Indian Journal of Exprimental Biology
Vol. 50, November 2012, pp. 771-775

Chronic fluoxetine treatment affects gene expression of catecholamine enzymes in the heart of depression model rats

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Received 27 April 2012; revised 3 August 2012

Depression is associated with increased risk of coronary heart diseases. Selective serotonin reuptake inhibitors (SSRIs) have been proved to be very effective in normalizing symptoms of depression, but the data on possible influence of these drugs on cardiovascular function is controversial. Applying Taqman RT-PCR assay, the effect of chronic treatment with a SSRI antidepressant fluoxetine has been investigated on gene expression of catecholamine biosynthetic enzymes in all four heart chambers of rats with signs of depression. Depression was induced by exposing the animals to chronic unpredictable mild stress (CUMS). Tyrosine-hydroxylase (TH) and dopamine-ß-hydroxylase (DBH) mRNA levels were decreased both in right and left atria, while phenylethanolamine N-methyltransferase (PNMT) mRNAs were increased in left atria and both ventricles of depression model rats. Fluoxetine elevated gene expression of TH and DBH in atria, but did not influence this process in the ventricles. Also, this antidepressant did not express a significant effect on the level of PNMT mRNA both in atria and ventricles. These results indicate that fluoxetine acted stimulating noradrenaline synthesis in the heart, which could lead to increased risk of heart disease.

Keywords: Antidepressant, Catecholamine enzymes, Depression, Gene expression, Heart

Chronic stress appears to be one of the most potent causes of depression[1,2]. Patients with depression have an increased activity of the hypothalamo-pituitary-adrenocortical and sympatho-adrenomedullary system, similar to those in the state of perceived stress, which could lead to the abnormalities in platelet function, autonomic tone and inflammation[3,4]. These numerous physiological changes could contribute to the development of cardiovascular disease and increased morbidity and mortality in patients with preexisting disease[5-7]. This raises the question of a possible efficacy of antidepressants in the treatment of heart diseases, because while treating heart patients with antidepressants alleviates the symptoms of depression, the effects of these drugs on cardiovascular function itself are far less clear. Many antidepressant medications have adverse cardiovascular effects that restrict their application, particularly to older patients[8-10]. Newer medications, such as selective serotonin reuptake inhibitors (SSRIs), not only increase brain serotonin levels, but also appear to improve behaviour[11] and normalize many physiological changes related to depression, including increased cortisol levels and improved heart rate variability[12-14]. However, SSRI drugs are also associated with cardiovascular risk[15,16].

The importance of catecholamines in augmenting cardiac function has been well established. Tyrosine hydroxylase (TH), a rate-limiting enzyme in the synthesis of catecholamines catalyzes conversion of tyrosine to dihydroxy-phenylalanine (DOPA) which is further converted into dopamine by a nonspecific enzyme, aromatic L-amino acid decarboxylase (AAAD). Dopamine, transferred from the cytoplasm into storage vesicles gets converted into noradrenaline by dopamine-ß-hydroxylase (DBH). Finally noradrenaline gets transformed into adrenaline by a soluble cytoplasmic phenylethanolamine N-methyltransferase (PNMT). These enzymes are present in different types of tissues and their gene expression can be modified by various stressors[17]. Chronic antidepressant treatments may alter catecholamine biosynthesis in brain[18,19]. However, possible effects of SSRI drugs on catecholamine biosynthesis in heart of animals with signs of depression are still unknown. To investigate these effects a rat model of depression induced by exposure

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of animals to chronic unpredictable mild stress (CUMS) has been employed. Animals subjected to CUMS had depression-like behavior and elevated plasma levels of corticosterone[20,21]. Grippo *et al.*[22] have found that these rats had increased blood pressure and tachycardia, making this model suitable for the studies of depression-induced cardiovascular diseases. Applying TaqMan RT-PCR, the influence of long-term treatment with fluoxetine, a serotonergic reuptake inhibitor has been estimated on gene expression of three catecholamine biosynthetic enzymes viz, TH, DBH and PNMT, in the right and left atria and ventricles of rats exposed to CUMS for 4 weeks.

Materials and Methods

Animals—Adult males Wistar rat weighing 280-320 g at the onset of experiments and maintained in a temperature-controlled room (21±1.0 °C) and 12:12 h L:D cycle, were used. The care was taken to minimize the pain and discomfort of the animals according to the recommendations of the Ethical Committee of the "Vinca" Institute, Belgrade, which are in accordance with the Guide for Care and Use of Laboratory Animals of the National Institute of Health, Bethesda, MD, U.S.A. Fluoxetine (fluxilan ®, Aeigis LTD, Cyprus) dissolved in sterile water and sonicated for approximately 10 min, was prepared *ex tempore*. Animals that were subjected to CUMS, according to method by Grippo *et al.*[22], received daily injections of vehicle (sterile water) or fluoxetine (10 mg/kg) by ip route during 4 weeks. Control group of animals also received daily injections of vehicle. The animals exposed to CUMS and the corresponding controls were decapitated under mild anesthesia (50 mg/kg, ip, ketamine), the right and left cardiac atria and ventricle rapidly dissected, frozen in liquid nitrogen and stored at -70 ºC until analyzed.

RNA isolation and cDNA synthesis—Total RNAs were isolated using TRIZOL reagent (Invitrogen, CA, U.S.A.). Reverse transcription was performed using Ready-To-Go You-Prime First-Strand Bead (AP, Biotech) and pd $(N)_6$ primer according to manufacturer's protocol.

Real-time RT-PCR—TaqMan PCR assays were carried out using Assay-on-Demand Gene Expression Products (Applied Biosystems, USA) for TH (assay ID: Rn 00562500_m1), DBH (assay ID: Rn 00565819_m1) and PNMT (assay ID: Rn 01495589_g1). The reactions were performed in a 25 μL reaction mixture containing 1x TaqMan Universal Master Mix with AmpErase UNG, 1x Assay Mix (Applied Biosystems) and cDNA template (10 ng of RNA converted to cDNA). PCR reactions were performed in ABI Prism 7000 Sequence Detection System at 50 ^{o}C for 2 min, 95 ^{o}C for 10 min, followed by 40 cycles at 95 ^{o}C for 15 sec and 60 ^{o}C for 1 min. A reference, endogenous control, was included in each analysis to correct the differences in the inter-assay amplification efficiency and all transcripts were normalized to cyclophyline A (assay ID: Rn 00690933_m1) expression. Quantification was done using the $2^{-\Delta\Delta Ct}$ method according to Livak and Schmittgen[23].

Statistical analysis—The results are reported as mean ± SE. Significance of the differences in gene expression levels of the examined catecholamine biosynthetic enzymes in the right and atrial appendage and ventricles of rats subjected to repeated antidepressants treatment was estimated by One-way ANOVA test. The Tukey *post-hoc* test was used to evaluate the differences between the groups. Statistical significance was accepted at $P<0.05$.

Results

The alterations in relative gene expression of catecholamine biosynthetic enzymes were investigated in right and left atria and ventricles after repeated fluoxetine treatment of adult rats exposed to CUMS for 4 weeks. A decrease of TH mRNA levels in the right and left atria was observed after chronic exposure to stress (by 32 and 65 %, respectively) (Fig. 1a). Fluoxetine considerably affected the expression of TH in the atria of stressed rats increasing gene expression of this enzyme by 21 and 106 % in the left and right atria, respectively. On the other hand, CUMS did not affect gene expression of TH in heart ventricles. Also, chronic application of the antidepressant, markedly elevated the expression of TH in both left and right ventricles (by 182 and 197%, respectively).

One-way ANOVA analysis also revealed significant stress-induced variations of DBH mRNA levels in atria. Similar to CUMS effect on TH gene expression, exposure of animals to this stress significantly decreased DBH mRNA levels in left and right atria (by 70 and 55%, respectively) (Fig. 1b). In contrast, fluoxetine increased DBH mRNA levels both in left and right atria (by 84 and 96%, respectively). Neither the CUMS procedure, nor the

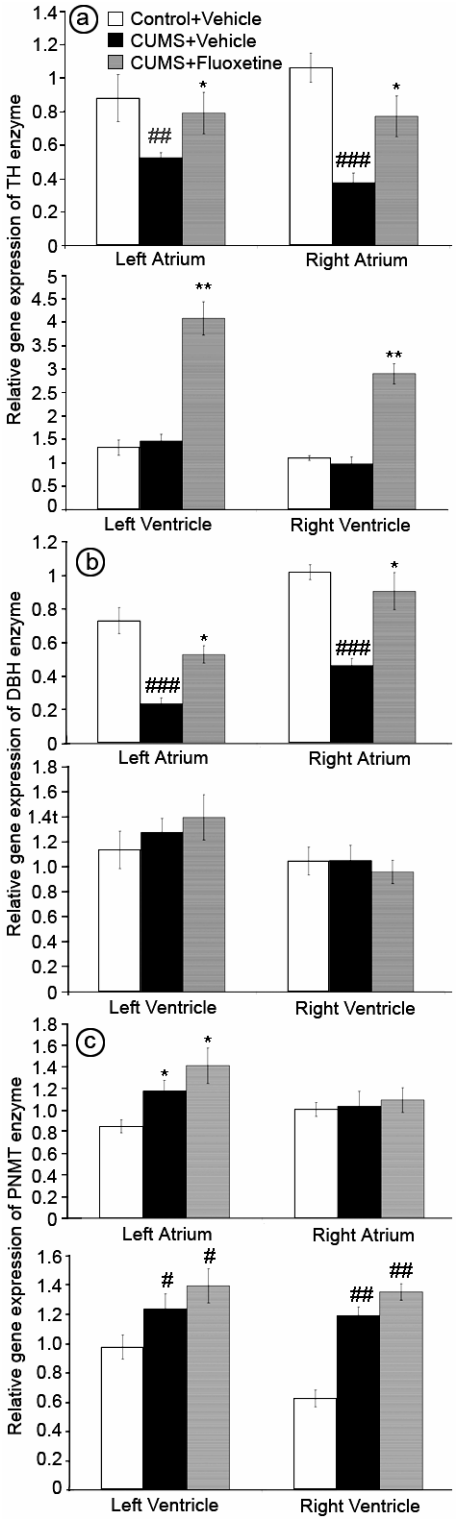

Fig. 1—Effect of chronic treatment of fluoxetine on (a) tyrosine hydroxylase (TH) mRNA levels (b) dopamine-ß-hydroxylase (DBH) mRNA and (c) phenylethanolamine N-methyltransferase (PNMT) mRNA levels in right and left heart atria and ventricle of rats exposed to CUMS for 4 weeks. The values are means ± SE of 6-8 rats. *P* values: #< 0.05; ##<0.01; ###<0.001 CUMS *vs.* unstressed control; *< 0.05; **<0.01 placebo *vs.* fluoxetine (Tukey test).

treatment with the antidepressant expressed a significant effect on relative gene expression of DBH in cardiac ventricles.

Analysis of the data indicated that the applied stressful procedure acted increasing PNMT mRNA levels by 39% in the left, while expressing no effect on this enzyme mRNA levels in right atria in comparison with the unstressed controls. Expression of the PNMT gene was also markedly increased both in the right and left ventricles (by 42 and 19%, respectively). Fluoxetine treatment, however, had no significant influence on the level of PNMT mRNA (Fig. 1c).

Discussion

There are numerous clinical studies demonstrating a significant advantage of SSRI antidepressants in the treatment of cardiovascular diseases. However, studies on the same topic using experimental animals have not been widely conducted. Grippo *et al.*[24] found that administration of fluoxetine to chronically stressed animals, only partially prevented the increase of both sympathetic activation and heart rate. In the present study the effects of chronic stress and fluoxetine treatment were determined on gene expression of three catecholamine synthesizing enzymes in right and left atria and ventricles of adult rats. The results showed that chronic stress led to a decreased TH and DBH mRNA levels both in the right and left heart atria, which might be interpreted as the adaptation of cardiomyocytes and protection against deleterious effects of noradrenaline excess released from stellate ganglia under stress conditions, as indicated by earlier studies[25,26]. In contrast, chronic application of the antidepressant increased the levels of TH and DBH mRNAs in the heart atria of CUMS rats. Fluoxetine had a similar effect in the atria of non-stressed animals[27]. Although SSRIs are potent inhibitors of neuronal serotonin uptake, the ability of fluoxetine to increase the extracellular concentrations of noradrenaline and dopamine, as well as of serotonin has been observed in prefrontal cortex[28,29]. Fluoxetine treatment was also shown to result in increased expression of the TH gene in the locus coeruleus[30,31]. Chronic application of fluoxetine led to an increased expression of these genes in the rat adrenal medulla[32]. It was confirmed that antidepressants could affect the transcription factors. Chronic application of SSRIs was shown to elevate CRE-dependent gene transcription in rat brain, that includes the genes for TH and DBH[33,34]. Shinkai *et al.*[35]

reported that milinacipran, a serotonin/noradrenaline reuptake inhibitor, activates TH through a p44/42 mitogen-activated protein kinase (MAPK)-dependent pathway in bovine adrenal medullary cell cultures. Rapid activation of this signaling pathway was also observed in the rat heart after acute immobilization stress[36]. In addition. Yasumoto *et al.*[37] suggested that fluoxetine has an ability to deplete catecholamines by inhibiting the activity of vesicular monoamine transporter.

Continuous release of catecholamines from vesicles could be the reason for the increased synthesis of noradrenaline in cardiomyocytes. Fluoxetine treatment, however, did not affect the expression of DBH gene in ventricles of stressed rats. Expression levels of DBH mRNA in ventricles were low, making it difficult to determine possible changes after treatment. Further, numerous genes are differentially expressed in mammalian atrial and ventricular cardiocytes, thus providing a better understanding of the molecular differences between these tissues[38,39]. It should be also underlined that fluoxetine did not affect the PNMT gene expression in rat heart. PNMT regulation was shown to be tissue specific. The PNMT gene contains glucocorticoid response elements (GREs). Tai *et al.*[40] reported that glucoccorticoids regulate PNMT gene transcriptionally and posttranscriptionally. Glucocorticoid treatment lowered the level of the intron-containing mRNA, increased the levels of the intronless mRNA, and the PNMT activity in the cardiac ventricle. Thus, unlike the adrenal gland, the heart regulates PNMT levels through alternative splicing[41]. Chronic fluoxetine treatment did not alter plasma corticosterone levels[42] and this could be the reason why a change in the PNMT mRNA levels was not observed.

These results raise the question of consequences which could result at the physiological level. Increased synthesis of catecholamines initiated by the antidepressant, may exert deleterious effects on cardiac structure and function. So far, two clinical studies reported increased blood pressure and heart rate in duloxetine and venlafaxine trials. The proposed mechanism for antidepressant-induced tachycardia is its effects on noradrenaline level[43,44]. Besides, Shyu *et al.*[45] found that noradrenaline at high concentrations stimulated apoptosis in rat neonatal cardiac myocytes *in vitro* and also, Neri *et al.*[46] showed that large catecholamine amounts may induce oxidative damages through reactive intermediates evolving by their auto-oxidation, thus causing a cardiotoxic effect. Therefore, increased gene transcription of TH and DBH in atria, suggesting an elevated noradrenaline synthesis indicates a possible increased risk of heart disease in depressed individuals treated with fluoxetine.

Acknowledgement

This work was supported by the Ministry of Education and Science of the Republic of Serbia, Contract No. 173044.

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