Pharmacology, Biochemistry and Behavior 153 (2017) 32–44

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Anxiogenic-like effects of fluoxetine render adult male rats vulnerable to the effects of a novel stress

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ARTICLE INFO

Article history:
Received 26 July 2016
Received in revised form 13 October 2016
Accepted 12 December 2016
Available online 14 December 2016

Keywords:
Fluoxetine
Stress
Stress-induced hyperthermia
Hypothalamic-pituitary-adrenal axis
CRH
POMC
Arcuate nucleus
Behavior
Anxiety
Food intake
Body weight
Energy homeostasis

ABSTRACT

Fluoxetine (FLX) has paradoxical anxiogenic-like effects during the acute phase of treatment. In adolescent (35 d-old) male rats, the stress-like effects induced by short-term (3 d–4 d) FLX treatment appear to involve up-regulation of paraventricular nucleus (PVN) arginine vasopressin (AVP) mRNA. However, studies on FLX-induced anxiety-like effects in adult rodents are inconclusive. Herein, we sought to study the response of adult male rats (60–65 d-old) to a similar FLX treatment, also investigating how the stressful component, inherent to our experimental conditions, contributed to the responses. We show that FLX acutely increased plasma corticosterone concentrations while it attenuated the stress-induced-hyperthermia (SIH) as well as it reduced (≈40%) basal POMC mRNA expression in the arcuate nucleus (ARC). However, FLX did not alter the basal expression of PVN-corticotrophin-releasing hormone (CRH), anterior pituitary-pro-opiomelanocortin (POMC) and raphe nucleusserotonin transporter (SERT). Nonetheless, some regressions point towards the plausibility that FLX activated the hypothalamic-pituitary-adrenal (HPA). The behavioral study revealed that FLX acutely increased emotional reactivity in the holeboard, effect followed by a body weight loss of ≈2.5% after 24 h. Interestingly, i.p. injection with vehicle did not have behavioral effects, furthermore, after experiencing the stressful component of the holeboard, the rats kept eating and gaining weight as normal. By contrast, the stress-naïve rats reduced food intake and gained less weight although maintaining a positive energy state. Therefore, on one hand, repetition of a mild stressor would unchain compensatory mechanisms to restore energy homeostasis after stress increasing the resiliency to novel stressors. On the other hand, FLX might render stressed adult rats vulnerable to novel stressors through the emergence of counter-regulatory changes, involving HPA axis activation and diminished sympathetic output may be due to reduced melanocortin signaling. Therefore, complex interactions between hypothalamic CRH and POMC might be determining the adaptive nature of the response of adult male rats to FLX and/or stress.

1. Introduction

The exact etiology of mood disorders is not fully known. Nevertheless, the dysfunction of the serotoninergic system and the stress-induced hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis are widely accepted as key contributors to both the pathogenesis and the symptomatology of these disorders (Lanfumey et al., 2008). Furthermore, both systems reciprocally interact regulating autonomic, neuroendocrine functions, and behavior (Chaouloff, 2000; Dinan, 1996; Lowry, 2002; Lowry et al., 2008).

Fluoxetine (FLX) is a selective serotonin (5-hydroxytryptamine, 5-HT) re-uptake inhibitor (SSRI) that is widely prescribed to treat disorders such as depression and anxiety in both pediatric and adult patients (Emslie et al., 2005). However, despite its therapeutic effectiveness, FLX has paradoxical acute anxiogenic effects (Bridge et al., 2007). It is generally acknowledged that the acute anxiogenic effects induced by FLX and other SSRIs might be the result of the acute increase in extracellular 5-HT concentrations. By contrast, the capacity of chronic SSRIs treatment to desensitize pre-synaptic 5-HT_{1A} inhibitory autoreceptors and, in consequence, to increase 5-HT release in terminal areas, such as the hippocampus and frontal cortex, has been associated with the delayed onset of the therapeutic antidepressant action of these drugs (for a review, see Blier et al., 1998; Hensler, 2003).

Rodent models have frequently been used to examine the effects of FLX and other SSRIs. We recently reported (Gomez et al., 2015) that in adolescent male rats, arginine vasopressin (AVP) and 5-HT may be critical mediating the anxiogenic/stress-like effects of FLX on physiological, neuroendocrine and behavioral variables. Nonetheless, it is important to remark that major maturational changes occur during adolescence on the serotoninergic system (Chen et al., 1997; Dinopoulos et al., 1997;

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http://dx.doi.org/10.1016/j.pbb.2016.12.007
0091-3057/

Tarazi et al., 1998; Knoll et al., 2000; Moll et al., 2000; Murrin et al., 2007) and on the HPA axis (Akana et al., 1999; Gomez and Dallman, 2001; Gomez et al., 2002; Gomez et al., 2004; Romeo and McEwen, 2006). Therefore, it is reasonable to expect that to some extent, the potential anxiety-related effects of FLX on adult male rats might be of different nature, intensity or mediated by the other mechanisms. In general, acute treatments with SSRIs in adult male rats have yielded inconclusive results regarding anxiety-related behaviors (for review, see Griebel and Holmes, 2013). For example, acute anxiogenic effects of SSRIs have been reported in adult rats in the elevated-plus maze (Griebel et al., 1994), in social interaction tests (Dekeyne et al., 2000), in novelty suppressed feeding (Bodnoff et al., 1989) as well as in exploratory activity in the holeboard (Belzung et al., 2001). Nonetheless, anxiolytic-like properties have been also reported in rats acutely treated with SSRIs (Inoue et al., 1996). Finally, lack of effects of acute treatment with SSRIs can be also found in the literature (Sánchez and Meier, 1997; Matto and Allikmets, 1999; Duxon et al., 2000). It is undoubtable that methodological differences such as the type of SSRI used, the doses and the routes of administration, the strain of the rats, gender, age, environmental/stress conditions as well as the animal model of anxiety in which animals are tested are critical contributors to this variability. In fact, experimental conditions appear to be crucial modifying the overall outcome of anxiety-related behaviors in rodents. Even subtle variations in the experimental environment interact with anxiety/emotionality behaviors (Izídio et al., 2005). Thus, mild manipulations such as repeated handling and/or i.p. injections with saline have a stressful component that modify behaviors as well as the effect of antidepressants such as imipramine and FLX in rats (Izumi et al., 1997; Silva and Brandão, 2000). Thus it appears undeniable that, at least at behavioral level, the pre-test history is a pivotal factor influencing anxiety-related behaviors in rodent models (Izumi et al., 1997; Silva and Brandão, 2000; Holmes and Rodgers, 2003). In this context, it is important to take into consideration the undirected susceptibility to change model proposed by Branchi (2011, 2013). The author postulates that SSRIs would not induced anxiogenic-like effects per se but they would rather have a permissive effect. This permissive effect would favor the emergence of unadaptive behaviors under stressful experiences, while, under non-stressful or under positive environments, would have beneficial/therapeutic effects. In this context, it is noteworthy that SSRIs as well as other serotoninergic agents modulate the basal and the stress-induced HPA axis activity (Dinan, 1996, Matheson et al., 1997, Gomez et al., 1998; Chaouloff, 2000, Jørgensen et al., 2002a, b) and, when acutely administered, SSRIs such as citalopram and FLX stimulate the rodent HPA axis (Jensen et al., 1999).

On the basis of all the aforementioned, our aim was to evaluate the effects of an acute/short-term treatment with FLX in adult (60–65 d-old) male rats from a more complete perspective by integrating physiological, neuroendocrine and behavioral variables. To this end, we performed two experiments resembling many aspects of those previously reported in a study with adolescent (35 d-old) male rats (Gomez et al., 2015). Nonetheless, herein we also studied, to which extent, even the mild stressor of daily saline injection might have an impact on the overall outcome of anxiety/emotional reactivity/stress-like profiles in our study.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Harlan Interfauna Ibérica, Barcelona, Spain) were used. At the time of initiating the experimental procedures, rats were 60-d-old young adults and their body weight was of 235.2 ± 1.7 g. To allow for more accurate measurements of food intake and further calculation of food efficiency rats were individually housed. The cages were made of transparent plexiglass (20 cm W × 36 cm L × 18 cm H) allowing the rats to have visual contact with rats housed in nearby cages and therefore reducing the potential stressful component of single housing. The rats were maintained in a standard 12:12 light–dark cycle (lights on at 08:00 h and off at 20:00 h) with the light period provided by a white dimmed light appropriated for the overall well-being of albino rats (Prager et al., 2011). The temperature of the room was 22 ± 2 °C. The rats were allowed to acclimate to the animal room conditions for 5 days before the experiments, and they were weighed once daily to allow adaptation to the experimental handling and to minimize further non-specific stress responses. Rats had free access to rodent chow (Global Diet 2014, Harlan Interfauna Ibérica, Barcelona, Spain) and tap water. All manipulations were performed between 08:00 and 13:00 h to minimize any circadian influence. The experimental procedures conducted in these studies were in compliance with The European Communities Council Directive 2010/63UE and it was approved by the Animal Research Ethical Committee of the Universidad Complutense de Madrid. All efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs and treatment

Fluoxetine hydrochloride (Sigma, Spain) was prepared daily in saline (0.9% NaCl) as vehicle (VEH), injected i.p. with a dose of 10 mg/kg body weight in a volume of 1 ml/kg body weight. Administration of FLX or VEH was performed once a day, at morning, for 3 consecutive days in experiment 1 and for 4 days in experiment 2.

2.3. Experimental procedures

2.3.1. Experiment 1: study of the effects of short-term FLX administration (10 mg/kg/day × 3 days) on HPA axis activity, rectal temperature changes, ARC-POMC and DRN-SERT mRNA expressions.

On experimental day 1, all rats were weighed and then gently restrained using a towel cloth to allow for rectal temperature recordings and tail blood sample collection. A thermocouple probe connected to a digital thermometer (TMP 812, LSI, Letica Scientific Instruments) was used to record rectal temperature. The probe was inserted up to 4 cm into the rectum, and steady temperature readouts were obtained within 10 s of the probe insertion. Simultaneously, collection of a blood sample of 300 μl from a cut made over a lateral tail vein (pre-injection, time 0). Immediately afterwards, half of the rats were injected with either VEH ($n = 6$) or FLX ($n = 6$). For each rat, the overall procedure lasted <2 min and then, the rats were returned to their home cages. Thirty and 60 min later, rectal temperature recordings and blood samplings were repeated. The repeated blood samplings were obtained by gently dislodging the clot that had formed over the initial cut. After the last rectal temperature recording and blood sampling, the rats were returned to their home cages and left undisturbed until next morning. This procedure was repeated on experimental days 2 and 3.

Blood samples obtained from the tail were collected into EDTA-coated capillary tubes (Sarstedt, Nümbrecht, Germany), kept on ice, and centrifuged at 4 °C. Aliquots of plasma were stored at −20 °C until corticosterone concentrations were measured.

On the morning of day 4, all rats were decapitated within 10 s after they had been taken from their cages. Trunk blood was collected in 10 ml plastic EDTA-containing tubes (Sarstedt, Nümbrecht, Germany), centrifuged at 4 °C, and plasma was stored in aliquots at −20 °C until determination of basal plasma corticosterone concentrations by radioimmunoassay (RIA).

Brains and pituitary were removed, frozen on dry ice, and stored at −80 °C until in situ hybridization histochemistry (ISHH) assays were performed. Adrenal glands, mesenteric and subcutaneous white adipose tissue depots were dissected, cleaned and weighed.

Body weight was recorded daily, in the morning. To measure the food intake, the same amount of food was added daily, at morning to all cages. The following days, the leftover food from the feeder on top metal grid was collected and weighed. In addition, the cage bedding