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Chronic treatment with fluoxetine modulates vascular adrenergic responses by inhibition of pre- and post-synaptic mechanisms

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ABSTRACT

Fluoxetine, a serotonin reuptake inhibitor (SSRI), has other effects in addition to blocking serotonin reuptake, including changes in the vasomotor tone. Whereas many studies focused on the acute effects of fluoxetine in the vasculature, its chronic effects are still limited. In the present study, we tested the hypothesis that chronic fluoxetine treatment modulates adrenergic vascular responses by interfering with post- and pre-synaptic mechanisms. Wistar rats were treated with vehicle (water) or chronic fluoxetine (10 mg/kg/day) for 21 days. Blood pressure (BP) and heart rate were measured. Vascular reactivity was evaluated in perfused mesenteric arterial beds (MAB) and in mesenteric resistance arteries. Protein expression by western blot analysis or immunohistochemistry, β-arrestin recruitment by BRET and calcium influx by FLIPR assay. Fluoxetine treatment decreased phenylephrine (PE)-induced, but not electrical-field stimulation (EFS)-induced vasoconstriction. Fluoxetine-treated rats exhibited increased KCl-induced vasoconstriction, which was abolished by prazosin. Desipramine, an inhibitor of norepinephrine (NA) reuptake, increased EFS-induced vasoconstrictor response in vehicle-treated, but not in fluoxetine-treated rats. Chronic treatment did not alter vascular expression of α_1 adrenoceptor, phosphorylation of PKCα or ERK 1/2 and RhoA. On the other hand, vascular contractions to calcium (Ca^{2+}) as well as Ca^{2+} influx in mesenteric arteries were increased, while intracellular Ca^{2+} storage was decreased by the chronic treatment with fluoxetine. *In vitro*, fluoxetine decreased vascular contractions to PE, EFS and Ca^{2+}, but did not change β-arrestin activity. In conclusion, chronic treatment with fluoxetine decreases sympathetic-mediated vascular responses by mechanisms that involve inhibition of NA release/reuptake and decreased Ca^{2+} stores.

1. Introduction

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) that, in addition to its antidepressant effect, has actions in the cardiovascular system. Fluoxetine interferes with cardiac contractility and heart rate as well as with atrium-ventricular conduction, producing a negative inotropic effect (Park et al., 1999), QT interval prolongation (Curtis et al., 2003) and arrhythmogenic effects, as tachycardia (Pacher and Kecskemeti, 2004). Fluoxetine also increases sympathetic activity in the heart and adrenal medulla by increasing tyrosine hydroxylase and dopamine beta-hydroxylase gene expression, which augments noradrenaline synthesis (Spasojevic et al., 2010) and stimulates vasopressin secretion, resulting in increased blood pressure (Lazartigues et al., 2000).

In elderly patients the chronic use of fluoxetine increases the susceptibility of developing hypertension (Hussein and Kaufman, 1994) and stroke (Hung et al., 2013).

In addition, fluoxetine treatment during pregnancy increases by approximately 10% the risk of gestational hypertension and preeclampsia (Toh et al., 2009) and the risk of the newborn to develop persistent pulmonary hypertension (Kieler et al., 2012).

Fluoxetine also inhibits the function of several receptors and ion channels, such as 5-HT_{2C} (Ni and Miledi, 1997) and 5-HT_3 receptors (Fan, 1994), nicotinic receptors (Maggi et al., 1998), sodium (Na^+) and potassium (K^+) voltage-dependent channels (Pancrazio et al., 1998; Perchenet et al., 2001; Thomas et al., 2002) and chloride (Cl^-) channels (Maertens et al., 1999), many of them directly involved in the

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regulation of vasomotor tone.

Although it is clear that fluoxetine produces cardiovascular effects, its actions in the cardiovascular system are not fully understood. In addition, whereas many studies focused on the acute effects of fluoxetine in the vasculature, chronic effects of this SSRI are still limited.

Crestani et al. (2011) investigating the effects of chronic administration of fluoxetine in rats, have demonstrated that fluoxetine alters pressor/depressor responses induced by the administration of vasoactive agents, indicating direct vascular effects. We have recently shown that chronic treatment with fluoxetine increases endothelium-dependent and -independent relaxation responses in rat mesenteric resistance arteries by mechanisms involving increased endothelial nitric oxide synthase (eNOS) activity, nitric oxide (NO) generation, and activation of calcium-activated potassium channels (K_{Ca}) (Pereira et al., 2015). In addition, Ribback et al. (2012) reported that fluoxetine decreases contractile responses of isolated rat aortic rings to different agonists, including norepinephrine (NA) and serotonin, by NO-mediated mechanisms.

Therefore, primarily based on the facts that acute administration of fluoxetine increases sympathetic activity and has direct effects in the vasculature, *e.g.* decreasing NA-mediated contractile response, we tested the hypothesis that chronic fluoxetine treatment decreases adrenergic vascular responses by interfering with both post- and pre-synaptic mechanisms. To address our hypothesis, we used two vascular preparations from Wistar rats chronically treated with vehicle or fluoxetine. The vascular preparations were submitted to adrenergic stimuli, α_1 adrenoceptor (α_1-AR) agonist and electrical-field stimulation (EFS) of sympathetic nerves, as well as to biochemical/molecular protocols to determine mechanisms by which chronic fluoxetine treatment alters vascular reactivity.

2. Material and methods

2.1. Animals

All experimental protocols performed in this study were approved by the Ethics Committee on Animal Experiments of the Ribeirao Preto Medical School, University of Sao Paulo (protocol. 013/2013) and are in accordance with the Guidelines of the National Council for Animal Experimentation Control (CONCEA).

Male Wistar rats, weighing 230–250 g were used in the present study. The animals were housed in high-top-filter cages (3 rats per cage – 48.3 cm× 33.7×25.3 cm) in a room with controlled humidity (45 ± 5%) and temperature (21 ± 2 °C), and light/dark cycles of 12 hs. Animals had free access to food (commercially available standard rat chow, Purina) and potable tap water.

2.2. Treatment with fluoxetine

Fluoxetine was administered (10 mg/kg/day) in the drinking water, which was daily changed (Alper, 1992; Lino-de-Oliveira et al., 2001). The average consumption of water by the rats was monitored for three days, and the concentration required to achieve a dose of 10 mg/kg/day was calculated. Treatment of rats with fluoxetine at the same dose (10 mg/kg/day) and same administration route, for 30 days, has been shown to produce clinically-relevant plasma concentrations (Able et al., 2014; McNamara et al., 2010). The bottles were light-protected to prevent degradation or oxidation of the drug. Rats were divided randomly into two groups: (I) vehicle, water for 21 days or (II) chronic fluoxetine (10 mg/kg/day for 21 days).

2.3. Determination of blood pressure and heart rate

Experiments were carried out in conscious, unrestrained rats, in their own cages, 24 h after surgery. The rats (n=20) were subcutaneously anesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg). A polyethylene catheter (PE-10, 0.61 mm outer diameter, inner diameter 0.28 mm, Clay-Adams) was implanted into the left femoral artery for direct measurement of arterial BP. The catheters was filled with heparinized saline (100 IU/ml), capped and tunnelled subcutaneously to the back of the neck. On the day of the experiment, the arterial catheter was connected to a pressure transducer (model DPT-100 Deltran; Utah Medical Products, Midvale, Utah) and the rats allowed to rest for 15–20 min. After the rest period, arterial pressure was continuously sampled (4 kHz) during 30 min, using a computer equipped with an analogic-to-digital interface (PowerLab/4SP; ADInstruments, Colorado Springs, CO).

2.4. Vascular reactivity – mesenteric arterial bed

Isolated and perfused MAB rat, which allows measurements of mesenteric vascular reactivity, were used as previously described by Macgregor, 1965. Briefly, rats were anaesthetized with a mixture of ketamine (50 mg/kg) and xylazine (10 mg/kg), given intraperitoneally; the abdominal cavity was opened and the intestinal loops were exposed. The superior mesenteric artery was dissected close to its origin in the abdominal aorta and cannulated with a PE-50 polyethylene catheter. The MAB was perfused with 1 ml Krebs Henseleit solution (KHS) [(in mmol/L): NaCl 120; KCl 4.7; KH_2PO_4 1.17; $MgCl_2$ 1.43; $NaHCO_3$ 25; Glucose 11; EDTA 0.03; $CaCl_2$ 3.0] containing heparin (500 IU). The intestinal loops were removed *en bloc*, the MAB was separated by cutting close to the intestinal loops, and the preparation was placed in a cuvette warmed to 37 °C. The cannulated superior mesenteric artery was coupled to a perfusion pump and the MAB was perfused with Krebs solution bubbled with 95% O_2 and 5% CO_2, pH 7.4, at a constant flow of 4 ml/min. A pressure transducer (Deltran® Disposable Pressure Transducer, 6069, Utah Medical) was coupled in a 'y' arrangement to the system for perfusion pressure recording. The pre-amplified and filtered outlet signal was coupled to the data acquisition system (AECAD 04F) connected to a computer, and stored for later analysis using the AQCAD software.

After a 30-min stabilization period, preparation steps were undertaken to perform dose-response curves. Mesenteric arterial beds were stimulated with increasing doses of phenylephrine [(PE) 300 pmol, 1, 3, 10, 30, 100, 300 nmol, 1, 3 and 10 µmol, administered *in bolus*] to produce contractions that were measured as increases in basal perfusion pressure. MABs were also stimulated with increasing concentrations of KCl (30, 45 and 60 mM) to produce contractions in the absence and presence of prazosin, an α_1 adrenoceptor antagonist (10 nM).

In a separate set of experiments, MAB were submitted to EFS. A bipolar electrode steel wire was placed around the superior mesenteric artery, stimulating the vessel wall and periarterial nerves. This electrode was connected to a current stimulator (AVS Avs-100C4-USB), releasing rectangular pulses of 5 ms duration, 34 V amplitude, and each stimulus train lasted 20 s (Salgado et al., 1992). The frequencies of stimuli ranged from 7 to 30 Hz to obtain the frequency-response curve vasoconstrictor (7, 10, 12, 15, 20, 25 and 30 Hz) in absence or presence of desipramine, an inhibitor of NA reuptake (10 nM) (Salgado et al., 1992). The interval for each train of stimuli was 3 min.

2.5. Vascular reactivity – isolated mesenteric resistance arteries

The method described by Mulvany and Halpern (1977) was used. The animals were euthanized and segments of third-branch mesenteric arteries, measuring about 2 mm in length, were mounted in a small vessel myograph (Danish Myo Tech, Model 620 M, A/S, Århus, Denmark). Arteries were maintained in a modified KHS [(in mmol/L) NaCl 130, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $NaHCO_3$ 14.9, Glucose 5.5, EDTA 0.03, $CaCl_2$ 1.6], at a constant temperature of 37 °C, pH 7.4, and gassed with a mixture of 95% O_2 and 5% CO_2.