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Flouxetine treatment acts selectively increasing myocardial ß$_1$-adrenoceptor mRNA expression in stress-induced depression

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Changes in gene expression of ß$_1$- and ß$_2$-adrenoceptors (ß$_1$- and ß$_2$-AR) in right and left atria and ventricles after fluoxetine treatment in stress-induced depression of adult rat males were studied. Elevated ß$_1$-AR mRNA levels in the left atria and significantly higher levels of ß$_2$-AR mRNA in the left atria and ventricles were observed in stress-induced depression in comparison with those of unstressed controls. Fluoxetine treatment led to increasing expression of ß$_1$-AR mRNA in the right atria and left ventricles, while the level of ß$_2$-AR mRNA remained unchanged. These findings suggest that fluoxetine therapy plays an important role in cardiac ß-adrenergic subsensitivity and gene regulation of ß-AR in animals with heightened sympathetic nervous activity.

1. Introduction

Chronic stress is a risk factor in the development of numerous psychopathological conditions including depression (Gao et al. 2009), known to be related to an increased risk of developing cardiovascular diseases in humans. Possible pathophysiological mechanisms leading to more frequent complications of coronary heart disease in patients with depression are not fully understood, but could be due, at least partly, to a higher sympatho-adrenergic stimulation. Serotonin reuptake inhibitors are widely used antidepressants for therapeutic treatment of mood disorders. This treatment of depressed patients has been found to lower sympathetic activation (Barton et al. 2007), whereas some authors have reported adverse effects (Dawood et al. 2007). Recently, we demonstrated that long-term fluoxetine treatment resulted in elevated levels of noradrenaline and adrenaline (Dronjak et al. 2007). Also, Blardi et al. (2005) observed increased levels of catecholamines in depressive patients receiving fluoxetine for 40 days. The autonomic nervous system directly regulates the contractility and frequency of heart by chemical signals, including neurotransmitters and hormones. Catecholamines released from autonomic sympathetic fibres produce a positive ionotropic chronotropic response acting *via* stimulation of ß-adrenergic receptors (ß-AR) which, once stimulated by coupling with catecholamines, trigger a series of intracellular reactions. Adrenergic receptors play an important role in physiological responses related to cardiac contraction and excitability. High levels of released catecholamines lead to myocardial ß-AR down-regulation, decrease of their density and desensitisation (Dong et al. 2010). The methods inducing depression-like states in experimental animals are generally accepted as valuable tools for the studies of affective disorders. Among these methods, chronic unpredictable mild stress (CUMS) model of depression has been frequently employed (Willner et al. 1992). Grippo et al. (2006) reported that CUMS model of depression characterises anhedonia and elevated sympathetic cardiac tone and that fluoxetine treatment was effective in preventing anhedonia, although only partially preventing cardiovascular consequences of CUMS. This prompted us to investigate changes in gene expression of ß$_1$- and ß$_2$-AR in right and left atria and venticles of adult rats exposed to CUMS model of depression after repeated treatment with fluoxetine, applying TaqMan RT-PCR assay.

2. Investigations, result and discussion

One-way ANOVA test revealed that CUMS expressed a significant effect on β_1-AR mRNA levels in left atria ($F_{(1,13)} = 12.83$, $p < 0.01$). Also, a significant effect of fluoxetine administration on relative gene expression of β_1-AR mRNA in the right atria ($F_{(1,13)} = 37.49$, $p < 0.001$) and in the left ventricles ($F_{(1,13)} = 15.03$, $p < 0.01$) was observed. *Post-hoc* analysis demonstrated that chronic fluoxetine administration acted increasing the expression of β_1-AR by 139% ($p < 0.001$) in the right atria and by 62% ($p < 0.01$) in the left ventricles of CUMS rats (Fig. 1).

One-way ANOVA test pointed that, while fluoxetine treatment alone expressed no effect, CUMS procedure significantly increased β_2-AR mRNA levels ($F_{(1,12)} = 5.41$, $p < 0.05$) in the left atria ($F_{(1.13)} = 15.83$, $p < 0.01$) and left ventricles (Fig. 2).

In this study, higher levels of ß$_1$-AR mRNA in left atria and significantly higher levels of ß$_2$-AR mRNA both in left atria and venticles were observed in animals exposed to CUMS in comparison with unstressed controls. Wallukat (2002) reported that over-expression of the ß$_1$-AR induced detrimental alterations of cardiac function in transgenic animals which developed heart failure accompanied by an increased mortality rate. Several authors working with transgenic mice demonstrated that over-expression of ß$_2$-AR markedly enhanced cardiac contractility without significant cardiac pathology (Gao et al. 1999; Milano et al. 1994). Fluoxetine treatment of CUMS rats led to

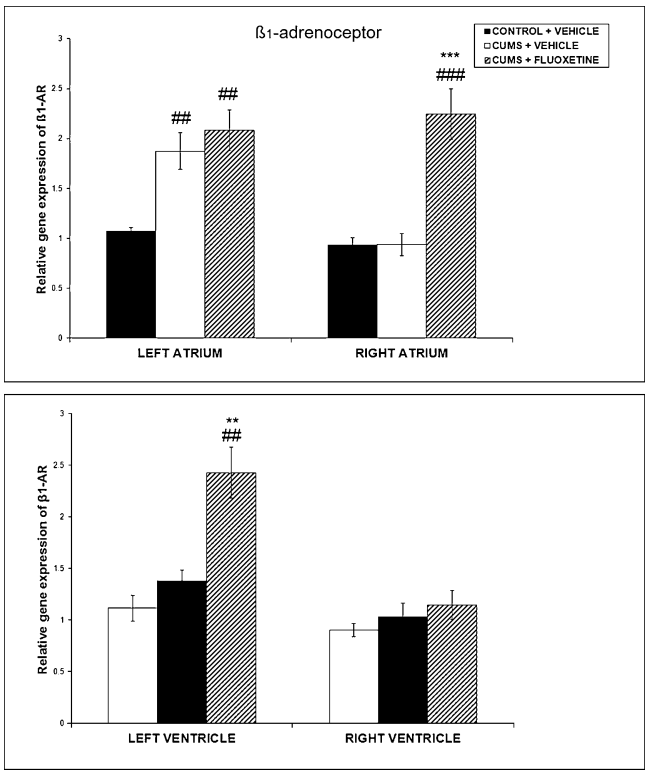

Fig. 1: The effects of chronic fluoxetine treatment on β1– adrenoceptors mRNA levels in left and right cardiac atrium and ventricle of CUMS rats. The values are means ± S.E.M. of 7 rats. Statistical significance: ## $p < 0.01$; ### $p < 0.001$ CUMS vs. control (Tukey-test); **$p < 0.01$; ***$p < 0.001$ fluoxetine vs. vehicle (Tukey-test). The final result was expressed as fold change relative to the calibrator and normalized to cyclophyline A.

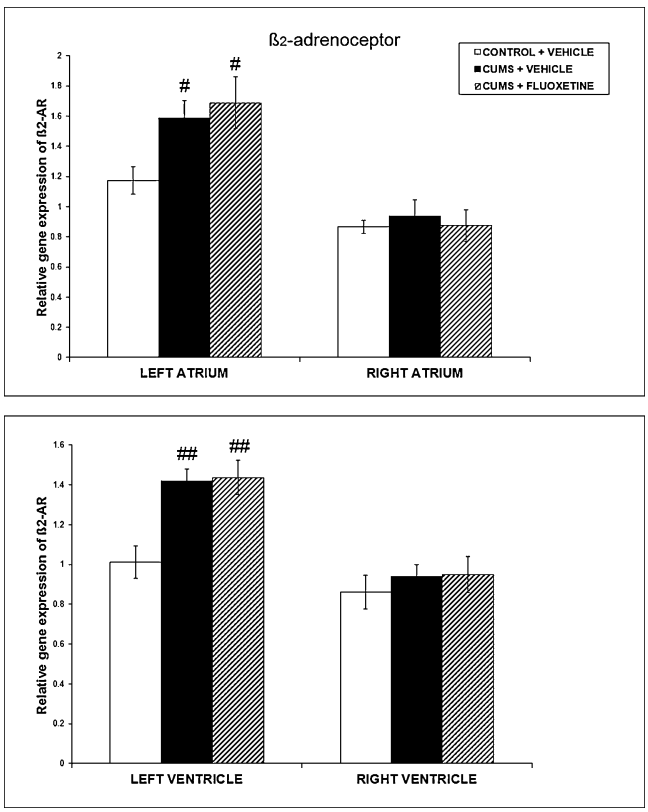

Fig. 2: The effects of chronic fluoxetine treatment on β2– adrenoceptors mRNA levels in left and right cardiac atrium and ventricle of CUMS rats. The values are means ± S.E.M.of 7 rats. Statistical significance: # $p < 0.05$; ## $p < 0.01$ CUMS vs. control (Tukey-test); The final result was expressed as fold change relative to the calibrator and normalized to cyclophyline A.

an increased expression of $ß_1$-AR mRNA in right atria and left venticles, whereas the level of $ß_2$-adrenoceptor mRNA remained unchanged. These findings suggest that fluoxetine treatment plays an important role in ß-adrenergic subsensitivity and gene regulation of ß-AR in animals with heightened sympathetic nerves activity. This gene regulation is subtype-selective and leads to increased steady-state levels of $ß_1$- but not of $ß_2$-AR mRNAs. As to our knowledge, this is the first report on the regulation of cardiac ß-AR at the mRNA level during fluoxetine treatment in stress-induced depression of adult rat males. The question arises why fluoxetine administration expressed no effect on $ß_2$-AR mRNA. The reason for that might be ascribed to the fact that glucocorticoids could affect expression of $ß_2$-AR through GRE sequences in promoter region. Myslivecek et al. (2003) reported a higher $ß_2$-AR density in ventricles of hydrocortisone-treated rats. In our earlier experiments, we demonstrated elevated plasma corticosterone in rats exposed to CUMS, while fluoxetine treatment acted decreasing the level of this hormone (Dronjak et al. 2007). The signal mediating the increase of specific $ß_1$-AR mRNA levels is still unknown. Activation of the ß-adrenergic system, specifically of adenylyl cyclase at the postreceptor level, may contribute to an increase of $ß_1$-AR mRNA levels as suggested by Strasser et al. (1990). This sensitisation of adenylyl cyclase is accompanied by a transient increase of intracellular cAMP levels, which may induce ß-AR transcription (Collins et al. 1989). The subtype-selective increase of $ß_1$-AR mRNA levels during fluoxetine treatment could be due to a transcriptional regulation. Alternately or additionally, the stability of mRNA may be regulated, as well. cAMP has been reported to influence both processes, *i.e.* to induce transcription and to stabilise specific mRNAs against degradation, as already shown for various enzymes (Hod and Hanson 1988). Further experiments are necessary to confirm our results and hypotheses and to determine the physiological consequences of the findings presented here. Although the molecular basis of the gene regulation of cardiac ß-AR in stress-induced depression during fluoxetine treatment remains to be elucidated, increased transcription of $ß_1$-AR provides an experimental opportunity to probe into further molecular mechanisms underlying the regulation of this receptor subtype.

3. Experimental

3.1. Animals and study design

Adult Wistar rat males weighing 280–320 g at the onset of experiments and maintained in a temperature-controlled room (21 ± 1.0 °C) and 12 h/12 h light/dark cycle, were used. Care was taken to minimise 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 subjected to CUMS according to the method by Grippo et al. (2006), received daily injections of vehicle (sterile water) or fluoxetine (10 mg/kg) by *i.p.* route for 4 weeks. Animals in the control group received daily injections of vehicle. After that, the animals exposed to CUMS and the corresponding controls were decapitated, the right and left cardiac atria and ventricles rapidly dissected, frozen in liquid nitrogen and stored at −70 °C until analysed.

3.2. 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.

3.3. Real-time RT-PCR

TaqMan PCR assays were carried out using Assay-on-Demand Gene Expression Products (Applied Biosystems, USA) for $β_1$–AR (Rn

00824536_s1) and β_2–AR (Rn 00560650_s1). 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 the ABI Prism 7000 Sequence Detection System at 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °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 normalised to cyclophyline A (ID:Rn 00690933) expression. Quantification was done using the $2^{-\Delta\Delta Ct}$ method according to Livak and Schmittgen (2001).

The results are reported as means ± S.E.M. Significance of the differences between the groups in gene expression levels of the examined ß-AR were estimated by One-way ANOVA test, followed by the Tukey *post hoc* test. Statistical significance was accepted at $p < 0.05$.

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