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Interaction of prenatal stress and morphine alters prolactin and seizure in rat pups

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HIGHLIGHTS

- Prenatal stress and morphine increased prolactin blood level in pup rats.
- Number of tonic–clonic seizure increased in stressed and morphine treated rats.
- Seizure score increased in morphine groups.
- Co-administration of morphine & stress attenuated morphine/stress-induced changes.

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ABSTRACT

Prenatal exposure to stress and morphine has complicated effects on epileptic seizure. In the present study, effect of prenatal forced-swim stress and morphine co-administration on pentylenetetrazol (PTZ) induced epileptic behaviors and prolactin blood level (PBL) was investigated in rat offspring. Pregnant Wistar rats were divided to four groups of control–saline, control–morphine, stressed–saline and stressed–morphine. In the stressed group, pregnant rats were placed in 25 °C water on gestation days 17, 18 and 19 (GD17, GD18 and GD19) for 30 min. In the morphine/saline group, pregnant rats received morphine (10, 12 and 15 mg/kg, IP, on GD17, GD18 and GD19, respectively) or saline (1 ml, IP). In the morphine/saline-stressed group, the rats received morphine or saline and then exposed to stress. On postnatal days 6 and 15 (P6 and P15), blood samples were obtained and PBL was determined. At P15 and P25, the rest of the pups was injected with PTZ to induce seizure. Then, epileptic behaviors of each rat were observed individually. Latency of first convulsion decreased in control–morphine and stressed–saline groups while increased in stressed–morphine rats compared to control–saline group on P15 ($P = 0.04$). Number of tonic–clonic seizures significantly increased in control–morphine and stressed–saline rats compared to control–saline group at P15 ($P = 0.02$). PBL increased in stressed–saline, control–morphine and stress–morphine groups compared to control–saline rats. It can be concluded that prenatal exposure of rats to forced-swim stress and morphine changed their susceptibility to PTZ-induced seizure and PBL during infancy and prepubertal period. Co-administration of morphine attenuated effect of stress on epileptic behaviors.

1. Introduction

Individuals are continuously exposed to potential disturbance of equilibrium in essential body functions. These potential disturbances may result in subjective state stress leading to a characteristic stress response, which aims to restore homeostatic control variable to demand [1]. One of the first steps in stress response is activation of the autonomic nervous system, which provides the individual with a means to quickly face a challenge. Stress also leads to activation of the hypothalamo–pituitary–adrenal (HPA) axis [2]. Prolactin blood level (PBL) has been shown to exert an anxiolytic and inhibitory tone on HPA axis activity in rats, showing stress tolerance effects [3]. It has been reported that PBL rises in stressful conditions and helps the organism to cope with the stressor [4]. Reduced emotional and neuroendocrine stress responses have been described in lactation, a time of high PBL [5]. On the other hand, there are many studies reporting that

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chronic morphine exposure alters postpartum PBL in adult female rats [6]. However, whether exposure to stress and morphine (alone or in combination with each other) during gestation affects PBL levels in offspring is not well known. Prolactin has also a well-known role in behavioral neurobiology [7–8] and may, at least in part, show a role in epileptic behaviors [9–11]. Rodent seizure models have revealed an important role for stress in promoting epilepsy. However, different stresses have different impacts on brain function and neuronal excitability [12], showing both pro-convulsive and anticonvulsive effects [13]. In previous studies we and others have shown that prenatal stress can potentiate epileptic behaviors and increase the susceptibility to seizures in offspring of rats [14–16]. Morphine abuse during gestation also produces long-term alterations in the CNS, alters the density of hypothalamic opioid receptors and shows different effects on seizure threshold and severity in rat progeny in a sex and age dependent manner [17–20]. On the other hand, the work of others showed that there is an interaction between morphine- and stress-induced behavioral changes in adult rats [21–23]. In this respect, it has been reported that the behavioral effects of swim stress are mediated in part through opioid receptors [24]. Swim-stress-induced analgesia through opioid receptors is also reported by several investigators [24–26]. Moreover, restraint stress, increase immobility in the swim test, and these effects can be blocked by the nonspecific opiate antagonist, naloxone [21,23]. However, whether exposure to morphine during fetal development affects stress responses is not well known. Previous studies suggest that the neural systems (norepinephrine and opioids) mediating stress responses are modified by prenatal exposure to opiates [21–23,27]. It has also been reported that many stressed humans have used or abused opiates to cope with stressful situations. Population of opiate addicted individuals is much higher in stressful than standard communities [28]. Although there are ample documents on stress/morphine impact on epilepsy, there is not any evidence of concomitant effect of prenatal exposure to morphine and stress on PTZ-induced seizure in rat pups. It is also important to investigate prolactin secretion and responses to challenges with stress and morphine and its probable role in epileptic behavior. Therefore, this study aimed to investigate the effect of prenatal forced-swim stress and morphine co-administration on PBL and PTZ-induced epileptic behaviors in rat pups at different time points.

2. Materials and methods

Male and female Wistar rats (200–250 g) were obtained from the animal facility at Urmia University of Medical Sciences, Urmia, Iran. They were 8 weeks old on delivery. The rats were housed in groups of four per cage and kept in standard conditions as follows: 12 h light/dark cycle, 22 ± 2 °C and food and water ad libitum. All the experimental protocols and procedures were complied according to guide lines of the 1975 declaration of Helsinki as reflected in the guidelines of the Medical Ethics Committee, Ministry of Health, I.R. Iran. Also, this study was approved by the regional Medical Ethics Committee in West Azerbaijan Province, I.R. Iran. All the female rats were mated at 12 weeks with a sexually experienced male of the same genotype. Each female was paired with one male at 8 am and checked for plugs at 3 pm. The pregnant rats were immediately moved to new cages. Four rats were housed per cage for the entire gestation period. The pregnant rats were divided to four groups (n = 7, in each group): control–saline (CS), control–morphine (CM), stressed–saline (SS) and stressed–morphine (SM). The stressed–morphine group was treated with 10, 12 and 15 mg/kg morphine sulfate (Temad, Tehran, Iran) intraperitoneally (IP) on gestation days 17, 18 and 19 (GD17, GD18 and GD19, respectively), prior to stress and then exposed to stress. The stressed–saline group received 1 ml saline IP on the same gestation days and then was exposed to stress. The control–morphine group was treated with morphine sulfate (similar to stressed–morphine group) and then transported to the experimental room on same gestation days and handled similarly to the stressed rats but were not exposed to stress. The control–saline group received saline and then transported to the experimental room and handled similarly to the stressed rats, without exposure to stress. Because in the present study effects of interaction between gestational morphine exposure and stress have been investigated, GD17-19 has been chosen. This gestational age as "late-gestational period" is important in developing the opioid system [29], hypothalamic–pituitary–adrenal axis (involved in stress) and nervous system [30]. According to previous studies, prenatal stress, particularly during the second and third weeks of pregnancy, may play an important role in increasing seizure vulnerability in rat offspring [16].

2.1. Forced-swim stress

The rats were forced to swim individually for 30 min in a plastic cylinder (50 cm high, 30 cm in diameter) filled to 30 cm with 25 ± 0.5 °C clear and fresh water [31–32]. Temperature of water was controlled by an automatic temperature controller (Campden instruments Ltd., UK). Period of each stress session was 30 min once per day between 9 and 11 am. Afterwards, the rats were dried by paper towels and returned to the home cage [32–33]. All the animals survived the experience and no additional follow-up care was required. Depth of water was chosen 30 cm to prevent the rats from standing up on their feet and tails.

2.2. Sample collection

After parturition, the pups in each litter were counted at 9 am on the first postnatal day (P1). The pups in each group were mixed and equally divided in the dams in case their birth date was the same. Each dam along with her pups was maintained in the individual cage [16]. At P6 and P15, blood samples were collected at 08:30 h from the 12 pups (n = 6, each sex; at each day) by direct heart puncture in all the experimental groups. Rats were anesthetized with ether before blood sample collection. Blood was collected in 1.5-ml EDTA-coated micro-centrifuge tubes, was kept on ice and was later centrifuged for 15 min at 9000 rpm at 3 °C. Its plasma was transferred to clean 1.5 ml micro-centrifuge tubes and stored frozen at −80 °C until PBL was determined. This hormone was measured using a commercial ELISA kit (Glory Science Company, Texas, U.S.A.).

2.3. Behavioral assessment

Different pups from each litter were used for behavioral studies in experimental groups at each day (P15 & P25). On P15, the pups (n = 6, each sex) were injected IP with PTZ 45 mg/kg in all the experimental groups. Following the injection, the animals were monitored for epileptic behaviors and behavior of each rat was observed and recorded for 120 min by a digital camera. Seizure rating was done using a previously defined scale [34]. In this scale, 0 = no response, 1 = ear and facial twitching, 2 = myoclonic jerks without rearing, 3 = myoclonic jerks with rearing, 4 = turning over onto one side with tonic–clonic seizures and 5 = turning onto back with generalized tonic–clonic convulsions. Other monitored parameters were latency to first convulsion and number of tonic–clonic convulsion. After completion of the behavioral testing on P15, the rats were killed using high doses of ether. The same protocol was carried out on P25 for the remaining rats (n = 6, for each sex) in all the experimental groups.

2.4. Statistical analysis

Normally distributed data related to PBL was analyzed using parametric techniques. Three-way ANOVA was performed for three factors of stress, morphine and sex. The data related to epileptic behaviors that were not normally distributed were analyzed using Mann–Whitney U test and/or Kruskal–Wallis one-way ANOVA. All the tests were run at critical significance level of $P < 0.05$. The results were expressed as mean ± SEM.

3. Results

3.1. Effect of prenatal stress and morphine on PBL

Data related to PBL were analyzed by three-way ANOVA for three factors of stress, morphine and sex. At P6, effect of stress was significant ($F(1,40) = 60.58$, $P < 0.001$), effect of morphine was significant ($F(1,40) = 25.63$, $P < 0.001$), and effect of sex also was significant ($F(1,40) = 7.06$, $P = 0.011$). Interaction of morphine * stress was significant ($F(1,40) = 15.39$, $P < 0.001$), interaction of stress * sex was significant ($F(1,40) = 19.62$, $P < 0.001$), but interaction of morphine * sex was not significant. Interaction of morphine * stress * sex was also significant $F(1,40) = 37.67$, $P < 0.001$). In general, PBL was higher in female than in male. At P15, effect of stress was significant ($F(1,40) = 9.3$, $P = 0.004$), effect of morphine was significant ($F(1,40) = 8.23$, $P = 0.007$), and effect of sex also was significant ($F(1,40) = 22.49$, $P < 0.001$). Interaction of morphine * stress was significant ($F(1,40) = 18.14$, $P < 0.001$), interaction of morphine * sex was significant ($F(1,40) = 8.94$, $P = 0.005$), but interaction of stress * sex, and interaction of morphine * stress * sex were not significant. Similar to values at P6, PBL was higher in female than in male at P15 (Figs. 1 and 2).

3.2. Behavioral alterations

Behavioral assessment was conducted at P15 and P25. First, data of both sexes were separately analyzed. There was no significant difference between male and female pups in the most seizure behaviors. Therefore, data of both sexes were mixed and analyzed together.

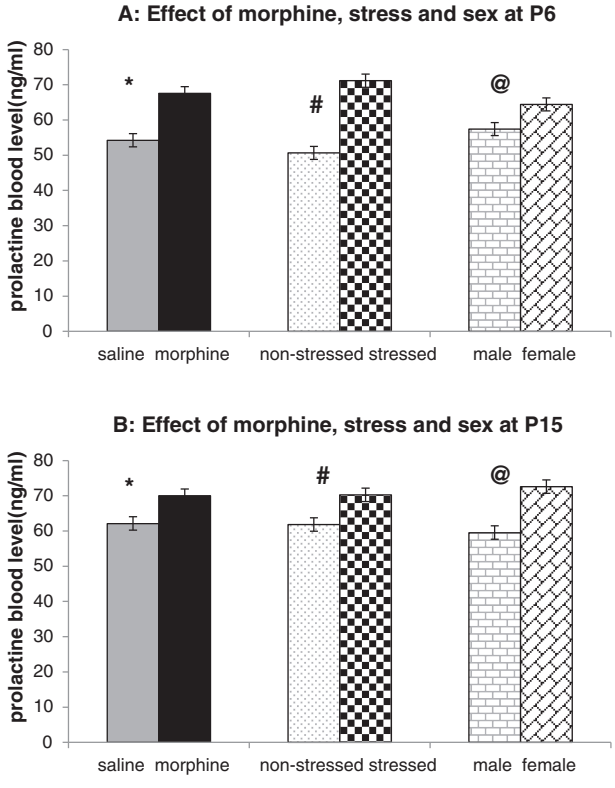

Fig. 1. Effect of prenatal exposure to forced-swim stress and co-administration of either saline or morphine on PBL (ng/ml) at p6 and P15 in rats. Panels A and B illustrate the effect of morphine, stress and sex on PBL at P6 and P15, respectively (three way ANOVA, each bar indicates n = 24). Panel A, * indicates significant difference with morphine ($P < 0.001$), # indicates significant difference with stressed ($P < 0.001$), and @ indicates significant difference with female pups ($P = 0.011$); Panel B, * indicates significant difference with morphine($P < 0.001$), # indicates significant difference with stressed pups ($P = 0.004$), and @ indicates significant difference with female pups ($P < 0.001$).

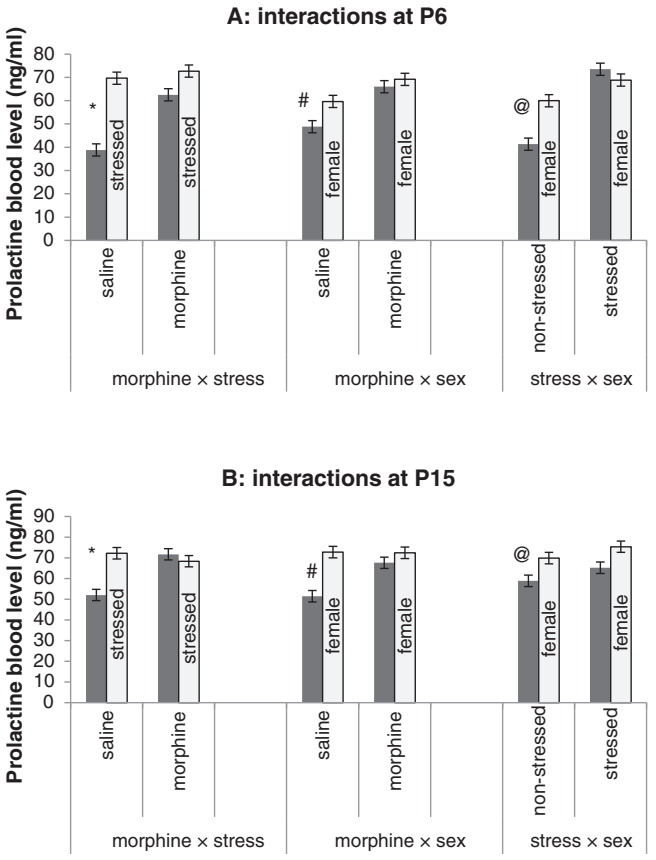

Fig. 2. Effect of prenatal exposure to forced-swim stress and co-administration of either saline or morphine on PBL (ng/ml) at p6 and P15 in rats. Panels A and B illustrate the effect of interaction of morphine * stress, morphine * sex, and stress * sex on PBL at P6 and P15, respectively (three way ANOVA, each bar indicates n = 12). Panel A, * indicates significant difference with all the groups related to morphine × stress ($P < 0.001$), # indicates significant difference with all the groups related to morphine × sex ($P < 0.01$), and @ indicates significant difference with all the groups related to stress × sex ($P < 0.001$); Panel B, * indicates significant difference with all the groups related to morphine × stress ($P < 0.001$), and # indicates significant difference with all the groups related to morphine × sex ($P < 0.01$), and @ indicates significant difference with stressed and non-stressed females ($P = 0.003$).

Number of tonic–clonic seizure significantly increased in CM and SS groups compared to the CS group at P15 ($P = 0.048$, Kruskal–Wallis and Mann–Whitney U). There was an insignificant increase of tonic–clonic number in stressed pups at P25. None of the SM pups showed tonic–clonic seizure at P15. This data was illustrated in Fig. 3A.

Latency of first convulsion significantly decreased in the CM group compared to CS at P15 ($P = 0.048$, Kruskal–Wallis and Mann–Whitney U). These changes were insignificant at P25 while co-administration of morphine with stress (SM group) increased first convulsion latency (Fig. 3B). None of the pups in the SM group showed convulsion during behavioral assessment (120 min) at P15 therefore, value of 121 min was assigned for this group.

Values in the seizure scale (method section) were calculated for each pup. For instance, if a pup showed behaviors of step 5, then its seizure score was $0 + 1 + 2 + 3 + 4 + 5 = 15$. Based on this calculation seizure score of the pups was obtained and compared (Kruskal–Wallis and Mann–Whitney U test) at different groups. There was no significant difference between the groups at P25, but the difference between groups was significant at P15. This result was shown in Fig. 3C.

4. Discussion

In the present study, pregnant rats were subjected to forced-swim stress, and morphine was co-administered to half of the rats while

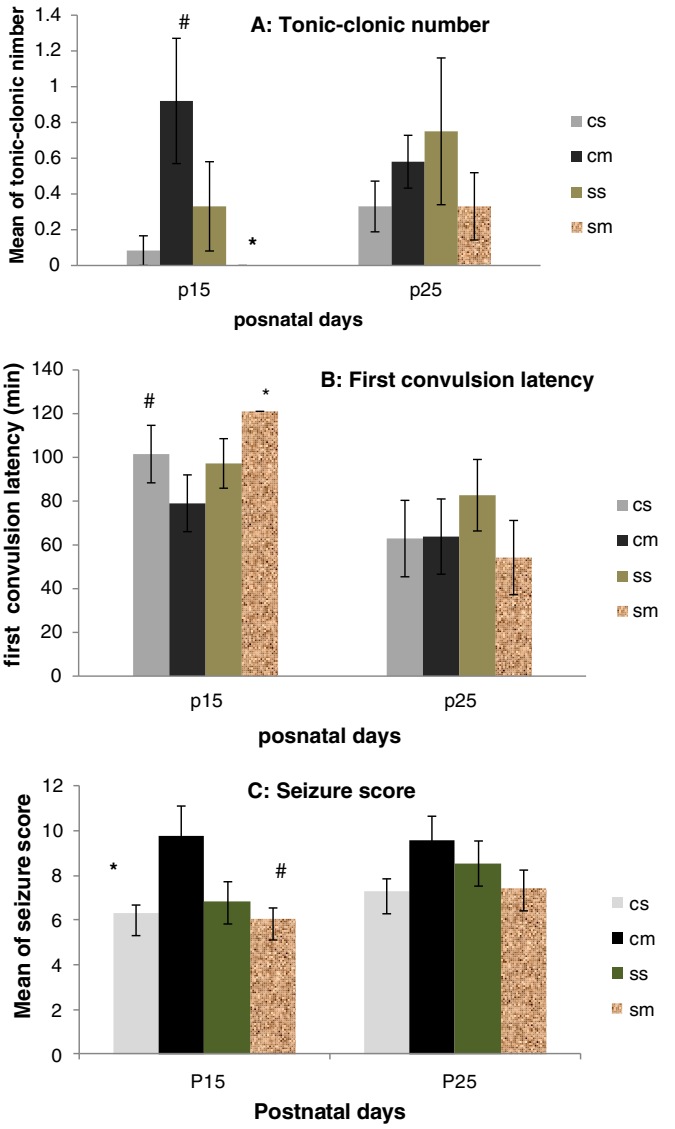

Fig. 3. Effect of prenatal exposure to forced-swim stress and co-administration of either saline or morphine on number of PTZ-induced tonic–clonic seizure (A), latency of first convulsion (B), and seizure score (C) in infant and prepubertal rats; Panel A: # indicates significant difference cm vs. cs ($P = 0.048$), * cm vs. sm ($P = 0.015$) at P15. There was no significant difference between the groups at P25; Panel B: # indicates significant difference cm vs. cs ($P = 0.048$), and * sm vs. all the groups ($P < 0.001$) at P15; Panel C: * indicates significant difference cs vs. cm ($P = 0.043$), and # sm vs. cm at P15 ($P = 0.014$).

another half received saline. The main findings of this study were that both stress and morphine increased PBL. The number of PTZ-induced tonic–clonic seizure also increased in stressed and morphine treated rats. Latency of first convulsion decreased in morphine groups. Co-administration of morphine with stress suppressed effect of forced-swim stress on PTZ-induced behaviors. These findings suggested that prenatal morphine and stress in combination with each other attenuated their separate effects on seizure behaviors in rat pups.

4.1. Effect of stress and morphine on PTZ-induced seizure

Prenatal stress (PS) potentiates seizure in different animal models. To confirm this point, it has been reported that prenatal restraint and predator stresses potentiate pilocarpine-induced seizure in rats [14–16]. PS significantly potentiates seizure parameters and facilitates occurrence of maximum seizures [16]. Presumably, this facilitation is caused by an imbalance between excitatory and inhibitory systems. There are neurobiological bases for stress-induced seizures: exposure to stressors evokes hippocampal plasticity, induces noradrenergic neurotransmission, and facilitates adrenocortical hormone activation. A stress-exposed organism appears to be at higher risk of seizure onset in the event of hippocampal imbalance, adrenergic loss of function or corticosteroid abundance [35]. On the other hand, prenatal morphine exposure also alters seizure susceptibility in the developing rat [17–19,27,34]. Previous studies have demonstrated that chronic opiate exposure during infancy may affect the developing central nervous system and alter the number of opioid receptors [36], which in turn affects seizure susceptibility [13,37]. In the current study, prenatal morphine exposure decreased latency of tonic–clonic seizure and onset time of seizure and also potentiated number of PTZ-induced tonic–clonic seizure in rat pups, at both P15 and P25, but with more severity at P15. The changing seizure sensitivity in the postnatal period may be part of a general reorganization in the structure and function of neurotransmitter/receptor expression and function and excitatory and inhibitory modulation from higher brain centers. Opioid system undergoes significant developmental regulation, observed to extremely occur within the first 2 weeks of life [38]. Previous studies have also shown the impact of sex on the dynamic interplay among stress, morphine exposure and convulsive's responsivity [17–18,39]. In our experiments one male and one female pup from each litter were assigned to each experimental group to investigate sex-dependent differences. Data of both sexes were separately analyzed. In spite of some differences between male and female rats in some seizure parameters, statistical analysis revealed no significant changes in most seizure behaviors, probably due to small sample size ($n = 12$ for each group, 6 male/6 female). Sex-dependent influence of prenatal stress on epileptic behavior could differ depending on models of epilepsy/stress that were used and experimental/animal conditions.

The present data indicated that prenatal exposure to morphine and stress separately potentiated PTZ-induced seizure while co-administration of morphine and forced-swim stress had an opposite effect, which indicated that morphine might suppress excitatory effect of stress on PTZ-induced seizure (Fig. 3C). Previous reports have defined the important role of endogenous opioids in modulating stress-associated behavior [40]. The release of β-endorphins in response to stress has been previously reported, which helps to cope with a stressor by inhibiting the over-activation of HPA axis [41–42]. Various clinical and preclinical studies have also documented the critical role of endogenous as well as exogenous opioids in modulating stress and stress-associated anxiety and disorder [43–44]. Furthermore, stress has been shown to modulate the response of opioids, including drug craving and reinstatement of drug of abuse [45–46].

4.2. Effect of stress and morphine on PBL

According to previous studies, maternal stress during pregnancy is associated with raised plasma levels of ACTH and PBL and may increase brain developmental delays in the offspring, reduce cerebral asymmetry and result in abnormalities in brain morphology. The present result was in agreement with the result of these investigations, indicating that PBL increased in the stressed groups compared with control rats, which is outlined in Fig. 1,B. It has also been suggested that mu opioid receptors are involved in the regulation of the anterior pituitary function, affecting the circulating levels of hormones produced by this gland [47]. There are ample evidences that morphine as a general agonist of opiate receptors increases PBL [48]. It has been reported that exposure to opiates during critical periods of prenatal development leads to long-lasting alterations in the neuroendocrine control systems of the animal. These alterations may then have significant consequences on the physiological maturation and adult behavior of the animal [49]. Recently, it was reported that chronic morphine administration induced higher prolactin concentrations than the control animals at mating, and at early and late pregnancy, while in rats receiving dextromethorphan co-administration,

the increase by morphine was not observed. This neurochemical results indicate that the effect of dextromethorphan may be partly through blocking the effect of morphine on inhibition of tuberoinfundibular dopaminergic neuronal activity [50]. It has been reported that D2 receptor stimulation by dopamine inhibits prolactin synthesis and release [51]. Considering this mechanism, morphine and nicotine could reduce the turnover and release of dopamine through occupying mu or kappa receptors of endorphins or through nicotinic cholinergic receptors presented on dopaminergic cells [52–53]. Result of the current study showed that PBL is higher in female than male, and stress/morphine increased PBL both in male and female but more severely in male rats. Because the mean basal levels of prolactin are higher in women than in men of the same age groups [56], it is likely that in female rat prolactin secretion is already potentiated by several sex-related factors, since stress/morphine may greatly promote PBL, an effect that would account for the increased efficacy of stress/morphine to induce elevated PBL in male.

In conclusion, this study indicated that prenatal exposure to stress, morphine and stress–morphine increased PBL in rat offspring, sex-dependently. Moreover, stress and morphine individually enhanced susceptibility to PTZ-induced seizure in rat offspring during infancy and prepubertal period, while co-administration of morphine with stress suppressed excitatory effects of stress on PTZ-induced seizures. These results suggest that opioids may play a role in modulating stress and stress-associated behaviors.

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