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Serotonin modulation in neonatal age does not impair cardiovascular physiology in adult female rats: Hemodynamics and oxidative stress analysis

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ABSTRACT

Aims: The present study investigates the effects of neonatal serotonin modulation in female rats on cardiac parameters related to hemodynamics and oxidative metabolism in the mature animal.
Main methods: Female Wistar rat pups were administered daily subcutaneous injections of fluoxetine (Fx-treated group) or vehicle solution (Ct-group) from the 1st to 21st day of life. At 60 days of age, animals from both groups were either used for cardiovascular evaluation or sacrificed for tissue collection for biochemical assays.
Key findings: We found that body weight in the Fx-treated group was less than that in the control. When analyzing hemodynamic parameters (i.e., arterial blood pressure, heart rate—HR, sympathetic and vagal tonus, or intrinsic HR), we did not observe significant difference in the Fx-treated group. Evaluating oxidative stress in brainstem and heart by measuring carbonyl content and malondialdehyde—MDA formation, we observe a decrease in carbonyl content only in the Fx-treated group (60.3%, in brainstem; 58.2%, in heart), without difference in the MDA levels. This observation is consonant with an increase in superoxide dismutase—SOD and catalase—CAT activity in brainstem and heart in the Fx-treated group (SOD: 82.7% and CAT: 23.7 in brainstem; SOD: 60.6%, and CAT: 40.7 in heart), with no changes in glutathione S-transferase activity and reduced glutathione levels. With regard to oxidative metabolism markers, citrate synthase activity was higher in brainstem in the Fx-treated group (20%).
Significance: Our data suggest that serotonin modulation by Fx-treatment at an early age does not induce hemodynamic alteration, although it modulates oxidative metabolism in cardiac-related tissues.

1. Introduction

Serotonin (5-hydroxytryptamine; 5-HT) was first discovered in the mid-50s as a neurotransmitter in the mammalian brain [1]. Only 2% of total body 5-HT is produced in the central nervous system (CNS) [2]. Though present in lesser amounts, 5-HT in the CNS is associated with a wide variety of functions, including control of arterial blood pressures [3,4]. Wolf et al. demonstrated that microinjection of 5-HT in the nucleus tractus solitarius (NTS) induced a hypertensive response [5]. In addition, Feldman and Galiano showed that microinjection of 5 nmol of 5-HT induces hypotension and bradycardia, postulating that 5-HT injection evokes vagal cardioinhibition and sympathetic withdrawal [6]. In agreement with these data, Callera et al. showed that unilateral microinjection of 5-HT in the picomolar range into the NTS of unanesthetized rats resulted in a dose-dependent decrease in mean arterial pressure (MAP) and heart rate (HR) which was blocked by a previous microinjection of 5-HT2A inhibitor (ketanserin) [7].

In addition to decreasing blood pressure following microinjection, 5-HT has also been shown to have a dual effect on vascular tone, inducing concentration-dependent vasoconstriction. Cohen et al. showed that the ED50 for 5-HT-induced contraction in the aorta and jugular vein was approximately 6.0 and 0.6 μM [8]. Later, Lai et al. also demonstrated an endothelium-independent 5-HT-induced contraction in the coronary artery [9]. Conversely, studies have shown that fluoxetine (Fx; inhibitor of serotonin transporter SERT) treatment for 21 days consecutively in adult rats can induce a mild hypertension due to an alteration in the baroreflex control of HR [10]. In support of this finding, recent studies have shown that exposure to Fx increases reactive oxygen species (ROS) levels and alters the antioxidant defense system in several tissues [11,12]. In agreement with these findings, studies have shown that an

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increase in ROS concentration in specific regions of the CNS can result in increased arterial blood pressure [13,14].

As mechanistic control of arterial blood pressure requires the participation of the CNS, which is vulnerable to environmental stimuli during the critical developmental period, it is very likely that the 5-HT system manipulation by Fx treatment during development could induce adaptation in hemodynamic and oxidative metabolism in brainstem and heart. 5-HT system modulation at an early age is not clinically irrelevant, as the serotonin reuptake inhibitor is widely used for the treatment of depressive disorders in women during pregnancy and in the postpartum period. Some authors demonstrate that Fx can cross human placenta and its presence in breast milk could induce harmful effects on developing fetuses and newborns [15,16]; thus, the evaluation of the hemodynamics and oxidative status in brainstem and heart in adult animal could provide further insight into serotonin modulation at a critical period of development. Thus, the aim of the present study was to investigate whether serotonin system manipulation by Fx treatment, during development, would affect hemodynamic response and oxidative metabolism in brainstem and heart in adult rat.

2. Material and methods

2.1. Animals

Wistar rats were maintained at a room temperature of 23 ± 1 °C, on a light–dark cycle of 12:12 h, with free access to water and food. The animals were assigned randomly to two groups with eight pups per litter 24 h after birth. In order to avoid any possible influence of the circadian rhythm on the measurements, all Fx and vehicle injections were administered between 7:00 and 8:00 AM. All surgical procedures and experiments were performed with the approval of the Animal Care Committee of the Federal University of Pernambuco, Brazil, under the process number 23,076.026644/2010-20 [17–19].

2.2. Pharmacological treatment

The experimental and control groups were administered a single daily subcutaneous injection of Fx (10 mg/kg, dissolved in saline solution, 10 ml/kg) or saline vehicle (NaCl 0.9%), approximately between 7:00 and 8:00 AM. The treatments were applied from the 1st to the 21st postnatal day (i.e., during the suckling period) [17–19].

2.3. Measurement of body weight

Body weights were determined from the 1st to the 21st day of life (i.e., birth to weaning) and also before sacrifice of the animals at 40 and 60 days of age. Body weights were recorded using an analytical balance accurate to 0.01 g [17–19].

2.4. Surgical procedure

Female Wistar rats at 59 days of age were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) for inserting arterial and venous cannulas into control (n = 8 in each group) and treated rats (n = 8 in each group) a day before cardiovascular evaluation, and all animals were administered a single injection of ketoprofen (4 mg/kg). While the rat was under anesthesia, polyethylene-tipped cannulas (PE-50 connected to PE-10, Clay Adams, NJ, USA) filled with heparin in normal saline (500 U/ml) were inserted into the left femoral artery and vein. The ends of the cannulas were tunneled subcutaneously and exteriorized at the back of the neck. After surgery for catheter implantation, each rat was maintained in a separate cage to avoid additional stress. On the day of the experiment, the arterial cannula was placed into a pressure transducer (SP844, AD instruments, MSW, Australia) and a pressure amplifier (ML866/P, AD instruments, MSW, Australia). The electrode cable and the arterial cannula were attached to extensions during the recording period, thus allowing each rat complete freedom of movement in its chamber.

2.5. Measurement of AP and HR

A day after catheter implantation, the AP and HR were recorded in conscious animals using the appropriate measurement system (LabChart 7 Pro; ADInstruments, Bella Vista, NSW, Australia). Briefly, each animal was placed in the recording chamber for a period of acclimatization (approximately 60 min). The pulsatile AP was recorded for an additional 30 min under basal conditions, and the values of the MAP and HR were calculated following measurements taken at a 5-min interval of this period.

2.6. Spectral analyses of cardiovascular variability

Cardiovascular autonomic evaluation was performed using frequency domain analysis of the HR and systolic arterial pressure (SAP) with an appropriate software program (CardioSeries-v.2.4) that integrates spectra in the low-frequency (LF) (0.2–0.75 Hz) and high-frequency (HF) bands (0.75–3 Hz). The LF/HF ratio of the variability was calculated for assessing the sympathetic and parasympathetic indices of the heart [20].

2.7. Evaluation of sympathetic and parasympathetic tonus and intrinsic HR

A muscarinic antagonist methylatropine, a parasympathetic blocker administered at 2 mg/kg, iv, and B-adrenergic antagonist propranolol, a sympathetic blocker administered at 4 mg/kg, iv, were used to evaluate the effects of parasympathetic and sympathetic tonus on HR, respectively. In the first group of animals, methylatropine was injected initially and the HR recorded for 15 min; then propranolol was injected, following which the HR was recorded for 15 min. In the second group, this sequence was reversed; the animals were initially administered propranolol and then methylatropine. After each sequence, the intrinsic HR (iHR) was calculated [21,22].

2.8. Tissue homogenate preparation

Brainstem and heart were collected quickly and stored at −20 °C. Tissue samples were placed in small glass homogenizers with extraction buffer containing protease and phosphatase inhibitors. The tissue was homogenized on ice and centrifuged at 1000 *g* for 10 min at 4 °C. The supernatant was collected and stored at −20 °C until assay [23,24].

2.9. Protein determination

Aliquots of tissue homogenate were used for measuring the total protein content as described by Bradford [25].

2.10. Evaluation of malondialdehyde production

Lipid peroxidation is a process under which reactive oxygen species attack lipids, especially polyunsaturated fatty acids. Among the many different aldehydes formed as secondary products during lipid peroxidation, malondialdehyde (MDA), appears to be the most mutagenic product of lipid peroxidation [26]. For the evaluation of lipid peroxidation, a total of 0.2 mg/ml homogenate was used to measure malondialdehyde production (MDA) following reaction with thiobarbituric acid (TBA) at 100 °C according to the method of Draper [27,28]. The results are expressed as nmol/mg protein.

2.11. Evaluation of protein oxidation

Reactive oxygen species can induce the oxidation of amino acid residues on proteins, thus yielding protein carbonyls. The protein carbonyl