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Contribution of oxidative stress and prostanoids in endothelial dysfunction induced by chronic fluoxetine treatment

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ABSTRACT

Objectives: The effects of chronic fluoxetine treatment were investigated on blood pressure and on vascular reactivity in the isolated rat aorta.
Methods and results: Male Wistar rats were treated with fluoxetine (10 mg/kg/day) for 21 days. Fluoxetine increased systolic blood pressure. Chronic, but not acute, fluoxetine treatment increased the contractile response induced by phenylephrine, serotonin (5-HT) and KCl in endothelium-intact rat aortas. L-NAME and ODQ did not alter the contraction induced by phenylephrine and 5-HT in aortic rings from fluoxetine-treated rats. Tiron, SC-560 and AH6809 reversed the increase in the contractile response to phenylephrine and 5-HT in aortas from fluoxetine-treated rats. Fluoxetine treatment increased superoxide anion generation (O_2^-) and the expression of cyclooxygenase (COX)-1 in the rat aorta. Reduced expression of nNOS, but not eNOS or iNOS was observed in animals treated with fluoxetine. Fluoxetine treatment increased prostaglandin (PG)$F_{2\alpha}$ levels but did not affect thromboxane (TX)B_2 levels in the rat aorta. Reduced hydrogen peroxide (H_2O_2) levels and increased catalase (CAT) activity were observed after treatment.
Conclusions: The major new finding of our study is that chronic fluoxetine treatment induces endothelial dysfunction, which alters vascular responsiveness by a mechanism that involves increased oxidative stress and the generation of a COX-derived vasoconstrictor prostanoid ($PGF_{2\alpha}$). Moreover, our results evidenced a relation between the period of treatment with fluoxetine and the magnitude in the increment of blood pressure. Finally, our findings raise the possibility that fluoxetine treatment increases the risk for vascular injury, a response that could predisposes to cardiovascular diseases.

1. Introduction

Cardiovascular mortality in patients taking psychotropic drugs is high. Tricyclic antidepressants (TCAs), among others, are associated with increased risk of cardiac arrhythmias and death [1]. Fluoxetine belongs to a class of new generation antidepressants which are collectively know as selective serotonin (5-HT) reuptake inhibitors (SSRIs). Those drugs are considered to be free from the cardiotoxic effects of TCAs. However, mounting evidences suggest that SSRIs induce cardiovascular dysfunction such as arrhythmias, electrocardiogram abnormalities and rest bradycardia [2–4]. Fluoxetine displayed potent inhibitory properties on Na^+, Ca^{2+} and K^+ channels in cardiac tissue in vitro [5]. More recently, treatment with fluoxetine for 21 days was showed to induce mild hypertension and enhanced baroreflex responses associated with bradycardia [6]. The cardiac effects of fluoxetine are well characterized, but information regarding the effects of SSRIs on the vasculature is limited.

Ungvari et al. [7] showed that fluoxetine induced endothelium-independent relaxation of isolated rat cerebral arteries. Moreover, fluoxetine inhibited the contraction induced by 5-HT, noradrenaline and Bay K 8644, a voltage-dependent Ca^{2+} channel opener, further suggesting that fluoxetine blocks Ca^{2+} channels in the vascular smooth muscle. Similar results were observed in arterioles from rat skeletal muscle where fluoxetine reduced intracellular Ca^{2+} concentration [8]. Although in vitro studies have shown that fluoxetine affects vascular reactivity to vasoconstrictor agents, there is no evidence on the effect of chronic fluoxetine treatment on vascular responsiveness to vasoactive agents.

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The vascular endothelium is important in the maintenance of the vascular tone since it is responsible for the production of endothelial-derived mediators involved in the contraction and relaxation of the vasculature [9]. Endothelial dysfunction results in impaired endothelium-mediated vasodilatation, increased vascular reactivity and is associated with several pathologies in the cardiovascular system, including hypertension [10]. Decreased nitric oxide (NO) bioavailability together with increased reactive oxygen species (ROS) generation contributes to the molecular events underlying endothelial dysfunction [9]. Interestingly, fluoxetine was described to reduce NO release by synovial and striatal cells [11,12]. Moreover, fluoxetine was also shown to reduce the expression of the enzyme NO synthase (NOS) in the rat hippocampus [13]. More recently, Göçmez et al. [14] suggested that chronic fluoxetine treatment impairs the synthesis or availability of NO in the *corpus cavernosum*. Another interesting observation is that fluoxetine induced ROS generation in human hepatocytes [15]. However, whether fluoxetine treatment increases ROS generation and reduces NO bioavailability in the vasculature remains elusive.

Since fluoxetine reduces NO bioavailability and increases ROS generation in different tissues, we hypothesized that fluoxetine treatment would induce endothelial dysfunction. Although the acute effect of fluoxetine in the vasculature in vitro was previously described, to the best of our knowledge, no studies have evaluated the effect of chronic fluoxetine treatment in the vasculature. In the present study, we investigated the effect of fluoxetine in the responsiveness of the isolated rat aorta and the mechanisms underlying such effect.

2. Methods

2.1. Experimental design

Male Wistar rats were housed under standard laboratory conditions with free access to food and water. The housing conditions and experimental protocols were approved by the Animal Ethics Committee of the University of São Paulo — Campus of Ribeirão Preto (#11.1.1593.53.9) and were performed in accordance with the Brazilian animal protection

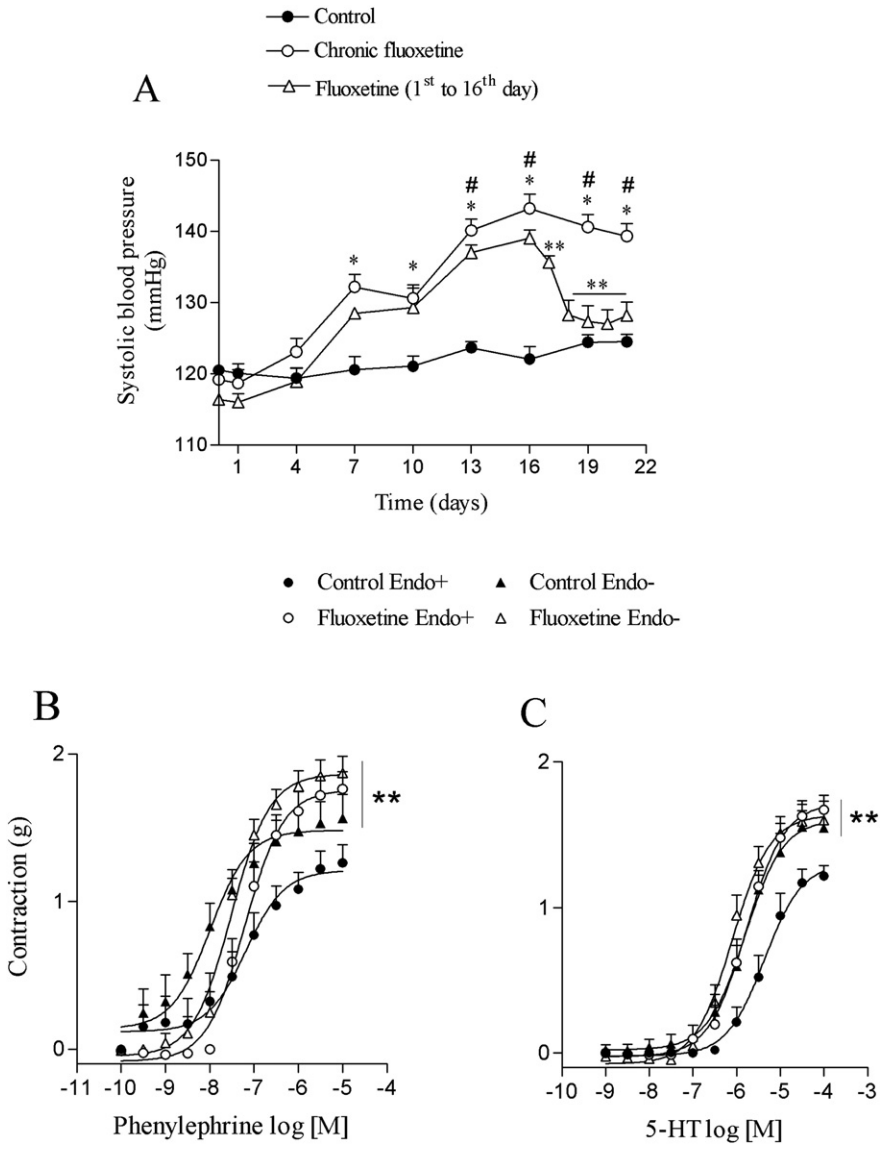

Fig. 1. Effect of chronic fluoxetine treatment on systolic blood pressure and aortic reactivity to phenylephrine and 5-HT. Systolic arterial pressure was evaluated by plethysmograph in 10 animals of each group (A). Concentration–response curves for phenylephrine (B) and 5-HT (C) were determined in endothelium-intact (Endo +) and endothelium-denuded (Endo −) rat aortic rings. Values are means ± SEM of 5 to 8 independent preparations. *Compared to control group; #compared to chronic fluoxetine group on the 7th and 11th days;**compared to chronic fluoxetine on the 16th day ($p < 0.05$, two-way ANOVA followed by Newman–Keuls multiple comparison test); **compared to control Endo + ($p < 0.05$, ANOVA followed by Newman–Keuls multiple comparison test).