Behavioural Brain Research 296 (2016) 408–417

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

Stress during the gestational period modifies pups' emotionality parameters and favors preference for morphine in adolescent rats

Luciana Taschetto Vey[a], Higor Zuquetto Rosa[c], Raquel Cristine Silva Barcelos[b], Hecson Jesser Segat[a], Vinícia Garzella Metz[c], Verônica Tironi Dias[b], Thiago Duarte[b], Marta M.M.F. Duarte[b,d], Marilise Escobar Burger[a,b,c,*]

[a] Programa de Pós Graduação em Bioquímica Toxicológica, Universidade Federal de Santa Maria (UFSM), Av. Roraima, 1000, Prédio 18, Cidade Universitária, CEP 97105-900 Santa Maria, RS, Brazil
[b] Programa de Pós Graduação em Farmacologia UFSM, Av. Roraima, 1000, Prédio 21, Cidade Universitária, CEP 97105-900 Santa Maria, RS, Brazil
[c] Departamento de Fisiologia e Farmacologia, UFSM, Av. Roraima, 1000, Prédio 21, Cidade Universitária, CEP 97105-900 Santa Maria, RS, Brazil
[d] Lutheran University of Brazil (ULBRA), Santa Maria, Brazil

HIGHLIGHTS

- Pups exposed to gestational stress (Gest-S) showed smaller body weight gain.
- Gest-S increased and post-NS decreased pups' corticosterone plasma levels.
- Gest-S-exposed rats showed increased emotionality and stress-related behaviors.
- Gest-S exposure increased morphine-conditioned place preference.
- Endogenous opiate system may be changed by Gest-S, altering responses to opiate drugs.

ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form 11 August 2015
Accepted 15 August 2015
Available online 20 August 2015

Keywords:
Addiction
Anxiety
Gestational stress
Post-natal stress
Morphine

ABSTRACT

Experimental animal studies have shown that early life periods are highly vulnerable to environmental factors, which may exert prolonged impact on HPA axis function and on subsequent neurochemical and behavioral responses in adulthood. Here we evaluated the influence of environmental stressful situations in two different early life stages on stress-related behaviors, and morphine-conditioned place preference (CPP), which is indicative of addiction. While in the gestational stress (Gest-S) dams were exposed to daily sessions of chronic mild stress (CMS) for 2 weeks, in the postnatal stress (post-NS) the offspring were exposed daily to neonatal isolation from postnatal day (PND) 2 to PND 9 for 60 min. Animals exposed to post-NS showed lesser anxiety in different behavioral paradigms (elevated plus maze—EPM and defensive burying test—DBT) as well as increased exploratory behavior (open-field task—OFT), and no preference for morphine in CPP. In contrast, animals exposed to Gest-S showed increased corticosterone plasma levels together with anxiety symptoms and greater preference for morphine following three days of drug withdrawal. Our findings indicate that the gestational period is critical for stress, whose effects may be manifest throughout life. On the other hand, post-NS can trigger neuroadaptations able to overcome emotional consequences of early life. We hypothesized that Gest-S is able to modify responses to opioids along adulthood, which may facilitate development of addiction to these drugs.

1. Introduction

Drug addiction has exerted a considerable impact on society, resulting in one of the biggest public health problems reaching different ethnic groups and social classes worldwide [1]. In 2007, the United Nations Organization (UNO) reported that about 172–250 million people have used an illicit drug worldwide. Among these drugs, marijuana has the highest annual prevalence of use

* Corresponding author at: Departamento de Fisiologia e Farmacologia-CCS, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil. Fax: +55 55 3220 8676.
E-mail address: mariliseeb@yahoo.com.br (M.E. Burger).

http://dx.doi.org/10.1016/j.bbr.2015.08.012
0166-4328/

(143–190 million people), followed by amphetamine, cocaine and opioids [2].

Over the past two decades, the use of opioid drugs has increased following different non-cancer chronic pains [3]. It has also been shown that only one in 6, or 17.3% of users of non-therapeutic opioids reported that they received the drugs through a medical prescription (NSDUH) [4]. Morphine, oxycodone and meperidine, besides heroin, which is illegal in most countries, are the opioids most commonly abused, and their continual use favors the development of addiction [5,6]. Morphine is clinically used in relieving moderate to severe pain, but its effectiveness is gradually lost with the rapid development of tolerance. In addition, chronic use of morphine leads to physical and psychological dependence, and its withdrawal triggers aversive symptoms known as abstinence syndrome [7]. Against this background, stressful experiences appear to have a strong influence on susceptibility to drug-taking behavior [8,9].

Consequences from adverse experiences in early postnatal life may be assessed through animal models, which include gestational stress (Gest-S) and post-natal stress (post-NS). While Gest-S involves maternal exposure to stressful environmental situations, in post-NS, pups are exposed to neonatal isolation, which is also a stressful environmental factor [10]. In this sense, adversities during pregnancy can compromise the development of the fetus, changing physiological and behavioral aspects of the offspring. In particular, Gest-S has been shown to produce behavioral and neuroendocrine changes, which may result in enhanced release of corticosterone due to maternal stress [11,12]. On the other hand, post-NS is also able to cause profound changes in the brain, some of which appear to be long lasting and possibly permanent. Experimental studies have confirmed clinical evidence of both gestational and early postnatal perturbations, which may exert prolonged impact on hypothalamic–pituitary–adrenal (HPA) axis function, and on subsequent neurochemical and behavioral responses to stress [13,14]. Such influences have been observed in humans, when early stress in the form of childhood abuse and neglect has been associated with increases in stress-reactivity and vulnerability to several psychiatric disorders and substance abuse later in adult life [15].

Considering the post-NS, prolonged periods of neonatal isolation have been related to disruption of dam-pup interaction, thus affecting the HPA axis responses to stress [16–19] with persistent consequences on the central nervous system (CNS) [20,21], modifying the neurobehavioral development and physiological plasticity [22]. Furthermore, prolonged maternal separation during the neonatal period is considered as the most powerful stressor to which rat pups can be exposed [23,24]. Of particular importance, neonatal stress has been described as affecting such brain neurotransmitter systems as dopamine and serotonin [25,26] and promoting dysfunctions in the endogenous opioid system, suggesting its involvement in brain reward patterns in drug abuse in animals.

So far, no studies have investigated influences of stressful situations on a comparative basis between the pre- and post-natal periods on the development of stress symptoms and opioids addiction. Experimentally, drug addiction has been explored through animal models, including conditioned place preference (CPP), which evaluates an animal's preference for the place where it received the drug [27]. According to a recent review [28], the CPP paradigm has acquired considerable importance, being routinely used to explore reinforcing effects from natural and pharmacological stimuli [29]. With this method, it is possible to identify reinforcing parameters, as well as aversive responses related to a determined treatment [30,31]. Thus, the CPP paradigm plays an important role in the study of addictive and potentially addictive drugs [32–34]. Against this background, this study was performed to evaluate the influence of environmental stressful situations in different early life periods (fetal and neonatal) on stress behaviors, and their consequences on addiction parameters after young animals' exposure to morphine.

2. Methods

2.1. Animals

Twelve female pregnant Wistar rats from the breeding facility of Universidade Federal de Santa Maria (UFSM), RS, Brazil, were individually kept in plexiglas cages with free access to food and water in a room with controlled temperature (22–23 °C) and on a 12 h-light/dark cycle with lights on at 7:00 a.m.. This study was approved by the Animal Ethical Committee of Universidade Federal de Santa Maria (27132-UFSM), affiliated to the Council for the Control of Animal Experiments (CONCEA), following international norms of animal care and maintenance.

2.2. Experimental procedures

Six dams were exposed to unpredictable stress protocol (gestational stress), which was held for two weeks. On postnatal day (PND) 1, male pups were identified and two animals of each mother were assigned to each experimental group, respectively, one pup for control ($n = 6$) and one pup for morphine ($n = 6$) group. The gender of the pups was distinguished by larger genital papilla and longer anogenital distance in male vs. female pups [35].

Animals were not handled until behavioral tests (PND 38). Another experimental group of six dams was not exposed to the stress protocol during the gestational period. One day after birth (PND 1) of the offspring, four male pups of each dam were randomly assigned to two experimental groups: unhandled (UH) and postnatal stress (Post-NS). Each of these groups (UH and post-NS) was re-assigned to control (one pup of each offspring, totaling $n = 6$) and morphine (one pup of each mother, totaling $n = 6$) groups. Pups' body weight was measured at PND 1 and PND 9, in order to assess body weight variation in this period. On PND 38, animals were initially subjected to behavioral assessments to evaluate anxiety parameters, locomotion and exploration, which were followed by the morphine-conditioned place preference (CPP) protocol, as shown in Fig. 1.

2.3. Gestational stress (Gest-S) procedure

Six pregnant rats were exposed daily to stress between gestational days 7 and 20. A modified version of the chronic mild stress (CMS) protocol first described by Willner et al. [36] was adopted. The stress protocol consisted of two or three different stressors following a semi-randomized schedule, including: damp sawdust, grouped housing, cage tilting (45°), lights on overnight, isolation, switching cages, and foreign object in cage for 14 consecutive days. This protocol did not involve any food or water deprivation [37]. The stressors were applied two or three times a day: morning and afternoon for 1 or 2 h and overnight. The procedures including the time and length of stressors are described in Table 1. All stressors were applied randomly to ensure unpredictability of the experiment [27].

2.4. Postnatal stress (Post-NS) procedure

Newborn litters found before 5 p.m. were considered to be born on that day (day 0). Pups were randomly assigned to one of two treatments: neonatal isolation or unhandled (UH) ($n = 16$). The treatments were distributed between litters to avoid differential maternal treatment within a litter and were carried out between 9 a.m. and 1 p.m.. For neonatal isolation, pups were removed from