Neuroscience 330 (2016) 50–56

PRENATAL STRESS ALTERS DIAZEPAM WITHDRAWAL SYNDROME AND 5HT1A RECEPTOR EXPRESSION IN THE RAPHE NUCLEI OF ADULT RATS

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Abstract—Early-life events have long-term effects on brain structures and cause behavioral alterations that persist into adulthood. The present experiments were designed to investigate the effects of prenatal stress on diazepam-induced withdrawal syndrome and serotonin-1A (5HT1A) receptor expression in the raphe nuclei of adult offspring. The results of the present study reveal that maternal exposure to chronic footshock stress increased the anxiety-like behavior in the prenatally stressed (PS) animals withdrawn from chronic diazepam (2.5 mg/kg/day i.p for 1 week). Moreover, prenatal stress induced a down-regulation of 5HT1A mRNA in the raphe nuclei of adult offspring. To our knowledge, this study is the first to demonstrate that maternal exposure to chronic footshock stress enhances diazepam withdrawal symptoms and alters 5HT1A receptor gene expression in the raphe nuclei of adult offspring. Thus, more studies are needed to clarify the mechanisms underlying the decrease of 5HT1A receptors expression in the raphe nuclei of PS rats.

Key words: prenatal stress, diazepam withdrawal, 5HT1A receptors, raphe nuclei.

INTRODUCTION

During prenatal period, the brain undergoes its most rapid growth. Chronic stress within this stage of life may therefore induce many persistent changes in brain structures and cause adverse behavioral and functional alterations that manifest later in life.

In humans, maternal stress during pregnancy has been identified as one of the risk factors for psychiatric disorders (Wadhwa et al., 2001; Maccari et al., 2003; Weinstock, 2008), as well as for cognitive impairments and language abilities (Koenig et al., 2002; Van den Bergh et al., 2008) and drug abuse disorders (Campbell et al., 2009).

Research in rodents has proven that prenatally stressed (PS) rats are characterized by a general impairment of the hypothalamo–pituitary–adrenal axis (Morley-Fletcher et al., 2003) and showed higher levels of anxiety (Vallée et al., 1997; Maccari et al., 2003; Lakehayli et al., 2015a), depression-like behavior (Alonso et al., 2000; Abe et al., 2007), alterations in social and sexual behavior (Weinstock, 2001) and significant phase advances in the circadian rhythms (Maccari et al., 1997). Moreover, numerous studies in animals demonstrate that early-life stress increases the abuse potential for a range of addictive drugs such as morphine (Yang et al., 2006), amphetamine (Deminiere et al., 1992), ethanol (Darnaudéry et al., 2007), MDMA (Morley-Fletcher et al., 2004), cocaine (Kippin et al., 2008), nicotine (Koehl et al., 2000; Said et al., 2015) and benzodiazepines (Lakehayli et al., 2015a). In addition, several lines of evidence show that dysfunctions in central monoamine neurotransmission particularly those related to 5HT, are involved in the regulation of stress and anxiety (Mann et al., 1995; Drevets et al., 2000; Roche et al., 2003). Many studies have also reported the involvement of raphe nuclei 5HT1A receptors in the pathogenesis of anxiety disorders (Andrews et al., 1994; File et al., 1996; Vicente et al., 2008). Furthermore, prenatal stress induces several functional and structural alterations in the monoamine neurotransmission (Weinstock, 1997; Huizink et al., 2004; Van den Hove et al., 2006), suggesting a central role for 5HT1A receptors in mediating the effects of prenatal stress exposure.

We have recently reported that electric footshocks during the last 10 days of pregnancy induce higher levels of anxiety, increases the sensitivity to benzodiazepines and up-regulated D2 receptor expression in the nucleus accumbens of adult offspring (Lakehayli et al., 2015a,b). The aim of the current study was to determine the long-term effect of prenatal stress on withdrawal syndrome by measuring the intensity of benzodiazepines withdrawal symptoms in PS rats in comparison with control rats using the water consumption paradigm. We also examined the impact of prenatal stress on the 5HT1A receptor expression in the raphe nuclei of adult offspring by quantitative Real-Time PCR.

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Abbreviations: ANOVA, analysis of variance; PS, prenatally stressed.

http://dx.doi.org/10.1016/j.neuroscience.2016.05.035
0306-4522/

EXPERIMENTAL PROCEDURES

Animals

Experiments were carried out on male and female Wistar rats (250–300 g; Laboratory of Pharmacology Casablanca). Rats were housed three or four per cage in a room with a 12-h light–dark cycle and a temperature-controlled environment (22 ± 1 °C). Animals used in these experiments were allowed *ad libitum* access to food and water, except as noted in the procedure.

Drugs treatments

Diazepam (Roche, Morocco), was obtained under solution form (1 g/100 ml), and diluted in saline (0.9% NaCl). Animals received saline (0.9% NaCl) or drug (Diazepam: 2.5 mg/kg) intraperitoneal injections, as appropriate in a volume of 5 ml/kg body weight of animal.

All experiments were approved by the Ethics Committee for biomedical research of the Faculty of Medicine and Pharmacy of Casablanca, Morocco.

Prenatal stress procedure

Virgin female rats were mated with male rats. Pregnant females were isolated in individual cages. Animals were subjected to PS according to our standard protocol (Lakehayli et al., 2015a,b; Said et al., 2015). Briefly, prenatal stress was carried out between days 10 and 20 of pregnancy. The stressed pregnant females were taken to an experimental box with a grid floor that allowed the delivery of 80 daily 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, whereas control dams were left undisturbed. After birth, offspring of each group were raise by their respective mothers (litters had been culled to eight pups). The pups were weaned at 21 days of age and housed in groups of three or four in a cage until the 80 days old when behavioral and molecular tests started. Offspring from the dams exposed to prenatal stress were used as the PS group, and offspring from control dams were used as control (C) group. A total of five randomly selected litters per group was used in this study. A maximum of two pups were taken for each experimental group from each litter to remove any litter effect. The experiments were carried out *during* the light phase of the light–dark cycle. Background white noise was continually used to cover possible surrounding noises.

Water consumption in a novel environment task

Anxiogenic-like effect of Diazepam withdrawal behavior was assessed by measuring water intake by rats in a novel environment in a Y-maze task as established before (Tazi et al., 1991; El Ganouni et al., 2004). The Y-maze apparatus, made of Plexiglas had three identical arms (50 cm long, 15 cm wide, 35 cm high). The floor and walls of two arms are black, while those of the third arm are white with a guillotine door used to separate arm 3 from the rest of the maze, and a light bulb (60 W) placed 40 cm above arm 3. A bottle could be connected to the end of arm 2 (black) or 3 (white). The amount of water drunk by each rat was measured by weighing the bottle before and after the session.

The behavioral test was conducted over two sessions (Fig. 1).

Training session. After the first week of handling, adult rats (80 days; $n = 60$) used in this experiment were randomly divided into four groups:

- PS-Vh ($n = 10$) and C-Vh ($n = 10$) rats treated with saline.
- PS-DZP ($n = 20$) and C-DZP ($n = 20$) groups treated with diazepam (2.5 mg/kg) once daily since the training session.

All rats were water deprived for 36 h before starting the training session which consisted of seven 10-min sessions performed 24 h apart, 30 min after diazepam or saline injection. During this period, the guillotine door was closed to prevent access to arm 3, and the water bottle was placed in the end of arm 2. The rats were placed individually at the end of arm 1 and allowed to run into arm 2 to drink from the water bottle. Water intake was measured by weighing the bottle before and after each session. However, the water intake exhibited only slight variations after the fifth training day, allowing the quantitative determination of a ‘‘plateau” of water consumption from day 5 to 7 of training.

Test session. On the test day (day 8), rats were randomized on the basis of water intake during the last training session (day 7) and divided into six groups ($N = 10$ for each group, Fig. 1). Diazepam groups were then treated with either an additional dose of diazepam (DZP-DZP group) or saline (diazepam-withdrawn groups: DZP-Vh). Saline groups (PS-Vh/Vh; C-Vh/Vh) were injected with saline and subjected to the same procedure.

The guillotine door was removed and the water bottle placed in the end of arm 3 (white). An additional bulb (60 W), was placed 40 cm above the floor of arm 3. Rats were placed in arm 1 and allowed to run within the Y maze to drink water in the unfamiliar arm (arm3) and water consumption was measured. This experimental procedure was previously shown to be very sensitive to anxiolytic drugs such as benzodiazepines (Tazi et al., 1991, 1992; El Ganouni et al., 1998, 2004).

Real-time quantitative PCR analysis

Adult control and PS offspring (80 days of age) were weighed and randomly assigned to either a saline or diazepam (DZP) group ($n = 6$ for each group). Saline groups received four daily injections of NaCl 0.9%, while the DZP-treated groups received four daily injections of diazepam (2.5 mg/kg). Twenty-four hours after the last injection, animals were sacrificed by decapitation; brains were quickly removed and placed on ice. Coronal sections (350-μm thickness) containing dorsal raphe nuclei and median raphe nuclei were identified by visual inspection with the aid of the Rat Brain Atlas