Effect of 6-Hydroxydopamine on the Gene Expression of Na^+/Ca^{2+} Exchanger in the Rat Heart

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Abstract. The Na^+/Ca^{2+} exchanger (NCX) is an important component of the process of excitation-contraction coupling in the heart muscle. The level of gene expression as well as transport activities of this membrane structure is changed under pathological conditions like ischemic injury, myocardial infarction or diabetes.

In this work we focused on the question whether the adrenergic modulation affects gene expression of the NCX in rat hearts. NCX mRNA levels were studied in the left cardiac atrium (divided into ganglionic and nonganglionic part) and also in the left ventricle of rats treated with 6-hydroxydopamine (6-OHDA) in control and stressed conditions. We have shown that administration of 6-OHDA decreases mRNA levels of NCX in both ganglionic and nonganglionic part of the left atrium and also in the left ventricle. This effect was not altered under combined administration of 6-OHDA and single immobilization stress.

These data suggest that an activation of the adrenergic system can potentiate gene expression of the cardiac NCX.

Key words: Na^+/Ca^{2+} exchanger — 6-hydroxydopamine — Cardiac atria — Cardiac ventricles

Introduction

The Na^+/Ca^{2+} exchanger (NCX) is the principal calcium extrusion mechanism in the heart muscle (Crespo et al. 1990). As a component of the excitation-contraction coupling in adult cardiac muscle, NCX is essential in the relaxation phase, which is accomplished by extrusion of Ca^{2+} from the cell (Blaustein and Lederer 1999). It was already shown that absence of sarcolemmal Na^+/Ca^{2+} exchange in NCX1 knock-out mice is embryonic lethal (Reuter et al. 2003). The mammalian NCX1

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gene contains six exons (A, B, C, D, E, F), which can be expressed as 16 alternatively spliced products. Schulze et al. (2002) described that exon A-containing transcripts are mostly present in excitable cells like cardiomyocytes and neurons, whereas exon B-containing transcripts can be found in non-excitable tissues as astrocytes and kidney cells. Exon D was present in all types of characterized tissues, and C–F exons are expressed in various cassette manners. Reuter et al. (2003) have also demonstrated that NCX1 products display differences in activation by protein kinase A and by $[Ca^{2+}]i$, in dependence of which exon (A or B) they contain.

In our previous work we have shown that gene expression of the NCX can be modulated by thyroid hormones (Hudecova et al. 2004) by losartan (antagonist of angiotensin receptors of type 1 (AT1-receptors); Krizanova et al. 2002) and by elevated glucocorticoid levels during repeated immobilization stress (Zacikova et al. 1999). Both stress and blocking of AT1-receptors affects gene expression of the NCX in the left, but not in the right ventricle of rat myocardium.

6-hydroxydopamine (6-OHDA) is neurotoxin that destroys catecholaminergic terminals (He et al. 2000). Administration of 6-OHDA to rats produces anatomical and functional noradrenergic denervation and induces compensatory hyperreactivity of the suprarenal chromaffin cells. These effects are manifested by the increase in the activity of catecholamine synthesizing enzymes (Kostrzewa and Jacobowitz 1974). In the rat heart, a significant increase in the phenylethanolamine N-methyltransferase (PNMT) activity was found in 6-OHDA treated rats compared to controls (Torda et al. 1987). Since increased PNMT activity results in altered levels of epinephrine, which is known to possess strong inotropic effect on the heart, we proposed that NCX that is directly involved in the process of excitation-contraction coupling, might be affected by administration of 6-OHDA. Therefore, in this study we investigated the effect of 6-OHDA on the gene expression of NCX in rat myocardial atria and ventricles.

Materials and Methods

Animals

Male Sprague-Dawley rats (cca 350 g, Charles River, Suzfeld, Germany) 4 months old were used. Prior to experiments, animals were housed for 1 week, four animals *per* cage in a controlled environment (22 ± 2°C, 12 h light/dark cycle, light 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. One experimental group of rats was treated two times (second administration after 24 h) by 6-OHDA, each dose was 100 mg *per* 1 kg of body weight. Second experimental group served as a control. Both groups were further divided into non-stressed subgroup and subgroup immobilized once for 2 h as described previously (Kvetnansky and Mikulaj 1970) and decapitated 3 h after the immobilization (IMO) termination. Each subgroup was composed of 10 animals. Animals were sacrificed by decapitation two weeks after the last 6-OHDA

administration; left ventricle and atrium were withdrawn, atrium was separated into ganglionic and nonganglionic part and immediately frozen in the liquid nitrogen.

RNA isolation and relative quantification of mRNA levels by RT-PCR

Population of total RNAs was isolated by TRI Reagent method (MRC Ltd.). Briefly, tissue samples were homogenized by tissue homogenizer (Biospec Products Inc.) in TRI Reagent and stored at room temperature for 5 min to permit the complete dissociation of nucleoprotein complexes. The homogenate was extracted by chloroform and RNAs in the aeqous phase were precipitated by isopropanol. RNA pellet was washed with 75 % ethanol and stored under 96 % ethanol at −70 °C. The purity and integrity of isolated RNAs was checked on GeneQuant Pro spectrophotometer (Amersham Biosciences). Reverse transcription was performed using 1.5 μg of total RNAs and Ready-To-Go You-Prime First-Strand Beads (Amersham Biosciences) with pd (N_6) primer. PCR specific for NCX1 was carried out afterwards using primers NCX1: 5′-AGG CGG CTT TTT TAC-3′ (position 1127–1145) and NCX2: 5′-CGA CTT CCA GAG-3′ (position 1286–1304, *Rattus norvegicus* GI 451571), giving a 177 bp fragment. After the initial denaturation (94 °C for 7 min), 30 cycles of denaturation at 94 °C for 1 min annealing at 48 °C for 1 min and polymerization at 72 °C for 1 min were performed. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression was used as a housekeeper gene control for semi-quantitative evaluation of PCR. Primers GA1: 5′-AGA TCC ACA ACG GAT ACA TT-3′ and GA2: 5′-TCC CTC AAG ATT GTC AGC AA-3′ (Terada et al. 1993) were used to amplify 309 bp fragment from each first strand sample. After the initial denaturation (94 °C for 7 min), 30 cycles of denaturation at 94 °C for 1 min annealing at 60 °C for 1 min and polymerization at 72 °C for 1 min were performed. Both products, NCX and GAPDH were analyzed in 2 % agarose gels.

Statistical analysis

Each value represents the average for 10 animals. Results are presented as a means ± S.E.M. Statistical differences among groups were determined by one-way analysis of variance (ANOVA). Statistical significance was defined as * $p < 0.05$. For multiple comparisons, an adjusted t-test with p values corrected by the Bonferroni method was used (Instat, GraphPad Software, USA).

Results

As determined by semi-quantitative RT-PCR, in ganglionic part of the left atrium the mRNA levels of NCX were significantly lower compared to nonganglionic part (51 ± 5 a.u. *vs.* 69 ± 6 a.u., $p < 0.05$). Administration of 6-OHDA decreases mRNA levels of NCX in both ganglionic (to 33 ± 4 a.u., $p < 0.05$) and nonganglionic (to 43 ± 4 a.u., $p < 0.01$) part of the left atrium (Figure 1). In accordance with our previous results, single immobilization for 2 h with subsequent 3 h rest in cages did not have any effect on mRNA levels of NCX neither in ganglionic nor

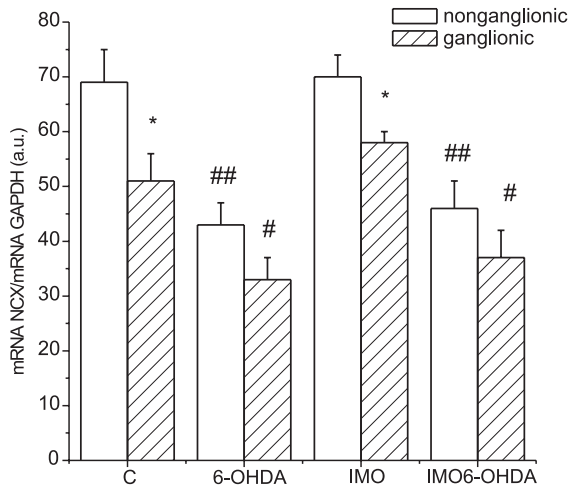

Figure 1. The mRNA levels of the Na^+/Ca^{2+} exchanger (NCX) in the left atria of rat myocardium. Empty columns represent gene expression of NCX (in arbitrary units) found in the nonganglionic tissue and slashed columns in the ganglionic tissue. After the administration of 6-hydroxydopamine (6-OHDA) and also after the combined effect of 6-OHDA and single immobilization (IMO) stress (IMO6-OHDA), decreased levels of NCX mRNA were observed in both ganglionic and nonganglionic parts of tissue. Single IMO stress did not affect expression of the NCX. Results are presented as a mean ± S.E.M. Statistical differences among groups were determined by ANOVA. Statistical significance between ganglionic and nonganglionic part was defined as * $p < 0.05$, significance of the effect of 6-OHDA compared to untreated controls is defined as # $p < 0.05$ and ## $p < 0.01$; C, control.

in nonganglionic part of the left atrium. Moreover, immobilization stress did not affect levels of mRNA in the group of rats treated with 6-OHDA and immobilized for 2 h compared to control 6-OHDA rats (46 ± 5 *vs.* 43 ± 4 a.u. in nonganglionic part of the left atrium and 37 ± 5 *vs.* 33 ± 4 a.u. in ganglionic part of the left atrium). Similar results were observed also in the left ventricle, where decrease of the NCX mRNA levels after 6-OHDA treatment was even more remarkable (from 45.7 ± 1.6 to 24.3 ± 2.9 a.u., $p < 0.001$; Figure 2).

Discussion

In this work we have shown that 6-OHDA significantly decreased gene expression of the NCX in the left cardiac atrium and ventricle. Decrease in the gene expression of this calcium transport system was more pronounced in ventricles compared to atria.

6-OHDA is a potent neurotoxin used extensively to produce chemical sympathectomy in experimental animals and an important tool for the understanding of

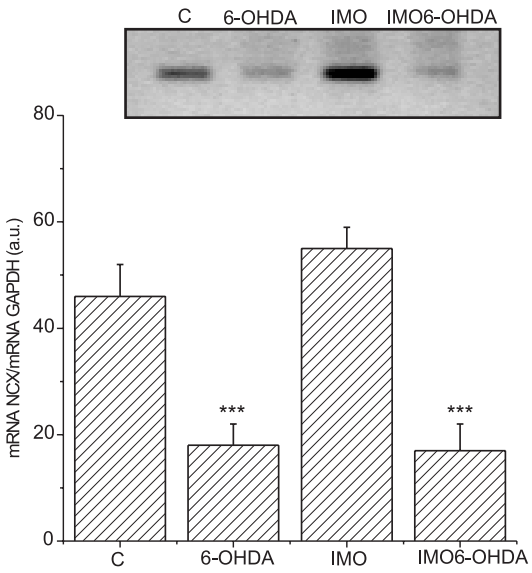

Figure 2. The mRNA levels of the Na^+/Ca^{2+} exchanger (NCX) in the left ventricles of rat myocardium. Significantly decreased levels of NCX mRNA (in arbitrary units) were observed in the groups of 6-hydroxydopamine (6-OHDA) and 6-OHDA with single immobilization (IMO) stress (IMO6-OHDA) treated animals. Single IMO stress did not change levels of the NCX mRNA in comparison to the control group of animals. Results are presented as mean ± S.E.M. Statistical differences among groups were determined by ANOVA. Statistical significance was defined as *** $p < 0.001$; C, control.

the adrenergic mechanism. 6-OHDA has also a teratogenic effect, and treatment of embryos resulted in high incidences of cardiac malformations, including ventricular septal lesions degenerative changes in the autonomic neurons, swollen mitochondria and cardiac hypertrophy (Ho and Duffield 2000). Left atrium, which is known to have higher amount of cardiac ganglia compared to the right atrium was divided into ganglionic and nonganglionic part. Since 6-OHDA is known to affect sympathetic terminals and not the neuronal cell bodies, decrease in mRNA levels of the NCX was the same in nonganglionic and ganglionic part of the left atrium.

Some calcium transport systems have already been described to be altered by 6-OHDA administration (Renaud et al. 1984; Skattebol and Triggle 1986). Binding of nitrendipine, the L-type calcium channel antagonist, was increased more than 30 % in the rat hearts with no change in affinity (Skattebol and Triggle 1986). L-type calcium channels are directly involved in the process of excitation-contraction coupling, and modulation of these systems generally results in an alteration of the cardiac contractility. NCX is a major calcium extrusion mechanism, which also plays an important role in the process of the excitation-contraction coupling in the heart. Major studies on the injured heart muscle describe massive elevation of NCX

mRNAs and protein (Wagner et al. 2003; Quinn et al. 2003; Taniguchi et al. 2004), which attributes to myocardial dysfunction. It is also known, that α-adrenergic stimulation is involved in the regulation of sarcolemmal NCX. Reinecke et al. (1997) described that phenylephrine – an α-adrenergic agonist increases significantly NCX mRNA levels in adult rat ventricular cardiocytes and α_1-adrenergic receptor antagonist prazosin can block this process. Since 6-OHDA destroys the transfer of signals through the α_1-adrenergic receptor, levels of NCX mRNA should be decreased in accordance to the results of Reinecke et al. (1997). The α_1-adrenergic activation is known to activate protein kinase C (Henrick and Simpson 1988), and to generate increased levels of inositoltrisphosphates, which induce the Ca^{2+} release from intracellular stores (Mann et al. 1992). Thus, decrease in NCXs after 6-OHDA administration might be a part of compensatory mechanism, which prevents the calcium overload from internal stores of the myocyte.

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