European Journal of Pharmacology 670 (2011) 527–533

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Cardiovascular Pharmacology

Chronic fluoxetine treatment alters cardiovascular functions in unanesthetized rats

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ARTICLE INFO

Article history:
Received 24 May 2011
Received in revised form 6 September 2011
Accepted 11 September 2011
Available online 19 September 2011

Keywords:
Chronic fluoxetine treatment
Hypertension
Arterial pressure
Heart rate
Baroreflex
Psychological stress

ABSTRACT

In the present study, we investigated the effects induced by fluoxetine treatment (10 mg/kg) for either 1 or 21 consecutive days on arterial pressure and heart rate basal levels, baroreflex activity, hemodynamic responses to vasoactive agents and cardiovascular responses to acute restraint stress. Mild hypertension was observed after 21 days of treatment, but not after administration for 1 day. Moreover, chronic treatment affected the baroreflex control of heart rate, which was characterized by a reduced reflex tachycardia and an enhanced bradycardiac baroreflex response. The pressor responses to systemic administration of the selective α_1-adrenoceptor agonist phenylephrine, as well as the depressor responses to systemic infusion of the nitric oxide donor sodium nitroprusside, were reduced after chronic fluoxetine treatment. Fluoxetine treatment for 21 days reduced both the pressor and tachycardiac responses evoked by acute restraint stress. In conclusion, the results indicate the development of mild hypertension after chronic fluoxetine treatment. This effect was followed by changes in the baroreflex control of heart rate and altered vascular responsiveness to pressor and depressor agents, which may explain, at least in part, the increase in arterial pressure. Chronic fluoxetine treatment also affected cardiovascular responses to restraint stress, thus indicating that fluoxetine may affect cardiovascular adaptation under conditions of stress.

1. Introduction

Fluoxetine and other selective serotonin reuptake inhibitors (SSRIs) have achieved such a phenomenal level of use mainly because of a supposed favorable safety and tolerability profile over tricyclic antidepressants (Gram, 1994). However, evidence suggests that SSRIs are associated with cardiovascular side effects (Pacher and Kecskemeti, 2004). Most notably, reports of arrhythmias, electrocardiogram abnormalities and rest bradycardia have been documented with the use of these substances (Ravina et al., 1998; Roose et al., 1998; Spier and Frontera, 1991). Moreover, the co-administration of fluoxetine and other drugs that interact with the serotoninergic system has been associated with the serotonin syndrome, which consists of a triad of symptoms including behavioral changes and autonomic instability (Radomski et al., 2000). This evidence of cardiovascular toxicity is further reinforced by *in vitro* studies demonstrating that fluoxetine, as well other SSRIs, affect membrane potentials and ion channels in cardiac and vascular cells (Pacher and Kecskemeti, 2004; Ungvari et al., 2000). Although these data indicate cardiovascular side effects associated with fluoxetine treatment, the actions of this drug on cardiovascular functions are not yet completely understood.

It has been described that impairment of baroreflex activity is associated with a number of cardiovascular diseases, such as heart failure, myocardial infarction and hypertension (Deck et al., 1992; Grassi et al., 2006; Osculati et al., 1990). Although the importance of the baroreflex in the control of cardiovascular activity has been shown, information regarding the effect of SSRIs on baroreflex activity are limited (Alper, 1992; Moffitt and Johnson, 2004).

The impairment of vascular reactivity to vasoactive agents has been proposed as an indicator of atherosclerosis (Harrison et al., 1987), and is related to the pathogenesis of hypertension (Panza et al., 1993; Resstel et al., 2006). Therefore, cardiovascular complications following fluoxetine treatment may be associated with changes in vascular reactivity. Indeed, *in vitro* studies have suggested that fluoxetine affects vascular reactivity to vasoconstrictor agents (Pacher et al., 1999; Ungvari et al., 1999). However, there is no evidence *in vivo* of the effect of fluoxetine treatment on vascular responsiveness to vasoactive agents.

Cardiovascular changes are part of the physiological response aimed to maintain homeostasis during exposure to stressful aversive situations (Ulrich-Lai and Herman, 2009). This adaptive mechanism is characterized by increases in arterial pressure and heart rate (Resstel et al., 2008). Although some studies have investigated the effect of

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doi:10.1016/j.ejphar.2011.09.030

chronic fluoxetine treatment on cardiovascular responses to stress, the results are contradictory (Grippo et al., 2006; Roche et al., 2007). Differences in the stressor employed and treatment duration may explain these contradictory results (McDougall et al., 2005). In addition, none of the aforementioned studies investigated the mechanisms responsible for the cardiovascular effects induced by fluoxetine in stressed and non-stressed animals.

So, in the present study, we investigated the effect of acute or multiple (i.e., daily for 21 consecutive days) systemic fluoxetine treatment on arterial pressure and heart rate basal levels, the baroreflex activity, and the hemodynamic responses to vasoactive agents in conscious rats; as well as in the cardiovascular responses elicited by an acute restraint stress.

2. Material and methods

2.1. Animals

Experimental procedures were carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto, which complies with the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society. Wistar rats weighing 250–260 g at the beginning of the experiments were used in the present experiments. Animals were housed in plastic cages in a temperature-controlled room at 25 °C in the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. They were kept under a 12:12 h light–dark cycle (lights on between 6:00 am and 6:00 pm) and had free access to water and standard laboratory food.

2.2. Fluoxetine treatment

Animals were randomly divided into four groups: (i) chronic vehicle (n = 9), daily intraperitoneal (i.p.) injections of vehicle (saline + 0.2% Tween-80, 1 ml/kg) for 21 days; (ii) chronic fluoxetine (n = 10), daily i.p. injections of fluoxetine (10 mg/kg) for 21 days; (iii) acute vehicle (n = 8), a single injection of vehicle (saline + 0.2% Tween-80, 1 ml/kg); and (iv) acute fluoxetine (n = 7), a single injection of fluoxetine (10 mg/kg) (Alper, 1992; Lino-de-Oliveira et al., 2001). Animals submitted to acute treatment were left undisturbed, except for cleaning the cages, in the Animal Care Unit for the same period as animals submitted to chronic treatments.

2.3. Surgical preparation

Twenty-four hours before the trial, rats were anesthetized with tribromoethanol (250 mg/kg, i.p.) and a catheter (a 4 cm segment of PE-10 heat-bound to a 13 cm segment of PE-50, Clay Adams, Parsippany, NJ, USA) was inserted into the abdominal aorta through the femoral artery, for arterial pressure and heart rate recording. A second catheter was implanted into the femoral vein for the infusion of vasoactive agents to evoke arterial pressure changes. Both catheters were tunnelled under the skin and exteriorized on the animal's dorsum. After the surgery, rats were treated with a streptomycin and penicillin polyantibiotic formulation (0.27 mg/kg, i.m.; Pentabiotico®; Fort Dodge, Brazil), to prevent infection, and received the non-steroidal anti-inflammatory drug flunixin meglumine (0.025 mg/kg, i.m.; banamine®; Schering Plough, Brazil) for postoperative analgesia.

2.4. Measurement of cardiovascular parameters

On the day of the experiment, the arterial cannula was connected to a pressure transducer and the pulsatile arterial pressure was recorded using an HP-7754A pre-amplifier (Hewlett Packard, Palo Alto, CA, USA) and an acquisition board (MP100A, Biopac Systems Inc, Goleta, CA, USA) connected to a personal computer. Mean arterial pressure, systolic arterial pressure, diastolic arterial pressure, and heart rate values were derived from pulsatile arterial pressure recordings.

2.5. Baroreflex assessment

The baroreflex was tested by intravenous infusion of either the selective α_1-adrenoceptor agonist phenylephrine (50 μg/ml/kg at 0.32 ml/min/kg) or the nitric oxide donor sodium nitroprusside (70 μg/ml/kg at 0.8 ml/min/kg), using an infusion pump (K.D. Scientific, Holliston, MA, USA) (Crestani et al., 2010; Head and McCarty, 1987; Resstel et al., 2006). Phenylephrine and sodium nitroprusside caused incremental pressor or depressor responses, respectively. Infusions of vasoactive drugs were randomized and lasted for 30–40 s, resulting in the injection of a total dose of 8–10 μg/kg of phenylephrine and 20–25 μg/kg of sodium nitroprusside.

2.6. Method of baroreflex evaluation

Baroreflex curves were constructed matching mean arterial pressure variations with heart rate responses. Paired values of mean arterial pressure (ΔMAP) and heart rate (ΔHR) variations were plotted to create sigmoid curves for each rat, which were used to determine baroreflex activity (Head and McCarty, 1987). Baroreflex analysis using sigmoid curves were characterized by four parameters: (i) P1 (bpm) lower heart rate plateau and P2 (bpm) upper plateau; (ii) heart rate range (bpm), i.e. difference between upper and lower plateau levels; (iii) median blood pressure (BP_{50}, mm Hg), which is the mean arterial pressure at 50% of the heart rate range; and (iv) average gain (G, bpm/mm Hg), which is the average slope of the curves between +1 and −1 standard derivations from BP_{50}. To analyze bradycardic and tachycardic responses separately, heart rate values matching 10, 20, 30 and 40 mm Hg of mean arterial pressure changes were calculated. Values were plotted to create linear regression curves for each rat and their slopes were compared to test changes in baroreflex gain.

2.7. Dose–response mean arterial pressure curves

The graded rises and falls in mean arterial pressure evoked respectively by increasing concentrations of phenylephrine and sodium nitroprusside produced by intravenous infusions were used to generate dose–response curves. In this manner, it was possible to generate dose–response curves that demonstrated the effect of fluoxetine treatment on pressor and depressor responsiveness. Dose–effect curves were generated for each vasoactive agent using mean arterial pressure values corresponding to cumulative recording times (each 5 s) after starting the infusion. The maximal effect (E_{max}) and the dose at 50% of the mean arterial pressure range (ED_{50}) for each vasoactive agent were compared in all experimental groups.

2.8. Acute restraint stress

Animals were submitted to restraint by placing each rat in a plastic cylindrical restraint tube (diameter ¼ 6.5 cm, length ¼ 15 cm), ventilated by holes (1 cm diameter) that comprised approximately 20% of the tube surface. Restraint lasted 60 min, after which the rats were returned to their home cages. Each rat was submitted to one session of restraint in order to avoid habituation.

2.9. Drugs

Phenylephrine hydrochloride ((R)-(−)-1-(3-hydroxyphenyl)-2-methylaminoethanol hydrochloride) (Sigma, St. Louis, MO, USA), sodium nitroprusside dihydrate ($Na_2[Fe(CN)_5NO]\cdot 2H_2O$; Sigma), and tribromoethanol (Br_3CCH_2OH; Sigma) were dissolved in saline

(0.9% NaCl). Fluoxetine (Sigma) was dissolved in saline + 0.2% Tween-80. Flunixin meglumine (Banamine®, Schering Plough, Brazil) and poly-antibiotic preparation of streptomycins and penicillins (Pentabiotico®, Fort Dodge, Brazil) were used as provided.

2.10. Experimental procedures

Animals were transferred to the experimental room in their home box. They were allowed 60 min to adapt to experimental room conditions such as sound and illumination before starting arterial pressure and heart rate recording. The experimental room was temperature controlled (25 °C) and was acoustically isolated from the other rooms. Constant background noise was generated by an air exhauster to minimize sound interference within the experimental room.

Animals in the groups for chronic vehicle (n = 9) and chronic fluoxetine (n = 10) received the 21st injection of their treatments, whereas those in the groups for acute vehicle (n = 8) and acute fluoxetine (n = 7) were treated with vehicle (i.p., 1 ml/kg) and fluoxetine (i.p., 10 mg/kg), respectively. Thirty min later, phenylephrine and sodium nitroprusside were randomly infused. Subsequently, animals were restrained for 60 min, after which they were returned to their home cages.

2.11. Data analysis

Data were expressed as means ± S.E.M. The basal values of arterial pressure and heart rate for each treatment, the E_{max} and ED_{50} of the mean arterial pressure responses caused by vasoactive agents, as well as the baroreflex parameters of linear and nonlinear curves were compared using two-way ANOVA with treatment regimen (acute or chronic) and treatment (vehicle or fluoxetine) as independent factors. When interactions between the factors were observed, groups were compared using Bonferroni's *post hoc* test. Nonlinear regression analysis was also used to compare mean arterial pressure changes caused by vasoactive drugs. Time-course curves of mean arterial pressure and heart rate changes to acute restraint stress were compared using three-way ANOVA with treatment regimen and treatment as independent factors, and time as repeated measurement. When interactions between the factors were observed, groups were compared using Bonferroni's *post hoc* test. Results of statistical tests with $P<0.05$ were considered significant.

3. Results

3.1. Effect of acute or chronic fluoxetine treatment on arterial pressure, heart rate, and body weight

There were significant effects of treatment regimen (SAP: $F_{(1,30)} = 23$, $P<0.0001$; DAP: $F_{(1,30)} = 29$, $P<0.0001$; MAP: $F_{(1,30)} = 27$, $P<0.0001$) and treatment (SAP: $F_{(1,30)} = 32$, $P<0.0001$; DAP: $F_{(1,30)} = 23$, $P<0.0001$; MAP: $F_{(1,30)} = 39$, $P<0.0001$), as well as treatment regimen × treatment interaction (SAP: $F_{(1,30)} = 12$, $P<0.005$; DAP: $F_{(1,30)} = 18$, $P<0.0002$; MAP: $F_{(1,30)} = 20$, $P<0.0001$) on systolic, diastolic, and mean arterial pressure baseline values (Fig. 1 and Table 1). *Post hoc* analyses indicated that fluoxetine treatment for 21 days, but not acute administration (SAP: $P>0.05$; DAP: $P>0.05$; and MAP: $P>0.05$), significantly increased systolic ($P<0.01$), diastolic ($P<0.01$) and mean ($P<0.01$) arterial pressure. Analyses of resting heart rate did not indicate effects of either treatment regimen ($F_{(1,30)} = 0.1$, $P>0.05$) or treatment ($F_{(1,30)} = 0.15$, $P>0.05$) (Fig. 1 and Table 1).

3.2. Effect of acute or chronic fluoxetine treatment in baroreflex control of heart rate

Nonlinear regression analysis of baroreflex activity indicated that fluoxetine treatment for 21 days affected P1 and P2 plateau, and BP_{50} (Fig. 2 and Table 2). There were significant effects of treatment regimen (P1: $F_{(1,30)} = 38$, $P<0.0001$; P2: $F_{(1,30)} = 47$, $P<0.0001$; BP_{50}: $F_{(1,30)} = 50$, $P<0.0001$) and treatment (P1: $F_{(1,30)} = 29$, $P<0.0001$; P2: $F_{(1,30)} = 23$, $P<0.0001$; BP_{50}: $F_{(1,30)} = 42$, $P<0.0001$), as well as treatment regimen × treatment interaction (P1: $F_{(1,30)} = 11$, $P<0.005$; P2: $F_{(1,30)} = 41$, $P<0.0001$; BP_{50}: $F_{(1,30)} = 33$, $P<0.0001$). *Post hoc* analyses revealed that fluoxetine treatment for 21 days, but not acute administration (P1: $P>0.05$; P2: $P>0.05$; and BP_{50}: $P>0.05$),

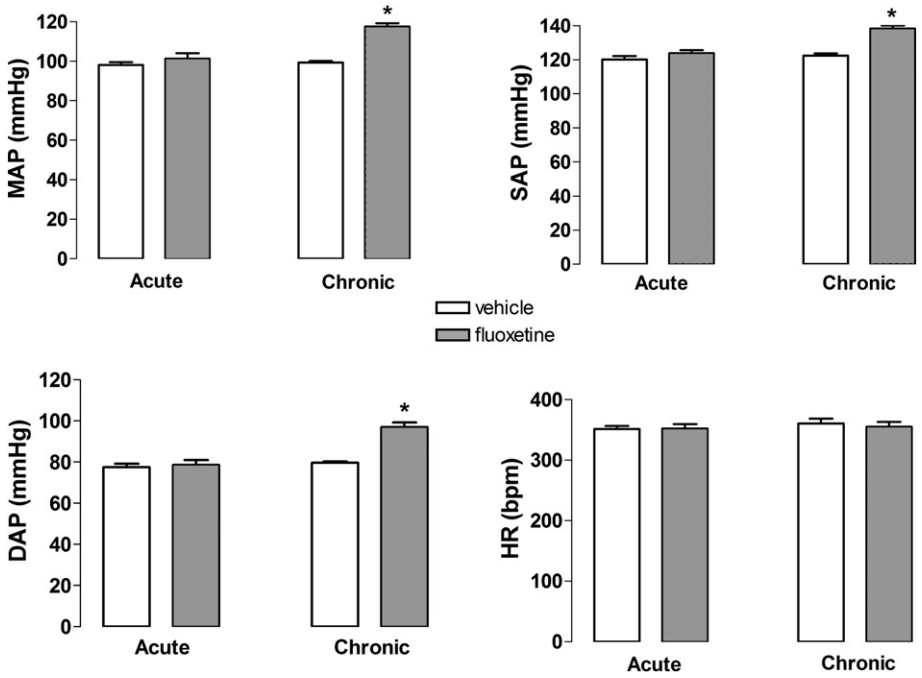

Fig. 1. Effect of acute or chronic (21 day) treatment with vehicle or fluoxetine on mean arterial pressure (MAP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and heart rate (HR). The bars represent the mean ± S.E.M. *$P<0.05$ vs chronic vehicle, two-way ANOVA followed by Bonferroni's *post hoc* test. Fluoxetine treatment for 21 days significantly increased basal parameters of systolic ($P<0.01$), diastolic ($P<0.01$) and mean ($P<0.01$) arterial pressure.

Table 1
Values of body weight, mean arterial pressure (MAP), systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and heart rate (HR) obtained from acute vehicle (n = 8), acute fluoxetine (n = 7), chronic vehicle (n = 9), and chronic fluoxetine (n = 10) rats. Value is the mean ± S.E.M.

Group	Body weight (g)	MAP (mm Hg)	SAP (mm Hg)	DAP (mm Hg)	HR (bpm)
Acute vehicle	415 ± 8	98 ± 1	120 ± 2	78 ± 2	352 ± 6
Acute fluoxetine	412 ± 6	102 ± 3	124 ± 3	79 ± 3	353 ± 8
Chronic vehicle	415 ± 10	99 ± 2	122 ± 2	80 ± 1	359 ± 8
Chronic fluoxetine	378 ± 5[a]	118 ± 2[a]	139 ± 3[a]	97 ± 3[a]	355 ± 8

[a] $P<0.05$ vs chronic vehicle, two-way ANOVA followed by Bonferroni's *post hoc* test.

Table 2
Parameters derived from sigmoidal baroreflex curves obtained from acute vehicle (n = 8), acute fluoxetine (n = 7), chronic vehicle (n = 9), and chronic fluoxetine (n = 10) rats. Value is the mean ± S.E.M.

Group	G (bpm/mm Hg)	P1 (bpm)	P2 (bpm)	HR range (bpm)	BP_{50} (mm Hg)
Acute vehicle	−1.1 ± 0.05	−40 ± 3	70 ± 3	109 ± 5	−6 ± 1
Acute fluoxetine	−1.2 ± 0.05	−46 ± 2	74 ± 5	118 ± 6	−5 ± 2
Chronic vehicle	−1.2 ± 0.1	−48 ± 4	69 ± 1	117 ± 4	−4 ± 2
Chronic vehicle	−0.9 ± 0.1	−74 ± 2[a]	40 ± 1[a]	114 ± 2	12 ± 1[a]

[a] $P<0.05$ vs chronic vehicle, two-way ANOVA followed by Bonferroni's *post hoc* test.

increased P1 ($P<0.001$) and BP_{50} ($P<0.001$), and decreased P2 ($P<0.001$) (Fig. 2 and Table 2). Analyses of heart rate range and gain did not indicate significant effects of either treatment regimen (heart rate range: $F_{(1,30)}=0.2$, $P>0.05$; gain: $F_{(1,30)}=3$, $P>0.05$) or treatment (heart rate range: $F_{(1,30)}=0.5$, $P>0.05$; gain: $F_{(1,30)}=3$, $P>0.05$) (Fig. 2 and Table 2).

The effect of arterial pressure increases or decreases on heart rate were analyzed separately using linear regression (Fig. 2B). Analyses of bradycardiac response slopes (acute vehicle: −1.2 ± 0.2 bpm/mm Hg; acute fluoxetine: -1.4 ± 0.1 bpm/mm Hg; chronic vehicle: −1.3 ± 0.1 bpm/mm Hg; chronic fluoxetine: −2.3 ± 0.1 bpm/mm Hg) indicated significant effects of treatment regimen ($F_{(1,30)}=14$, $P<0.0005$) and treatment ($F_{(1,30)}=21$, $P<0.0001$), as well as treatment regimen × treatment interaction ($F_{(1,30)}=9$, $P<0.005$). *Post hoc* analyses revealed that fluoxetine treatment for 21 days ($P<0.001$), but not acute administration ($P>0.05$), significantly increased reflex bradycardia (Fig. 2B). Analyses of tachycardiac response slopes (acute vehicle: −1.8 ± 0.1 bpm/mm Hg; acute fluoxetine: −1.9 ± 0.2 bpm/mm Hg; chronic vehicle: −1.8 ± 0.1 bpm/mm Hg; chronic fluoxetine: −1.0 ± 0.05 bpm/mm Hg) also indicated significant effects of treatment regimen ($F_{(1,30)}=17$, $P<0.0003$) and treatment ($F_{(1,30)}=11$, $P<0.003$), as well as treatment regimen × treatment interaction ($F_{(1,30)}=17$, $P<0.0003$) (Fig. 2B). *Post hoc* analyses revealed that fluoxetine treatment for 21 days ($P<0.001$), but not acute treatment ($P>0.05$), significantly decreased reflex tachycardia (Fig. 2B).

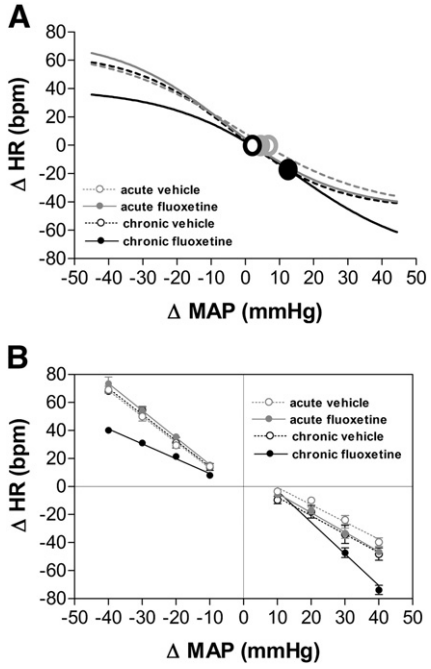

Fig. 2. (A) Sigmoid baroreflex curve correlating mean arterial pressure change (Δ MAP) and heart rate response (Δ HR) of acute vehicle (n = 8) (○, $r^2=0.93$), acute fluoxetine (n = 7) (◦, $r^2=0.92$), chronic vehicle (n = 9) (○, $r^2=0.90$) and chronic fluoxetine (n = 10) (•, $r^2=0.95$) treated rats. Symbols on curves indicate the respective BP_{50}. (B) Linear regression of baroreflex curves correlating mean arterial pressure change (Δ MAP) and heart rate response (Δ HR) of acute vehicle (○), acute fluoxetine (◦), chronic vehicle (○) and chronic fluoxetine (•) treated rats. Correlation r^2 values for bradycardic regression curves were 0.81, 0.86, 0.60 and 0.90 for data of acute vehicle, acute fluoxetine, chronic vehicle, and chronic fluoxetine treated rats, respectively. Correlation r^2 values for tachycardic regression curves were 0.92, 0.90, 0.89 and 0.95 for data of acute vehicle, acute fluoxetine, chronic vehicle, and chronic fluoxetine treated rats, respectively. Increase or decrease in the mean arterial pressure was induced by the i.v. infusion of phenylephrine or sodium nitroprusside, respectively.

3.3. Effect of acute or chronic fluoxetine treatment on the pressor dose–response curve for phenylephrine and depressor dose–response curve for sodium nitroprusside

Phenylephrine, a selective α_1-adrenoceptor agonist, dose-dependently induced pressor responses in all experimental groups (Fig. 3). Analyses of E_{max} of dose–response curves indicated significant effects of treatment regimen ($F_{(1,30)}=11$, $P<0.003$) and treatment ($F_{(1,30)}=11$, $P<0.003$), as well as treatment regimen × treatment interaction ($F_{(1,30)}=4$, $P<0.01$) (Table 3). *Post hoc* analyses revealed that fluoxetine treatment for 21 days ($P<0.001$), but not acute administration ($P>0.05$), significantly reduced pressor response (Fig. 3 and Table 3). There were no significant effects on the ED_{50} of the dose–response curve ($F_{(1,30)}=0$, $P>0.05$) (Table 3).

Sodium nitroprusside, a nitric oxide donor, dose-dependently reduced arterial pressure in all experimental groups (Fig. 3). There were significant effects of treatment regimen (ED_{50}: $F_{(1,30)}=60$, $P<0.0001$; E_{max}: $F_{(1,30)}=53$, $P<0.0001$) and treatment (ED_{50}: $F_{(1,30)}=15$, $P<0.0005$; E_{max}: $F_{(1,30)}=42$, $P<0.0001$), as well as treatment regimen × treatment interaction (ED_{50}: $F_{(1,30)}=15$, $P<0.0005$; E_{max}: $F_{(1,30)}=42$, $P<0.0001$) on the ED_{50} and E_{max} of the dose–response curve for sodium nitroprusside. *Post hoc* analyses indicated that fluoxetine treatment for 21 days (ED_{50}: $P<0.001$; E_{max}: $P<0.001$), but not acute treatment (ED_{50}: $P>0.05$; E_{max}: $P>0.05$), significantly reduced sodium nitroprusside depressor response (Fig. 3 and Table 3).

3.4. Effects of acute or chronic fluoxetine treatment on cardiovascular responses to acute restraint stress

Three-way ANOVA performed for cardiovascular responses to acute restraint indicated significant effects of treatment regimen (mean arterial pressure: $F_{(1,450)}=28$, $P<0.001$; heart rate: $F_{(1,450)}=37$, $P<0.001$), treatment (mean arterial pressure: $F_{(3,450)}=207$, $P<0.001$; heart rate: $F_{(3,450)}=307$, $P<0.001$), and time (mean arterial pressure: $F_{(14,459)}=46$, $P<0.0001$; heart rate: $F_{(14,450)}=102$, $P<0.001$) (Fig. 4). There were also significant treatment regimen × treatment × time interaction (mean arterial pressure: $F_{(42,450)}=3.3$, $P<0.0001$; heart rate: $F_{(42,450)}=5.1$, $P<0.0001$). *Post hoc* analyses revealed that fluoxetine treatment for 21 days (mean arterial pressure: $P<0.0001$; heart rate: $P<0.0001$), but not acute treatment (mean arterial pressure: $P>0.05$; heart rate: $P>0.05$), reduced both pressor and tachycardiac responses to acute restraint stress (Fig. 4).

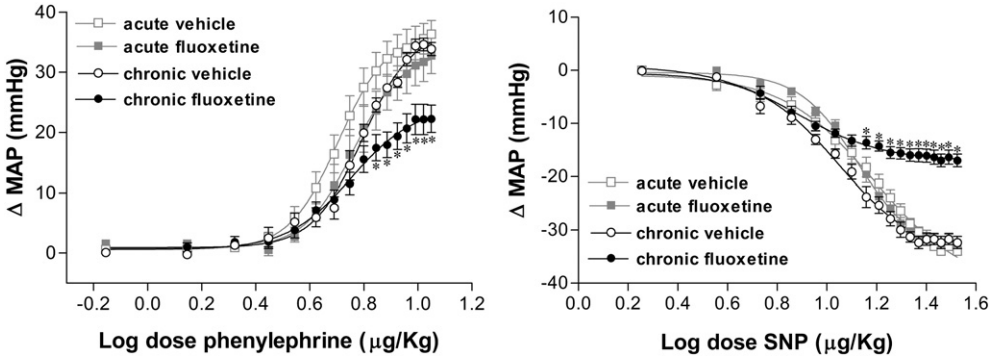

Fig. 3. Changes in mean arterial pressure (ΔMAP) evoked by increasing concentrations of the selective α_1-adrenoceptor agonist phenylephrine and the nitric oxide donor sodium nitroprusside (SNP) in acute vehicle (n = 8), acute fluoxetine (n = 7), chronic vehicle (n = 9), and chronic fluoxetine (n = 10) treated rats. Circles represent the mean and bars the S.E.M. *P<0.05 compared to vehicle, two-way ANOVA followed by Bonferroni's *post hoc* test. Fluoxetine treatment for 21 days significantly reduced both pressor response following phenylephrine (P<0.001) and depressor response following sodium nitroprusside (P<0.001).

4. Discussion

It has been reported that fluoxetine lowers arterial pressure in hypertensive rats (Fuller et al., 1979). In the present study, we report the development of mild hypertension after fluoxetine treatment for 21 days in normotensive rats. To our knowledge, this is the first study to show an increase in arterial pressure basal levels in healthy animals. This result corroborates with clinical reports of increased arterial pressure associated with fluoxetine treatment in elderly and cardiac patients (Hussein and Kaufman, 1994; Roose et al., 1998). However, the increase in arterial pressure contrasts with previous preclinical studies showing that fluoxetine treatment for 4, 16 or 28 days did not affect cardiovascular basal parameters in rats (Grippo et al., 2006; Moffitt and Johnson, 2004). The reasons for the discrepancy are not clear. Nevertheless, it is interesting to note that basal values of mean arterial pressure reported in these previous studies were higher (110–130 mm Hg) than those observed in the control animals of the present study (~100 mm Hg) (Grippo et al., 2006; Moffitt and Johnson, 2004). Moreover, in the previous studies cardiovascular activity was measured 24 h after the last injection of fluoxetine. This difference in experimental protocol may be relevant, since plasma fluoxetine levels decrease 90% 8 h after administration (Liu et al., 2005). In our protocol, cardiovascular activity was investigated 30 min after the 21st injection, thus addressing effects of fluoxetine treatment in cardiovascular functions during chronic treatment. Since the fluoxetine metabolite norfluoxetine is still present in the plasma 48 h after treatment (Durand et al., 1999), our results, together with previous data that investigated cardiovascular activity 24 h after the last injection, suggest that fluoxetine, and not its metabolite, is important in cardiovascular consequences of chronic fluoxetine treatment.

The mechanism by which chronic fluoxetine treatment might induce an increase in arterial pressure is unknown. Our data showed that either acute or chronic fluoxetine treatment did not affect resting heart rate, thus suggesting that heart rate changes do not mediate the elevation of arterial pressure in fluoxetine-treated rats. Similarly, previous studies reported that resting heart rate was not affected by chronic fluoxetine treatment in rats (Alper, 1992; Grippo et al., 2006; Moffitt and Johnson, 2004).

An impaired baroreflex control of heart rate has been associated with hypertension in humans and animal models of hypertension (Grassi et al., 2006; Irigoyen and Krieger, 1998). Acute fluoxetine treatment did not affect baroreflex control of heart rate. However, we have observed that tachycardiac responses to blood pressure decreases caused by intravenous infusion of sodium nitroprusside were inhibited, whereas bradycardiac responses evoked by blood pressure increases caused by intravenous infusion of phenylephrine were enhanced after fluoxetine treatment for 21 days. It was previously reported that fluoxetine treatment for 4, 16 or 27 days did not affect cardiac baroreflex response (Alper, 1992; Moffitt and Johnson,

Table 3
Maximal effect (E_{max}) and ED_{50} values for phenylephrine (phenyl) and sodium nitroprusside (SNP) dose–response curves obtained from acute vehicle (n = 8), acute fluoxetine (n = 7), chronic vehicle (n = 9) and chronic fluoxetine (n = 10) rats. Value is the mean ± S.E.M.

Group	Phenyl		SNP	
	ED_{50}	E_{maxl}	ED_{50}	E_{max}
Acute vehicle	0.6 ± 0.03	36 ± 3	1.1 ± 0.05	−33 ± 1
Acute fluoxetine	0.7 ± 0.04	33 ± 3	1.1 ± 0.02	−33 ± 2
Chronic vehicle	0.7 ± 0.03	33 ± 1	1.0 ± 0.05	−32 ± 1
Chronic fluoxetine	0.6 ± 0.04	21 ± 2[a]	0.8 ± 0.03*	−16 ± 1[a]

[a] P<0.05 vs chronic vehicle, two-way ANOVA followed by Bonferroni's *post hoc* test.

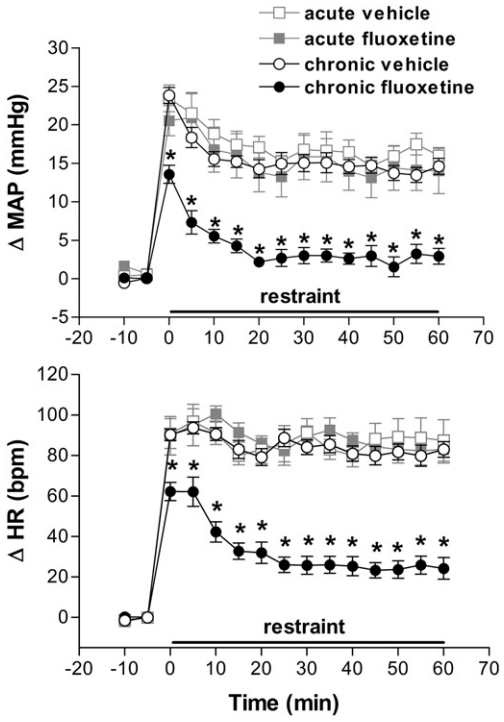

Fig. 4. Time-course of mean arterial pressure (ΔMAP) and heart rate (ΔHR) changes during acute restraint stress in acute vehicle (n = 8), acute fluoxetine (n = 7), chronic vehicle (n = 9), and chronic fluoxetine (n = 10) treated rats. Circles represent the mean and bars the S.E.M. *P<0.05 vs chronic vehicle, three-way ANOVA followed by Bonferroni's *post hoc* test. Fluoxetine treatment for 21 days significantly reduced both the pressor (P<0.001) and tachycardic (P<0.001) response to acute restraint stress.

2004). As stated earlier, differences in experimental protocol may explain the discrepancy, since in these previous studies baroreflex activity was studied 24 h after the last injection of fluoxetine. The baroreflex bradycardiac and tachycardiac responses are mainly mediated by cardiac parasympathetic and sympathetic stimulation, respectively (Head and McCarty, 1987). Consequently, our data support the idea that chronic fluoxetine treatment inhibits the sympathetic component of baroreflex activity and facilitates the parasympathetic component of baroreflex activity. Considering that the impairment of the baroreflex control of heart rate in hypertension extends to the tachycardiac response evoked during baroreflex unloading (Grassi et al., 2006), the decreased tachycardiac baroreflex response could play a role in the elevation of arterial pressure in fluoxetine-treated rats. However, present results do not link the change in bradycardiac baroreflex response following fluoxetine treatment with the alteration of arterial pressure.

Several mechanisms may explain the effect of fluoxetine on baroreflex activity. It was previously reported that fluoxetine treatment for 21 days increased the number of cells showing Fos-like immunoreactivity, a marker of neuronal activation, in several central nervous system areas involved in control of autonomic activity (Lino-de-Oliveira et al., 2001). Considering that these structures are involved in the cardiac baroreflex response, the central action of fluoxetine could mediate the change in baroreflex activity. *In vitro* studies indicated that fluoxetine inhibited L-type Ca^{2+} channels in cardiac cells (Park et al., 1999), thus suggesting that this drug could play an important role in reducing HR, cardiac contractility, and atrio-ventricular conduction. Therefore, although chronic fluoxetine treatment does not affect resting heart rate, a direct action of the drug in the heart could facilitate reflex bradicardia and inhibit tachycardiac response.

In opposition to tricyclic antidepressants, SSRIs have little affinity for α-adrenoceptors (Wong et al., 1983). Therefore, reduction in pressor response to phenylephrine reported in the present study after chronic fluoxetine treatment is not associated with blockade of vascular α_1-adrenoceptors by fluoxetine. In fact, there is a negative correlation between the pressor response to phenylephrine and baroreflex control of heart rate (Goldstein, 1983; Gordon et al., 1981). Thus, the decreased responsiveness to phenylephrine could be related to the enhanced baroreflex activity. Nevertheless, other fluoxetine effects may also explain the reduced phenylephrine responsiveness. The reduction in phenylephrine response corroborates with *in vitro* results reporting that fluoxetine antagonizes arterial constrictions to norepinephrine and serotonin (Pacher et al., 1999; Ungvari et al., 1999). It has been documented that fluoxetine blocks entry of Ca^{2+} into arteriolar smooth muscle cells, most likely by inhibiting L-type Ca^{2+} channels (Pacher et al., 1999; Ungvari et al., 2000). It is interesting to note that tricyclic antidepressants also produce relaxation in vascular smooth muscle cells (Vila et al., 1999), probably by inhibiting Ca^{2+} entry (Auguet et al., 1986). On the basis of these results, a smooth muscle-relaxing effect, by interfering with Ca^{2+}-entry, seems to be a general characteristic of the monoamine-uptake inhibitor compounds.

Impairment of vascular relaxation responses due to chronic fluoxetine treatment may be responsible, at least in part, for the development of fluoxetine-induced hypertension. In the present work, the hypotensive response induced by sodium nitroprusside was decreased after fluoxetine treatment for 21 days. It has been proposed that hypertension is associated with impaired endothelial function, which can be explained by a decrease in nitric oxide generation, or by an enhanced inactivation of nitric oxide after its release from endothelial cells (Panza et al., 1993). Moreover, other mechanisms may be postulated, including altered guanylate cyclase activity or other down-stream nitric oxide pathways. Although reduction of intracellular calcium in smooth muscle cells is an important mechanism by which nitric oxide dilates blood vessels (Akata, 2007), a fluoxetine-mediated decrease of vascular reactivity to sodium nitroprusside seems not to be related to fluoxetine influence on vascular L-type Ca^{2+} channels, since inhibition of this channel augments the relaxing capacity of nitric oxide (Van Hove et al., 2009).

Cardiovascular changes are part of the physiological response during aversive stimuli, and they are aimed at maintaining homeostasis (Ulrich-Lai and Herman, 2009). In the present study, fluoxetine treatment for 21 days reduced cardiovascular responses to acute restraint stress. Present results contrast with those of Grippo et al. (2006), who reported an increased tachycardiac response to air-jet stress after fluoxetine treatment for 28 days. Since differences in cardiovascular responses to restraint stress and air-jet stress have been reported (McDougall et al., 2005), the use of different stressors may explain the discrepancy. It has been proposed that a gating mechanism is operative in the central nervous system that determines the cardiovascular response to aversive stimuli (Dampney et al., 2008; Ulrich-Lai and Herman, 2009). Fluoxetine treatment induces changes in the restraint-induced increase in Fos-like immunoreactivity in several areas of the central nervous system involved in autonomic control (Lino-de-Oliveira et al., 2001), suggesting that a change in the pattern of central activation could mediate the fluoxetine influence on cardiovascular responses to restraint stress.

In summary, the present results provide an advance in our understanding of the cardiovascular effects of fluoxetine. Our results show the development of mild hypertension after fluoxetine treatment for 21 days. Alterations in baroreflex activity and impairment of vascular relaxation responses to vasodilator agents may be responsible, at least in part, for the development of fluoxetine-induced hypertension. However, mechanisms involved in the mild hypertension following fluoxetine treatment deserve further investigation. The present study also shows that chronic fluoxetine treatment alters the pressor responsiveness to vasoactive agents, further indicating that the cardiovascular system counteracts the effects induced by fluoxetine using mechanisms involved in the maintenance of vascular tonus. Finally, fluoxetine treatment for 21 days reduced cardiovascular responses to restraint-stress, thus suggesting that fluoxetine may affect adaptation under conditions of stress. Clinicians should be more vigilant about these potential adverse reactions, especially in patients with cardiovascular disorders. The SSRIs have achieved such phenomenal usage mainly because of a favorable safety and tolerability profile. However, it is important for clinicians to have an awareness of the adverse effects and not assume that SSRIs are devoid of potential medical complications.

Acknowledgments

The authors wish to thank L.H.A. de Camargo and S. Guilhaume for technical help. The present research was supported by grants from FAPESP (2009/03187-9; 2009/18372-6; 2010/16192-8; 2011/07332-3), CNPq (470042/2009-5 and 305996/2008-8).

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