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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.