Modulation of expression of the sigma receptors in the heart of rat and mouse in normal and pathological conditions

M. Novakova[1], V. Bruderova[2], Z. Sulova[2], J. Kopacek[3], L. Lacinova[2], R. Kvetnansky[4], A. Vasku[5], P. Kaplan[6], O. Krizanova[2] and D. Jurkovicova[2]

[1] *Department of Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
[2] *Institute of Molecular Physiology and Genetics, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovakia*
[3] *Institute of Experimental Endocrinology, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovakia*
[4] *Institute of Virology, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Bratislava, Slovakia*
[5] *Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic*
[6] *Department of Biochemistry, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia*

Abstract. The aim of the present work was to study the effect of various stressors (hypoxia, cold, immobilization) on the gene expression of sigma receptors in the left ventricles of rat heart.

We have clearly shown that gene expression of sigma receptors is upregulated by strong stress stimuli, such as immobilization and/or hypoxia. Nevertheless, cold as a milder stressor has no effect on sigma receptor's mRNA levels. Signalling cascade of sigma receptors is dependent on IP_3 receptors, since silencing of both, type 1 and 2 IP_3 receptors resulted in decreased mRNA levels of sigma receptors.

Physiological relevance of sigma receptors in the heart is not clear yet. Nevertheless, based on the already published data we can assume that sigma receptors might participate in contractile responses in cardiomyocytes.

Key words: Sigma receptors — IP_3 receptors — Stress — Gene expression

Introduction

Sigma receptors were reported for the first time in 1976 (Martin et al. 1976), but till 1992 they were considered a mere subtype of opioid receptors (Quirion et al. 1992). This misconception was due to their as a rule high affinity binding of various substances of opioid character. Numerous pharmacological studies and cloning of sigma receptors in late nineties provided the ultimate confirmation of them as a new, structurally and functionally autonomous class of receptors (Hanner et al. 1996). Three subtypes of sigma receptors, so far described, differ mainly by their pharmacological profile.

Correspondence to: Olga Krizanova, Institute of Molecular Physiology and Genetics, Centre of Excellence for Cardiovascular Research, Slovak Academy of Sciences, Vlárska 5, 833 34 Bratislava 37, Slovakia
E-mail: olga.krizanova@savba.sk

The subclassification of sigma receptors is based on differences in ligand binding. Originally, two subtypes of sigma receptors were proposed: sigma 1 binding site or high affinity sigma receptor (binding dextromethorphan, (+)-pentazocine, (+)-NANM, and carbetapentane with moderate to high affinity) and sigma 2 binding site or low affinity sigma receptor (binding the same compounds with low affinity) (Zhou and Musacchio 1991; Bowen et al. 1993). The possible existence of the third sigma binding site was discussed already in the early nineties (Wu et al. 1991; Zhou and Musacchio 1991; Connick et al. 1992). This novel subtype of sigma receptor in rodent brain closely resembles histamine H(1)-type receptor and its activation might bring about synthesis of dopamine (Wyrick et al. 1995; Booth et al. 1999). The information about sigma 2 and 3 subtypes is merely restricted to their pharmacological profile and putative function. On the other side, the sigma 1 receptor subtype has been studied intensely concerning its gene and its expression.

Sigma receptors bind with a high affinity to a number of heterogeneous exogenous ligands (de Costa and He 1994). Numerous novel highly specific ligands of sigma receptors are introduced statedly (Maeda et al. 2000; Marazzo et al. 2001). Most of them belongs to psychotropic drugs and also addictive drugs, such as cocaine. Some ligands of sigma receptors might be of some relevance even in the treatment of these addictions (Matsumoto et al. 2003). Although the conclusive identification of endogenous sigma ligand remains unsettled, it may be characterized as a lipophilic substance, very likely one of neurosteroids (Meyer et al. 1998). Intracellular localization of sigma receptors on the endoplasmic reticulum, from which the ligand-receptors complex translocates into the surface membrane, is in the accordance with the lipophilic character of the ligand (Monassier and Bousquet 2002). In addition, the structure of sigma receptors as revealed by cloning and cDNA analysis is closely related to the structure of sterol C8-C7-isomerase, a key enzyme in postqualene sterol biosynthesis (Moebius et al. 1997).

The cloning studies revealed that the receptor protein consists of 223 amino acids (Kekuda et al. 1996; Seth et al. 1997, 1998; Prasad et al. 1998;), is linked to the G-protein and very likely possesses a single transmembrane span, which probably would make it easier to oscillate between the reticular and plasma locations. Sigma receptors isolated from different tissues and in different species exhibit high identity and very high homology (Monassier and Bousquet 2002).

Sigma receptors were originally described in the central nervous system, afterwards they were found in rather high densities in variety of peripheral tissue – nerves, ovaries, testes, placenta kidney, liver, etc. (Su and Junien 1994). With respect to this wide spread to various tissues, effects of the sigma binding and implicitly putative functions of sigma receptors vary greatly – from memory and cognitive functions to sperm motility. It is worth to mention that hypothetically, sigma signalling functionally links the immune, nervous and endocrine systems (Su 1991).

Cardiac muscle belongs to tissues equipped with sigma receptors in high densities. It has been reported that the rat myocardium contains both sigma 1 and sigma 2 subtypes (Novakova et al. 1995). Effects of highly specific sigma receptor ligands as well as those clinically used were tested in isolated rat cardiomyocytes and the sigalling pathway of sigma 1 receptor has been described (Novakova et al. 1998). Inositol 1,4,5-trisphosphate (IP_3) receptors are undoubtely involved in the signalling cascade of sigma receptors. Exposure of isolated cardiac myocytes from adult rat ventricles to nanomolar concentrations of aryl ethylene diamine-related sigma receptor ligands BD-737 and BD-1047 caused elevation in the IP_3 production, which in turn potentiates the systolic release of calcium from sarcoplasmic reticulum calcium stores (Novakova et al. 1998).

The aim of the present study was to study the effect of various stressors (hypoxia, cold, immobilization) on the gene expression of sigma receptors in the left ventricles of rat heart. Since aging is the important factor affecting the gene expression of IP_3 receptors in the heart (Kaplan et al. 2007), we compared gene expression of sigma receptors in left ventricles of differently aged rats. Mutual interplay of sigma receptors and IP_3 receptors of the type 1 and/or 2 was studied on isolated cardiomyocytes, where each type of IP_3 receptors was silenced.

Materials and Methods

Animals

All used protocols were approved by the Ethic Committee of Masaryk University, Brno, Czech Republic. In presented experiments, Male Sprague-Dawley (Suzfeld, Germany), Wistar-Kyoto (WKY; Prague, Czech Republic) rats, spontaneously hypertensive (SHR; Suzfeld, Germany) rats and mice inbred strain C57B1/129SV were used. Prior to experiments, animals were maintained under controlled environmental conditions (22 ± 1°C, 12 h light/dark cycle, light on at 06:00 a.m.). Food and water were available *ad libitum*. Rats and mice were sacrificed by decapitation. Whole hearts were withdrawn and washed out with cold physiological solution. Left ventricles were withdrawn, stored frozen at –70°C and used for the experiments.

In short-term hypoxia experiments, male mice were exposed to 8% of oxygen in the hypoxic chamber during 6 h. Immobilization stress was performed as described by Kvetnansky and Mikulaj (1970). Male Sprague-Dawley rats (280–320 g) were exposed to single or seven times repeated immobilization stress. Animals were immobilized for 2 h and decapitated after 3 h of rest. Repeated immobilization stress was achieved by immobilizing animals for seven consecutive days. Separate group of male Sprague-Dawley rats was exposed to cold stress performed in the cold room under constant temperature 4°C and standard light and humidity conditions for 7 days. In aging experiments, male WKY rats – 6- (cca 350 g), 15- (cca 420 g) and 26- (cca 480 g) month-old were used, being divided into three goups according to their age. Each group was composed of 6 animals that were decapitated after 5 min halothane anesthesia (3% halothane in oxygen/nitric oxygen, 1 : 2) on corresponding date. Antioxidant flavonoid quercetin (20 mg/l kg of body weight *per* day) was administrated to 4 and 12 weeks-old WKY rats (200–250 g) and SHR rats (200–250 g) in 0.1 $mol \cdot l^{-1}$ phosphate buffer pH 6.0 instead of drinking water during 4 weeks. Control animals drank phosphate buffer. The pilot study, where one group of animals drank water and

second group the phosphate buffer, showed no differences in the spent volumes of both fluids.

Preparation of cardiomyocytes

Male Wistar rats (220–270 g) were anaesthetized with Pentobarbital (SPOFA, United Pharmaceutical Works, Czech Republic), in the dose of 10–20 mg/100 g of body weight, and Heparin (LECIVA, Czech Republic; 0.2 ml) was added. The heart was rapidly excised and placed into a cold KREBS solution (5°C). The aorta was then cannulated and the heart perfused for 3 min with 50 ml of KREBS solution (37°C, 1 mmol·l^{-1} $CaCl_2$, flow rate 10 ml *per* min, 30 ml of total volume) to washout the blood and to stabilize the heart. After 3–5 min, perfusion was switched to calcium-free KREBS solution (50 ml of total volume) for 5 min. Afterwards, heart was perfused with collagenase A (Roche Diagnostics, Germany) in albumin for 5–20 min. When the heart became swollen and turned slightly pale, the enzymatic digestion was stopped, atria were removed and the heart was placed in the Petri dish containing HEPES solution with 2% albumin, minced with scissors and individual cardiomyocytes were obtained by repeated gentle sucking with pipette. The cell suspension was filtered through mull, placed in a conical tube and centrifuged (250 rev./min). The supernatant was then removed, myocytes resuspended in albumin-free HEPES and calcium added to final concentration of 0.4 mmol·l^{-1}.

Preparation of primary cardiomyocyte culture

Primary cardiomyocyte culture was prepared as described in Tillinger et al. (2006). Briefly, isolated cells were placed into the pre-treated flasks and kept in specific medium designed for cardiomyocytes only: EX-CELL 320 with HEPES and L-glutamine (JRH Biosciences, USA). Insulin (15 μg/ml), Penstrephten (100 U/ml), non-essential amino acids (0.1 mmol·l^{-1}), retinoic acid (1 μmol·l^{-1}), and 10% fetal calf serum were added (all from Gibco). The cells were allowed to settle to the flask bottom for two days and then the medium was changed repeatedly, usually every two days. The cells were then let to stabilize and grow continually for couple of weeks.

Experiments were performed in cultures growing for at least 2 weeks in strictly controlled conditions. At this moment, only cardiomyocytes are present in the culture. Viability of the cells in culture was regularly tested by immunohistochemic methods.

Gene silencing

Silencing experiments were performed on primary cardiomyocyte culture. For gene silencing, pre-designed siRNAs for the type 1 and type 2 IP_3 receptors (Ambion, Inc.) were used. As a negative control the Silencer^{TM} Negative Control #1 siRNA was used. The sequence has no significant homology to any known gene sequences from mouse, rat or human and is non-toxic to cells. Tested amounts of siRNAs were 1.2; 2.5 and 3.75 μg and for negative control 1.2 and 2.5 μg. Before the use, siRNAs were annealed in the annealing buffer according to the procedure recommended by provider (Ambion, Inc.). For transfection of cardiomyocytes the Gene Silencer siRNA Transfection Kit was used (Gene Therapy Systems, Inc.). After the transfection, the cells were cultivated 5 h in serum-free DMEM medium and after that, in the same medium containing 10% fetal calf serum up to 72 h.

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

Population of total RNAs was isolated by TRI Reagent (MRC Ltd.). Briefly, tissue samples were homogenized by tissue homogenizer (Biospec Products Inc.) in TRI Reagent and after 5 min the homogenate was extracted by chloroform. RNAs in the aqueous 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 (GE healthcare-Life Sciences). Reverse transcription was performed using 1.5 μg of total RNAs and Ready-To-Go You-Prime First-Strand Beads (GE healthcare-Life Sciences) with pd(N_6) primer. PCRs were calibrated for the optimal number of cycles to ensure linear range of amplification. PCR specific for sigma receptors (accession number GI 38541100) was carried out afterwards using primers Sig1A (5` GAG GCA ACA GCT GTG GAG TG `3) and Sig1B (5` AAA GAG GTA GGT GGT GAG CTC `3). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH, accession number GI 56187) (Terada et al. 1993) expression using primers GA1 (5` AGA TCC ACA ACG GAT ACA TT `3) and GA2 (5` TCC CTC AAG ATT GTC AGC AA `3) was used as a housekeeper gene control for semi-quantitative evaluation of PCR. Used primer sequences amplified fragments of 185 bp for sigma receptor and 309 bp for GAPDH. In hypoxia experiments as additional control mitochondrial ribosomal protein L19 (MRPL, accession number GI 71361654, Szabo et al. 2004) was amplified using primers MRPL1 (5` GGG ATT TGC ATT CAG AGA TCA G 3`) and MRPL2 (5` GGA AGG GCA TCT CGA AAG 3`), giving amplification product of 182 bp. PCR specific for sigma receptors as well as for GAPDH started by initial denaturation at 94°C and was followed by 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. PCR specific for MRPL started by initial denaturation at 94°C and was followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min and polymerization at 72°C for 1 min. PCRs were terminated by final polymeriza-

tion at 72°C for 7 min. All PCR products were analyzed on 2% agarose gels.

Statistical analysis

Each value represents an average of minimum 6 animals. Results are presented as a mean ± S.E.M. Statistical differences among groups were determined by one-way analysis of variance (ANOVA). Statistical significance was defined as * $p < 0.05$ – ** $p < 0.01$. For multiple comparisons, an adjusted *t*-test with *p* values corrected by the Bonferroni method was used (Statistica 7, StatSoft, Czech Republic).

Results

Short-term hypoxia (8% oxygen for 6 h) significantly increased mRNA levels of sigma receptors in the left ventricle of rat hearts, compared to ventricles from the control rats (Figure 1, Hy: 3.25 ± 0.14 *vs.* C: 2.12 ± 0.29 a.u.).

Moderate stressor cold had no significant effect on the gene expression of sigma receptors in the rat left ventricles even after 7 days of exposure compared to non-exposed controls (Figure 2, COLD7: 15.18 ± 1.35 *vs.* C: 12.6 ± 0.65 a.u.). On the contrary, single and seven-times repeated immobilization stress increased the sigma receptor's mRNA signal in the left heart ventricle more then 2-times compared to non-exposed control (Figure 2, left; IMO1: 22.8 ± 2.04 and IMO7: 24.7 ± 2.32 *vs.* C: 12.6 ± 0.65 a.u.). To ensure that the effect observed on whole left ventricle is from cardiomyocytes, we isolated ventricular cardiomyocytes from hearts of 7-times

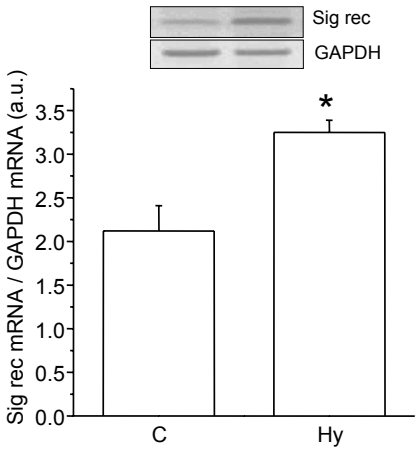

Figure 1. Comparison of the sigma receptors mRNA level in left ventricle of control (C) and hypoxic (Hy) mice. Gene expression was measured after reverse transcription by PCR and evaluated semiquantitatively to GAPDH. In the upper part of the graph, the typical result from the gel is shown. Each column is displayed as mean ± S.E.M. and represents an average of 6 animals. Statistical significance * represents $p < 0.05$.

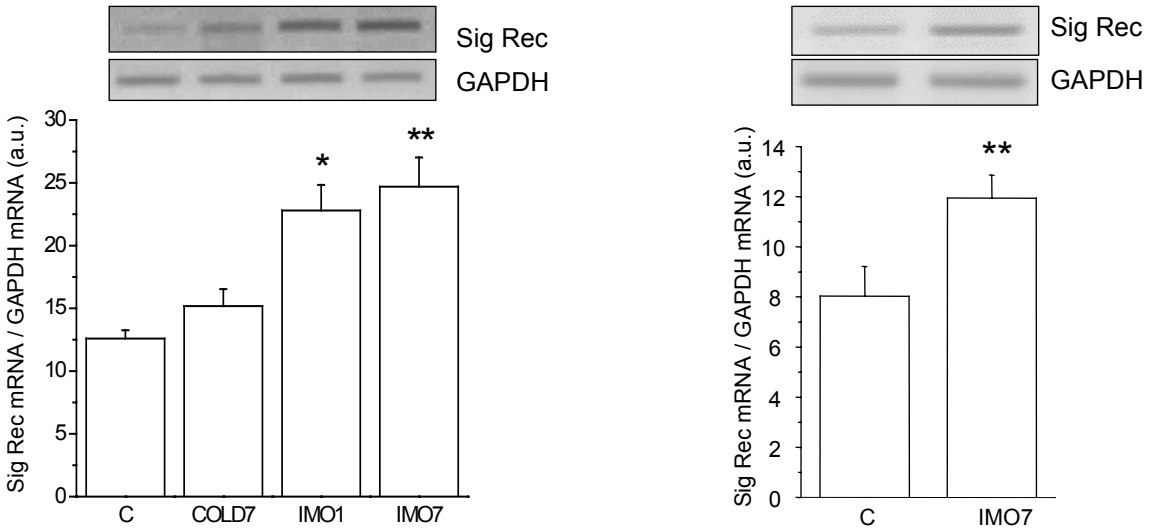

Figure 2. Comparison of sigma receptors mRNA levels in the left ventricle of cold (COLD7) and immobilization stress (IMO1 – 1 day and IMO7 – 7 days) exposed rats (left) and in myocytes isolated from left ventricle of seven-times immobilized rats (right). In the upper part of the left panel, representative results from gels are shown. The mRNA levels of the sigma receptors in left ventricle were not affected by 7 days of cold exposure but immobilization increased mRNA level of sigma receptors after both, single and repeated exposure compared to unaffected controls (C). Sigma receptor mRNA level was significantly elevated also in myocytes isolated/purified from left ventricles of seven times immobilized rats. Results are presented as mean ± S.E.M. Statistical differences among groups were determined by one-way analysis of variance (ANOVA). Statistical significance between control and stressed rats was determined as * $p < 0.05$ and ** $p < 0.01$.

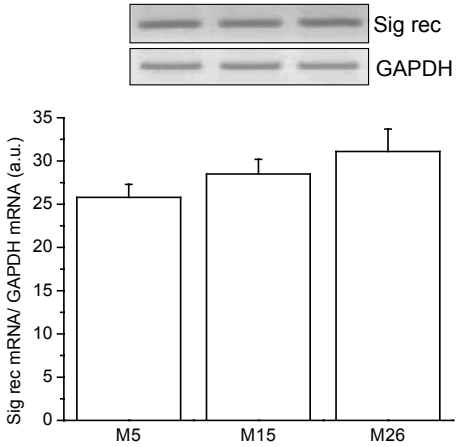

Figure 3. Comparison of mRNA levels of the sigma receptors in 5- (M5), 15- (M15) and 26- (M26) month-old rat left ventricles. In the upper part of the graph, typical result from gel is shown. Each column is displayed as mean S.E.M. and represents and average of 6 animals. Statistical significance was calculated compared to 5-month-old rats, no significant change of the gene expression by aging was observed.

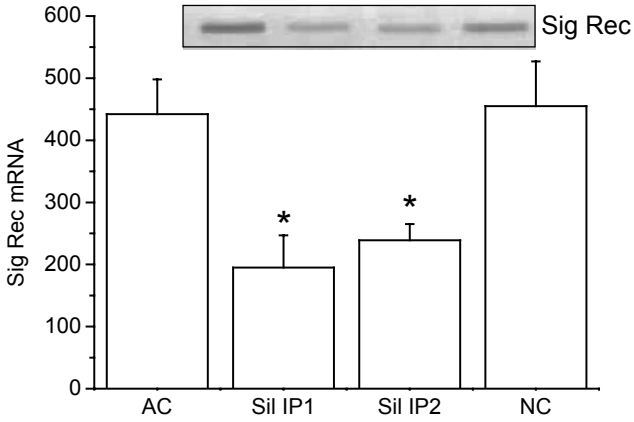

Figure 5. Levels of the sigma receptors mRNA in siRNA treated isolated/purified cardiomyocytes. Cells were treated with IP_3R1 siRNA (Sil IP_1), IP_3R2 siRNA (Sil IP_2) and negative control siRNA (NC). Silencing of both, IP_3 receptors type 1 and 2, significantly decreased the level of mRNA of sigma receptors in isolated/purified left ventricular cardiomyocytes. All these cell groups were compared relatively to control cells without any siRNA (AC). Results are expressed as mean ± S.E.M. Statistical significance * $p < 0.01$.

immobilized rats, where we observed the same effect (Figure 2, right; IMO7: 11.9 ± 0.92 *vs.* C: 8.03 ± 1.19 a.u.).

In addition, we were interested whether gene expression of the sigma receptors is changed by the process of aging in left ventricle of rat hearts. Our results showed that no significant differences in the mRNA levels of sigma receptors were observed among 5-months (M5), 15-months (M15) and 26-month-old (M26) rats (Figure 3, M5: 25.8 ± 1.5 *vs.* M15: 28.5 ± 1.7 and M26: 31.1 ± 2.6 a.u.). Thus, aging has no significant effect on the level of gene expression of sigma receptors.

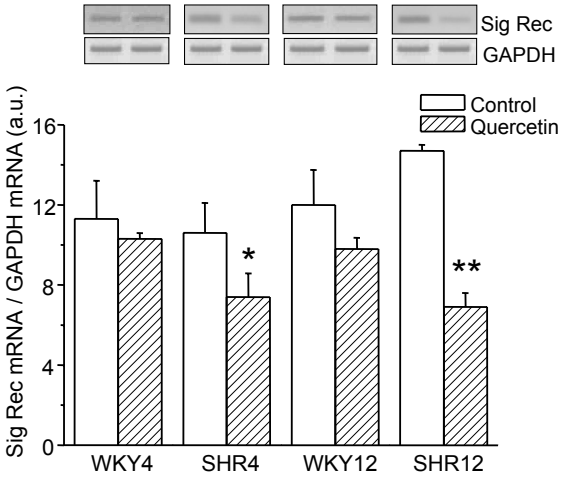

Figure 4. Effect of antioxidant quercetin on the mRNA levels of the sigma receptors. Each column is displayed as mean ± S.E.M. and represents an average of 6 animals. Quercetin did not affect gene expression of sigma receptors in 4 (WKY4) and 12 (WKY12) weeks old WKY rats, but significantly decreased mRNA levels in SHR rats 4 and also 12 weeks old (SHR4 and SHR12, respectively). In the upper part of the graph, typical result from gel is shown. Statistical significance between control and quercetin administered groups * represents $p < 0.05$ and ** $p < 0.01$.

Also, no significant difference in left ventricular mRNA of sigma receptors was observed between 4-months old and 12-month old WKY rats and SHR rats (Li et al. 2000). Nevertheless, antioxidant quercetin significantly decreased mRNA of sigma receptors in the left ventricle of SHR, independently of the age (Figure 4; SHR4: 7.4 ± 1.18 *vs.* 10.6 ± 1.5 a.u.; SHR12: 6.9 ± 0.7 *vs.* 14.7 ± 0.3 a.u.). When quercetin was administered to Wistar-Kyoto rats of the same age groups, no differences in sigma receptors gene expression compared to controls were observed (WKY4: 10.3 ± 0.29 *vs.* 11.3 ± 1.9 a.u.; WKY12: 9.8 ± 0.56 *vs.* 12.0 ± 1.75 a.u.).

Silencing of IP_3 receptors of type 1 and 2 resulted in marked decrease of mRNA levels of sigma receptors (Figure 5) compared to both, absolute untreated control and negative control with scrambled siRNA (AC 442 ± 56 a.u.; IP_3R1sil 165 ± 47 a.u.; IP_3R2sil 239 ± 26 a.u. and NC 455 ± 72 a.u.).

Discussion

In this work we have clearly shown that gene expression of sigma receptors is upregulated by strong stress stimuli, such as immobilization and/or hypoxia. Nevertheless, cold as a milder stressor has no effect on sigma receptor's mRNA.

Signalling cascade of sigma receptors is dependent on IP_3 receptors, since silencing of both type 1 and 2 IP_3 receptors resulted in decreased mRNA levels of sigma receptors.

Physiological relevance of sigma receptors is not clear yet. Sigma receptors have been implicated in modulation of a number of biochemical, physiological and behavioral processes in the central nervous system, as well as in the endocrine, immune and gastric systems (Novakova et al. 1998). The importance of sigma receptors was described mainly in the brain. Decreased number of sigma receptors in the brains of schizophrenic patients has been reported repeatedly (Helmeste et al. 1996; Weismann et al. 1996). Later, some authors reported association between schizophrenia and two sigma 1 receptor gene variants (Ishiguro 1998; Ohmori et al. 2000). On the contrary to these findings, more recent papers report that there is no association between the sigma receptor type 1 gene variants and schizophrenia (Uchida et al. 2003; Satoh et al. 2004). Another study has been focused on functional polymorphisms in sigma 1 receptor gene associated with alcoholism (Miyatake et al. 2004). Authors have identified the allele and the haplotype, which can be considered as a possible protective factor for the development of alcoholism. Moreover to this finding, it has been reported that a variant of the sigma receptor type 1 gene is a protective factor for Alzheimer disease – the TT-241-240p2 haplotype of sigma 1 receptor gene decreases expression of the gene and thus may have a protective role against susceptibility to Alzheimer disease (Uchida et al. 2005).

Sigma receptors have also been implicated in the regulation of the cardiovascular system, where sigma 1 receptor transcripts have been found mainly in parasympathetic intracardiac neurons. Here we present that sigma 1 receptors are expressed also in cardiomyocytes. This observation is in a good agreement with the physiological measurements of other laboratories, where they found that in cardiomyocytes, nanomolar concentrations of the prototypic sigma receptor ligands exerted effects on contractility, calcium transients, calcium fluxes and also beating rate (Ela et al. 1994; Novakova et al. 1995). In intracardiac neurons, activation of sigma 1 receptors depresses the excitability of cells and is thus likely to block parasympathetic input to the heart (Zhang and Cuevas 2005). In the heart, ligands for opioid and sigma receptors improve cardiac electrical stability in rat models of post-infarction cardiosclerosis and stress (Lishmanov et al. 1999). Taking together, there is still considerable speculation about the role of sigma receptors in the cardiovascular system. Although the endogenous ligand responsible for activating sigma receptors under physiological and pathophysiological conditions remains to be determined, there are data suggesting that some putative sigma receptor's ligands may affect the heart and coronary vasculature. Activation of sigma 1 receptors in these cells is likely to attenuate parasympathetic input to the heart, and consequently, to affect cardiovascular function (Zhang and Cuevas 2005). Increased gene expression of the sigma receptors during stress exposure might thus have impact on cardiac function. Nevertheless, precise function of the sigma 1 receptors especially in cardiomyocytes remains to be elucidated.

Spare information is available in the field of gene expression and sigma receptor's modulation under various physiological and pathophysiological conditions. A few studies were reported on age related changes in number of sigma 1 receptors and this research has been focused only on the brain tissue. Ishiwata et al. (2003) reported increase in number of both sigma 1 and sigma 2 receptor subtypes in the aged rat brains using radioligand binding assay. The increased number of both receptor subtypes in the aged rats compensate for the lowered affinity. This result contrasts strikingly with the age-dependent decrease in the dopaminergic, cholinergic and glutamatergic receptors reported before in various studies. Another supporting evidence of this finding is the paper by Kawamura et al. (2003) where the authors report a significant increase in binding potential for highly specific sigma ligand in the aged monkey brain. However, their conclusion – that the sigma 1 receptor binding sites increased in the aging process of the monkey brain – is somewhat disputable since their evidence is only indirect, based on the quantitative analysis of the binding to sigma 1 receptors with positron emission tomography and they did not take in consideration possible increased affinity of the receptors rather than their increased number. In our experiments on rats we have shown that aging slightly, but gradually increased gene expression of sigma 1 receptors in the left ventricle.

There is a receptor system, which is linked to sigma receptors exclusively – it is in fact united with them. Hayashi and Su (2001) found that sigma 1 receptors form a trimeric complex with other two proteins on the endoplasmic reticulum: the IP_3 receptor and the ankyrin isomer 220. Ankyrins are a family of cytoskeletal adaptor proteins, which interconnect membrane proteins with the spectrin-based cytoskeleton (Bennett and Stenbuck 1979). Upon the stimulation elicited by sigma 1 receptor agonist, sigma 1 receptor – ankyrin dissociates as a dimer from IP_3 receptors, which remain on the endoplasmic reticulum, and translocates to the plasma membrane and nucleus (Hayashi et al. 2000). Sigma receptor antagonists indirectly block this process by causing dissociation of sigma 1 receptor from ankyrin, which remains coupled to IP_3 receptors on the endoplasmic reticulum. Analogically, sigma ligands increased intracellular level of the IP_3 in cultured myocytes (Novakova et al. 1998). It seems that sigma 1 receptors and their associated ligands help create a "supersensitized state" to facilitate the amplification of IP_3 signalling at the endoplasmic reticulum (Su and Hayashi 2003). Nevertheless, none of the above mentioned studies has definitely demonstrated, which subtype of sigma receptors mediated these effects. Based on this knowledge we silenced type 1 and/or type 2 IP_3 receptors

in primary cardiomyocyte's culture and studied the gene expression of sigma receptors. No changes in morphology were observed in silenced cells compared to controls. Nevertheless, sigma receptors were downregulated, when either type 1 or type 2 IP_3 receptors was silenced. These results supported the proposal that both types of IP_3 receptors might be regulated by sigma 1 receptors.

In summary, several lines of evidence have suggested that sigma receptors are involved in calcium regulation. Besides, IP_3 receptors, M-methyl-D-aspartate-induced calcium signalling in primary rat neurons (Hayashi et al. 1995; Klette et al. 1997) and calcium signalling through the L-type calcium channels (Hayashi et al. 2000) was observed. In this paper we have shown that both types – type 1 and also type 2 IP_3 receptors regulates the gene expression of sigma 1 receptors in the heart. Sigma 1 receptors reside in close proximity to IP_3 receptor at the endoplasmic reticulum (Tsao and Su 1996). Together with ankyrin they can play important roles in controlling various cell functions. Nevertheless, further experiments are required to clarify the exact function.

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