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Chronic fluoxetine treatment enhances sympathetic activities associated with abnormality of baroreflex function in conscious normal rats

Ling-Zong Hong[a,b,*], Keh-Feng Huang[c], Siu-Wan Hung[d,e], Li-Te Kuo[f,g]

[a] Department of Medical Research, Taichung Veterans General Hospital, Taichung, Taiwan
[b] Department of Food Science, Yuanpei University of Medical Technology, Hsinchu, Taiwan
[c] Department of Applied Chemistry, Providence University, Taichung, Taiwan
[d] Department of Radiology, Taichung Veterans General Hospital, Taichung, Taiwan
[e] School of Medical Imaging and Radiological Sciences, Chung Shan Medical University, Taichung, Taiwan
[f] Department of Otolaryngology Head and Neck Surgery, Taichung Veterans General Hospital, Taichung, Taiwan
[g] School of Medicine, China Medical University, Taiwan

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ABSTRACT

Data regarding the effects of selective serotonin reuptake inhibitors (SSRIs), which are a common type of antidepressants, on cardiovascular autonomic function are inconsistent. The present study was conducted to determine the effects of chronic fluoxetine, an SSRI, on blood pressure, cardiac autonomic nervous activities and baroreflex control of heart rate. Male Sprague-Dawley rats were treated with fluoxetine (10 mg/kg day, p.o.) or saline for 14 weeks. Baroreflex function was determined by the sigmoid logistic method based on the heart rate responses to changes in blood pressure elicited by phenylephrine or sodium nitroprusside infusions. Cardiac sympathetic and parasympathetic tones were determined after methylatropine and propranolol treatments. Vascular responsiveness to acetylcholine, phenylephrine and sodium nitroprusside, and cardiac responsiveness to isoproterenol were determined after ganglionic blockade. Chronic fluoxetine treatment increased plasma levels of adrenaline and noradrenaline, but not nitric oxide. Elevation of blood pressure and heart rate by chronic fluoxetine was accompanied by baroreflex resetting and depressed baroreflex sensitivity. Elevated heart rate was mediated by enhanced sympathetic and depressed parasympathetic tones. The lowered baroreflex sensitivity might be attributed to attenuation of the parasympathetic component of baroreflex function. Chronic fluoxetine also diminished cardiac and vascular responsiveness to isoproterenol and acetylcholine, respectively. The plasma levels of adrenaline and noradrenaline were highly correlated with blood pressure, heart rate and baroreflex sensitivity. In conclusion, our results demonstrate that chronic fluoxetine treatment in normal rats induced predominant sympathoexcitation and depressed parasympathetic activity leading to mild hypertension, tachycardia, and impairment of baroreflex function.

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) are a class of compounds that increase the extracellular levels of the neurotransmitter serotonin by inhibiting its reuptake into the presynaptic cells and consequently enhance the availability of serotonin for the serotonergic system. Because the disruption of serotonergic neurotransmission plays a major role in the aetiology of depressive disorders (Bloom and Kupfer, 1995), SSRIs are widely prescribed in the treatment of depression and are noted for their safety and tolerability (Jakubovski et al., 2016).

In addition to their antidepressant effect, SSRIs also have beneficial effects on cardiovascular function. SSRIs have been shown to improve flow-mediated vascular dilatation (Pizzi et al., 2009), reduce basal sympathetic activity (Barton et al., 2007), and facilitate the recovery rate of cardiac autonomic function in depressed patients after acute myocardial infarction (McFarlane et al., 2001). However, SSRIs also seem to have adverse effects on the cardiovascular system, such as resting bradycardia (Roose et al., 1998), electrocardiogram abnormalities (Pacher and Kecskemeti, 2004), and the lowering of cardiac parasympathetic activity in depressed patients (Dawood et al., 2007). Impairment in cardiovascular autonomic function was frequently observed and correlated with increased risk of sudden cardiac death in depressed individuals (Penninx, 2017). Studies on the effects of SSRIs on cardiovascular autonomic function were inconsistent.

Fluoxetine, a member of the SSRI class, is widely prescribed for the

* Corresponding author at: Department of Medical Research, Taichung Veterans General Hospital, 1650 Taiwan Boulevard Sect. 4, Taichung 40705, Taiwan.
E-mail address: lzhong@vghtc.gov.tw (L.-Z. Hong).

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treatment of depressive disorders (Benfield et al., 1986). Evidence indicates that fluoxetine may cause vasodilatation by activating muscarinic receptors and inhibiting acetylcholinesterase activities (Ofek et al., 2012), and enhancing nitric oxide (NO) bioavailability (Pereira et al., 2015). Fluoxetine also inhibits sodium, calcium, and potassium channels in cardiac tissue *in vitro* (Pacher and Kecskemeti, 2004), implying that it could alter cardiac electric properties. However, in conscious normotensive rats, fluoxetine treatments for various durations (4 days to 5 weeks) showed inconsistencies in blood pressure response, while the resting heart rate (HR) and baroreflex sensitivity remained unchanged (Crestani et al., 2011; Grippo et al., 2006; Henze et al., 2013; Moffitt and Johnson, 2004).

Resting HR is dependent on the balance of cardiac parasympathetic and sympathetic activities. Baroreflex function, which is mediated by the cardiac and vascular autonomic nervous system, is an important physiological mechanism for regulating cardiovascular function. It has been suggested that long-term administration and adaptive changes are required for fluoxetine to induce apparent therapeutic effects (Grippo et al., 2006; Hantsoo et al., 2014; Roose et al., 1998). It is not clear how baroreflex function would respond to a long-term of fluoxetine treatment as in clinical practice. Moreover, the underlying mechanisms of changes in baroreflex function in response to fluoxetine treatment would be worthy of further evaluation.

The present study, therefore, was designed to determine the chronic effect of fluoxetine on blood pressure, cardiac autonomic nervous activities and baroreflex function. Moreover, the possible roles of related humoral factors, such as NO, adrenaline and noradrenaline, were also studied.

2. Materials and methods

2.1. Animals and preparation

Male Sprague-Dawley rats weighing 250–300 g were purchased from the National Laboratory Animal Centre (Taipei, Taiwan), which is certified by the Association of Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). The rats were housed in individual cages with a 12 h:12 h dark-light cycle and maintained at a constant temperature of 22 ± 2 °C. Rats were allowed free access to the regular chow. All animal procedures and the experimental protocols in the present study were approved by the Institutional Animal Care and Use Committee, Taichung Veterans General Hospital, Taichung, Taiwan (ethical committee authorization La-100881), in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication no. 85-23, revised 1996).

The rats were randomly assigned into two groups treated with either fluoxetine (10 mg/kg·day, p.o.; fluoxetine hydrochloride, Prozac®; Patheon, Bourgoin-Jallieu, France; n = 16) or saline (the controls; n = 16) for 14 consecutive weeks. Blood samples were withdrawn via the tail vein between 9:00 and 11:00 a.m. after a 12-week treatment period. Plasma samples were separated into several aliquots and stored at − 80 °C for later analyses. On the next day, the rats were anesthetized by chloral hydrate (400 mg/kg, i.p.; Sigma-Aldrich, St. Louis, MO, USA). A vascular catheter (Micro-Renathane tube, MRE 040, 1.02 mm outer diameter × 0.64 mm inner diameter) was implanted in the left femoral artery for later blood pressure measurements, and another vascular catheter (MRE 033, 0.84 mm outer diameter × 0.36 mm inner diameter) was implanted in the right femoral vein for drug administration. The catheters were filled with heparinized saline (20 U/ml), exteriorized through the dorsal mid-scapular region of the animal, and covered with a stainless-steel extension spring. After surgery, the rats were given nalbuphine hydrochloride (0.2 mg/kg, i.p.; Bain®; Genovate Biotechnology, Taipei, Taiwan) for postoperative analgesia. The rats were allowed to recover for a minimum of 5 days. During the recovery period, rats were monitored for signs of infection, body weight gain, behavior, and food and water intake. Only animals that were apparently healthy, showing no signs of pain and infection, moving freely and actively, and able to gain weight to the pre-surgical levels were used for the experiments. The treatment of fluoxetine was continued during these recovery and experiment periods.

2.2. Measurement of blood pressure and HR

In experiments, the arterial catheter was connected to a pressure transducer (Gould Statham P23Db; Gould, Cleveland, OH, USA). The signal was transferred to a pulsatile arterial blood pressure signal (Pressure Processor amplifier; Gould) and simultaneously digitized and recorded at a sampling rate of 200 Hz, using the AcqKnowledge data acquisition system (MP150, Biopac Systems; Goleta, CA, USA) connected to a computer. The mean arterial blood pressure (MAP) and HR were analyzed and triggered from pulsatile arterial blood pressure signals. The rats were left in the experimental cage for at least 1 h before the experiment. The rats were conscious and allowed to move freely during the experiment. The experimental environment was kept as quiet as possible to avoid any interference with blood pressure and HR. Baseline measurements for systolic arterial blood pressure (SAP), diastolic arterial blood pressure (DAP), MAP, and HR were recorded for 10 min before drug administration.

2.3. Construction of baroreflex function curves and evaluation of baroreflex sensitivity

Baroreflex control of the HR was evaluated by the HR responses to the increases or decreases in MAP by intravenous infusion of either phenylephrine (20 μg/kg, i.v.; Sigma-Aldrich) or sodium nitroprusside (25 μg/kg, i.v.; Sigma-Aldrich) in the rats. Infusions of phenylephrine or sodium nitroprusside (100 μg/ml, 180–200 μl/min) were carried out in random order to raise or decrease MAP by approximately 50 mmHg over 30–40 s. A recovery period of 20 min was allowed for MAP and HR to return to base values before administration of the next drug.

The changes in MAP and the consequent reflex responses of HR during phenylephrine or sodium nitroprusside infusions were acquired every 1 s from the threshold to the saturation points. The MAP and HR data were fitted to a sigmoid logistic function using a nonlinear regression program (SigmaPlot version 10.0). The sigmoid logistic equation was applied for least-squares estimation of four nonlinear parameters (Kent et al., 1972). Briefly, $HR=P1+P2/[1+\exp^{P3(MAP-P4)}]$, where P1 is the minimum HR (lower plateau), P2 is the HR range, P1+P2 is the maximum HR (upper plateau), P3 is the curvature coefficient which is independent of range and P4 is the MAP at the midpoint of the HR range (MAP_{50}). The maximum gain ($Gain_{max}$) or peak slope of the curve was determined from the first derivative of the above equation. The $Gain_{max}$ was presented as the baroreflex sensitivity. The baroreflex function curves (sigmoid MAP-HR response curve) were constructed by averaging the four parameters of the sigmoid logistic equation for all curves and using the mean parameters to reconstruct a single curve.

2.4. Measurement of cardiac sympathetic and parasympathetic tones

After the MAP and HR returned to baseline level, cardiac sympathetic and parasympathetic tones were determined in the same rats based on the HR responses to methylatropine bromide (atropine; a muscarinic receptor blocker, 4 mg/kg, i.v.; Sigma-Aldrich) and propranolol (a β-adrenoceptor blocker, 5 mg/kg, i.v.; Sigma-Aldrich), respectively (Hsieh and Hong, 2008). One-half of the rats in each group were given propranolol followed by atropine treatments, and the other half of the rats were treated with atropine followed by propranolol. The intrinsic heart rate (IHR) was determined after administration of both