Am J Transl Res 2016;8(7):3148-3158
www.ajtr.org /ISSN:1943-8141/AJTR0029575

Original Article

Effects of immobilizations stress with or without water immersion on the expression of atrial natriuretic peptide in the hearts of two rat strains

Jana Slavikova[1,2], Eliska Mistrova[1,2], Vera Klenerova[3], Peter Kruzliak[4], Martin Caprnda[5], Sixtus Hynie[3], Pavel Sida[3], Magdalena Chottova Dvorakova[1,2]

[1]Department of Physiology, Charles University in Prague, Faculty of Medicine in Pilsen, Pilsen, Czech Republic; [2]Biomedical Centre, Charles University in Prague, Faculty of Medicine in Pilsen, Pilsen, Czech Republic; [3]Institute of Medical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine and General University Hospital, Charles University in Prague, Prague, Czech Republic; [4]Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic; [5]2nd Department of Internal Medicine, Faculty of Medicine, Comenius University and University Hospital, Bratislava, Slovakia

Received March 31, 2016; Accepted June 8, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Atrial natriuretic peptide (ANP) is produced and released by mammalian cardiomyocytes and induces natriuresis, diuresis, and lowering of blood pressure. The present study examined localization of ANP and a possible role of the hypothalamic-pituitary-adrenal axis (HPA) activity on the expression of proANP gene in the heart. The Sprague Dawley (SD) and Lewis (LE) rat strains were used. The animals were exposed to the two types of stress: immobilization and immobilization combined with water immersion for 1 hour. Localization of ANP was detected by immunohistochemistry and expression of the proANP mRNA by real-time qPCR in all heart compartments of control and stressed animals after 1 and 3 hours after stress termination (IS1, IS3, ICS1, and ICS3). Relatively high density of ANP-immunoreactivity was observed in both atria of both rat strains. In control rats of both strains, the expression of the proANP mRNA was higher in the atria than in ventricles. In SD rats with the intact HPA axis, an upregulation of ANP gene expression was observed in the right atrium after IS1, in both atria and the left ventricle after IS3 and in the left atrium and the left ventricle after ICS3. In LE rats with a blunted reactivity of the HPA axis, no increase or even a downregulation of the gene expression was observed. Thus, acute stress-induced increase in the expression of the proANP gene is related to the activity of the HPA axis. It may have relevance to ANP-induced protection of the heart.

Keywords: Atrial natriuretic peptide- Stress- Heart- Real-time qPCR

Introduction

Cardiac natriuretic peptides (NPs) are involved in the regulation of blood pressure and fluid homeostasis [1]. Three peptides, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C- type natriuretic peptide (CNP) are synthesized by three separate genes and then stored as three prohormones [2]. ANP and BNP are primarily synthesized in the heart atria and released by atrial wall stress and stretch [3], whereas CNP is synthesized in the vascular endothelium. ANP and BNP are also synthesized by the ventricles, but to a significantly lesser degree than by the atria. In addition, NPs were shown to be produced in several non-cardiac sites including the central nervous system, liver, gastrointestinal tract, kidneys and gonads [4].

The biological activities of the cardiac ANP and BNP are similar. They serve to counterbalance the activity of systems that tend to increase the extracellular fluid volume and blood pressure, such as the renin-angiotensin system and the sympathetic nervous system [5]. Under chronic hemodynamic overload, cardiac ANP and BNP expression, synthesis and secretion are increased through both mechanical and neuroendocrine stimuli [6].

Acute stress triggers the brain noradrenergic system which interacts with the hypothalamic-

pituitary-adrenocortical (HPA) axis, the major neuroendocrine effector of the stress response [7]. As such, activation of the HPA axis may play an important integrative role in coping and adaptation to stress [8]. Stressor experiences may contribute to various cardiovascular disturbances such as ventricular arrhythmia, increased coronary vasoconstriction, heart rate and blood pressure [9, 10]. These pathological conditions were associated with increased plasma NPs levels [11, 12]. It was also shown that emotional stressors and chronic insults increased heart gene expression of NPs coupled with an increased ANP release [13-15]. However, little is known about the role of the HPA system activity on the expression of NPs in the heart in acute stress conditions. It is also still unclear whether a change in the expression of NPs in cardiomyocytes could participate in the acute stress response.

This study was conducted to asses: a) the localization of ANP in the rat heart atria by immunohistochemistry and b) the proANP mRNA expression in all rat heart compartments by real-time reverse transcription followed by polymerase chain reaction (RT-PCR). The studies were performed in control and stressed animals. Given the potential links between the activity of HPA axis and natriuretic peptides, we used the two rat strains differing in the magnitude of their HPA response to stress: Sprague-Dawley (SD) rats, a widely studied comparator strain, and Lewis (LE) rats, which show a blunted HPA response to stress [16]. All groups of animals were exposed to the two types of acute stress: immobilization and immobilization combined with cold in a single session.

Materials and methods

Animals

Sprague-Dawley (SD) and Lewis (LE) adult male rats were used (Charles-River Laboratories, Sulzfeld, Germany). Their average starting body weight was about 200 grams. Animals had free access to a standard pellet food and water. Rats were housed five per cage (42 x 26 cm^2) and maintained on a standard 12 h light/12 h dark cycle, at a constant temperature (22 + 1°C) and relative humidity (50-70%). Behavioral tests were performed from 8 a.m. to 1 p.m. Treatment of rats was in accordance with the recommendations of the European Community Guide for the Care and the Use of Laboratory Animals. The study was approved by the Ethical Review Committee, 1st Faculty of Medicine, Charles University in Prague.

Stress procedure

Animals of both strains were exposed to the two types of acute stressors. Immobilization alone (IS) was applied by fixing the front and hind legs of the rat with adhesive plaster with mull inside to avoid pain. Then the animal was immobilized in a snug-fitting plastic mesh. This mesh was bent to conform to the size of individual animal and a bandage fixed this shape of mesh. When combined immobilizations with water immersion (ICS), stressed rats were immersed to water bath (22°C) in such a way that upper ¼ of the animal was outside of water. After stressor exposure for 60 minutes, the rats were dried and returned to home cages for 60 minutes (IS1, ICS1) or 180 minutes (IS3, ICS3). Control animals remained untreated and were used directly after their removal from the home cages [17, 18].

Sample preparation

Rats were killed by decapitation. For RNA isolation, hearts were rapidly excised, rinsed with ice-cold saline, freed of connective tissue and fat and divided into the left atrium with the interatrial septum (LA), right atrium (RA), and free walls of the left (LV) and right (RV) ventricles. The samples were than frozen in liquid nitrogen and stored in -80°C until RNA isolation. All the procedures lasted less than 15 minutes from killing the rat until freezing the tissues. For immunofluorescence, hearts were incubated for 18 hours in Zamboni's fixative (15% picric acid and 4% paraformaldehyde in 0.1 M phosphate buffer; pH 7.3), washed in 0.1 M sodium phosphate buffer, cryoprotected in 2.3 M sucrose for 24-48 h. Specimens were frozen in liquid nitrogen.

Immunofluorescence

Frozen tissues from four hearts per experimental group were cut with a cryostat (Leica CM1850, Germany); 10-μm-thick sections were placed onto gelatinized slides and stored until incubated. After 30 minutes of preincubation with normal goat serum (diluted 1:25 with PBS), the sections were incubated with primary antibodies. Immunofluorescence was performed

using rabbit antibody against ANP (1:100, Millipore, Temecula, CA, USA) for overnight at room temperature in a moist chamber. After thorough washing, primary antisera were directly labeled (1 hour, ambient temperature) with the secondary goat anti-rabbit serum conjugated to Texas Red (1:200, Calbiochem, Darmstadt, Germany). Such directly labeled specimens were used for double incubation with mouse monoclonal antibody against protein gene product (PGP) 9.5 (a general marker for neurons; 1:100, UltraClone Limited, UK). Following this incubation at room temperature overnight, the second incubation was carried out with biotinylated goat anti-mouse IgG (1:200, 1 h, ambient temperature, Sigma, St. Louis, MO, USA) followed by fluorescein isothiocyanate (FITC) labeled streptavidin (1:200, 1 hour ambient temperature, Calbiochem, San Diego, CA, USA). The omission of individual primary antisera in all samples served as negative controls. After careful washing, the preparations were mounted in glycerol and buffered at pH 8.6 with diazobicyclooctan (DABCO, Sigma, USA). Sections were evaluated using an epifluorescence microscope equipped with appropriate filter combinations (Olympus BX60, Germany). Some slides were after that stained by alum hematoxylin solution for 10 minutes, washed and evaluated in order to verify localization of immunopositivity.

Real-time RT-PCR

Total RNA was extracted from all prepared samples (n = 6 per group) by TRI reagent (Sigma, Prague, Czech Republic) according to the manufacturer's instructions. The purity and integrity of the RNA preparations were checked spectrophotometrically and by agarose gel electrophoresis. Contaminating DNA was destroyed with 1 U DNase/μg of total RNA (Invitrogen, Carlsbad, CA, USA). RNA was converted to cDNA using Superscript RNase H-Reverse Transcriptase III (200 U; Invitrogen) for 50 minutes at 42°C. Single-strand cDNA was synthetized from 2 μg of total RNA. Real-time analysis was carried out on an iCycler (Bio-Rad, Prague, Czech Republic). The primers were designed to amplify the sequence corresponding to nucleotides 172-242 (forward: CAACACAGATCTGATGGATTTCA, reverse: CCTCATCTTCTACCGGCATC) of the published rat pro-ANP cDNA sequence (Genbank Accession No. NM_012612.2), to nucleotides 873-969 (forward: TTCCTTCCTGGGTATGGAATC, reverse: GTTGGCATAGAGGTCTTTACGG) of the published β-actin cDNA (Genbank Accession No. NM_031144), to nucleotides 650-782 (forward: GATGAAGCCATTGCTGAACTTG, reverse: CTCCCGCTTCTGCTTCGTCTCC) of the published rat tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide (Ywhaz) cDNA sequence (Genbank Accession No. NM_013011.3), and to nucleotides 908-1057 (forward: GGCTGTAGCGTGCCAGTAGCAG, reverse: TGGACCATCTTCTTGCTGAACA) of the published rat hydroxymethylbilane synthase (Hmbs) cDNA sequence (Genbank Accession No. NM_013168.2). Classical PCR reactions were first conducted to confirm the specificity of primers. The expected PCR products were isolated by agarose gel electrophoresis, eluted, and cloned. The plasmids containing the cloned fragments were serially diluted from 5×10^3 to 5×10^8 molecules/μl and used as templates in quantitative real time PCR reactions to establish the standard curves and assess efficiency of reaction. Reactions were performed in a 25 μl reaction mixture containing either 5 μl of diluted cDNA or plasmid standard or water, 1 μl (20 nmol/l) of each primer, 5.5 μl of ultrapure water and 12.5 μl iQ SYBR Green Supermix (Bio-Rad). The PCR reactions were performed as follows: denaturation at 95°C for 10 minutes followed by 45 cycles of amplification (denaturation: 95°C for 30 seconds; primer annealing: 58°C for 25 seconds and primer extension: 72°C for 20 seconds). Each run was completed with a melting curve analysis in order to confirm the specificity of amplification and lack of primer dimers. After amplification, the samples were slowly heated from 65°C to 95°C with continuous reading of fluorescence to obtain a melting curve. Each pair of primers yielded a single peak in the melting curve and a single band of the expected size in agarose gel.

Reactions for all samples were performed in triplicate and from these replicates, averages were calculated and expressed as real quantitative cycle (C_q; $\Delta C_q = C_{q\ target} - C_{q\ reference}$) and reported as normalized ΔC_{qs}. Freely available computational program BestKeeper, excel-based tool using pair-wise correlations, was used for identifying the optimal normalization gene among a set of candidates reference genes as was in detail explained by the authors

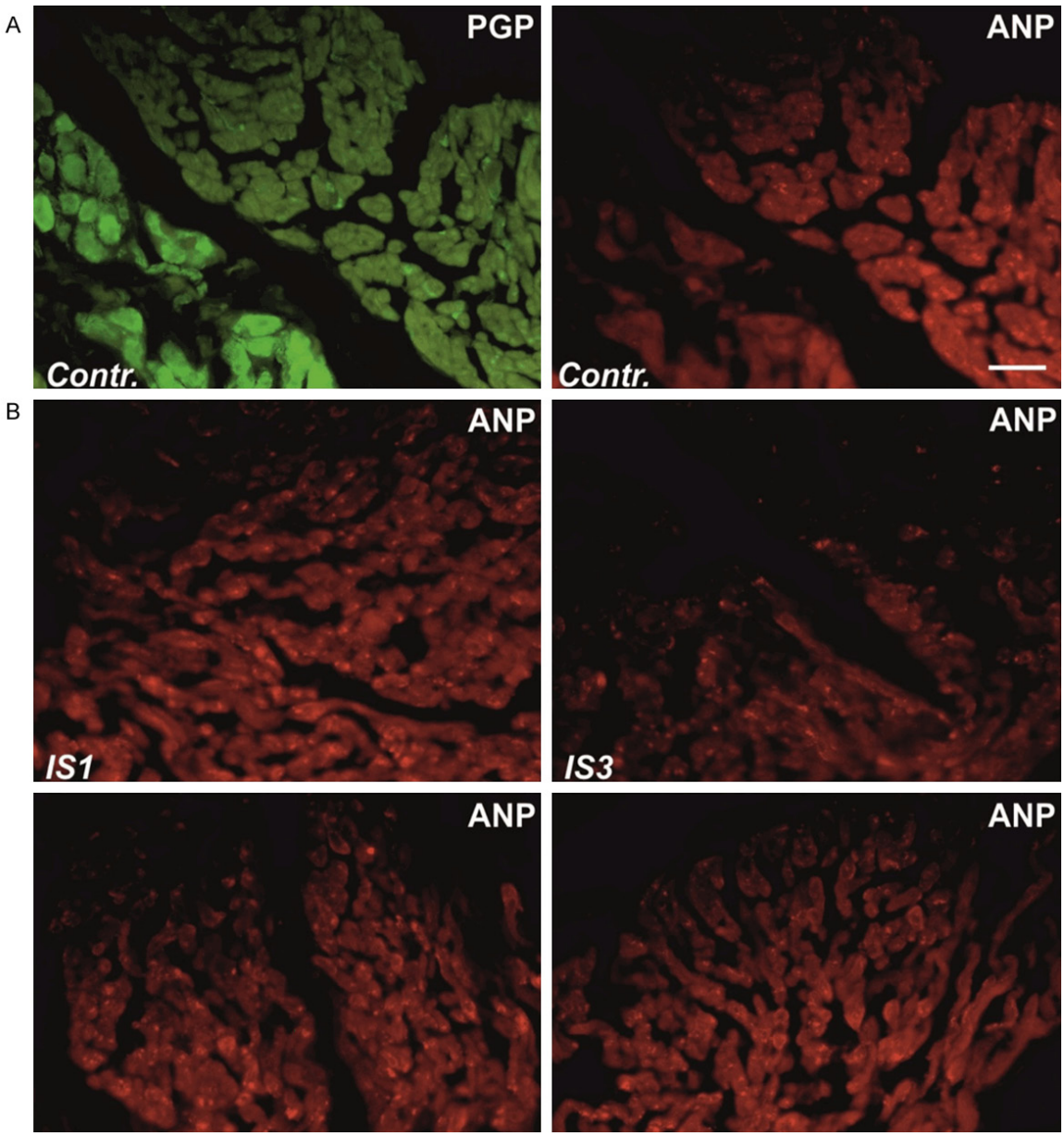

Figure 1. A. Immunoreactivity (IR) for PGP 9.5 and ANP in the same field in section of the left control atrium of SD rats. A ganglion containing large-diameter neurons is seen. PGP 9.5-IR visualized by FITC labelled streptavidin is located in neuron cell bodies of a cardiac ganglion and in varicosities of nerve terminals within the myocardium. In contrast, the localization of ANP-IR conjugated to Texas Red is completely different. It is shown in coarse granules within the cytoplasm of cardiomyocytes. Bar represents 100 μm. B. Immunoreactivities (IR) for ANP by the use of antibody conjugated with Texas Red in atrial sections of stressed SD rats for 1 hour according the protocols: immobilization and immobilization + cold after 1 or 3 hours (IS1, IS3, ICS1, ICS3). ANP-IR is seen in coarse granules within the cytoplasm of cardiomyocytes. Quantitative analysis of ANP-IR in sections of preparations from different stress protocols was not carried out. Bar represents 100 μm.

[19]. The expression level of the β-actin gene was used to normalize for differences in input cDNA while it has been shown as the most stable gene out of the tested reference genes-Hmbs, Ywhaz and β-actin in our experimental conditions. The quantification analysis of the data was performed by using the Optical System Software (Bio-Rad). To analyze relative expression of the proANP gene in separated heart compartments in control (unstressed) rats, as well as in stressed animals following formula has been used: Normalized amount =

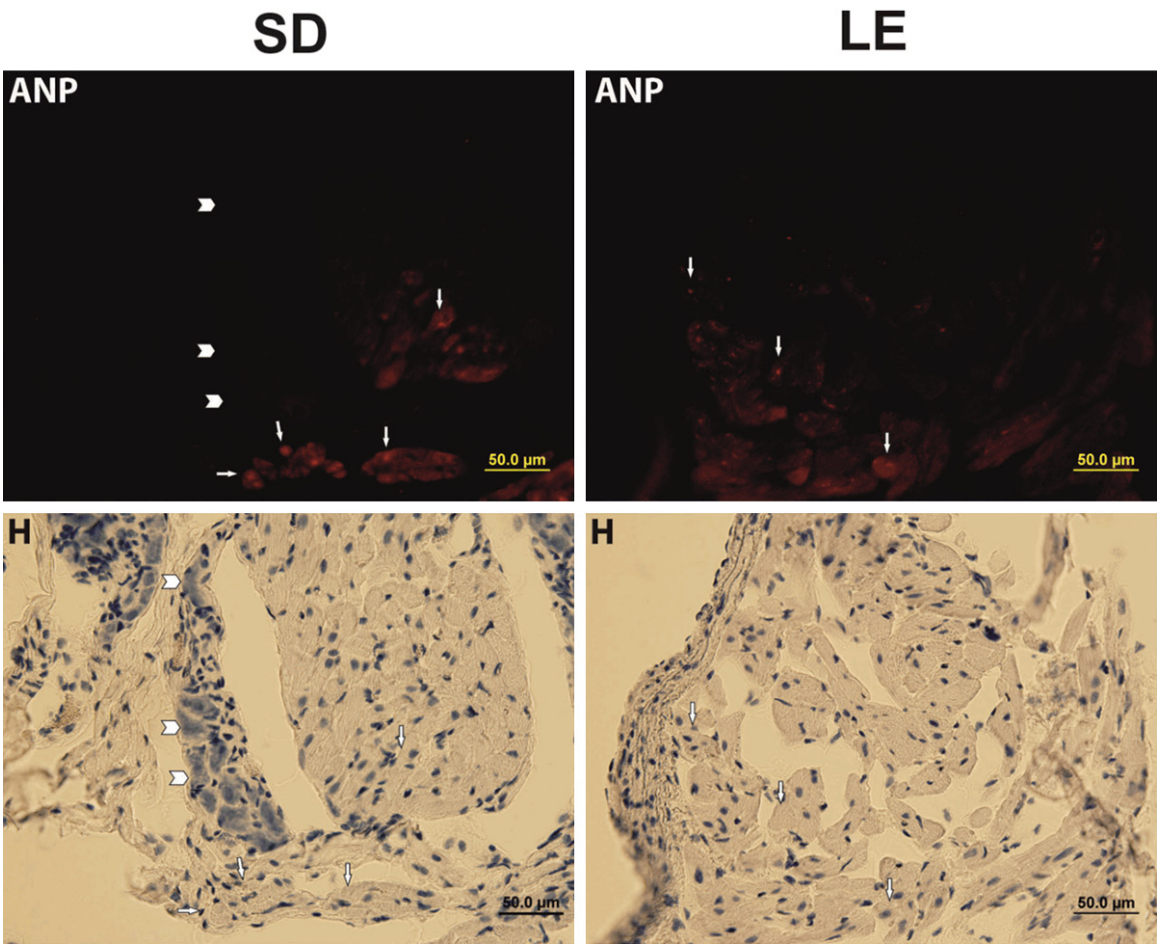

Figure 2. Immunoreactivity (IR) for ANP and histochemical staining in control atrium of SD and LE rats to verify localization of ANP-IR. ANP-IR is seen in coarse granules within the cytoplasm of cardiomyocytes (arrows) in atria of both rat strains. Large-diameter neurons (arrowheads) are not ANP-IR.

$(1 + E)^{Cq\ reference\ transcript}/(1 + E)^{Cq\ target\ transcript}$. The control group was used as a calibrator for comparison of gene expression level in every sample from stressed animals.

Data analysis

Results from separated heart compartments of control animals are presented as ΔC_q and RE ± SEM. To compare levels of the ANP mRNA expression in corresponding compartments of control SD and LE rats, mean values from SD rats were used as comparators. All results were statistically analyzed by the non-parametric Kruskal-Wallis test followed by the Mann-Whitney test. The analysis was performed using the software package STATISTICA Cz, version 7 (StatSoft CR, Prague, Czech Republic). The results were considered significantly different when $P < 0.05$.

Results

ANP immunoreactivity

ANP immunoreactivity (IR) was evaluated in all heart compartments of control and stressed animals. Relatively high density of ANP-IR was observed in both atria of the two rat strains, SD and LE ones. However, no specific staining was seen in any of the two ventricles (not shown). Method of double labeling with the two antibodies (against ANP and PGP 9.5) in the same section of preparations was used to confirm the pattern of distribution of ANP in the cytoplasm of cardiomyocytes in comparison with the distribution of a general neuronal marker PGP 9.5 within the cell bodies of neurons of a cardiac ganglion and in the varicosities of nerve terminals (**Figure 1A**). Since the method used is basically qualitative, no

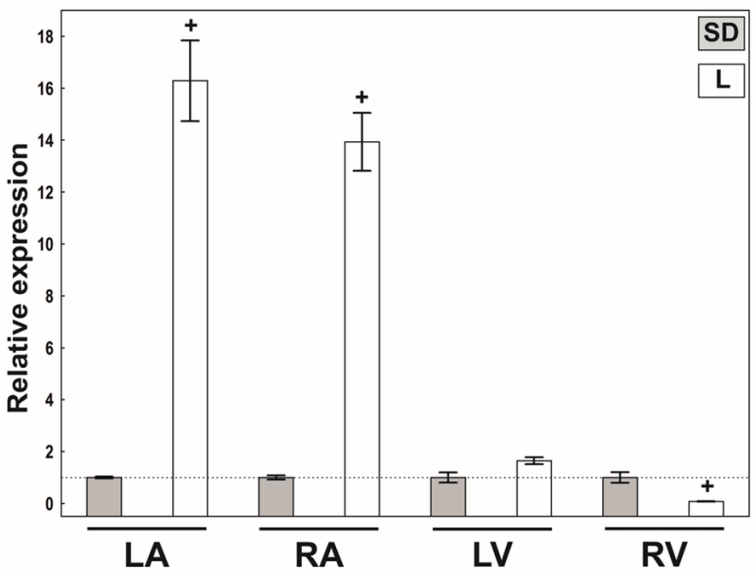

Figure 3. Real-time RT-PCR of ANP mRNA levels in corresponding heart compartments of control Sprague-Dawley (SD) and Lewis (LE) rats. Data are presented as a relative expression ± SEM. Mean values from SD rats were used as a calibrator. *p < 0.005 (Mann-Whitney-test) compared to corresponding heart compartment in SD rats. N = 4-6 in each group.

quantitative changes of ANP immunoreactive granules among preparations from control and stressed animals were observed (**Figure 1A** and **1B**). Subsequent staining of these sections with alum hematoxylin solution demonstrates that ANP-IR is present in cardiomyocytes (**Figure 2**).

ANP mRNA expression

The proANP mRNA expression was detected in all rat heart compartments: left atrium (LA), right atrium (RA), left ventricle (LV) and right ventricle (RV) in control and stress-induced animals of the two rat strains with a different activity of the HPA axis, SD and LE rats.

In control SD and LE animals, relative expressions were much higher in atria than in ventricles. We used relative expression of LV as a comparator, since in SD rats it contained the smallest amount of mRNA for proANP. In SD rats, rank order of the proANP gene expression was: LA = 50.8 ($p > 0.005$) > RA = 35.9 ($p > 0.005$) > RV = 3.3 ($p > 0.005$) > LV = 1, while in LE rats, it was LA = 501.5 ($p > 0.005$) > RA = 302.9 ($p > 0.005$) > LV = 1 > RV = 0.27 ($p > 0.01$). Thus, relative expression of the proANP mRNA level in each heart compartment was significantly different from all other compartments in both strains.

When comparing levels of the proANP gene expression between corresponding heart compartments in control SD and LE rats, the mean values of relative expression in individual compartments of SD rats were used as comparators. With the exception of RV in LE rats, all other heart compartments of this strain showed significantly higher expressions of proANP mRNA levels than those of SD rats: namely LA (16.3-fold), RA (13.9-fold), and LV (1.7-fold). The proANP relative expression made just 0.14-fold in the RV of LE rats compared to SD rats (**Figure 3**).

We also investigated the effect of the two types of stress on the proANP gene transcripts in both SD and LE rats. Immobilization by itself led to increased expression of the gene in SD rats, which was observed after 1 hour (IS1) in RA as well as after 3 hours (IS3) after the stress exposure in LA, RA and LV. Also, combination of immobilization with immersion to water had similar effect in LA and LV 3 hours (ICS3) after the stress exposure. However, in LE rats, no change in any compartment was observed with the exception of RA. This compartment, however, responded to both types of stress (IS and ICS) by a significant decline of the proANP mRNA expression, which was observed just 1 hour after the stress exposure (**Figure 4**). Additionally, we observed a tendency to decreasing of the gene expression in RV of both rat strains, however statistical significance was not reached. Comparison of the expressions in the same compartments of both rat strains under stress conditions showed that since in LA of SD rats the expression raised, it did not reach the levels of expressions in LA of LE animals. On the contrary, the observed down-regulation of the ANP expression in RA of LE rats did not lead to comparable expression in both rat strains, while the expression was higher in LE rats than in SD rats 1

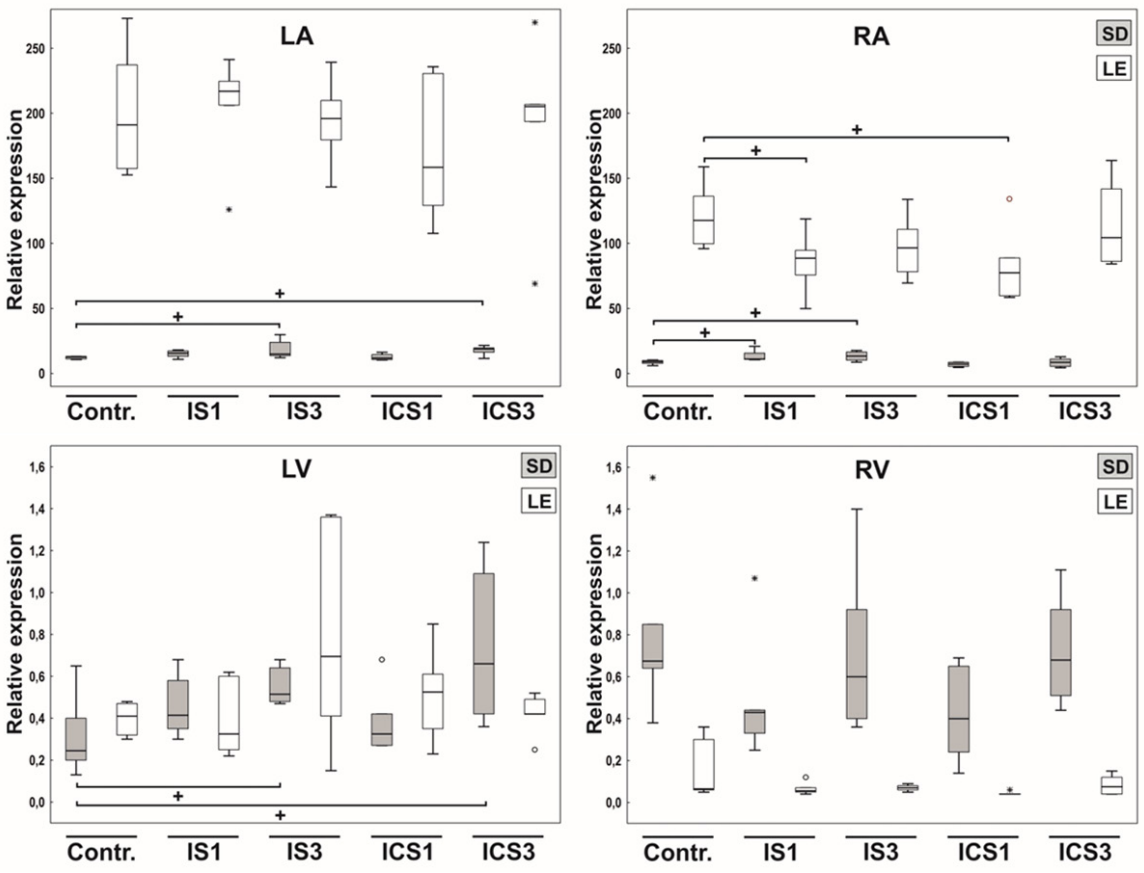

Figure 4. Real-time RT-PCR. Effect of acute restraint stressors on relative expression of proANP mRNA levels in four heart compartments of Sprague Dawley (SD) and Lewis (LE) rats. Data are presented as relative expression values (compared to β-actin) in corresponding heart compartments. Percentiles 0, 25, 50, 75 and 100 are presented in box plots; small circles and asterisks represent single data beyond 3x S.D. Abbreviations: IS1, IS3 are immobilization stresses and ICS1, ICS3 are immobilization stresses combined with immersion of rats to water. $^{+}p < 0.05$ (Mann-Whitney-test) compared to corresponding control group. N = 4-6 in each group.

hour after immobilization and/or combined stress application (**Figure 4**).

Discussion

Our study confirms the previous findings, that ANP is stored in atrial cardiomyocytes in the specific atrial granules (for a historical review see [20]. The proANP gene encodes for the synthesis of a 126-amino acids (aa) prohormone. Within this prohormone are several peptide hormone: the first 30 aa of the prohormone (long-acting natriuretic hormone; LANH), aa 31-67 (proANP 31-67; vessel dilator), aa 79-98 (proANP 79-98; kaliuretic peptide), and aa 99-126 (ANP, which is synonymous with its other terminology as atrial natriuretic hormone, ANH). Four peptide hormones constitute greater than 98% of the circulating ANP which exert significant diuretic, natriuretic, kaliuretic, and blood-pressure-lowering properties in animals and humans [6, 21, 22].

We have determined the expression of the proANP gene transcripts separately in all heart chambers in the two rat strains, SD and LE rats. Our results confirm the previous observations, that the greater part of the proANP gene expression occurs in atria of the heart in comparison with ventricles [23, 24]. The relative proANP mRNA expression in control SD rats was 36 to 51-fold higher in the right and left atrium, respectively, than in the left ventricle. In control LE rats, the difference between the expression in atria and ventricles was even higher; 303 to 501-fold in the right and left atrium, respectively, when compared with the left ventricle. In addition, the atria of control LE rats exhibited much higher expression of the proANP gene than the atria of control SD rats.

SD rats represented a comparative strain with the intact HPA system. Stress is known to activate this system and thus elicits the secretion of vasopressin (ADH) and corticotropin-releasing hormone (CRH) into hypophyseal portal vessels, which evokes increased adrenocorticorticotropin (ACTH) secretion by the anterior pituitary [25, 26], resulting in elevated plasma levels of corticoids. In addition, levels of catecholamines increase in the peripheral circulation [27]. The resulting effect is retention of salt and water and an increased vascular tone with increased blood volume and blood pressure [28].

Fink et al. [29] discovered that ANP is an important neurohormone which acts centrally to suppress stress-induced ACTH release. They also reported that ANP reduces negative feedback control of ACTH release mediated by glucocorticoid receptors. In addition, Franci et al. [25] showed that ANP also inhibits stress-induced vasopressin release which could account for the inhibitory action of ANP on stress-induced ACTH release. The results of these studies appear to indicate that an increased release of ANP may dampen a number of responses to stressful stimuli.

In our experiments, immobilization stress increased expression of the proANP mRNA in SD rats significantly (by 59%) in the right atrium only. A tendency to an increase was also observed in the left atrium (by 22%) and the left ventricle (by 89%) when determined 1 hour after stress exposure. An increase persisted over 3 hours after stress exposure, being significantly pronounced in the right atrium (by 53%), the left atrium (by 48%), and the left ventricle (by 127%). Combination of immobilization with immersion to water caused a significant increase of the proANP mRNA expression in the left atrium (by 67%) and the left ventricle (by 187%) only 3 hours after stress exposure. Since higher expression of the proANP gene potentially elevates a depletable pool of ANP in the heart [30], an increased plasma level of ANP may exhibit the protective effect on the heart and circulation due to the peptide suppression of stress-induced ACTH release [29] in combination with its diuretic, natriuretic and vasodilatory actions [31, 32].

LE rats however, exhibit a defect in the hypothalamic response to stress in the form of reduced synthesis and secretion of CRH, leading to reduced plasma ACTH release from the pituitary gland and corticosterone from the adrenal cortex [16, 33]. In our experiments, restraint stressors did not change the proANP gene level in any heart compartment of LE rats which may be attributed to hyporesponsiveness of the HPA axis of this strain. Moreover, the significant decline by 29% and 32% in the right atrium 1 and 3 hours after stress exposure, respectively, was observed. However, much higher levels of the pro ANP gene expression in both atria of control LE rats in comparison with SD rats were observed which probably reflect a role of ANP in body fluid homeostasis also in this strain.

It has been reported that the production and expression of ANP in atria and ventricles is differentially regulated. In atria, ANP expression appears to be governed by mechanical stimuli whereas in ventricles, it seems to be mainly dependent on the neuroendocrine environment [23, 34-36]. In our experiments the greatest increase in the proANP gene expression was found in the left ventricles of stress-induced SD rats in which the endocrine environment of cardiomyocytes is changed. Effects of hormones on regulation of the proANP gene expression were observed after the administration of mineralocorticoids and glucocorticoids. Ballerman et al. [37] administered deoxycorticosterone (DOCA) to rats and found plasma ANP levels and the atrial proANP mRNA content increased in animals retaining sodium in response to DOCA after 12 hours. Administration of dexamethasone, a potent glucocorticoid receptor agonist, increased proANP mRNA levels in both atria and ventricles of the rat approximately twofold. Additionally, norepinephrine stimulates the proANP mRNA expression and secretion [38]. The enhancement appears to result mainly from transcriptional activation of the proANP gene [39].

However, molecular mechanisms of control or stress-induced ANP gene expression and its relationship to other genes and proteins in myocardium which may undergo a pathogenesis of different cardiovascular disturbances have been only partially revealed. ANP mRNA was shown to be upregulated in the rat left ventricle in response to immobilization stress and reached a maximum 3 hours following the onset of the stress. In addition, colocalization

of the ANP mRNA and one of the typical cellular stress-induced substance, c-Fos protein, was observed which suggest that c-Fos can be involved in the upregulation of ANP [13]. On the other hand, the immobilization stress did not activate another transcriptional factor, the c-Jun-N-terminal kinase, as seen in ischemia-reperfusion [40, 41]. Thus, transcriptional control involved in ANP mRNA expression in different types of stress, diseased states and also in control individuals of different strains have still to be elucidated.

Conclusion

In summary, the study demonstrated inter-strain differences of the proANP mRNA expression to the two types of acute stress, immobilization and immobilization combined with water immersion. In SD rats with the intact HPA axis, both types of stress led to an upregulation of ANP gene expression with a maximum in the left ventricle 3 hours after stress exposure. In LE rats with a blunted reactivity of the HPA axis, no increase or even a downregulation of the gene expression was observed. Thus, stress-induced increase in the expression of the pro ANP gene is related to the activity of the HPA axis. Augmented expression and production of ANP can reduce both the cardiac preload and afterload thus contributing to the maintenance of cardiovascular homeostasis.

Acknowledgements

This work was supported by the Charles University Research Fund (project number P36 and 25) and by the National Sustainability Program I (NPU I) Nr. LO1503 provided by the Ministry of Education Youth and Sports of the Czech Republic.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Peter Kruzliak, Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Palackeho tr. 1946/1, 612 42 Brno, Czech Republic. E-mail: kruzliakpeter@gmail.com; Dr. Magdalena Chottova Dvorakova, Department of Physiology, Faculty of Medicine in Pilsen, Charles University in Prague, alej Svobody 76, 301 00 Pilsen, Czech Republic. E-mail: magdalena.dvorakova@lfp.cuni.cz

References

[1] Nakao K, Ogawa Y, Suga S, Imura H. Molecular biology and biochemistry of the natriuretic peptide system. I: Natriuretic peptides. J Hypertens 1992; 10: 907-912.

[2] Potter LR, Yoder AR, Flora DR, Antos LK, Dickey DM. Natriuretic peptides: their structures, receptors, physiologic functions and therapeutic applications. Handb Exp Pharmacol 2009; 191: 341-366.

[3] Edwards BS, Zimmerman RS, Schwab TR, Heublein DM, Burnett JC Jr. Atrial stretch, not pressure, is the principal determinant controlling the acute release of atrial natriuretic factor. Circ Res 1988; 62: 191-195.

[4] McGrath MF, de Bold AJ. Determinants of natriuretic peptide gene expression. Peptides 2005; 26: 933-943.

[5] Schlueter N, de Sterke A, Willmes DM, Spranger J, Jordan J, Birkenfeld AL. Metabolic actions of natriuretic peptides and therapeutic potential in the metabolic syndrome. Pharmacol Ther 2014; 144: 12-27.

[6] McGrath MF, de Bold ML, de Bold AJ. The endocrine function of the heart. Trends Endocrinol Metab 2005; 16: 469-477.

[7] Tsigos C, Chrousos GP. Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res 2002; 53: 865-871.

[8] Koob GF. Corticotropin-releasing factor, norepinephrine, and stress. Biol Psychiatry 1999; 46: 1167-1180.

[9] Dalack GW, Roose SP. Perspectives on the relationship between cardiovascular disease and affective disorder. J Clin Psychiatry 1990; 51: 4-11.

[10] Hatton DC, Brooks V, Qi Y, McCarron DA. Cardiovascular response to stress: baroreflex resetting and hemodynamics. Am J Physiol 1997; 272: R1588-1594.

[11] Grabie N, Hsieh DT, Buono C, Westrich JR, Allen JA, Pang H, Stavrakis G, Lichtman AH. Neutrophils sustain pathogenic CD8+ T cell responses in the heart. Am J Pathol 2003; 163: 2413-2420.

[12] Tavener SA, Kubes P. Cellular and molecular mechanisms underlying LPS-associated myocyte impairment. Am J Physiol Heart Circ Physiol 2006; 290: H800-806.

[13] Ueyama T, Senba E, Kasamatsu K, Hano T, Yamamoto K, Nishio I, Tsuruo Y, Yoshida K. Molecular mechanism of emotional stress-induced and catecholamine-induced heart attack. J Cardiovasc Pharmacol 2003; 41: S115-118.

[14] Ueyama T. Emotional stress-induced Tako-tsubo cardiomyopathy: animal model and mo-

lecular mechanism. Ann N Y Acad Sci 2004; 1018: 437-444.
[15] Wann BP, Audet MC, Gibb J, Anisman H. Anhedonia and altered cardiac atrial natriuretic peptide following chronic stressor and endotoxin treatment in mice. Psychoneuroendocrinology 2010; 35: 233-240.
[16] Gomez F, De Kloet ER, Armario A. Glucocorticoid negative feedback on the HPA axis in five inbred rat strains. Am J Physiol 1998; 274: R420-427.
[17] Klenerova V, Kaminsky O, Sida P, Krejci I, Hlinak Z, Hynie S. Impaired passive avoidance acquisition in Sprague-Dawley and Lewis rats after restraint and cold stress. Behav Brain Res 2002; 136: 21-29.
[18] Skopek P, Hynie S, Chottova-Dvorakova M, Sida P, Slavikova J, Mistrova E, Klenerova V. Effects of acute stressors on the expression of oxytocin receptor mRNA in hearts of rats with different activity of HPA axis. Neuro Endocrinol Lett 2012; 33: 124-132.
[19] Pfaffl MW, Tichopad A, Prgomet C, Neuvians TP. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper--Excel-based tool using pair-wise correlations. Biotechnol Lett 2004; 26: 509-515.
[20] de Bold AJ. On the shoulders of giants: the discovery of atrial natriuretic factor. Can J Physiol Pharmacol 1987; 65: 2007-2012.
[21] Vesely DL, San Miguel GI, Hassan I, Schocken DD. Atrial natriuretic hormone, vessel dilator, long-acting natriuretic hormone, and kaliuretic hormone decrease the circulating concentrations of CRH, corticotropin, and cortisol. J Clin Endocrinol Metab 2001; 86: 4244-4249.
[22] Piechota M, Banach M, Jacon A, Rysz J. Natriuretic peptides in cardiovascular diseases. Cell Mol Biol Lett 2008; 13: 155-181.
[23] Ogawa T, Linz W, Stevenson M, Bruneau BG, Kuroski de Bold ML, Chen JH, Eid H, Scholkens B A, de Bold A J. Evidence for load-dependent and load-independent determinants of cardiac natriuretic peptide production. Circulation 1996; 93: 2059-2067.
[24] Ruskoaho H. Cardiac hormones as diagnostic tools in heart failure. Endocr Rev 2003; 24: 341-356.
[25] Franci CR, Anselmo-Franci JA, McCann SM. The role of endogenous atrial natriuretic peptide in resting and stress-induced release of corticotropin, prolactin, growth hormone, and thyroid-stimulating hormone. Proc Natl Acad Sci U S A 1992; 89: 11391-11395.
[26] Buynitsky T, Mostofsky DI. Restraint stress in biobehavioral research: Recent developments. Neurosci Biobehav Rev 2009; 33: 1089-1098.
[27] Kvetnansky R, McCarty R, Thoa NB, Lake CR, Kopin IJ. Sympatho-adrenal responses of spontaneously hypertensive rats to immobilization stress. Am J Physiol 1979; 236: H457-462.
[28] Espiner EA. The effects of stress on salt and water balance. Baillieres Clin Endocrinol Metab 1987; 1: 375-390.
[29] Fink G, Dow RC, Casley D, Johnston CI, Bennie J, Carroll S, Dick H. Atrial natriuretic peptide is involved in the ACTH response to stress and glucocorticoid negative feedback in the rat. J Endocrinol 1992; 135: 37-43.
[30] Mangat H, de Bold AJ. Stretch-induced atrial natriuretic factor release utilizes a rapidly depleting pool of newly synthesized hormone. Endocrinology 1993; 133: 1398-1403.
[31] Saito Y, Nakao K, Nishimura K, Sugawara A, Okumura K, Obata K, Sonoda R, Ban T, Yasue H, Imura H. Clinical application of atrial natriuretic polypeptide in patients with congestive heart failure: beneficial effects on left ventricular function. Circulation 1987; 76: 115-124.
[32] Yoshimura M, Yasue H, Morita E, Sakaino N, Jougasaki M, Kurose M, Mukoyama M, Saito Y, Nakao K, Imura H. Hemodynamic, renal, and hormonal responses to brain natriuretic peptide infusion in patients with congestive heart failure. Circulation 1991; 84: 1581-1588.
[33] Sternberg EM, Glowa JR, Smith MA, Calogero AE, Listwak SJ, Aksentijevich S, Chrousos GP, Wiilder RL, Gold PW. Corticotropin releasing hormone related behavioral and neuroendocrine responses to stress in Lewis and Fischer rats. Brain Res 1992; 570: 54-60.
[34] de Bold AJ, Bruneau BG, Kuroski de Bold ML. Mechanical and neuroendocrine regulation of the endocrine heart. Cardiovasc Res 1996; 31: 7-18.
[35] Bianciotti LG, De Bold AJ. Effect of selective ET(A) receptor blockade on natriuretic peptide gene expression in DOCA-salt hypertension. Am J Physiol Heart Circ Physiol 2000; 279: H93-H101.
[36] Bianciotti LG, de Bold AJ. Modulation of cardiac natriuretic peptide gene expression following endothelin type A receptor blockade in renovascular hypertension. Cardiovasc Res 2001; 49: 808-816.
[37] Ballermann BJ, Bloch KD, Seidman JG, Brenner BM. Atrial natriuretic peptide transcription, secretion, and glomerular receptor activity during mineralocorticoid escape in the rat. J Clin Invest 1986; 78: 840-843.
[38] Kuwahara K, Nakao K. Regulation and significance of atrial and brain natriuretic peptides as cardiac hormones. Endocr J 2010; 57: 555-565.

[39] Gardner DG, Gertz BJ, Deschepper CF, Kim DY. Gene for the rat atrial natriuretic peptide is regulated by glucocorticoids in vitro. J Clin Invest 1988; 82: 1275-1281.

[40] Mizukami Y, Yoshioka K, Morimoto S, Yoshida K. A novel mechanism of JNK1 activation. Nuclear translocation and activation of JNK1 during ischemia and reperfusion. J Biol Chem 1997; 272: 16657-16662.

[41] Mizukami Y, Yoshida K. Mitogen-activated protein kinase translocates to the nucleus during ischaemia and is activated during reperfusion. Biochem J 1997; 323: 785-790.