Gene Expression of the Phenylethanolamine N-Methyltransferase is Differently Modulated in Cardiac Atria and Ventricles

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Abstract. Phenylethanolamine N-methyltransferase (PNMT) is a final enzyme in catecholamine synthesizing cascade that converts noradrenaline to adrenaline. Although most profuse in adrenal medulla, PNMT is expressed also in the heart, particularly in cardiac atria and ventricles. In atria, the PNMT mRNA is much more abundant compared to ventricles. In present study we aimed to find out whether there is a difference in modulation of the PNMT gene expression in cardiac atria and ventricles. We used three methodological approaches: cold as a model of mild stress, hypoxia as a model of cardiac ischemic injury, and transgenic rats (TGR) with incorporated mouse renin gene (mREN-2)27, to determine involvement of renin-angiotensin pathway in the PNMT gene expression. We have found that PNMT gene expression was modulated differently in cardiac atria and ventricles. In atria, PNMT mRNA levels were increased by hypoxia, while cold stress decreased PNMT mRNA levels. In ventricles, no significant changes were observed by cold or hypoxia. On the other hand, angiotensin II elevated PNMT gene expression in ventricles, but not in atria. These results suggest that PNMT gene expression is modulated differently in cardiac atria and ventricles and might result in different physiological consequences.

Key words: PNMT — Cardiac atria — Cardiac ventricles — Hypoxia — Cold stress — Renin

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Introduction

Catecholamines are proposed to play a crucial role in augmenting cardiac function. The most important regulator of cardiac function is noradrenaline, since it is a main mediator of the sympathetic nerve's involvement in the heart (Esler et al. 1990). Majority of the noradrenaline in the blood plasma is released from the sympathetic neuronal endings in the whole body, while bulk of the adrenaline is released from the adrenal medulla (Esler et al. 1990). In the heart, most of the adrenaline originates from the circulation, from where it is trapped by sympathetic endings. Nevertheless, synthesis of the adrenaline was proved also in the rat heart (Elayan et al. 1990; Kennedy and Ziegler 2000).

Phenylethanolamine N-methyltransferase (PNMT, EC 2.1.1.28) is the terminal enzyme of the catecholaminergic pathway converting noradrenaline to adrenaline. Although PNMT's activity is largely restricted to the adrenal medulla, where a big portion of body's adrenaline is synthesized, low PNMT activities were determined in various tissues, e.g. heart (Ebert et al. 1996; Kennedy and Ziegler 2000), spleen (Pendleton et al. 1978; Kennedy et al. 1995), kidney (Kennedy et al. 1995), lung, thymus and different parts of brain (Pendleton et al. 1978). In majority of these tissues, gene expression of the PNMT was also detected (Andreassi et al. 1998; Krizanova et al. 2001; Jelokova et al. 2002; Warthan et al. 2002; Kubovcakova et al. 2006).

The PNMT mRNA levels are highly modulated by glucocorticoids, since the PNMT promoter contains glucocorticoid responsive elements (GREs) (Sabban and Kvetnansky 2001). Besides GREs, several AP2 motifs and two overlapping SP1/EGR1 motifs are also located in PNMT promoter region. Interestingly, synergistic activation of PNMT promoter-given reporter activity by EGR1, AP2 and glucocorticoid receptor has been observed (Wong et al. 1998). We have already shown that response of PNMT gene expression in adrenalectomized and subsequently immobilized rats is different in atria and ventricles (Krizanova et al. 2001). We proposed that glucocorticoid regulation in the PNMT promoter region in atria and ventricles might be different.

Angiotensin II stimulates catecholamine secretion from adrenal medulla by increasing activity of two catecholamine biosynthetic enzymes, tyrosine hydroxylase and PNMT (Stachowiak et al. 1990). Long-term increases in the activities of these enzymes produced by angiotensin could result from their enhanced synthesis, as indicated by the increases in relative abundances of tyrosine hydroxylase mRNA and PNMT mRNA (Stachowiak et al. 1990). These results support the evidence of the control of secretory responses of adrenal medulla by angiotensin in a long-term manner.

Based on our previous observation on different regulation of the PNMT gene expression in rat cardiac atria and ventricles of adrenalectomized and immobilized rats, the objective of this study was to determine possible differences in the modulation of PNMT mRNA levels in cardiac atria and ventricles by other stimuli.

We also tested possible involvement of renin-angiotensin pathway on PNMT gene expression.

Materials and Methods

Cold stress

Male Sprague-Dawley rats (cca 250 g, Charles River, Suzfeld, Germany) 3 months old were used. Prior to experiments, animals were housed at least 1 week, four animals *per* cage in a controlled environment (22±2℃, 12 h light/dark cycle, lights on at 6.00 a.m.). Food and water were available *ad libitum*. The Ethic Committee of the Institute of Experimental Endocrinology (SAS, Bratislava, Slovakia) approved all presented experiments.

As a model of cold stress, animals were subjected to the cold room at constant temperature 4℃ for 7 days. Control animals were kept at room temperature. Afterwards, rats were sacrificed by decapitation, hearts were rapidly removed, left atria (LA) and left ventricles (LV) withdrawn and immediately frozen in liquid nitrogen and stored at −70℃ until the assay.

Hypoxia

Ten male mice C57B1/129SV (25–30 g, approximately 2 months old) were used in the experiment. Hypoxic stress was performed in a hypoxic chamber, where 5 mice were exposed to 8% oxygen (equivalent to oxygen content in 2500 m beyond the see level) for 6 h, while other 5 mice were kept in normoxic environment and served as a control. Afterwards, mice were sacrificed by decapitation, hearts were rapidly removed, LA and LV withdrawn and immediately frozen in liquid nitrogen and stored at −70℃ until the assay.

Transgenic rats (mREN-2)27

For studies of the renin-angiotensin pathway's involvement, transgenic rats (TGR) (mREN-2)27 were used. This strain has been established by introducing the murine renin *Ren-2* gene into the genome of the rat with the use of microinjection techniques (Mullins et al. 1990). In heterozygous male TGR (mREN-2)27, systolic blood pressure increases at 5 weeks of age and maximum systolic values (240 mm Hg) are reached at 10 weeks of age. Homozygous TGR (mREN-2)27 rats develop even higher blood pressure values reaching 300 mm Hg and exhibit a high mortality rate if not treated with antihypertensive medication (Langheinrich et al. 1996). Therefore, in our experiments we used heterozygous male TGR (mREN-2)27 rats which, together with control Sprague-Dawley rats, were obtained from the Institute of Clinical and Experimental Medicine (Prague, Czech Republic) and breeding pairs came from the Max-Delbruck-Center of Molecular Medicine (Berlin, Germany). Rats were sacrificed at the age of 11 weeks.

RNA isolation and relative quantification of mRNA levels by reverse transcription with subsequent polymerase chain reaction (RT-PCR)

Total RNA from frozen heart tissue and cardiomyocytes was isolated by TRI Reagent method (Molecular Research Center Ltd.). The purity and integrity of isolated RNAs was checked on GeneQuant Pro spectrophotometer (Amersham Biosciences Ltd., UK). Reverse transcription was performed using 2 μg of total RNAs and Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences Ltd., UK) with pd(N_6) primer (Amersham Biosciences Ltd., UK). PCR specific for PNMT was carried out as described previously (Krizanova et al. 2001) using primers PT1: 5′-TAC CTC CGC AAC AAC TAC GC-3′ (position 1,171–1,190) and PT2: 5′-AAG GCT CCT GGT TCC TCT CG-3′ (position 1,904–1,923), yielding a 260-bp fragment. As a housekeeper of the gene control for semi-quantitative evaluation of PCR glyceraldehyde 3-phosphate dehydrogenase (GAPDH), primers GA1: 5′-AGA TCC ACA ACG GAT ACA TT-3′ and GA2: 5′-TCC CTC AAG ATT GTC AGC AGC AA-3′ were used to amplify a 309-bp fragment from each first strand sample. For hypoxia, mitochondrial ribosomal protein L (MRPL) housekeeper (MRPL1: 5′-GGGATTTGCATTCAGAGATCAG-3′ and MRPL2: 5′-GGAAGGGCATCTCGAAAG-3′; 182 bp product) was used. PCR products were analyzed on 2% agarose gels.

Statistical analysis

Each value represents the average of 5–6 animals. Results are presented as means ± S.E.M. Statistical differences among groups were determined by one-way analysis of variance (ANOVA). Statistical significance $p < 0.05$ was considered to be significant (Statistica, version 7, StatSoft, CZ).

Results

Seven days exposure to cold significantly decreased PNMT mRNA levels in the LA (from 0.46 ± 0.03 a.u. in controls to 0.33 ± 0.04 a.u. in cold stressed rats; $p < 0.01$) and also in the right atrium (RA; from 0.41 ± 0.02 a.u. in controls to 0.23 ± 0.03 a.u. in cold stressed rats, $p < 0.001$; Figure 1). No significant difference was observed in the LV (from 0.4 ± 0.05 a.u. in controls to 0.38 ± 0.04 a.u. in stressed rats, $p =$ n.s.) and right ventricle (RV; from 0.34 ± 0.05 a.u. in controls to 0.36 ± 0.05 a.u. in stressed rats, $p =$ n.s.; Figure 1).

Hypoxia for 6 h significantly increased PNMT mRNA levels in LA and RA compared to normoxic controls (LA: 0.20 ± 0.06 a.u. in normoxic controls and 0.56 ± 0.05 a.u. in hypoxic rats, $p < 0.001$; RA: 0.24 ± 0.01 a.u. in normoxic controls and 0.42 ± 0.03 a.u. in hypoxic rats, $p < 0.001$; Figure 2). Nevertheless, PNMT mRNA levels in ventricles were not affected by hypoxic stimulus (LV: 0.24 ± 0.01 a.u. in normoxic controls and 0.26 ± 0.05 a.u. in hypoxic rats, $p =$ n.s.; RV: 0.28 ± 0.02 a.u. in normoxic controls and 0.24 ± 0.02 a.u. in hypoxic rats, $p =$ n.s.; Figure 2).

Involvement of renin-angiotensin system in a modulation of the PNMT gene expression was tested using TGR (mREN-2)27. No significant changes in the

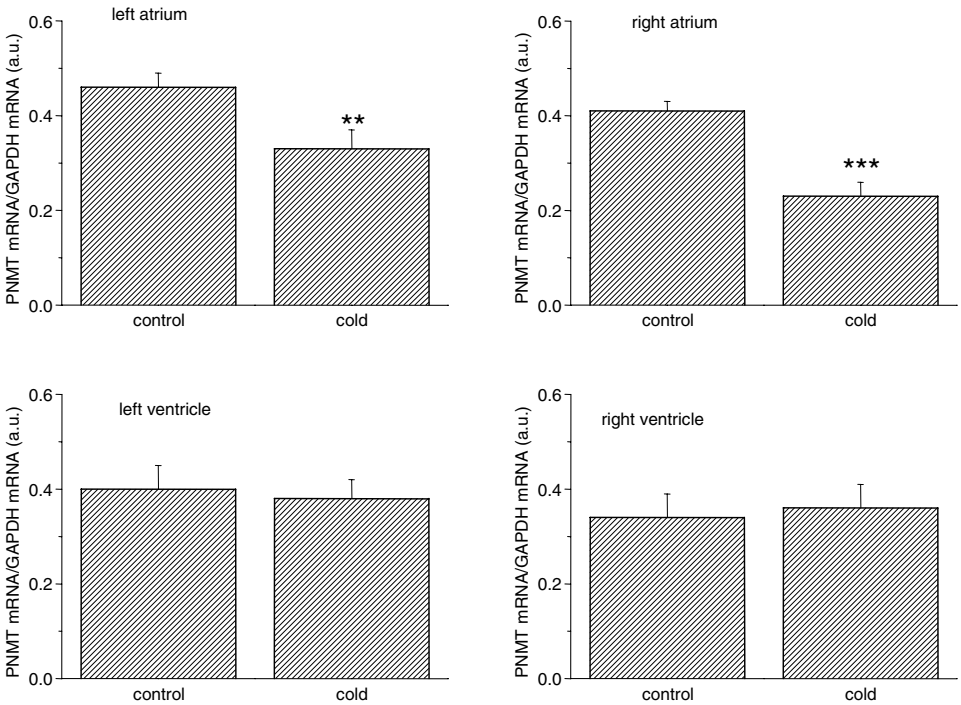

Figure 1. Gene expression of the PNMT in cardiac atria and ventricles in control conditions and after the 7-days exposure to cold. In both left and right atrium, PNMT mRNA levels were significantly decreased after the exposure to cold compared to controls. No changes were observed in left and right ventricle. Results are displayed as mean ± S.E.M. and each column represents an average of 6 animals. ** $p < 0.01$, *** $p < 0.001$.

PNMT mRNA levels were observed in atria (LA: 0.81 ± 0.06 a.u. in controls and 0.87 ± 0.05 a.u. in TGR; RA: 0.53 ± 0.05 a.u. in controls and 0.61 ± 0.05 a.u. in TGR; Figure 3). However, rapid increase was observed in LV and RV (LV: 0.52 ± 0.04 a.u. in controls and 0.83 ± 0.04 a.u. in TGR, $p < 0.01$; RV: 0.58 ± 0.04 a.u. in controls and 0.90 ± 0.06 a.u. in TGR, $p < 0.01$; Figure 3).

We also tried to determine protein levels by Western blot analysis using specific antibody against PNMT (Protos Biotech Corporation, NY, USA). Although we got a nice signal for adrenal medulla as a positive control, no signal was observed for cardiac tissue (not shown), probably because PNMT protein levels in the heart are below the detection limit.

Discussion

Recently it has been accepted that PNMT is expressed also in the heart. First studies describing cardiac PNMT mRNA levels were performed in fetal rat hearts

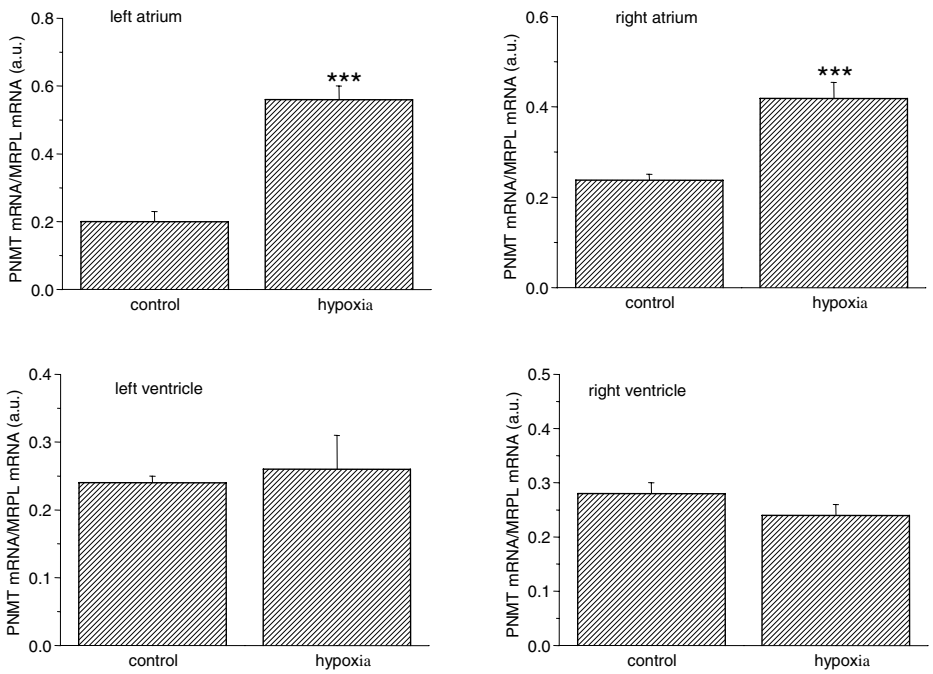

Figure 2. Gene expression of the PNMT in cardiac atria and ventricles in normoxic conditions and after the 6-h exposure to hypoxia. In both left and right atrium, PNMT mRNA levels were significantly increased after the exposure to hypoxia compared to controls. No changes were observed in left and right ventricle. Results are displayed as mean ± S.E.M. and each column represents an average of 5 animals. *** $p < 0.001$.

(Ebert et al. 1996), where the PNMT mRNA was found in special cardiac cells, capable of adrenergic paracrine signaling in mammalian hearts – intrinsic cardiac adrenergic cells (Huang et al. 1996). These cells are specialized nonneuronal cardiocytes, which might provide an alternative adrenergic supply to maintain cardiac contractile and pacemaker function at rest and during stress in the absence of sympathetic innervation (Zhuang et al. 2005). In 2001 we have shown for the first time that PNMT mRNA is expressed in both cardiac atria and ventricles of adult rats (Krizanova et al. 2001), with much higher predominance in atria than in ventricles. Since cardiac atria are rich in cardiac neuronal ganglia, we proposed that majority of PNMT mRNA comes from neuronal cells in the heart. Presence of PNMT in intrinsic cardiac neurons could point to a role of adrenaline in the neural integration, which occurs in the local cardiac ganglia (Slavikova et al. 2003). Nevertheless, we have found PNMT mRNA levels in the punches from heart after the heart transplantation, where no innervation occurs (Goncalvesova et al. 2004). Also, dual origin of the cardiac PNMT mRNA could be supported by a fact that

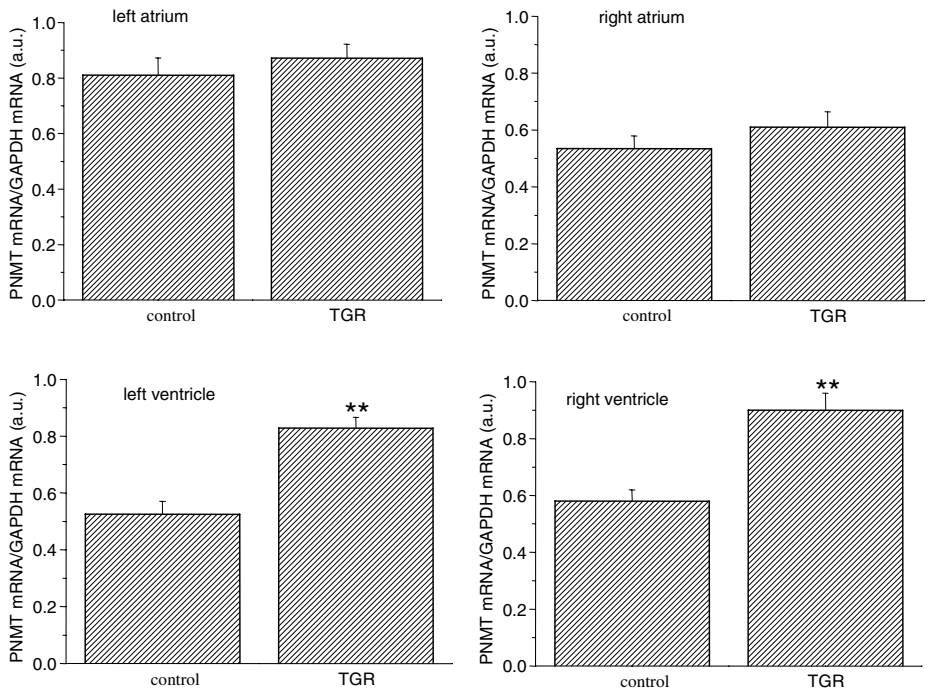

Figure 3. Gene expression of the PNMT in cardiac atria and ventricles in control rats and TGR (mREN-2)27 rats with incorporated murine renin gene. In both left and right atrium, no significant changes in PNMT mRNA levels were observed. In left and right ventricle, significantly higher levels of PNMT mRNA were observed in TGR (mREN-2)27, compared to control rats. Results are displayed as mean ± S.E.M. and each column represents an average of 6 animals. ** $p < 0.01$.

immobilization stress provoked different effect on the PNMT mRNA in ganglionic and nonganglionic part of atrium (Kvetnansky et al. 2004). Very recently we have shown that PNMT mRNA is expressed also in cardiomyocytes (Tillinger et al. 2006). Taking together, it becomes clear that PNMT in the heart is expressed in at least two different types of cells in the heart. Our further experiments were based on this observation. We proposed that modulation of the PNMT gene expression might differ according to its source. Therefore, we applied two different models of stress – cold and hypoxia – and we compared modulation of the PNMT mRNA levels with corresponding controls. We have found that cardiac atria were vulnerable to both stress stimuli, while cardiac ventricles did not respond to neither cold exposure, nor to hypoxia. Differences in the response of the PNMT mRNA to both these stimuli in cardiac atria might be due to activation of different pathways by cold and hypoxia. Thus, from these results it is highly probable that modulation of the PNMT gene expression in atria and ventricles might be also different. This

proposal is in accordance with our previous observations, where we have already shown that depletion of glucocorticoids by adrenalectomy prevents immobilization-induced increase in PNMT mRNA in atria, but not in ventricles (Krizanova et al. 2001). Thus, glucocorticoids are involved in immobilization-induced increase in the PNMT mRNA in atria, but not in ventricles.

There is ample evidence indicating that renin-angiotensin system may play a role in reflex stimulation of the sympathoadrenal catecholamine cells. Angiotensin has been shown to enhance catecholamine secretion by acting directly on sympathetic neurons (Peach 1977; Kawasaki et al. 1982). Stachowiak et al. (1990) have found that angiotensin increases activities of two major catecholamine biosynthetic enzymes – tyrosine hydroxylase and PNMT. Long-term increase in PNMT produced by angiotensin could result from its enhanced synthesis, as indicating by the increase in relative abundances of PNMT mRNA in adrenal medulla (Stachowiak et al. 1990). In the heart, significant increase in PNMT gene expression due to increased angiotensin II production was observed in ventricles, but not in cardiac atria.

In summary, these results support our previous hypothesis about different modulation of the PNMT gene expression in atria and ventricles. Physiological relevance of our observation is not known yet. Nevertheless, it seems that in atria, PNMT mRNA is much more vulnerable to different stress stimuli and thus might cope with equalization of stress-induced changes, while PNMT in ventricles might be involved in modulation of the basic metabolic demands of the heart. However, this hypothesis remains to be verified.

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