced tolerance in the both stress groups as compared with the sulpiride and dextromethorphan. However, this decrease was much greater in physical stress group (Figure 2).

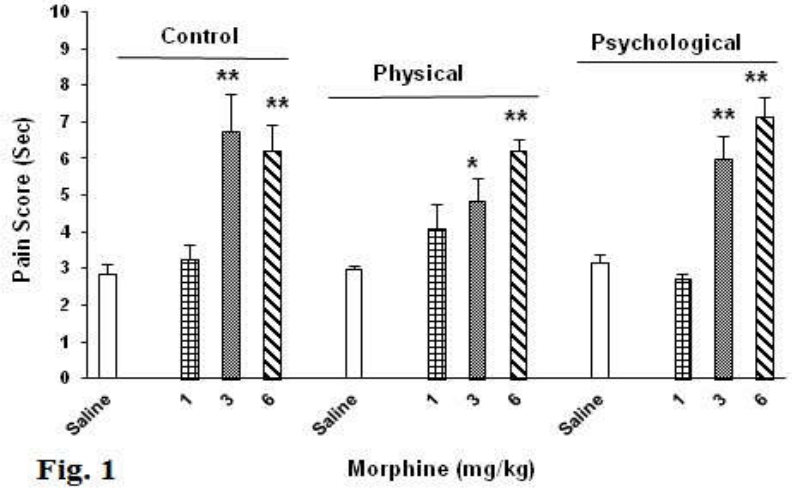

Figure 1: Evaluation of analgesic effects of morphine in F2 generation of mice. In order to determine of the dose responses to morphine, animals in physical, psychological and control groups divided in 4 subgroup and then were received saline, morphine (1, 3 and 6 mg/kg) respectively and the analgesic effect measured by Tail-Flick method. There were 6 mice in each subgroup. Results are expressed as Mean±SE.*p<0.05, indicates a significant difference with saline animals. **p<0.01, indicates a significantly with saline.

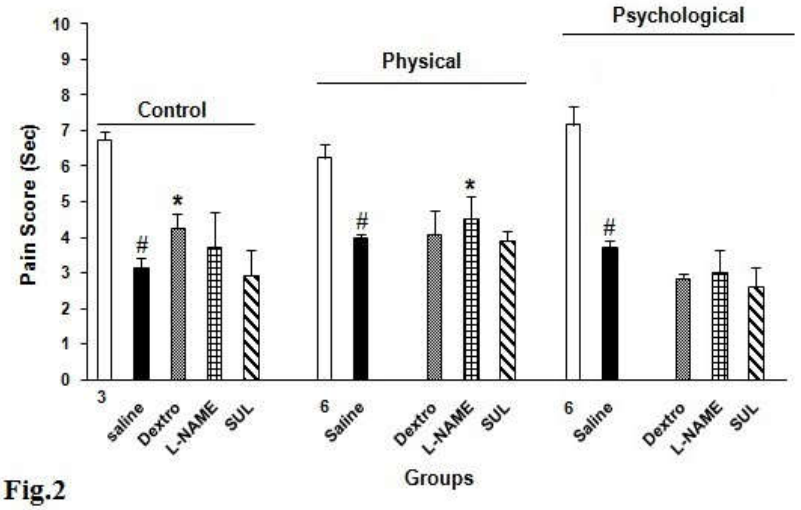

Figure 2: Evaluation of Dextromethorphane, L-NAME, Sulpiride on morphine-induced tolerance in F2 generation of mice.

In order to second generation response to morphine analgesic effects, the animals in stress and control groups divided in 5 subgroups and then animals in each subgroup, three consecutive days, morning and evening, were received the drug and then 30 minutes later, 50 mg/kg morphine (to induce tolerance) the fourth day (the test day) received 6 mg/kg morphine and then experimented with Tail-Flick method. Each sub group had at least 6 mice. The numbers of 3 and 6 in groups indicated acute administration of effective dose of morphine in the control and stressed groups, respectively. Results are expressed as Mean±SE.*p<0.05, indicates a significant difference with saline animals. #p<0.05, indicates a significant

difference with acute administration of morphine (that is 3mg/kg in control group and 6 mg/kg in both stressed groups.

DISCUSSION

The present study has been designed to investigate the role of maternal psychological and physical stress on the morphine-induced analgesia in the F2 generation of mice. Our data showed that both maternal psychological and physical stress did not change pain-threshold in the F2 generation of mice. However, the effective dose of morphine-induced analgesia in the control group (3 mg/kg)was less than the both stressed groups (6 mg/kg) (Figure 1). Also, it seems that both of the maternal psychological and physical stress can change the analgesic effects of morphine and also effective dose of morphine for analgesia in F2 generation of mice. Several studies reported that anti-nociceptive system is one of the important systems that affected by stress and stress can activates this system and relieves the pain[9, 17].For example, Ashkinazi observed that the prolonged psychological stress leads to lower the threshold of pain perception in humans. It seems that this decrease is probably due to the decrease of the brain opioid system activities [6, 18]. However in our results, controversy with these reports, psychological and physical stress did not change threshold of pain (Figure 1).But, it seems that the potency of morphine for induction of analgesia is different in control and stressed groups. Because the effective dose of morphine for induction of analgesia in both stressed group (6 mg/kg) was more than control group (3 mg/kg). In accordance with the previous studies, our data showed that effective dose of morphine for analgesia was 3 mg/kg in control group [19, 20], but the effective dose in both stressed groups (psychological and physical stress) was 6 mg/kg. Some evidences revealed that morphine-induced analgesia modulated by spinal and supra-spinal opioids pathways such as PAG, which severely monitored by glutamate and also NO system[11]. Therefore, in the present study by inhibiting glutamate and nitric oxide systems, we examined the role of maternal psychological and physical stress on the morphine-induced tolerance, using of NMDA receptor antagonist (Dextromethorphan), NO synthase inhibitor (L-NAM) and dopamine (sulpiride) receptor inhibitor. In current study, chronic administration of morphine induced tolerance to analgesic effect of morphine in all groups. In addition, we investigated the effects of three main systems, dopamine, and glutamate and nitric oxide on morphine-induced tolerance. It is known that the intra-spinal injection NMDA receptor agonist causes hyperalgesia in the rat [21].Also, application of the NMDA receptors antagonist (Dizocipline) reduced the hyperalgesia in neuropathic animals[22]. Chronic administration of a non-competitive antagonist of NMDA (MK-801) increased morphine-induced analgesia and decreased the morphine-induced tolerance [23, 24].Then, it is highly likely that NMDA receptors plays an important role in the development of opioid-induced tolerance, dependence and withdrawal [21, 25].In the present study, application of dextromethorphan decreased tolerance against the analgesic effects of morphine only in the control group, but doesn't affect the physical and psychological stressed groups. Since, dextromethorphan could not change morphine-induced tolerance in the both physical and psychological stressed groups as compared with control group. It seems that glutamate system has changed in stressed groups. In addition of NMDA receptors, NO is also involved in the mechanisms of pain[12, 26]. So, our results showed that administration of L-NAME (10 mg/kg) significantly reduced morphine-induced tolerance only in the physical stressed group. Then, it seems that only maternal physical stress changed NO pathways in F2 generation of mice in current study. Some evidences reported that NO inhibitors reduced the opioids-induced tolerance[27].Also, it has been showed that administration of L-NAME or L-NA prevented the development of tolerance to the analgesic effect of morphine[13, 28]. Thus,our result are in accordance with these studies that indicate NO may play animportant role in the development of morphine-induced tolerance. Furthermore, several studies suggest that dopaminergic system also plays an important contribution in the morphine-induced tolerance, dependence and withdrawal symptoms[29].It has been shown that application of D_1 and D_2dopaminic receptors antagonist (perphenazine) reduced opioid-induced tolerance [30]. Also, it is known that eticloride (dopamine D_2 receptor antagonist) but not Sch23390 (dopamine D_1 receptor antagonist), significantly reduced tolerance to the analgesic effects of morphine [24]. Several evidences showed that chronic administration of morphine increased activity of CaMkII. Moreover, inhibition of spinal and supra-spinal CaMkII did not only prevent but also reverses the tolerance to analgesia and opioid physical dependence in several rodents models [12].Yang et al. in 2011 showed that haloperidol (D_2 receptor antagonist) can reduce tolerance and dependence on analgesic effect of morphine by CaMkII activity inhibition [31].Surprisingly, in the present study we observed administration of sulpride(as a dopamine D_2 receptor antagonist)did not change morphine-induced tolerance in both stressed groups and also in the control group.

CONCLUSION

In summary, it seems that the physical and psychological stress during pregnancy produces permanent changes in different parts of central nervous system in the second generations. It is highly likely that opioid analgesic systems change by physical and psychological stress during pregnancy.

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