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Research article

Prenatal stress alters sensitivity to benzodiazepines in adult rats

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HIGHLIGHTS

- Prenatal stress leads to a greater propensity toward drug-seeking behavior.
- Prenatal stress enhances diazepam conditioned place preference.
- Prenatal stress increased sensitivity to the anxiolytic effects of diazepam.

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ABSTRACT

In rats, prenatal stress (PS) induces persistent changes in the brain that eventually can be translated in altered behavior leading to a greater consumption of psychostimulants in the offspring during adulthood. Though many studies have been carried on the effects of PS on stimulant drug responsiveness, little is known about susceptibility to benzodiazepines dependence in this animal model.

We hereby examined the long-lasting impact of PS exposure during the last 10 days of pregnancy on the vulnerability to benzodiazepine addiction in adult rats. In addition, we also investigated the link between PS and the sensitivity to anxiolytics.

Our results reveal that PS offspring exhibited a significantly greater preference to the diazepam-paired side than control offspring in the conditioned place preference. Importantly, we found that PS enhanced the anxiolytic effects of diazepam in the elevated plus maze paradigm.

This work demonstrates that PS increased the abuse potential of benzodiazepines and the sensitivity to anxiolytic drugs in offspring of stressed mothers. Thus, investigating the interactions among addiction and PS may contribute to a better understanding how early life events modify neural circuitry and thereby behavior.

1. Introduction

It is well known that exposure to stress during pregnancy in rats produces physiological and behavioral alterations in the offspring [1]. Retrospective studies in humans suggest that children of mothers who experienced stress during gestation exhibit alterations in early motor development [2] and an increased vulnerability to neuropsychiatric disorders in later life such as anxiety [3], depression [4], autism [5], schizophrenia [6], and substance abuse disorders [7].

In rodents, many animal models have been established to study the long-term effects of prenatal stress. These include restraint [8], chronic variable stress [9,10], immobilization [11], social isolation stress [12], and electric foot shocks [13–16]. Furthermore, animal studies investigating the effects of prenatal stress (PS) have shown that repeated maternal exposure to stress during the last week of gestation lead to a hyper-reactive hypothalamo–pituitary–adrenal axis [17], high levels of anxiety [18], learning and memory impairments [19]. In addition, PS enhances the reinforcing and locomotor-sensitizing properties of amphetamine [20] as well as the reinstatement of cocaine-seeking after extinction training [21]. PS also augments the rewarding properties of morphine [22] and ethanol [23], suggesting that early-life stress increases the abuse potential for arrange of addictive drugs [7].

Although a large amount of information might be available on the effects of PS on stimulant drug responsiveness, little is known about vulnerability to benzodiazepine addiction in this animal model. Nevertheless, there are no studies definitively linking PS and vulnerability to benzodiazepine addiction. In this study, we first determined the impact of PS on the susceptibility to

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benzodiazepine dependence. Then, we examined whether PS may affect the sensitivity of anxiolytics such as benzodiazepine.

2. Materials and methods

2.1. Animals and drugs

Experiments were carried out on 100 male and female Wistar rats (Laboratory of Pharmacology Casablanca), weighing 200–250 g. They were housed three per cage and allowed free access to food and water. The housing room was maintained under constant temperature (22 ± 1 °C) and lighting conditions (12 L:12 D cycle). All experiments were approved by the Ethical Committee for biomedical research of the Faculty of Medicine and Pharmacy of Casablanca, Morocco.

2.2. Drugs

Diazepam (Roche, Morocco), obtained under solution form (1 g/100 ml), was diluted in 0.9% NaCl. Animals received saline (0.9% NaCl) or drug (diazepam, DZP) via intraperitoneal (i.p.) injections, as appropriate in a volume of 5 ml/kg body weight of animal.

2.3. Prenatal stress procedure

Pregnant female rats were randomly divided into two groups: prenatal stress (PS) and control (Ctrl) groups. Stress was performed during the last ten days of pregnancy in which the neural development of the fetus is supposed to occur in rats [24].

Stressed dams were taken to an experimental box with a grid floor that allowed delivering daily 80 electric shocks (0.5 mA, for 5 s, 1–2 min apart) on a random basis during 100 min sessions carried out between 08:00 and 16:00 h. Control females were left undisturbed in their home cages.

After birth, the litter sizes were recorded and adjusted to the same litter size (8 pups per litter). All offspring were fostered by their own mothers. No differences in litter sizes were found between stressed and non-stressed animals (Mean ± SEM: 10.2 ± 0.6 and 9.8 ± 0.5 pups/litter for controls and prenatally stressed, respectively). The pups were weaned at 21 days of age and housed in groups of three per cage until the 80 days old when behavioral tests started. A total of 5 randomly selected litters was assigned to each treatment group. A maximum of three pups per litter were used for each experimental group to avoid any litter effect. Different animals were used in each behavioral test to avoid the confounding factors to the minimum. All behavioral experiments were carried out during the light phase of the light-dark cycle.

2.4. Conditioned place preference test (CPP)

The apparatus of conditioned place preference consisted of black and white chambers (60 × 30 × 30 cm), having distinct floor texture (smooth floor in the black but rough floor in the white chamber) and a third (neutral) gray compartment measuring 12 × 16 × 36 cm. The three chambers were separated by guillotine doors. The CPP method employed was according to that used by Spyraki and Fibiger [25].

The experiment included three phase: The Preconditioning phase, the Conditioning phase and the post-conditioning phase. Between trials on all experimental days, all compartments were cleaned and wiped dry. A ceiling mounted video camera was used to record the time in each compartment.

The effects of two doses of diazepam On the CPP paradigm were investigated in order to determine the most suitable dose to assess the rewarding properties of diazepam in PS rats.

Table 1
Conditioned place preference induced by diazepam.

Group	Conditioning score (sec)
Saline	98.8 ± 8.26
DZP 2,5 mg/kg	172.5 ± 19.63*
DZP 5 mg/kg	173.7 ± 22.78*

Values represent mean ± SEM time (sec) conditioning score.
* $p < 0.05$ compared with saline group.

The groups of rats injected with diazepam at both doses tested showed a greater preference to the diazepam-paired side ($p < 0.05$) (Table 1). However, no dose-related effect was observed, which may be attributable to the sedative effect of diazepam at the larger dose.

According to these data, we decided for the next CPP experiment to choose diazepam at a dose of 2.5 mg/kg in order to prevent its sedative effect. This dose has already been used by others in the same experimental paradigm [25,26].

Thus, control ($N = 20$) and PS offspring ($N = 20$) used in the CPP experiment were randomly assigned to receive one of the following treatments: saline (Ctrl/Saline: $N = 10$; PS/Saline: $N = 10$) or 2.5 mg/kg of diazepam (Ctrl/DZP: $N = 10$; PS/DZP: $N = 10$).

2.4.1 Preconditioning phase

During this phase (3 days), rats were placed into the neutral compartment and allowed to freely explore all the apparatus for 15 min. On the 3rd day, the time spent in the black and white compartments was recorded. The average time spent by each animal on day 3 in the compartment assigned for diazepam conditioning was defined as the baseline preference for this compartment.

2.4.2. Conditioning phase

After the preconditioning phase, animals were subjected to eight conditioning trials held on eight consecutive days.

On days 4, 6, 8 and 10, diazepam was administered to Ctrl-DZP and PS-DZP groups, while vehicle was administered to saline groups. 20 min later rats were confined to the white compartment for 30 min.

On days 5, 7, 9 and 11, all groups were injected with vehicle and confined to the black compartment.

2.4.3. Post-conditioning phase

On day 12, all rats were given a saline injection and placed in the neutral compartment with free access to the whole apparatus for a 15 min period. The time spent on the two main compartments (white and black) was recorded. The conditioning score was defined as the time spent in the compartment associated with diazepam injection during the post-conditioning day (day 12) minus the baseline preference time for the same compartment (day 3).

2.5. Elevated plus maze test (EPM)

The EPM test is a widely used paradigm to investigate anxiety-related behavior [27,28] which has been validated in rats [28]. It is based on the test-induced conflict between aversion of being exposed to an open and elevated platform and motivation to explore the new environment. Thus, more animal are anxious, less they will explore open arms.

The EPM used was made of painted wood and consisted of two open arms (10 × 50 cm) and two enclosed arms (10 × 50 × 45 cm high walls) extending from a central platform (10 × 10 cm) and elevated 50 cm above the floor.

Before each trial, the maze was cleaned with an alcoholic solution and dried with a towel. At the beginning of the test, each rat was placed on the central platform, facing an open arm during 10 min.