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Intrauterine and lactation exposure to fluoxetine blunted in the offspring the aortic adaptive response induced by acute restraint stress

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ABSTRACT

Selective serotonin reuptake inhibitors are the most widely prescribed antidepressants to women during pregnancy. Maternal treatment with fluoxetine can expose fetuses and neonates to higher levels of serotonin that plays a role in stress response. Thus, the aim of the study was to evaluate whether maternal treatment with fluoxetine interferes with aorta reactivity of adult male offspring after acute restraint stress. Wistar rats were gavaged with fluoxetine (5 mg/kg/day) or water (control) during pregnancy and lactation. The experiments were performed in adult male offspring, treated or not with reserpine (4 mg/Kg, ip, 28 h before the experimental protocol). Fluoxetine and control rats were submitted to a single restraint stress session (ST) for 1 h. Curves to phenylephrine were performed in thoracic aorta with endothelium. Aortic nitric oxide (NOx) were evaluated by the Griess method. The aortic contraction induced by phenylephrine was similar between control and fluoxetine rats. The acute stress reduced contraction in aorta of control ST compared to control, and L-NAME equaled this response. In fluoxetine rats, ST did not change the aortic constriction. Reserpine treatment restored the vasoconstriction in control ST, but did not interfere with aortic contraction in control, fluoxetine or fluoxetine ST. The NOx concentration was higher in aortas from control ST than control rats, and reserpine reduced NOx levels of control ST. The NOx concentration was similar between fluoxetine and fluoxetine ST rats, treated or not with reserpine. In conclusion, maternal treatment with fluoxetine blunted acute restraint stress-induced NO system activation and aortic adaptation in adult offspring.

1. Introduction

According to the "Developmental Origins of Health and Disease" (DOHaD) theory, events such as illness or exposure to xenobiotics that occur during intrauterine and neonatal development can later influence the individual's health in adulthood (Gluckman et al., 2009; Sinclair et al., 2007). Women are likely to use antidepressants during pregnancy and/or breastfeeding for two different reasons: if they are already using the drug before becoming pregnant and the treatment cannot be discontinued under the risk of relapse or recurrence of the disease or if they fall ill during pregnancy or breastfeeding and need to start using medication (Andrade et al., 2008).

Nowadays, selective serotonin reuptake inhibitors (SSRIs) are the most widely prescribed antidepressants to pregnant women due to their efficacy associated with few side effects (Barbey and Roose, 1998; Gentile, 2005) even though they have been associated with an increased risk of pregnancy complications such as preterm birth (Wisner et al., 2009).

Fluoxetine is one of the most commonly used SSRIs to treat depression and anxiety disorders (Zohar and Westenberg, 2000), and in the management of neuropathic pain (McQuay et al., 1996) and it has been used during pregnancy and lactation. This drug acts by inhibiting the serotonin reuptake and increasing its concentration in the synaptic cleft. The pharmacokinetics of fluoxetine have already been widely described as has its ability to cross the human placenta (Heikkinen et al., 2002) and be excreted in the breast milk recognized (Hendrick et al., 2001), exposing the fetus and neonate to supraphysiological serotonin levels (Bonnin and Levitt, 2011).

Since its discovery, serotonin has been reported to play a role in stress response (Chaouloff et al., 2015). In fact, offspring exposure to fluoxetine during pregnancy and lactation presents decreased corticosterone plasmatic level (Pawluski et al., 2012) and lower activation of

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the basolateral amygdala in response to an acute restraint stress (Francis-Oliveira et al., 2013), indicating that modifications in the serotonergic system homeostasis during early development can influence both basal and stress-induced activity of the hypothalamus–pituitary–adrenal axis.

Acute restraint stress induces hypo-reactivity to alpha-1 adrenergic agonists in the aorta of rats by mechanisms involving nitric oxide (NO) and sympathetic systems activation (Cordellini and Vassilieff, 1998). However, as far as we know, the consequences of intrauterine and lactation exposure to fluoxetine on vascular response induced by acute restraint stress have not been evaluated. Thus, the present study aimed to evaluate whether maternal treatment with fluoxetine during gestation and lactation can interfere with aorta reactivity after acute restraint stress in rats. The role of NO and sympathetic nervous systems were also evaluated.

2. Materials and methods

2.1. Animals

Male and female naive Wistar rats were mated (three females and one male per cage) overnight. Gestational day 0 (GD0) was diagnosed if there were spermatozoa and oestrus-phase cells in vaginal smears. Dams were housed singly and randomly divided into the control (n = 18) or fluoxetine (n = 12) group. Control dams were gavaged daily with tap water whereas the fluoxetine dams were gavaged with Fluoxetine (5 mg/kg, Daforin® oral solution EMS Laboratory, Brazil) diluted in tap water. The fluoxetine dose of 5 mg/kg/day was chosen based on previous studies from our group and also considering the absence of toxicity for dams and litters (Francis-Oliveira et al., 2013; Higashi et al., 2016). Dam weights were obtained every three days throughout pregnancy and lactation for dose adjusting. The birth day was denominated post-natal day (PND) 0, and at PND 4 the litter was reduced to ten pups (sex balanced) to avoid bias related to availability of fluoxetine via lactation. Considering that, a previous study from our group demonstrated that male offspring exposed to FLX presented behavioral alteration after acute restraint stress (Francis-Oliveira et al., 2013) in the present study just the male progeny was applied. At PND 21, male pups were weaned and housed in five male animals per cage. For the present study 30 fluoxetine and 50 control male offspring were used. All animals had free access to water and regular chow (Nuvital, Curitiba, Brazil). The rats were maintained at 21 ± 2 °C on a 12:12 h light–dark cycle (lights on at 06:00 a.m.). Different littermates (one male) from each litter were used for each evaluation conducted. All experimental protocols were approved by the State University of Londrina Ethics Committee for Animal Research (CEUA no.16166.2012.12).

2.2. Stress induction

At PND 75–80, male rats from both groups were submitted to 1 h restraint stress, which consisted of immobilization in metal conical tubes with holes to allow ventilation (Francis-Oliveira et al., 2013). To evaluate the role of the sympathetic system in the vascular response, 24 h before the stress induction, a single dose of reserpine (Res) (Merck®, 4 mg/kg, i.p.), a catecholamine depleting drug (Navarro-Oliveira et al., 2000), was administrated to rats from all groups.

2.3. Aorta reactivity

Immediately after stress exposure, the thoracic aorta was removed from anesthetized rats (sodium thiopental 40 mg/kg, i.p. – Cristália, Brazil). Segments of the thoracic aorta (4 mm in length), free of connective tissue, with (Endo+) or without (Endo-) endothelium, were mounted in a tissue chamber with Krebs-Henseleit solution (containing in mM: 118 NaCl, 4.7 KCl, 25 $NaHCO_3$, 2.5 $CaCl_2 \cdot 2H_2O$, 1.2 KH_2PO_4, 1.2 $MgSO_4 \cdot 7H_2O$, 11 glucose, and 0.01 EDTA), gassed with 95% O_2–5% CO_2, and maintained at a resting tension of 1.5 g at 37 °C at pH 7.4, as previously published by our group (Higashi et al., 2016). To test the catecholamine depletion, in rats treated with reserpine, tyramine (100 μM, Calbiochem®) was added to the aortic rings bath. Next, to test the smooth muscle viability, the aortic rings were stimulated with KCl (90 mM). To evaluate the endothelium integrity, the aortic rings were contracted with phenylephrine and acetylcholine (0.1 mM), an endothelium-dependent vasodilator. Endothelium was considered preserved if acetylcholine promoted 70–80% relaxation and removed if acetylcholine promoted less than 5% relaxation. Cumulative concentration-effect curves to phenylephrine (1 nM to 30 μM), acetylcholine (1 nM to 30 μM), or sodium nitroprusside (1 nM to 30 μM) were performed in aortic rings. The aortic response to phenylephrine was also evaluated in the presence of N-nitro-L-arginine methyl ester (L-NAME − 1 μM), a NO synthase (NOS) inhibitor. The maximal response (MaxR) to phenylephrine was expressed in grams of tension (g) and the MaxR to the vasodilators was expressed as a percentage of phenylephrine-induced contraction. The log of drug concentration resulting in 50% of the MaxR (pD2) was calculated using non-linear regression analysis (GraphPad Prism software, USA).

2.4. NO levels in aortic tissue

Aortic tissue was obtained from anesthetized rats (sodium thiopental 40 mg/kg, i.p. – Cristália, Brazil). The NO levels were assessed on the basis of nitrite and nitrate concentration according to the Griess reaction, supplemented by the reduction of nitrate to nitrite with cadmium (Navarro-Gonzálvez et al., 1998). The aortas were subjected to homogenization in phosphate-buffered saline to the final concentration of 100 mg of wet weight tissue/mL of saline (Panis et al., 2011). The results are reported as percentage of NO metabolites (NOx) in the control group.

2.5. Statistical analysis

Initially, an exploratory analysis was conducted to evaluate normality of each variable and homogeneity of variance among the groups. Since criteria for normality and homogeneity of variances were reached ($P > 0.05$), the variables were evaluated by one-way ANOVA (MaxR and pD2 to vasodilators and to phenylephrine in Endo- aortic rings) or three-way (MaxR and pD2 to phenylephrine in the Endo+ aortic rings and NOx aortic levels). For three-way ANOVA, the factors were: exposure (control or fluoxetine), inhibitors (H_2O, reserpine or L-NAME), and stress. If three-way ANOVA indicated significant interaction between factors, all data were analyzed together by one-way ANOVA complemented with Bonferroni's test. Conclusions were established at $P \leq 0.05$ and only biologically important differences described. The results are shown as mean ± standard error of the mean (S.E.M.).

3. Results

3.1. Fluoxetine early exposure blunted the aortic hypo-contraction after stress

Phenylephrine induced concentration-dependent contraction in Endo+ (Figs. 1 and 2) and Endo- (Fig. 3) aortic rings isolated from all groups.

Three-way ANOVA indicated an interaction between the factors exposure and stress [$F(1, 94) = 3.50$, $P = 0.05$] and stress and reserpine [$F(1, 39) = 5.22$; $P = 0.025$]. One way ANOVA indicated that MaxR to phenylephrine was different between the control, fluoxetine, control ST, and fluoxetine ST rats [$F(3, 52) = 7.59$, $P = 0.0003$]. Bonferroni's test showed a decrease in MaxR to phenylephrine in Endo + aortic rings isolated from control ST rats when compared with Endo + aortic rings from control rats (Table 1, Fig. 1A). Also, in fluoxetine