

REGULATION OF CATECHOLAMINE-SYNTHESISING ENZYMES AND β -ADRENOCEPTORS GENE EXPRESSION IN VENTRICLES OF STRESSED RATS

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Short title: Catecholamine enzymes and β -adrenoceptors in stressed rats

Summary

Stress exposure activates the sympathoneural system, resulting in catecholamine release. Chronic stress is associated with development of numerous disorders, including cardiovascular diseases. Here we investigated the expression of mRNAs for catecholamine biosynthetic enzymes tyrosine-hydroxylase, dopamine- β -hydroxylase and phenylethanolamine N-methyltransferase, and for β_1 - and β_2 -adrenoceptors in the right and left ventricles of rats exposed to chronic unpredictable mild stress. The tyrosine-hydroxylase and dopamine- β -hydroxylase mRNA levels were not affected by stress, whereas the phenylethanolamine N-methyltransferase mRNA levels significantly increased in both right and left ventricles. No changes in β_1 -adrenoceptor mRNA levels in either right or left ventricles were observed. At the same time, stress produced a significant increase of β_2 -adrenoceptor mRNA levels in left ventricles. These results suggest that elevated expression of phenylethanolamine N-methyltransferase in both ventricles and β_2 -adrenoceptor genes in left ventricles could provide a molecular mechanism that leads to altered physiological response, which is important for the organism coping with stress.

Key words: chronic stress, ventricles, catecholamine enzymes, β -adrenoceptors, gene expression

Introduction

Clinical studies have confirmed the correlation of stressful events and pathophysiology observed in depression (Kendler *et al.* 1999). Numerous authors reported that depression and chronic stress are common causes of cardiovascular diseases (Brosschot *et al.* 2007; Whang *et al.* 2009). Also, it has been shown that some individuals are more vulnerable to mild but prolonged stressors (Kessler 1997; Hammen 2005) and chronic unpredictable mild stress (CUMS) became one of widely used stress models. Recently, we demonstrated that rats subjected to CUMS evoked depression-like behavior and had elevated plasma levels of corticosterone (Dronjak *et al.* 2007; Spasojevic *et al.* 2008). Others have found that rats subjected to CUMS had increased blood pressure and tachycardia, making this model suitable for the studies of stress-induced cardiovascular diseases (Grippeo *et al.* 2002).

Changes in neuroendocrine function may play a role in linking responsiveness to stressors and cardiovascular function. The evidence suggests that altered activity of autonomic nervous system in depressed patients might be responsible for the increased risk of mortality in individuals with cardiovascular diseases. High plasma catecholamine levels were confirmed as a significant risk predictors of cardiovascular mortality (Peng *et al.* 2006). Catecholamines increase the cardiac output through their positive effects on chronotropism, inotropism and lusitropism, and also through control of cardiomyocyte growth and death, thus contributing to cardiac remodeling (Santos and Sadari-Bratfisch 2006). Recently, the cells exhibiting all monoaminergic features were described in the heart of both adult rodents and humans (Huang *et al.* 1996). These cells might help to regulate cardiac function in parallel with the autonomic nervous system. They are capable of catecholamine biosynthesis in order

to maintain myocardial contractility in the resting state and during stress (Zhuang *et al.* 2005).

The effects of chronic stress are associated with alterations in gene expression of catecholamine biosynthetic enzymes: tyrosine hydroxylase (TH), dopamine- β -hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT). According to previous studies, the PNMT gene expression is localised not only in cardiac ganglion cells, but also in a wide range in cardiomyocytes (Kennedy *et al.* 1995). Recently, we have found that TH and DBH mRNAs are present in the ventricles of adult rats (Gavrilović *et al.* 2010). Sympathetic nervous system plays a role in the control of cardiac function by stimulating adrenergic receptors (ARs). Both β_1 - and β_2 -subtypes of these receptors are present in healthy human heart in the ratio of approximately 5:1 (Bengel and Schwaiger 2004). Their stimulation results in increased heart rate and force of contraction. Brunner-La Rocca *et al.* (2001) also demonstrated that chronic adrenoceptor stimulation could have a direct cardiotoxic effect.

The aim of the present study was to determine whether the stress-induced depression leads to changes in gene expression of three catecholamine biosynthetic enzymes and two β -adrenoceptors in right and left cardiac ventricles of adult rats, that could affect normal cardiovascular function.

Material and methods

Animals

Adult, 11-week-old Wistar rat males, maintained under standard laboratory conditions with water and food *ad libitum* in the groups of four individuals *per cage* were used. All experiments were conducted in accordance with the rules of animals

care proposed by the Serbian Laboratory Animal Science Association. In these experiments, 16 rats were used. One group of animals was subjected to CUMS for 4 weeks. The CUMS procedure, a slight modification of the method by Grippo *et al.* (2002) was designed to maximize the unpredictable nature of the stressors. The CUMS groups were exposed to the following stressors at random order: continuous illumination (24 h), continuous darkness (24 h), 40° cage tilt along the vertical axis (17 h), crowding (8 rats *per* cage, 24 h), soiled cage (300 ml water spilled onto the bedding, 17 h), restraint in a small cage (1 h), cold room (4 °C, 7 h), individual housing (24 h), forced running (15 min) and food and water deprivation (7 h). Animals were also maintained on a reversed light/dark cycle from Friday evening to Monday morning. The CUMS procedure applied for a single week was repeated during the following 4 weeks. After that, the animals exposed to CUMS and the corresponding controls were decapitated, the right and left heart ventricles rapidly dissected, frozen in liquid nitrogen and stored at -70 °C until analyzed.

Real-time RT-PCR

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. TaqMan PCR assays were carried out using Assay-on-Demand Gene Expression Products (Applied Biosystems, USA) for TH (Rn00562500_m1), DBH (Rn00565819_m1), PNMT (Rn01495589_g1), β_1 -AR (Rn 00824536_s1), and β_2 -AR (Rn 00560650_s1) The reactions were performed in a 25 μ l 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 sec 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 normalized to cyclophilin A (ID:Rn 00690933) expression. Quantification was done using the $2^{-\Delta\Delta C_t}$ method according to Livak and Schmittgen (2001).

Statistical analysis

The results are reported as means \pm S.E.M. Significance of the differences in gene expression levels were estimated by two-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

CUMS did not lead to significant changes in relative gene expression of TH and DBH in the rat ventricles. The relative gene expression of TH enzyme in right ventricle was 1.09 ± 0.07 in controls and 0.97 ± 0.15 in stressed animals and in left ventricle was 1.31 ± 0.17 in controls and 1.45 ± 0.15 in stressed animals. The relative DBH-mRNA levels in left and right ventricles of control animals was 1.04 ± 0.13 and 1.05 ± 0.11 , respectively, whereas in stressed animals it was 1.17 ± 0.11 and 1.14 ± 0.14 . In contrast, *post-hoc* analysis showed that this stressful procedure increased the PNMT mRNA levels both in the right and left ventricles (by 42%, $p < 0.01$ and 19%, $p < 0.05$, respectively) (Fig. 1).

The gene expression of β_1 -ARs remained unchanged in the ventricles after 4 weeks of exposure to CUMS (Fig. 2). However, a significant difference in β_2 -AR

gene expression between right and left ventricles ($F_{(1,16)}=18,82$, $p<0.001$) was observed. Chronic stress increased the expression of β_2 -AR by 40% ($p < 0.01$) in the left ventricles, but expressed no significant effect on gene expression of this receptor in the right ventricles (Fig. 3).

Discussion

In the present study, we investigated changes in gene expression of three catecholamine synthesising enzymes in right and left ventricles of rats exposed to CUMS for 4 weeks. Our results demonstrated that CUMS did not affect the levels of TH and DBH mRNAs, while increasing the level of PNMT mRNA in the heart ventricles. Single or repeated 7-day-immobilisation of adult rats also produced an increase of PNMT mRNA levels both in heart atria and ventricles (Kvetnansky *et al.* 2004; Micutkova *et al.* 2004). According to Kuroko *et al.* (2007), myocardial ischemia leads to accumulation of large noradrenaline amounts released from sympathetic nerve endings in interstitial space. This results in the stimulation of increased noradrenaline reuptake in cardiomyocytes, as a form of defense against excessive stimulation of β -ARs, but also in the stimulation of adrenaline synthesis through PNMT activity. Kaye *et al.* (1995) confirmed previously possible non-neuronal origin of cardiac adrenaline synthesis and release, which could further facilitate toxic effects and lead to the development of arrhythmia in the ventricles. It was noticed that after myocardial infarction, plasma and ventricular adrenaline levels were markedly increased (Ganguly *et al.* 1997). Neri *et al.* (2007) showed that large catecholamine amounts may induce oxidative damages through reactive intermediates evolving by their auto-oxidation, independently of their effects on the receptors, thus causing a cardiotoxic effect.

Released catecholamines act increasing contractility in atria and ventricles through their binding to postsynaptic β -receptors that have chronotropic, dromotropic and inotropic effects (Riemann *et al.* 2003; Bengel and Schwaiger 2004). In our experiments, a significant increase of β_2 -ARs mRNA levels was recorded in left ventricles of rats exposed to CUMS for 4 weeks. Also, these animals were shown previously to have elevated plasma corticosterone levels (Dronjak *et al.* 2007). Glucocorticoids are known to affect expression of these receptors through GRE sequences in promotor region. Mysliveček *et al.* (2003) observed that rats treated with hydrocortisone had a higher density of β_2 -ARs in the ventricles comparing to the corresponding control. Activation of cardiac β_2 -ARs may contribute to an additional increase in heart rate and/or contractility (Khamssi and Brodde 1990). Ventricular cardiomyocytes stimulation of adult rat β_2 -adrenoceptors was reported to inhibit apoptosis (Communal *et al.* 1999). Liggett *et al.* (2000) demonstrated that heart tolerates enhanced contractile function *via* 60-fold β_2 -AR overexpression without detriment, but higher levels of expression lead to cardiomyopathy. Also, chronic β_2 -AR stimulation with a selective agonist elicits a significant cardiac hypertrophy and impaired cardiac function (Gregorević *et al.* 2005). Therefore, a small increase in expression of this receptor, observed in CUMS animals, may play a protective role.

However, prolonged exposure to stress could express detrimental cardiovascular effects. Our results showed that despite identical changes in the ventricular expression of catecholamine synthesising enzymes, changes in β -receptor mRNA levels were visible only in the left ventricles. This is in accordance with the report of Wang *et al.* (2006) who also observed different response of the same receptor class in the left and right ventricles. While α_1 -AR stimulation leads to negative inotropic effect in the right ventricle, it expressed a positive inotropic effect

in the left ventricles. Besides, these results may also indicate a higher sensitivity of the left ventricles to stress comparing to that of the right ventricles. Yalçın *et al.* (2010) have shown that there is a regional left ventricular hypercontractility and dysfunction in response to acute and chronic stress. In addition, Li *et al.* (2001) reported that the noradrenaline transporter expression is higher in the right cardiac sympathetic ganglia of adult rats than in the left ones. Therefore, a greater capacity of noradrenaline uptake in the right ventricles may contribute to the maintenance of right ventricular function under pathological conditions.

In conclusion, our results indicate that CUMS causes elevated expression of PNMT-mRNA in both ventricles and β_2 -AR-mRNA in left ventricles. It is reasonable to suggest that such gene and tissue specific response could be of the physiological relevance for the organism coping with stress. However, prolonged exposure to stress can result in pathophysiological changes of heart. Therefore, these results also provide an insight into the molecular mechanisms that may indicate a connection between altered mood and cardiovascular dysfunction.

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LEGENDS FOR FIGURES

Fig. 1. Chronic unpredictable mild stress-related (CUMS) changes in phenylethanolamine N-methyltransferase (PNMT) mRNA levels in right and left ventricle of adult rat males. The values are means \pm S.E.M. of 6-8 rats. Statistical significance: * $p < 0.05$; ** $p < 0.01$ (Tukey test) CUMS vs. control. The final result was expressed as fold change relative to the calibrator and normalized to cyclophyline A.

Fig. 2. Chronic unpredictable mild stress-related (CUMS) changes in β_1 -adrenoceptors mRNA levels in right and left ventricle of adult rat males. The values are means \pm S.E.M. of 6-8 rats. The final result was expressed as fold change relative to the calibrator and normalized to cyclophyline A.

Fig. 3. Chronic unpredictable mild stress-related (CUMS) changes in β_2 -adrenoceptors mRNA levels in right and left ventricle of adult rat males. The values are means \pm S.E.M. of 6-8 rats. Statistical significance: ** $p < 0.01$ (Tukey test) CUMS vs. control. The final result was expressed as fold change relative to the calibrator and normalized to cyclophyline A.

Fig 1.

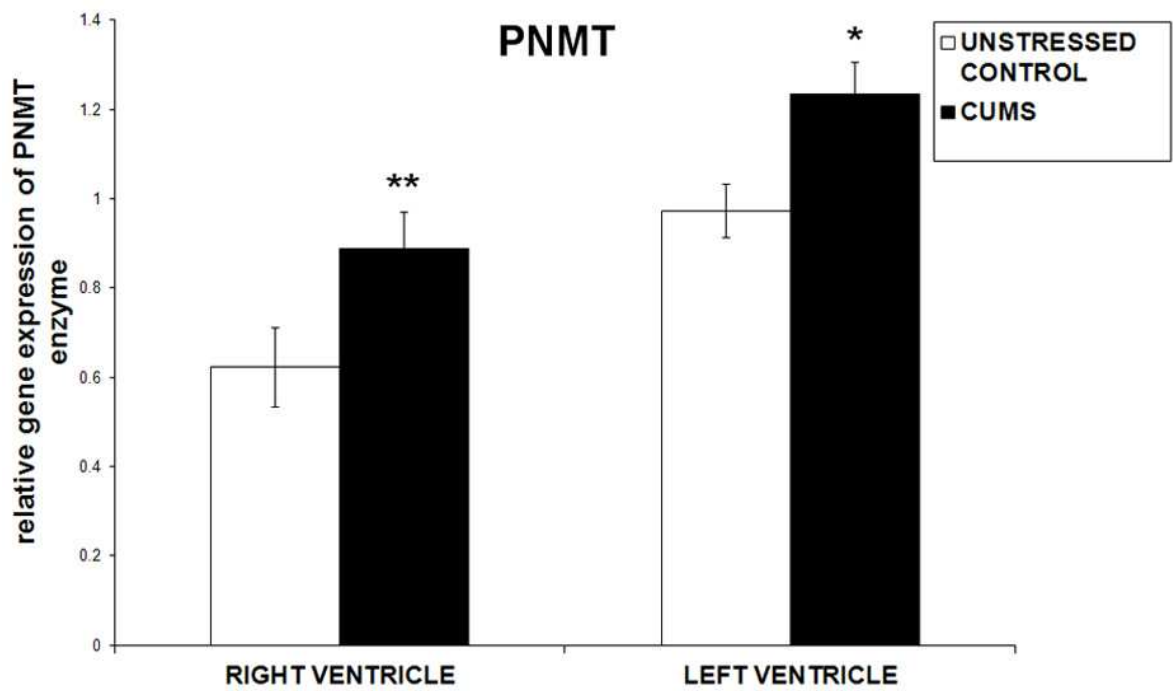


Fig. 2.

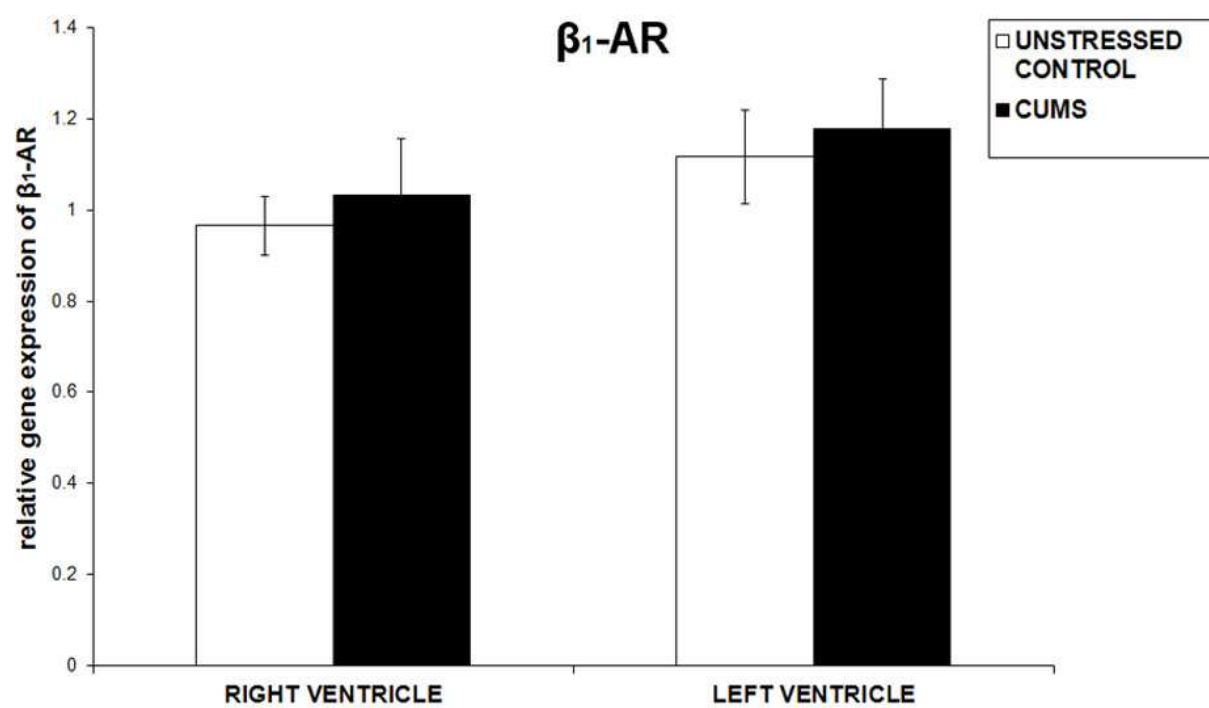


Fig. 3.

