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Deteriorated gene expression of selected calcium transporters in streptozotocin-induced diabetic hearts of Wistar rats

Original research article/Review

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Abstract **Aim:** The aim is to identify the possible changes in the expression of genes, that regulate calcium homeostasis in cardiomyocytes in diabetes mellitus.
Methods: Male Wistar rats were randomized into two experimental protocols: short-term 5-days streptozotocin-induced diabetes protocol with 20 weeks old animals at the end of the protocol (total N = 20) and long-term 4-weeks protocol with 18 weeks of age at the end of the protocol (total N = 38). 50 mg/kg of streptozotocin (STZ) was administered in both protocols by a single intraperitoneal injection in 0,1M citrate buffer (pH = 4.5). Control group (CON) received only vehiculum. Gene expressions in samples of left heart ventricle were measured by RT-qPCR method.
Results: The expression of SERCA2a in short-term protocol was decreased. In long-term protocol, decreased SERCA2a, TRPC4 and TRPC6 mRNA levels were observed (*p < 0.05). SERCA2a and TRPC4 mRNA levels exhibited statistical monotonic correlation in STZ-treated group in long-term protocol.
Conclusions: In diabetes mellitus, the calcium homeostasis in cardiomyocytes is altered and there could be a relation between alteration of internal sarcoplasmatic stores and store-operated calcium entry.

Keywords *TRPC – diabetes – SERCA2a – calcium – heart ventricles*

INTRODUCTION

Diabetic cardiomyopathy was previously associated with altered calcium handling. Decreased sarcoplasmatic load and sarco/endoplasmatic calcium ATPase (SERCA2a) activity contribute to intracellular calcium overload, that is associated with excitation-contraction coupling and other deterioration of cardiac function in diabetic models (Turan and Dhalla, 2014). TRPC (Transient receptor potential – canonical) channels are non-voltage-gated calcium channels that could be activated by intracellular calcium stores and take a part in the development of hypertrophy and heart failure (Watanabe et al., 2008). However, little is known about the contribution of cardiac TRPC channels in diabetes mellitus.

METHODS

Animals and experimental design

All the experimental procedures involving the use of experimental animals were approved by the State Veterinary and Food Administration of the Slovak Republic and by the Ethics Committee of the Faculty of Pharmacy, Comenius University in Bratislava. All the animals were kept under standard conditions and received standardized rodent chow and water ad libitum. Male Wistar rats (Department of Toxicology and Laboratory Animal breeding, Dobra Voda, Slovak Republic) were randomized into two experimental protocols: short-term 5 days streptozotocin-induced diabetes with animals 20 weeks of age at the end of the protocol (total N = 20) and long-term 4 weeks streptozotocin-induced diabetes with animals 18 weeks old at the end of protocol (total N = 38). 50 mg/kg of streptozotocin (STZ; Chemos CZ s.r.o., Czech Republic) was administered in both protocols by a single intraperitoneal injection in 0,1M citrate buffer (pH = 4.5). Control group (CON) received only vehiculum.

Glucose measurements and termination

In short term 5 days protocol, the postprandial glucose blood levels were measured from inferior vena cava, post mortem. In long term 4 weeks protocol, the standard rat laboratory chow

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was removed 12 hours before blood glucose measurement on the last day of experimental protocol. Water was provided ad libitum the whole time. Blood glucose levels were measured with point of care glucose testing device (Accutrend Plus system; Cobas/Roche Diagnostics International, Switzerland) from lateral tail vein after tail nicking. After the experimental protocol, animals were sacrificed by asphyxiation in carbon dioxide and samples of the left ventricular free-wall were dissected for RNA isolation.

RNA isolation and RT-PCR

Total RNA was isolated from left ventricular samples using Tri-Reagent^{Ô} (Sigma-Aldrich, USA). The quality of isolated RNA was verified with agarose gel electrophoresis and microspectrophotometric analysis (NanoDropND-1000, Thermo Scientific, USA). Reverse transcription was performed using the High-Capacity cDNA KIT with RNAse inhibitor (Applied Biosystems, USA). Real-time polymerase chain reaction (PCR) was performed using SYBR Green detection (SYBR Select Master Mix, Life Technologies, USA) on StepOnePlusÔ Real-Time PCR System (Life Technologies, USA) according to the manufacturer's instructions. Expressions of natriuretic peptide A (ANP), sarco/endoplasmatic reticulum calcium ATPase 2a subunit (SERCA2a) and transient receptor potential canonical 4 and 6 (TRPC4, TRPC6) were evaluated using gene-specific primers. The results were normalized to the expressions of endogenous reference genes (Beta-2 microglobulin, B2M; hypoxanthine phosphoribosyltransferase 1, HPRT1). The following primers sequence were used: HPRT1 (forward: 'CAGCTTCCTCCTCAGACCGCTTT', reverse: 'TCACTAATCACGACGCTGGGACTG'), B2M (forward: 'ATGGAGCTCTGAATCATCTGG', reverse: 'AGAAGATGGTGTGCTCATTGC'), ANP (forward: 'GGGGGTAGGATTGACAGGAT', reverse: 'GGATCTTTTGCGATCTGCTC'), SERCA2a (forward: 'CCCGAAACTACCTGGAGCCTGCA', reverse: 'ATGCACGCACCCGAACACCC'), TRPC4 (forward: 'AGCCCAGCGGAGAGAAGCAG', reverse: 'GGTCTGGGCACCGAGACACC'), TRPC6 (forward: 'TCGTGGCGCATCCGAACTGC', reverse: GGAGGAGCTTGGTGCCTTCAAATCT'). All primers (Sigma-Aldrich, USA) were verified to yield a single PCR product with the correct molecular weight, and the absence of signal was verified when reverse transcription was omitted.

Computational and statistical analysis

Mean PCR efficiency estimates (E) per amplicon and quantification cycle (Cq) values per sample were determined with LinRegPCR software (version 2015.0) and efficiency corrected relative expression ratios were calculated (all reactions had E > 1.8 and Cq < 35). All results were reported as mean ± standard error of mean (SEM). Comparisons between the means of two groups were performed by Student's t test for normally distributed data or Mann-Whitney test for non-parametric data, $p < 0.05$ considered as statistically significant. Statistical dependence between gene expression values of the two genes was evaluated by the Spearman's rank correlation coefficient (rho). Data were handled by GraphPad Prism (GraphPad Software Inc., version 6).

Table 1. Relative mRNA levels of ANP, SERCA2a, TRPC4 and TRPC6 genes in left ventricle samples divided in control group (CON) and group with streptozotocin-induced diabetes (STZ). Values are mean ± SEM. (p < 0.05 vs. CON).*

Target gene	5 days protocol		4 weeks protocol	
	CON 5D (n = 10)	STZ 5D (n = 10)	CON 4W (n = 16)	STZ 4W (n = 12)
ANP	1.00 ± 0.09	0.97 ± 0.21	1.00 ± 0.01	4.36 ± 0.51*
SERCA2a	1.00 ± 0.06	0.67 ± 0.03*	1.00 ± 0.04	0.68 ± 0.05*
TRPC4	1.00 ± 0.16	0.77 ± 0.10	1.00 ± 0.09	0.58 ± 0.11*
TRPC6	1.00 ± 0.09	0.87 ± 0.06	1.00 ± 0.08	0.65 ± 0.05*

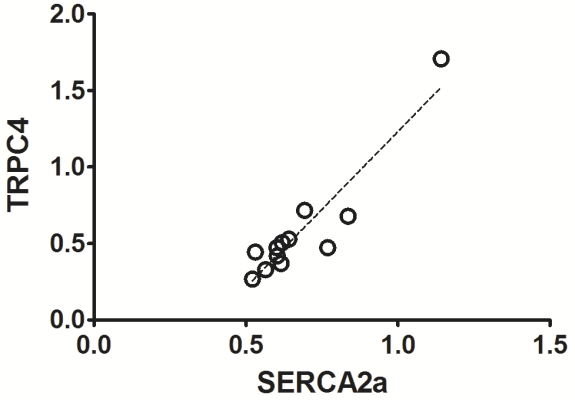

*Figure 1. Association between mRNA levels of SERCA2a and TRPC4 in STZ-treated group after 4 weeks experimental diabetes. Spearman's rank correlation coefficient rho = 0.82 (*p < 0.01).*

RESULTS

Objective of our study was to monitor the development of changes in abundance of selected calcium transporters in a model of streptozotocin-induced simulation of type I diabetes mellitus from the short term 5 days protocol to a longer follow-up after 4 weeks from diabetes induction. Actual diabetes in treated animals was confirmed against the criterion of preprandial and postprandial blood glucose measurement (≥ 7.0 mmol/l and ≥ 11.1 mmol/l, resp.).

Postprandial blood glucose levels in 5 days protocol were 21.39 ± 1.55 mmol/l vs. 36.78 ± 1.76 mml/l in control and STZ-treated group resp. (* $p < 0.0001$). Unusually, high levels in control group were the consequence of post mortem measurement after asphyxiation ion CO_2 and probable adrenalin-induced release of glucose from liver. However, the difference between the groups is self-evident. Postprandial glycaemia was also higher in 4 weeks protocol: 13.92 ± 2.74 mmol/l in STZ-treated group, vs. 6.19 ± 0.50 mmol/l of CON (* $p < 0.05$). Potential for diabetes related cardiac deterioration was estimated by myocardial expression of ANP, increased quantities were observed after 4 weeks, not in short term protocol. The expression of SERCA2a 5 days after STZ injection was decreased in contrast with other measured genes, where the expression levels remained unchanged. However, we found decreased mRNA levels of SERCA2a, with TRPC4 and TRPC6 after 4 weeks of experimental diabetes (Tab. 2). In addition, SERCA2a and TRPC4 mRNA level exhibited statistical monotonic correlation in STZ-treated group in 4 weeks experimental protocol (Figure 1).

DISCUSSION

Several pathways or mechanisms contribute to the development of diabetic cardiomyopathy. One of the hypothesis is alteration in calcium homeostasis. We set up in our experimental design to assess the development of changes in mRNA expression of selected calcium handling transporters from short lasting streptozotocin-induced type 1 diabetes mellitus (DM) to a prolonged untreated diabetes. We observed, that the first changes in calcium transporter gene expression occur in short-term of DM, when the expression of SERCA2a, as one of the most important channel in intracellular calcium homeostasis, is downregulated and this downregulation is preserved also in long-term stages of DM. This decline of expression is in agreement with the findings of Zhao et al. (2014), who found out progressive decrease of SERCA2a expression during 12 weeks of experimental diabetes. Interestingly, decrease in SERCA2a expression preceded the increase of ANP expression, as a marker of nonspecific cardiac damage, that is present after 4 weeks of experimental diabetes. TRPC channels were previously associated with cardiac remodelling and the development of heart failure (Eder and Molkentin, 2011). We propose that TRPC4 and TRPC6 channels could also play a role in diabetic cardiomyopathy. We found both downregulated, however, only after 4 weeks of experimental diabetes. We also looked if mRNA expressions of TRPC4 channel, that is responsible for store-operated calcium entry and SERCA2a as a major regulator of sarcoplasmatic internal calcium stores, could correlate. We found that the expressions of these two agents significantly correlate. This suggested that the intracellular stores and store-operated calcium entry could be altered, and these two aspects could be related.

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Impact of nitrate therapy on the expression of caveolin-1 and its phosphorylated isoform in lungs in the model of monocrotaline induced pulmonary hypertension

Original research article/Review

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Abstract Aim: Nitric oxide signalling pathway showed to be one of the crucial factors in the treatment and pathogenesis of pulmonary arterial hypertension. The aim of this study was to determine the effect of administration of inorganic nitrate, $NaNO_3$, on the expression of caveolin-1 and its phosphorylated isoform (pTyr14Cav-1) in lungs in the experimental model of monocrotaline induced pulmonary hypertension.
Methods: 10 weeks old male Wistar rats were subcutaneously injected with 60 mg/kg dose of monocrotaline (MCT) or vehicle (CON). Twelve days after the injection, part of the MCT group was receiving 0.3 mM $NaNO_3$ (MCT+N0.3) daily in the drinking water and rest was receiving 0.08% NaCl solution. Four weeks after MCT administration, the rats were sacrificed in CO_2. Protein expression in lungs was determined by western blot.
Results: We observed a significant decrease in the caveolin-1 expression and a significant shift towards the expression of pTyr14Cav-1 in the group treated with nitrate ($p < 0.05$).
Conclusion: $NaNO_3$ administration affected the expression of caveolin-1 and the ratio of its active (phosphorylated) isoform increased.

Keywords *Pulmonary hypertension – monocrotaline – inorganic nitrate – Caveolin-1 – lung*

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a rare disease with prevalence and incidence in Europe ranging from 15–60 patients per million and 5–10 patients per million per year, respectively (Galiè et al., 2016). In this disease, endothelial dysfunction and vascular remodelling cause pulmonary vascular resistance and increased pulmonary pressure (Lai et al., 2014). The mean pulmonary arterial pressure is elevated above 25 mm Hg at rest, while the pulmonary artery wedge pressure is not higher than 15 mm Hg (Galiè et al., 2016). Due to this pressure overload, the right ventricle hypertrophies and failure occurs (Archer et al., 2010).

The median life expectancy in adults without treatment intervention is approximately 2.8 years (Clapp & Gurung, 2015). However, there are therapeutic approaches that decrease pulmonary pressure, improve right heart function, exercise capacity, quality of life and slow down the progression of the disease (Lai et al., 2014). Specific pharmacotherapy targets three main pathways, affecting vascular tone and vascular remodelling: endothelin-1, prostacyclin and nitric oxide (NO) pathway (Humbert et al., 2004). Currently, endothelin receptor antagonists, prostacyclin analogues, prostacyclin receptor agonists, phosphodiesterase type 5 inhibitors and guanylate cyclase stimulators are used in treatment (Galiè et al., 2016). Despite availability of these therapeutic options, PAH remains incurable (Montani et al., 2014). Therefore, it is vital to look for novel treatment strategies.

Several investigations showed the vasodilatory effect of nitric oxide in the pulmonary circulation and its pathway has been explored for potential therapeutic interventions ever since (Thenappan & Weir, 2017). NO is synthesized by NO synthases (NOS). There are three isoforms of NOS – endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS). NO synthesized by eNOS is thought to be the major source of nitric oxide in lung circulation (Klinger & Kadowitz, 2017). However, an NOS-independent generation of NO – by reduction from nitrates and nitrites also occurs. Dietary or

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endogenous nitrates require the presence of commensal bacteria in the gastrointestinal tract for its reduction to nitrites, which can be then transformed to NO by various pathways involving myoglobin, haemoglobin, ascorbate, xanthine oxidoreductase or polyphenols (Lundberg et al., 2008).

Caveolin-1 (Cav-1) is a vital structural protein found in the invaginations of the plasmatic membrane – caveolae, and its function is to stabilize several proteins and regulate them through protein-protein interactions (Mathew, 2014). Cav-1 is also important for cellular transport, cholesterol homeostasis (Chettimada et al., 2015), regulation of vascular permeability, cell proliferation and apoptosis (Mathew, 2014). Moreover, Cav-1 inhibits eNOS activity, and therefore NO production, by binding to its domain (Fleming & Busse, 1999). Phosphorylation on Tyr-14 promotes and enhances the eNOS-Cav-1 binding (Chen et al., 2012).

In this study, we tested the hypothesis that administration of inorganic nitrate could induce changes in eNOS activity through expression of Cav-1 and pTyr14Cav-1.

METHODS

Experimental Design

Ten weeks old male Wistar rats were subcutaneously injected either with monocrotaline (Sigma-Aldrich, USA) in dose of 60 mg/kg (MCT) or vehicle (CON) as in the previous study (Malikova et al., 2016). Twelve days after the application, part of the MCT group started receiving 0.3 mM $NaNO_3$ (CentralChem, Slovakia) (MCT+N0.3) in the drinking water, while the rest was receiving 0.08% NaCl solution. Four weeks after MCT administration, the rats were sacrificed by CO_2 asphyxiation and the tissue samples from lungs were isolated.

Protein Expression

The lung samples were homogenized in the presence of liquid nitrogen, centrifuged at 12,560 g for 15 minutes and then the supernatant was collected. The concentration of samples was determined by BCA method (Pierce BCA Protein Assay, Thermo Fischer, USA). Individual samples were subjected to electrophoresis in polyacrylamide gel. Subsequently, the proteins were transferred to a polyvinylidene difluoride membrane and incubated overnight with the primary antibodies (BD Biosciences, USA) at 4°C. Afterwards, the membranes were incubated for 1 hour at room temperature with horseradish peroxidase-conjugated secondary anti-mouse antibody (Jackson ImmunoResearch, UK). For chemiluminescent detection, Pierce ECL Western Blotting Substrate (Thermo Scientific, USA) was used and the density of bands was determined by Optiquant program. The values of bands optical density were normalized by β-actin values.

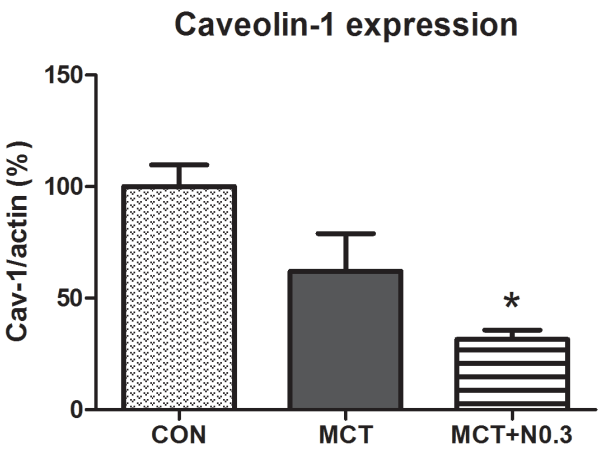

Figure 1. Protein expression of caveolin-1 in lung. Average ± SEM; ($P < 0.05$ vs. CON).

Statistical Analysis

Acquired data were analysed by the Shapiro-Wilk normality test. Normally distributed data were evaluated by ANOVA, with Tukey´s post-hoc test. Non-parametric data were evaluated by Kruskal-Wallis test and Wilcoxon post-hoc test. Results were expressed as mean ± SEM. *P* value of < 0.05 was considered statistically significant.

RESULTS

Protein expression of caveolin-1 in lung was significantly decreased in MCT+N0.3 compared to the control group ($P < 0.05$ vs. CON) (Fig. 1). We did not observe any significant changes in the expression of phosphorylated isoform of the protein (pTyr14Cav-1); however, there was a non-significant increase in the group treated with nitrate ($P = 0.068$ vs. CON) (Fig.2). The ratio pTyr14Cav-1/Cav-1 was significantly increased in MCT+N0.3 ($P < 0.05$ vs. CON) (Fig. 3).

DISCUSSION

The therapy with 0.3 mM $NaNO_3$ did not prevent an increase of the right ventricular pressure and cardiac hypertrophy induced by monocrotaline (data not shown). However, it affected the expression of caveolin-1 isoforms in lungs. The nitrate-nitrite-NO pathway works with NOS pathway in parallel but, in cases of decreased oxygen availability, the first process is enhanced (Lundberg et al., 2008). We hypothesised that an increased nitrate intake would cause a shift in eNOS activity.

Monocrotaline decreased the caveolin-1 expression in pulmonary arterial endothelial cells (Mathew et al., 2004)

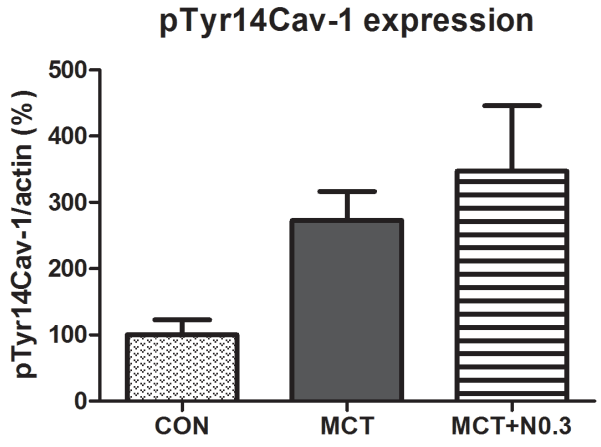

Figure 2. Protein expression of pTyr-14Cav-1 in lung.

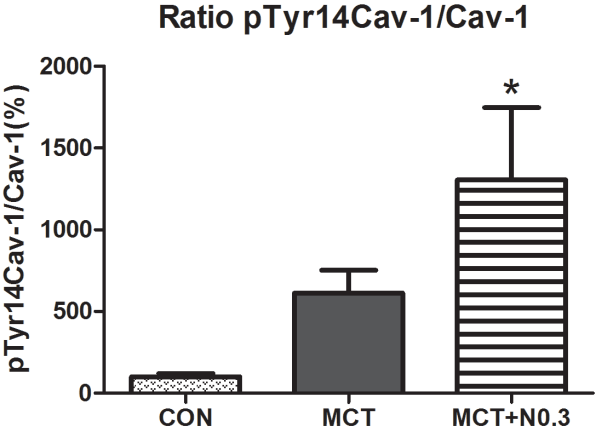

Figure 3. Ratio of pTyr14Cav-1/Cav-1 in lung. Average ± SEM; (P < 0.05 vs. CON).

and in lung tissue of rats (Haga et al., 2015). We observed non-significant decrease of Cav-1 expression in MCT group as well (by 38%, $P = 0.063$ vs. CON). Zhao et al. demonstrated that knock-out of Cav-1 in mice caused persistent eNOS activity, which led to nitration of protein kinase G (PKG) and subsequently to pulmonary hypertension. Nitration of PKG occurs after NO reacts with superoxide and forms peroxynitrite (Zhao et al., 2009). Under certain pathological states, termed as uncoupling of eNOS, this enzyme is able to produce reactive oxygen species (ROS) such as oxygen radicals (Mathew, 2014).

Moreover, Cav-1 interacts with bone morphogenetic protein receptor type II (BMPR-II). Mutations of *BMPR2* gene can be found in 75% of cases of hereditary PAH (Austin et al., 1993). Cav-1 is necessary for signal transduction and localization of BMPR-II (Wertz & Bauer, 2008). Cav-1 also inhibits a number of proliferative pathways (Mathew, 2011).

We surprisingly noted even more profound decrease of Cav-1 expression in MCT+N0.3 group ($P < 0.05$ vs CON) than in the MCT group. This could suggest a noxious effect of nitrates supplementation, regarding Cav-1. However, there are reports stating that the increase of Cav-1 in pulmonary arterial smooth muscle cells contributes to pathophysiology of PAH (Patel et al., 2007). Regarding this point of view, decrease of Cav-1 might be a positive feature and a compensatory mechanism.

As noted before, Cav-1 inhibits eNOS activity, after phosphorylation on its tyrosine residue (Chen et al., 2012). Moreover, phosphorylation on Tyr-14 is also necessary for the interaction with BMPR-II (Wertz & Bauer, 2008). We observed a non-significant shift towards the expression of pTyr14Cav-1 in the MCT group and a significant increase of pTyr14Cav-1 expression in the group treated with nitrate ($P < 0.05$ vs. CON). This suggests a possibly decreased eNOS activity after nitrate administration. That might be a positive feature, since there is a possibility that the enzyme produces detrimental ROS in the pathological setting of PH. Increase in Cav-1 phosphorylation could also possibly show tendency to enhance signalling through BMPR-II pathway, which was found to be disrupted in PAH (Morrell, 2006).

CONCLUSION

In conclusion, we demonstrated that 0.3mM dose of nitrates decreased the expression of caveolin-1 in the lung tissue of monocrotaline-treated rats. However, the ratio of active, phosphorylated form of this protein (pTyr-14Cav-1) and Cav-1 significantly increased after the therapy, suggesting an inhibition of endogenous production of NO through eNOS-caveolin binding and the potentiation of BMPR-II pathway.

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The Modulation Of Detrusor Contractility By Agents Influencing Ion Channel Activity

Original research article/Review

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Abstract **Background:** This study specified the role of several significant ion channels regulating the metabolism of calcium ions in contraction and relaxation of human detrusor muscle in order to identify possible target for future drugs that are capable of treating diseases resulting from impaired detrusor activity, e.g. overactive bladder. Although this disease can be successfully treated with muscarinic receptor antagonists or β_3 agonist, many patients may not be suitable for chronic therapy, especially due to the relatively high side effects of the treatment.
Material and Methods: The study used the isolated detrusor tissue samples, which were obtained from the macroscopic healthy tissue of urinary bladder from 19 patients undergoing a total prostatectomy because of localized prostate cancer. Each biological sample was prepared into 8 strips. We used oxybutynin and mirabegron as control drugs and several blockers of specific subtypes calcium and potassium ion channels as tested substances. The contractility of bladder was investigated by an organ tissue bath method in vitro and contraction was induced by carbachol.
Results: The amplitude of contraction was successfully decreased by positive control drugs and, from tested agents, the comparable effect had the substance capable of influencing IP_3 receptors and Orai-STIM channels and combination consisting of drugs possessing an inhibitory effect on IP_3 receptors, L- and T-type voltage-gated calcium channels and Orai-STIM channels.
Conclusion: The present work represents a new finding about handling Ca^{2+} in urinary bladder contraction and pointed to a dominant role of IP_3 receptor-mediated pathway in the regulation of Ca^{2+} metabolism, which may represent a future strategy in pharmacotherapy of impaired detrusor activity.

Keywords *Overactive bladder – calcium channels – smooth muscle reactivity*

INTRODUCTION

Overactive bladder (OAB) is specifically defined by the International Continence Society as an urinary urgency, usually accompanied by frequency and nocturia, with or without urgency urinary incontinence, in the absence of urinary tract infection or other obvious pathology (Gormley et al., 2015). OAB occurs in both men and women and it has a significant impact on the quality of life (Sharaf and Hasim, 2017). The impaired physiological contractile or relaxing function of the detrusor muscle is regarded as the most significant reason for OAB.

The relaxation of detrusor is mediated by a tonic release of norepinephrine, which activates β_3 adrenergic receptors and also by intrinsic urinary bladder (UB) smooth muscle (SM) properties. The contraction is achieved by acetylcholine, which activates UB SM, mainly M3 muscarinic receptors (Andersson, 2015), and can be reversed either by muscarinic receptor antagonists (e.g. oxybutynin, tolterodine, fesoterodine or trospium) or β_3 receptor agonists, e.g. mirabegron (Cernecka et al., 2015).

Several treatment options are available for OAB, including bladder and behavioural training, pharmacologic treatment, and surgical therapies. The antimuscarinics used to treat OAB are recognised to be effective in the improvement of OAB symptoms, but the associated side effects such as dry mouth, constipation, headache, and blurred vision occurred relatively often, leading to the discontinuation of therapy (Maman

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et al., 2014; Rajalabaya et al. 2016). The approval of the β_3-adrenoceptor agonist mirabegron has added a new class of pharmacotherapy for OAB. In 12-week trials, mirabegron (25, 50, and 100 mg) demonstrated significant reductions, compared with placebo, in micturition and incontinence episode frequency, with an incidence of antimuscarinic-associated adverse events similar to placebo (Abrams et al., 2015). However, patients who use mirabegron could suffer from hepatotoxic, cardiovascular and CNS toxic adverse effects (Sharaf and Hasim, 2017).

So there is still a need to find other treatment options. From literature, the importance of calcium ions (Ca^{2+}) is well known for an appropriate contractile function of the detrusor muscle. Increase in intracellular Ca^{2+}, resulting in contraction, occur primarily due to extracellular Ca^{2+} entry through plasmalemmal ion channels or release of Ca^{2+} from the endoplasmic reticulum (ER). In addition to Ca^{2+} homeostasis, administration of both pharmacological groups, generally used for OAB treatment, influence transport of Ca^{2+} through the plasma membrane (mirabegron) or its release from ER (antimuscarinics) (Cernecka et al., 2015). The generation and propagation of UB SM action potential results especially from Ca^{2+} entry through L-type voltage-gated ion channels (VGCC) (Parajuli et al., 2016). Several recent studies documented that M3 receptor-mediated detrusor contractions also require Ca^{2+} influx via L-type VGCC (Hedge, 2006). T-type channel activity is important at membrane potentials near the resting level (Sui et al., 2007). Nonselective cation channels, such as transient receptor potential (TRP) channels, significantly contribute to extracellular Ca^{2+} entry pathways in SM cells.

In response to a variety of stimuli, Ca^{2+}metabolism-controlling channels in the ER, i.e. ryanodine receptors (RyRs) and inositol-trisphosphate receptors (IP_3 Rs), mediate the efflux of Ca^{2+} from the ER into the cytoplasm of the cell (Hill-Eubanks et al., 2011). The antimuscarinic agents inhibit processes triggered by the activation of M3 receptors, especially emptying of Ca^{2+} from SR through the activation of IP_3 Rs. Large-conductance calcium-activated potassium channels (BK^+_{Ca}) stimulated by β3 agonists indirectly inhibit L-type VGCC (Sutovska et al., 2007). There is also the strong evidence that BK^+_{Ca} channel activity is inhibited upon the activation of M3 receptors and this mechanism contributes to suppressed UBSM contraction on administered antimuscarinics (Parajuli et al., 2016).

Extracellular Ca^{2+} influx in response to the depletion of intracellular Ca^{2+} stores, a process termed store-operated Ca^{2+} entry (SOCE), is known to play an important role in a number of cell types. The ubiquitously expressed STIM proteins serve as ER Ca^{2+} sensors and members of the Orai family (Feske et al., 2016) of transmembrane proteins as the entities responsible for mediating Ca^{2+} entry. Several morphological studies have shown that STIM and Orai family members are expressed in SM, and, under the test conditions, are capable of functionally coupling store depletion to extracellular Ca^{2+} entry (Hill-Eubanks et al., 2011). The Orai-STIM pathway role was examined in the rat model of OAB (Zhao et al., 2014), but not in humans.

MATERIAL AND METHOD

The primary objective of the current study was to evaluate the potential efficacy of different modulators of ion channel activity and their combinations in the suppression of UBSM activity compared with mirabegron and oxybutynin monotherapy.

All processes were approved by the Institutional Ethics Committee of the Jessenius Faculty of Medicine registered in the Institutional Review Board or the Institutional Ethic Board Office (IRB 00005636) in accordance with Slovakian and European legislation (decision No. EK1249/2013, EK1880/2016). Patient recruitment was conducted by using information sheets, and written informed consent was obtained. The samples of urinary bladder were collected in cooperation with the Clinic of Urology. Samples were collected from 19 male patients undergoing total prostatectomy for localized carcinoma (cT2N0M0) without OAB symptoms, systemic chronic disease, and therapy influencing ion channels activity administered systemically, neurogenic bladder and/or inflammation of lower and upper urinary tract before operation. Urinary bladder specimen was immersed into Krebs-Henseleit's buffer of the following composition (nM): NaCl, 112.9; KCl, 4.7; $CaCl_2$, 2.8; $MgSO_4$, 0.5; $NaHCO_3$, 24.9; and glucose, 11.1.

UBSM reactivity was evaluated using well-described organ tissue bath methodology (Franova et al., 2009). Prior to drug administration, urinary bladder samples were incubated for 1 hour in Krebs-Henseleit solution. Carbachol (1μM) was applied directly into the chamber to induce SM contractions. Cumulative doses (1μM, 10μM, 100μM and 1 mM) of the tested substances able to modulate transport of Ca^2+ ions**,** i.e. diltiazem, SKF96365 (1-[β-(3-(4-methoxyphenyl)propoxy)-4-methoxyphenethyl]-1H-imidazole hydrochloride**)**, 2APB (2-aminoethoxydiphenyl borate), potassium channels opener NS1619, their combinations and reference drugs mirabegron and oxybutynin were injected into the chamber 5 min after carbachol application. The amplitude of UBSM contraction was recorded and data were normalized on the weight of each sample.

2APB and SKF 96365 were purchased from TOCRIS (USA), and all other drugs from Sigma Aldrich (SR). 2APB was dissolved in 10% DMSO, and the remaining chemicals in water for injection.

Statistical analysis was performed in R (R Core Team 2015), using libraries WRS2 (Mair et al., 2016) and car (Fox and Weisberg, 2011). Normality of the data was assessed by quantile-quantile plots with bootstrap confidence intervals. Since the normalized amplitudes of contraction were not found to be Gaussian, the robust ANOVA was used for evaluation. Post-hoc confidence intervals were adjusted to control the family-wise error rate. Results with *p*-value below 0.05 were considered statistically significant.

RESULTS

From each urinary bladder sample 8 specimens were prepared and we tested the following monotherapies and combination (all in concentration c=1μM – 1mM): diltiazem (DIL), SKF96365 (SKF), 2APB, SKF + DIL, SKF + DIL + 2APB and SKF + DIL + 2APB + NS1619.

According to the obtained data, 2APB had a similar relaxing effect compared to reference drugs oxybutynin (OXY) and mirabegron (MIR) with effective onset seen in the lowest tested concentration of drugs (Fig. 1) and relatively low variability in responses (Fig. 2). DIL and SKF in monotherapies had a similar effect, which was lower than both positive controls, but their combination did not augmented decrease of UBSM contractility. Adding 2APB and both 2APB and NS1619 in combination with DIL + SKF led to statistically significant improvement of the relaxing effect. However, the effect of SKF+DIL+2APB combination was still significantly lower than that of MIR (Figs. 1 and 2).

DISCUSSION

The involvement of Ca^{2+} ion channels, such as VGCC (L-type and T-type), IP_3 Rs and SOCE (TRPC superfamily and Orai-STIM channels) and K^+ channels, especially BK^+_{Ca}, in UB SM contractility was examined using the pharmacological method in the present study. The individual ion channels activity was modulated by DIL, SKF, 2APB, NS1619 and their combinations and detrusor contractility was determined by the organ tissue bath method. This method was previously described in detail by Franova et al. 2009 and is generally used to test the efficacy of drugs that modulate SM tone in, for example, airways SM, uterine SM as well as UB SM.

The reference drugs OXY and MIR, clinically used for OAB treatment, effectively and dose-dependently decreased the contractile curve of isolated UB SM induced by carbachol with only insignificant differences of the effectiveness. As the mechanism of action of OXY and MIR is eminently regulated by Ca^{2+} and K^+ ions, this strongly supported the importance of calcium and potassium ion channels in detrusor physiology.

The results showed a significant role of IP_3 Rs and Orai-STIM channels in the process of contraction and relaxation of UB SM, because the efficacy of the tested drug 2APB was comparable to the generally used OXY and MIR. 2APB is used regularly in the research on the manipulation of intracellular Ca^{2+} release. It was initially found to block IP_3 Rs and was later shown to inhibit SOCE dose-dependently, independent of IP_3 Rs inhibition (DeHaven et al., 2008). Regardless it was expected that either monotherapy by DIL and SKF or by their combination did not cause significant a decrease in the amplitude of contraction induced by carbachol. We presumed especially a synergic effect of SKF+DIL combination because of the influencing T- and L-type VGCC as well as Orai-STIM channels. However, SKF is able to also block BK^+_{Ca} in higher concentrations, which probably explains such an effect (Tanahashi et al., 2016). A combination of SKF+DIL+2APB possessed significantly higher effects than SKF+DIL and, moreover, the isolated UBSM relaxation as similar to that on OXY. However, the effectiveness comparable to MIR alone was achieved by a combination of drugs SKF+DIL+2APB+NS1619.

CONCLUSIONS

Our experimental results confirmed the key role of IP_3 Rs, Orai-STIM and BK^+_{Ca} channels in the detrusor muscle physiology and pathophysiology. Furthermore, drugs able to inhibit IP_3 Rs, such as 2APB or agents influencing Orai-STIM and BK^+_{Ca} channels should be useful in new strategies drawn for the development of OAB treatment. Other possible approach should be the optimization of current treatments aimed at maintaining the balance between K^+ and Ca^{2+} ions, which seems to be essential for the right function of UB SM contractility.

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Effect of high-fat-fructose diet on synaptic plasticity in hippocampus and lipid profile of blood serum of rat: pharmacological possibilities of affecting risk factors

Original research article/Review

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Abstract The aim of this study was to determine pharmacological possibilities of influencing the risk factors of metabolic syndrome (MetS). Hypertriacylglycerolemic (HTG) rats fed with high-fat-fructose diet (HFFD) were used as a model of the MetS. Wistar rats fed with standard diet were used as negative control group. HTG rats fed with HFFD for 8 weeks were used as positive control group. The effects of atorvastatin and SMe1EC2 were tested. The compounds were administered to the HTG rats after 5 weeks of HFFD, once a day for 3 weeks. After 8 weeks, the blood serum lipid profile and electrophysiology of neurotransmission in hippocampal sections were evaluated *in vitro*. SMe1EC2 and atorvastatin had a significant effect on total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-cholesterol) and atorvastatin had a significant effect on triacylglycerols (TGs). SMe1EC2 improved the long-term potentiation (LTP) course in the hippocampus.

Keywords *metabolic syndrome – high-fat-fructose diet – hippocampus – cholesterol – triacylglycerols – rat*

INTRODUCTION

Metabolic syndrome (MetS) is a cluster of risk factors, including visceral obesity, insulin resistance, dyslipidemia, and hypertension, that, in conjunct, can lead to the development of type 2 diabetes mellitus and cardiovascular diseases (O'Neil and O'Driscoll, 2014). The risk of developing MetS depends on both genetic and life-style factors. The main risk factor for the development of MetS is physical inactivity and food consumption based on large amounts of fat and sugars (Janczura et al., 2015). Some risk factors for MetS are associated with the development of dementia and exert a negative influence on cognitive functions. It is not clear whether MetS as a whole syndrome or its individual components are more likely to contribute to the decline in cognition and development of dementia (Launer et al., 1995; Qiu et al., 2005). MetS is associated with impaired microstructural integrity of white brain mass in the parahippocampal gyrus and the hippocampus. In addition, MetS may lead to reduced hippocampal volume and increased cerebrospinal fluid volume (Yau et al., 2012; Alfaro et al., 2016). In rats, changes in hippocampal neuronal plasticity were also confirmed as related to MetS (Treviño et al., 2017). There is a great number of potential links between MetS and cognitive impairment. These include inflammation in the nervous system, oxidative stress, abnormal lipid metabolism in the brain, and impaired vascular reactivity. Insulin resistance can affect synaptic plasticity through damage to insulin-sensitive processes, which are responsible for neuronal survival, learning, and memory (Liu et al., 2015; Yates et al., 2012). Treatment that exists in this area is focused on influences of individual components of MetS. In MetS, several components are presented at the same time. Their treatment individually represents an increase in the number of undesirable effects of all concomitant medications. The aim of our work was to find whether the tested substances would affect several comorbidities associated with MetS.

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METHODS

Male hypertriacylglycerolemic (HTG) rats (n = 40) and Wistar rats (n=10) were divided into 5 groups: Wistar rats, the negative control group fed with standard diet; HTG rats, the positive control group fed with high-fat-fructose diet (HFFD); the HTG group fed for 8 weeks with HFFD and treated from the 6th to 8th week with atorvastatin (25 mg/kg); the HTG group fed for 8 weeks with HFFD and treated from the 6th to 8th week with SMe1EC2 with lower dosage (0.5 mg/kg); and the HTG group fed for 8 weeks with HFFD and treated from the 6th to 8th week with SMe1EC2 with higher dosage (25 mg/kg). The substances were administered intraperitoneally once a day at 9:00 a.m. for 3 weeks. Methylcellulose (*vehiculum*) was administered to both the control groups of rats. Standard rodent diet was produced by the certified producer of pellets at the Department of Toxicology and Breeding of Laboratory Animals, Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Dobra Voda, Slovakia, which is registered under number α SK 100089, the code 6147. The composition of the standard diet is given as follows: wheat, processed animal protein, oats, barley-corn, extruded lucerne, oil soybean meat, wheat bran, wheat germs, mineral mix, vegetable oil, sodium chloride. Vitamins added per 1 kg of the standard diet included the following: 20,000 IU of E672 vitamin A, 2,000 IU of E671 vitamin D3, and 70 mg of vitamin E; aminoacids added per 1 kg of the standard diet included the following: 1.2 g of DL-methionine and 0.8 g of L-lysine. Analytical components used in the standard diet included the following: 19.10% of nitrogen substances, 3.60% of fiber, 5.10% of oil and fat, 5.85% of ash, and 9.10% of humidity. High-fat diet (HFD) was enriched with about 1% of cholesterol, 7.5% of lard, and 10% of fructose. Rats had free access to water and food pellets and were kept on a 12-h/12-h light/dark cycle. All procedures involving the animals were performed in compliance with the Principles of Laboratory Animal Care issued by the Ethical Committee of the Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences and by the State Veterinary and Food Administration of Slovakia. The rats were decapitated under short ether anesthesia. Biochemical determination of lipid profile was performed on blood serum sample of each rat. Electrophysiological measurement of neurotransmission was performed on slices of left hippocampus of each rat. Lipid profile was determined by enzymatic photometric kit assay *in vitro*. Total cholesterol (TC), low-density lipoprotein cholesterol (LDL-cholesterol), high-density lipoprotein cholesterol (HDL-cholesterol), and triacylglycerols (TG) were examined. Neurotransmission was determined by recording and digitizing electrically induced responses of hippocampus. We used artificial cerebrospinal fluid (ACSF) composed of 124 mmol/l of NaCl, 3.3 mmol/l of KCl, 1.25 mmol/l of KH_2PO_4, 2.4 mmol/l of $MgSO_4$, 2.5 mmol/l of $CaCl_2$, 26 mmol/l of $NaHCO_3$, and 10 mmol/l of glucose and saturated with 95% O_2 + 5% CO_2, at pH 7.4. Synaptic plasticity was evoked using high-frequency stimulation (HFS) of Schaffer collaterals. Hippocampal slices (400-μm thick) were stimulated by bipolar stainless steel wire electrode. Electrically evoked responses were recorded using glass microelectrode filled with ACSF (3-5 MΩ) in the *stratum radiatum* of the rat hippocampus. At the beginning of the measurement, the stimulus intensity was reduced to obtain 30–50% of maximal excitatory postsynaptic potential (EPSP) at stimulus frequency of 0.033 Hz as the so-called baseline response. After 10–15 min of a stabilization period, long-term potentiation (LTP) of neuronal transmission was induced by a single train of HFS (100 Hz, 1 s). After an LTP induction, further evoked responses were recorded at baseline stimulus frequency (0.033 Hz) for the next 40 min. Average baseline response recorded during 10 min before HFS was normalized to 1, and consequently, all further responses were recalculated to normalized values.

STATISTICAL EVALUATION

The data were statistically evaluated using the InStat software version 2.05 (GraphPad) and GraphPad Prism Software (GraphPad, La Jolla, USA). Data were expressed as means ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) was used to evaluate (1) the difference among all the experimental groups (using the Tukey–Kramer multiple comparison test) and (2) the difference obtained compared to the control groups (using the Dunnett multiple comparison test). The limit of $p<0.05$ was considered a statistically significant difference.

RESULTS

In biochemical determination of the lipid profile, TG levels before the diet were elevated in HTG rats compared to the Wistar group, which is consistent with their genetic status. Diet and administration of SMe1EC2 did not affect the TG levels. In the atorvastatin-treated group, there was a decrease in TG levels compared to their pretreatment level (Fig. 1A). HFFD in combination with hypertriacylglycerolemia increased the levels of TC and LDL-cholesterol in blood serum compared to Wistar rats. The administration of atorvastatin and SMe1EC2 at a lower dose resulted in the reduction of the increase in the levels of TC (Fig. 1B) and LDL-cholesterol (Fig. 1C). The higher dose of SMe1EC2 did not affect the lipid profile of blood serum. HDL-cholesterol levels were significantly reduced by the use of HFFD in combination with hypertriacylglycerolemia. Treatment did not affect the HDL-cholesterol (Fig. 1D). In electrophysiological measurements, HFFD in combination with hypertriacylglycerolemia deteriorate the course of LTP and administration of

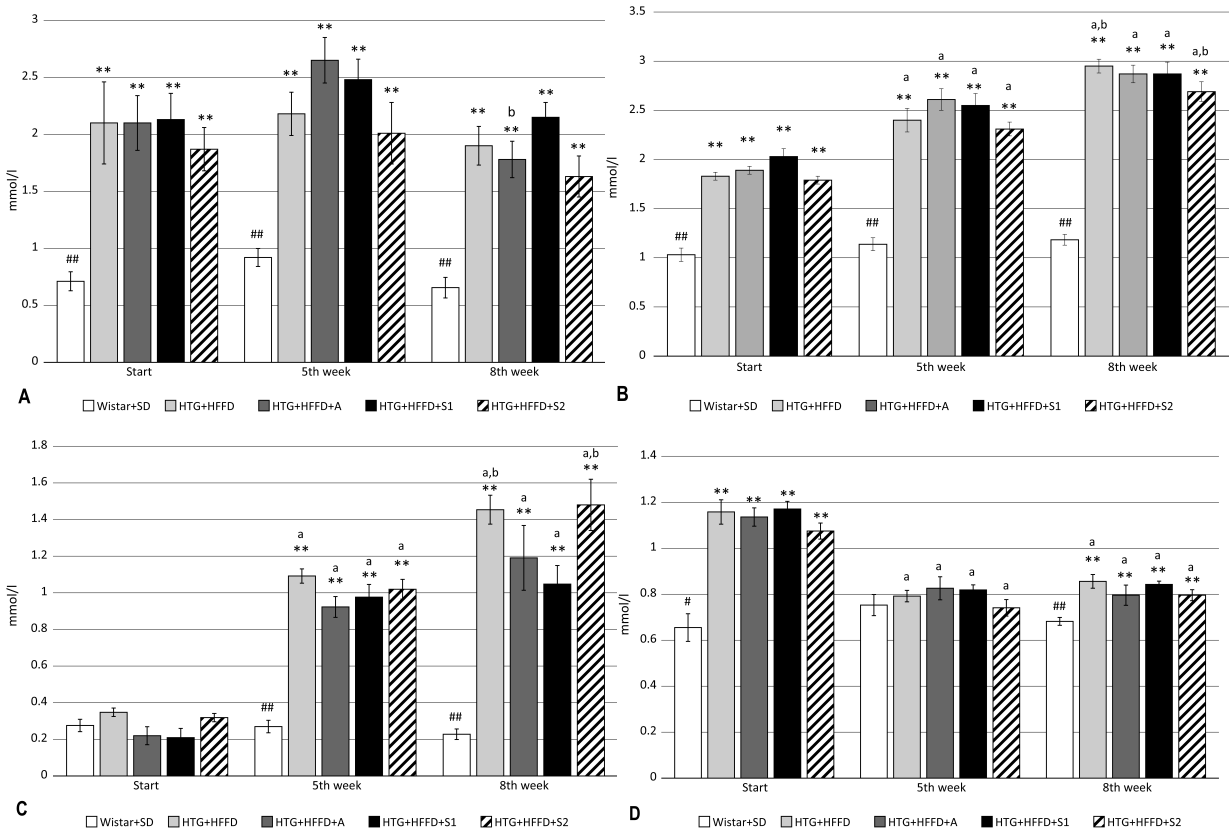

*Figure 1. Lipid profile (mmol/l) in blood serum during the 8-week diet. (A) Levels of triacylglycerols (TG) before the diet were elevated in hypertriacylglycerolemic (HTG) rats compared to Wistar group. High-fat-fructose diet (HFFD) and administration of SMe1EC2 did not affect the TG levels. Atorvastatin decreased TG levels compared to their pretreatment level. (B) HFFD in combination with hypertriacylglycerolemia increased the levels of total cholesterol (TC) in blood serum compared to Wistar rats. The administration of atorvastatin and SMe1EC2 at a lower dose resulted in the reduction of the increase in TC levels. The higher dose of SMe1EC2 did not affect the levels of TC in blood serum. (C) HFFD in combination with hypertriacylglycerolemia increased the levels of LDL-cholesterol in blood serum compared to Wistar rats. The administration of atorvastatin and SMe1EC2 at a lower dose resulted in the reduction of the increase in LDL-cholesterol levels. The higher dose of SMe1EC2 did not affect the levels of LDL-cholesterol in blood serum. (D) HDL-cholesterol levels were significantly reduced by the use of HFFD in combination with hypertriacylglycerolemia. Wistar-SD, the negative control group fed with standard diet; HTG-HFFD, the positive control group of HTG rats fed with HFFD; HTG+HFFD+A, HTG rats fed with HFFD and treated from the 6th to 8th week with atorvastatin (25 mg/kg); HTG+HFFD+S1, HTG rats fed with HFFD and treated from the 6th to 8th week with SMe1EC2 with lower dosage (0.5 mg/kg); HTG+HFFD+S2, HTG rats fed with HFFD and treated from the 6th to 8th week with SMe1EC2 at higher dosage (25 mg/kg). Each group consisted of 10 rats. The values on the chart are arithmetic means ± SEM. The limit of $p<0.05$ was considered as a statistically significant difference. * significant difference compared to Wistar (before diet, after 5 weeks of diet [before treatment], after 8 weeks of diet [after 3 weeks of treatment]); # significant difference compared to the HTG-HFFD group (before diet, after 5 weeks of diet [before treatment], after 8 weeks of diet [after 3 weeks of treatment]). a, significant difference between values of the same group compared to values before diet; b, significant difference between values of the same group compared to values after 5th week of diet.*

atorvastatin and SMe1EC2 at both doses tested resulted in improvement in the course of LTP (Fig. 2).

DISCUSSION

MetS is a worldwide health problem that can lead to cardiovascular and cerebrovascular diseases. We studied the effect of HFFD on lipid profile of blood serum and neurotransmission in the hippocampus of HTG rats. The main feature of HTG rats are elevated TG levels that are present from 10 weeks of their age, which may further lead to the development of dyslipidemia characterized by decreased HDL-cholesterol levels and elevated LDL-cholesterol levels (Štolba et al., 1992). The HFFD causes non-alcoholic liver steatosis (Chukijrungroat et al., 2017), insulin resistance, and increases in cholesterol levels in

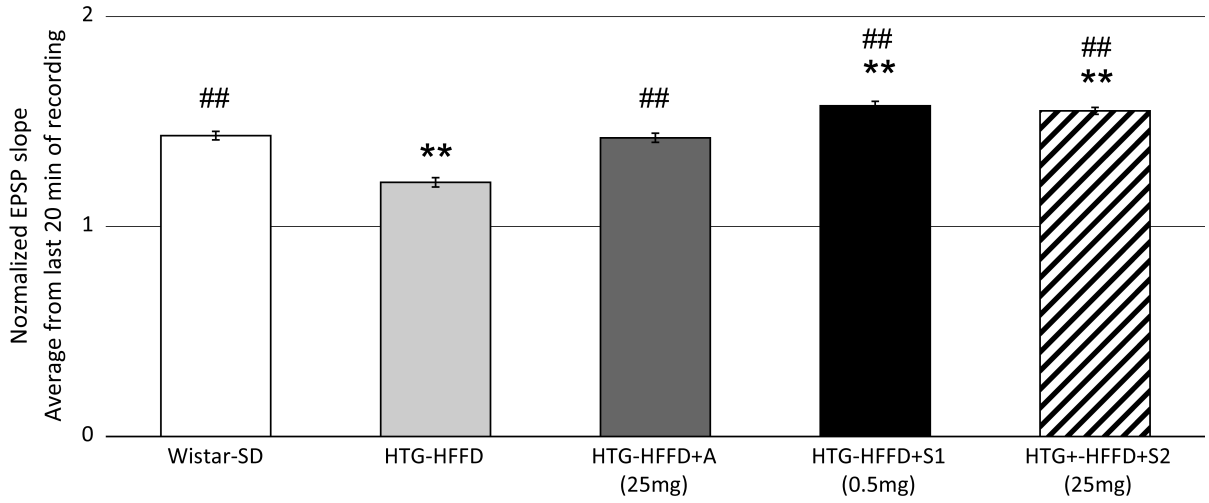

*Figure 2. Electrophysiological measurement of long-term potentiation (LTP) in hippocampal stratum radiatum. High-fat-fructose diet (HFFD) in hypertriacylglycerolemic (HTG) rats deteriorated the course of LTP and the administration of atorvastatin and SMe1EC2 at both doses tested resulted in an improvement in the course of LTP. Wistar-SD, the negative control group fed with the standard diet, n = 10 hippocampal slices; HTG-HFFD, the positive control group of HTG rats fed with HFFD, n = 14 hippocampal slices; HTG+HFFD+A, HTG rats fed with HFFD and treated from the 6th to 8th week with atorvastatin (25 mg/kg), n = 13 hippocampal slices; HTG+HFFD+S1, HTG rats fed with HFFD and treated from the 6th to 8th week with SMe1EC2 at lower dosage (0.5 mg/kg), n = 14 hippocampal slices; HTG+HFFD+S2, HTG rats fed with HFFD and treated from the 6th to 8th week with SMe1EC2 at higher dosage (25 mg/kg), n = 15 hippocampal slices; each group consisted of 10 rats. The values on the chart are arithmetic means ± SEM. The column chart shows arithmetic means ± SEM of normalized EPSP slope recorded during last 20 min. The limit of $p<0.05$ was considered as a statistically significant difference. * Difference is significant compared to Wistar rats; # difference is significant compared to the HTG-HFFD group.*

blood plasma (de Sousa Rodrigues et al., 2017). Recently, it was reported that levels of TC, TG, and LDL-cholesterol were higher and levels of HDL-cholesterol were significantly reduced in Sprague-Dawley rats fed with HFD (Jia et al., 2013). Furthermore, rats of Fischer 344 strain fed with HFD had elevated levels of TC, TG, LDL-cholesterol, and even HDL-cholesterol after diet (Granholm et al., 2008). Krishna et al. (2015) found that HFD consumption altered synaptic plasticity, evidenced by significant reductions in LTP magnitude. Valladolid-Acebes et al. (2012) also published an attenuation of the LTP response in the radiatum layer in rats fed with HFD. In our work, we found that HFFD increased the levels of TC and LDL-cholesterol and decreased the levels of HDL-cholesterol in blood serum of HTG rats. Levels of TG before the diet were elevated in HTG rats compared to Wistar group, and the diet did not affect these values further. In electrophysiological measurement we found that HFFD diet deteriorated the course of LTP.

We also investigated the effect of SMe1EC2 and atorvastatin treatment on lipid profile and hippocampal neurotransmission in HTG rats fed with HFFD. We chose the pyridoindole antioxidant SMe1EC2 because of its wide spectrum of protective action. According to previous studies conducted at the Slovak Academy of Sciences, SMe1EC2 is not toxic and has neuroprotective and cardioprotective effects (Horáková and Štolc, 1998). SMe1EC2 reduced the serum levels of TC and TG (Bezek et al., 2010). Its effect on plasma lipid levels is comparable to fenofibrate (Kyseľová et al., 2010). The effect of SMe1EC2 was compared with the effects of atorvastatin, which is clinically used as lipid-lowering agent. Atorvastatin is an HMG-CoA reductase inhibitor effectively reducing LDL-cholesterol levels and TG levels and increasing HDL-cholesterol concentrations (Cornier et al., 2008). Atorvastatin acts as an immunomodulator by inhibiting the activation of T-lymphocytes. It reduces oxidative stress and improves endothelial function. Statins act as anti-inflammatory agents by several mechanisms (Meyer-Sabellek and Brasch 2006). In our study, SMe1EC2 at a lower dose and atorvastatin caused a reduction in the increase in the levels of TC and LDL-cholesterol and atorvastatin induced a decrease in the levels of TG, which is consistent with previous studies with HFD without fructose. Furthermore, SMe1EC2 at a lower dose improved the course of LTP at the rat hippocampus. This could be due to its neuroprotective as well as antioxidant effects. These results indicate that SMe1EC2 could be promising in the treatment of MetS-related disorders. This has, however, to be verified in further studies.

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kardiovaskulárnych a cerebrovaskulárnych ochorení a farmakologické možnosti ich ovplyvnenia/ Risk factors of cardiovascular and cerebrovascular diseases and pharmacological possibilities of their influence.

ABBREVIATIONS

ANOVA – Analysis of variance
HDL-cholesterol – high-density lipoprotein cholesterol
HFD – high-fat diet
HFFD – high-fat-fructose diet
HTG – hypertriacylglycerolemic
LDL-cholesterol – low-density lipoprotein cholesterol
LTP – long-term potentiation
MetS – metabolic syndrome
n – number
TC – total cholesterol TG – triacylglycerols

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Effect of pre-gestational stress and prenatal venlafaxine administration on cardiovascular system of rat offspring

Original research article/Review

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Abstract

A number of pregnant women all over the world suffer from depression and are treated during gestation with antidepressants, mostly with selective serotonin reuptake inhibitors or selective serotonin and norepinephrine reuptake inhibitors. Exposure to prenatal stress is also a great risk factor for a developing fetus and could be responsible for altered fetal development or various neurobehavioral disturbances of a child. Administration of selective serotonin and norepinephrine reuptake inhibitor venlafaxine is associated with various cardiovascular adverse effects, such as tachycardia, increased blood pressure, arrhythmias and hypertensive crisis. The aim of this study was to focus on the effect of pre-gestational chronic mild unpredictable stress and/or administration of antidepressant venlafaxine (10 mg/kg/day, p. o.) on specific parameters, determining the function of the cardiovascular system of male and female rat offspring. Blood pressure and standard ECG were recorded in the offspring. Exposure to pre-gestational stress did not cause significant changes in the systolic, diastolic blood pressure and pulse frequency either in males or in females, compared to the unexposed control animals. Pre-gestational stress caused the shortening of QT interval and prolongation of QRS complex duration in males. On the other hand, in females, the effects of pre-gestational stress were potentiated by the administration of venlafaxine and resulted in elevated systolic and diastolic blood pressure, prolonged QT interval and shortened QRS complex duration, compared to the control. In conclusion, the female rat offspring are more sensitive to exposure to pre-gestational, to chronic mild unpredictable stress and venlafaxine.

Keywords *Venlafaxine – pre-gestational stress – blood pressure – ECG*

INTRODUCTION

Depression is a stress-related, affective disorder, with prevalence of 10–18% among pregnant women all over the world. Exposure to prenatal stress represents a great risk factor for developing pregnancy diseases (preeclampsia, miscarriages, gestational hypertension and hypoxia of the fetus), altered fetal development, low birth weight and a range of neurobehavioral disturbances of a child, including autism, anxiety and depression (St-Pierre et al., 2016; Kiryanova et al., 2017; Gemmel et al., 2018).

During gestation, 2–3% women are treated with antidepressants (AD), mostly with selective serotonin reuptake inhibitors (SSRI) or selective serotonin and norepinephrine reuptake inhibitors (SNRI). These substances act by blocking the serotonin transporter (5-HTT) and increasing the extracellular level of serotonin (Hanley & Oberlander, 2013). Being relatively small molecules, SSRIs/SNRIs cross the placenta and are able to affect the fetal serum levels of serotonin (Kim et al., 2006). Prenatal exposure to SSRIs is connected with an increased risk of neonatal AD withdrawal syndrome, neurobehavioral alterations, persisting pulmonary hypertension, low birth weight and cardiovascular abnormalities (Chambers et al., 1996; Kallen, 2004; Zeskind & Stevens, 2004; Moses-Kolko et al., 2005; Chambers et al., 2006; Louik et al., 2007). According to Furu et al. (2015), the most frequent adverse effects of AD are cardiovascular defects, however, the mechanism of these defects is still unclear.

Venlafaxine belongs to the group of SNRIs and acts by the inhibition of both serotonin and norepinephrine reuptake (Kivrak et al., 2014) and has been used for depression therapy since 1994. Venlafaxine administration is associated mostly

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with mild adverse effects, such as tachycardia, increased blood pressure (BP), arrhythmias, headache, dizziness, dry mouth and is also reported as a cause of hypertensive crisis (Khurana & Baudendistel, 2003; Ciuna et al., 2004; Johnson et al., 2006; Howell et al., 2007). Premedication of rats with venlafaxine in a single dose 30 mg/kg, 24 hours before isolation of the heart and perfusion according to Langendorff, caused significantly increased incidence of ventricular tachycardia and QTc interval prolongation in spontaneously beating isolated heart (Vicen et al., 2016). Administration of venlafaxine in pregnancy affects both maternal and fetal levels of serotonin. Serotonin, a neurotransmitter involved in brain and heart development, is also involved in the functional regulation of cardiovascular system (CVS), including vasoconstriction, cardiomyocyte apoptosis, incidence of arrhythmias, such as tachycardia (Gregs et al., 2009).

Although numerous studies have examined various animal models of prenatal stress (Newport et al., 2002; Mueller & Bale, 2006; Mueller & Bale, 2008; Huang et al., 2012) and the effect of prenatal AD use on rat offspring (Chambers et al., 2006; Forcelli and Heinrichs, 2008; Dubovický et al., 2012; Gemmel et al., 2018), the majority of them are using SSRIs, mostly with the focus on their neurobehavioral effects. Pre-clinical and clinical studies have found a correlation between emotional stress during pregnancy and increased incidence of neurological and behavioral disorders in offspring (Kapoor et al., 2009; Li et al., 2010; Huang et al., 2010; Huang et al., 2012). Few studies have also shown, that male and female animals are differently affected by stress and outcomes of male offspring of stressed mothers may vary from that of females (Kiryanova et al., 2017). To our knowledge, there have not been studies examining the simultaneous exposure to prenatal stress and SNRIs.

Therefore, the purpose of our study is the analysis of the effect of pre-gestational chronic mild unpredictable stress (Bögi et al., 2018) and/or administration of antidepressant venlafaxine on the function of CVS of rat offspring of both genders.

MATERIAL AND METHODS

Animals

The experiments were conducted in compliance with the Principles of Laboratory Animal Care issued by the Ethical Committee of the Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine (CEM), Slovak Academy of Sciences, Bratislava, and the experimental design was approved by the State Veterinary and Food Administration of the Slovak Republic (Protocol No.: 1030/16/221). In this study, the female Wistar rats were obtained from the Department of Toxicology and Laboratory Animal Breeding Station of Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Sciences, Dobra Voda, Slovakia. Rats had free access to standard rat chow and water and were kept in a room with controlled temperature (20–24 °C), and relative humidity (50–60%), with 12/12 hour light/dark cycle. Half the number of the animals (age: 3 months, n = 30) were exposed to chronic mild unpredictable stress for 2 weeks in total. Animals were exposed 2 times per day to following stressors: 1. Overcrowding (6 rats in 1 cage for 24 h), 2. Damp bedding (8 h, 500 ml of tap water), 3. Food deprivation (12 h), 4. Rotation of the cage (cages were reversed front to back once in 12 h), 5. Water deprivation (5 h), 6. Decline of the cage (45 degree, 12 h), 7. Swim stress (vertical cylindrical glass container – 45 cm high, Ø 25 cm, filled with tap water at temperature 26 ± 1 °C). Stressors were picked up randomly and animals were exposed to 2 different stressors each day (Csatlósová et al., 2018).

Three weeks after the stress conditions females were mated with age-matched males in the ratio 1 male : 3 females. Day 0 of gestation was determined by the presence of sperms in vaginal smears. Pregnant rats were housed in plastic cages, 3 per cage. On the 15th day of gestation, the females were separated and housed individually. Vehicle or venlafaxine (10 mg/kg/day, Chemos, Regenstauf, Germany) were applied twice a day *per os* (on biscuits) to dams that were divided into the following groups: non-stress + vehiculum, non-stress + venlafaxine, stress + vehiculum, stress + venlafaxine. Venlafaxine was administered to dams for 74 days in total.

Offspring were separated from their mothers on the 21st day *postpartum* and divided, according to their exposure to pre-gestational stress or prenatal venlafaxine administration and according to gender, into the following groups: non-stress + vehiculum male (NSKM, n = 5), non-stress + venlafaxine male (NSVM, n = 5), stress + vehiculum male (SKM, n = 5), stress + venlafaxine male (SVM, n = 5), non-stress + vehiculum female (NSKF, n = 6), non-stress + venlafaxine (NSVF, n = 6), stress + vehiculum female (SKF, n = 6), stress + venlafaxine female (SVF, n = 6).

Blood pressure and ECG measurement

The experiments were realized in offsprings´ in *"in vivo"* conditions, while the age of the offspring was between 110–120 days. Blood pressure (BP) was measured in un-anaesthetized rats using non-invasive tail cuff method. Animals were prewarmed at 37 °C for 10 min and BP was measured at the dorsolateral tail artery by tail cuff MLT125R Cuff & Transducer, using NIBP Controller (ADInstruments, Spechbach, Germany) and Powerlab 8/30 (ADInstruments, Spechbach, Germany) (Fig. 1). Consequently, the standard ECG was monitored in animals using Seiva EKG Praktik Veterinary (Seiva s.r.o., Prague, Czech Republic) (Kráľová et al., 2008). Stored recordings were statistically analyzed in Excel (Microsoft Excel, Version 15.40) and all the data are presented as mean ± SEM; for statistical comparison between the groups, the Student's t-test was used. After the functional measurements, the hearts were excised under general anesthesia.

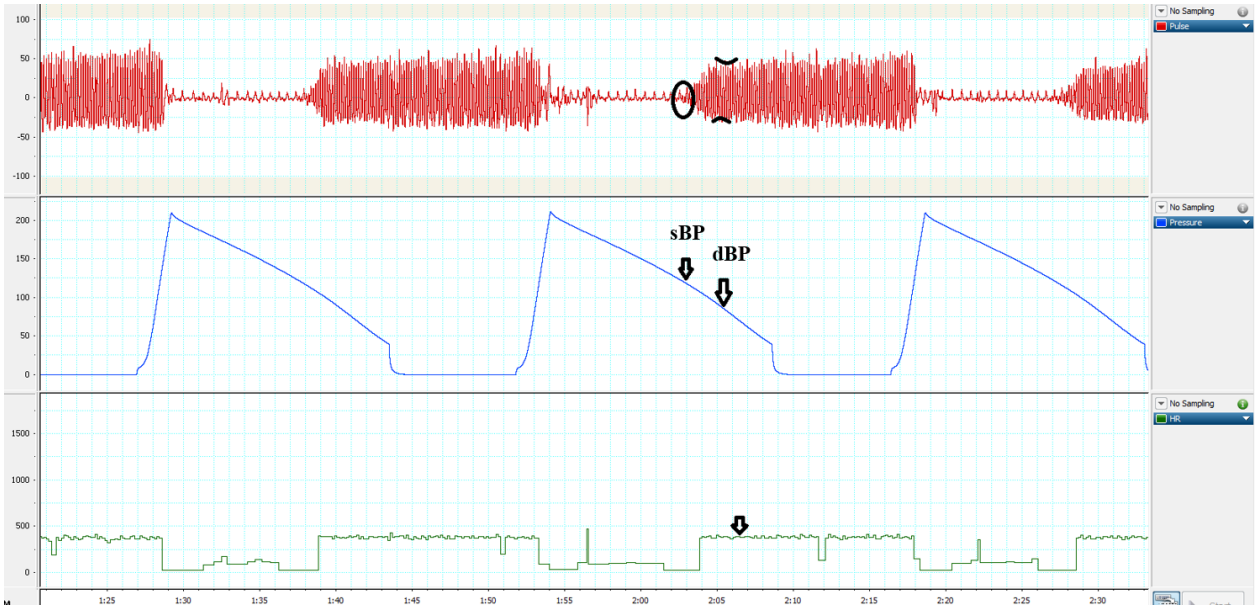

Figure 1. Representative original recording of blood pressure (BP) obtained by using non-invasive tail cuff method in a rat. Upper red track shows signal from the pressure sensor, characteristic for typical BP "spindles". The beginning of the spindle corresponds with the systolic BP (sBP, elypsis). After reaching the maximum amplitude, we can find a small decrease, which represents a value of diastolic BP (dBP, small black curves above and below the spindle). The middle blue curve represents pressure changes in the cuff and on the lower green track, the pulse frequency of the rat is seen (modified according to Vicen, 2016).

RESULTS

Analysis of BP (Fig. 2a) showed that exposure to pre-gestational stress modified systolic and diastolic BP in both males and females. Venlafaxine alone caused either non-significant decrease in systolic and diastolic BP in males, and non-significant increase in females. Pre-gestational stress potentiated the effect of venlafaxine (SV group) in females and manifested as a significant increase in systolic and diastolic BP compared to SKF group. In both genders, pre-gestational stress induced a non-significant increase of pulse frequency (PF) compared to control (males: NSKM – 339.76 ± 2.94 beats/min, SKM – 343.09 ± 6.8 beats/min; females: NSKF – 350.01 ± 4.80 beats/min, SKF – 373.62 ± 8.56 beats/min). Administration of venlafaxine to dams exposed to stress did not induce changes in PF of their offsprings.

Standard ECG recordings analysis from anesthetized rats, was focused on the selected parameters, like QT interval and QRS complex duration and showed changes in cardiac electrical activity induced by exposure to pre-gestational chronic unpredictable mild stress or prenatal administration of venlafaxine (Fig 2b).

In males, the exposure to pre-gestational stress led to non-significant shortening of QT interval compared to non-stressed control. Venlafaxine alone did not affect the duration of QT interval, but was able to suppress the effect of pre-gestational stress. QRS complex was significantly prolonged by 64% in male rats prenatally exposed to venlafaxine (NSVM) compared to the control (NSKM) (NSKM – 14.48 ± 0.97 ms; NSVM – 23.8 ± 0,71 ms, respectively). Stress combined with venlafaxine (SVM) did not cause further prolongation of QRS complex duration, however, mild shortening was found.

In females, the duration of QT interval was not affected by exposure to pre-gestational stress. Prenatal administration of venlafaxine induced non-significant prolongation of QT interval in females not exposed to pre-gestational stress and this effect was augmented by stress, as this change was significant (SKF – 54.8 ±0.78 ms; SVF – 61.44 ± 2.66). We did not observe changes in QRS complex duration induced by stress or venlafaxine, but their combination led to significant shortening compared to control (SKF – 21.64 ± 0.74 ms; SVF – 16.04 ± 1.86 ms).

After functional measurements, the hearts were excised under general anesthesia and weighed. The body weight of males and females, 355.95 ± 5.65 g, 216.28 ± 3.46 g, respectively, was not affected either by stress or by venlafaxine separately. Heart weight decreased after prenatal exposure to venlafaxine in the male and female venlafaxine groups. Significant decrease in heart weight was observed in the male venlafaxine group NSVM (1.021 ± 0.056 g) compared to the control NSKM group (1.224 ± 0.054 g) and also in the female venlafaxine group SVF (0.761 ± 0.031 g) compared to control SKF group (0.841 ± 0.025 g).

DISCUSSION

Exposure to pre-gestational stress did not cause significant changes in systolic, diastolic BP and PF either in males or in females, compared to the unexposed animals. Disturbances in cardiac electrical activity caused by pre-gestational stress

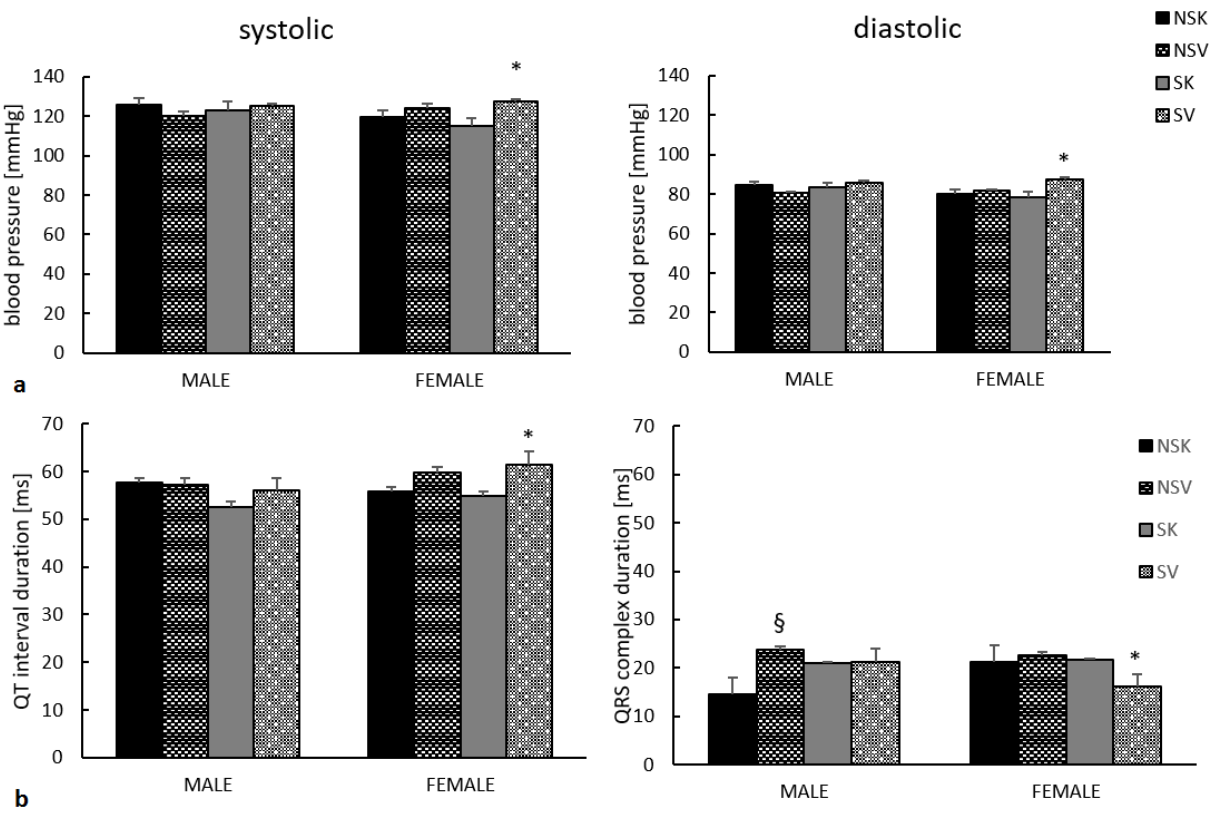

*Figure 2. Part **a**. The effect of pre-gestational stress and/or venlafaxine on blood pressure of rat offspring. NSK – non-stress + vehiculum, NSV – non-stress + venlafaxine, SK – stress + vehiculum, SV – stress + venlafaxine. Data are expressed as mean ± SEM, significance *p ≤ 0,05 for SV vs. SK females. Part **b**. The effect of pre-gestational stress and/or venlafaxine on QT interval and QRS complex duration of rat offspring. NSK – non-stress + vehiculum, NSV – non-stress + venlafaxine, SK – stress + vehiculum, SV – stress + venlafaxine. Data are expressed as mean ± SEM, n = 5–6, significance §p ≤ 0,05 for NSV vs. NSK males; *p ≤ 0,05 for SV vs. SK females.*

were reflected as non-significant shortening of QT interval and prolongation of QRS complex duration in males, but not in females. On the other hand, in females, the effects of pre-gestational stress were aggravated by administration of venlafaxine and resulted in elevated systolic and diastolic BP, prolonged QT interval and shortened QRS complex duration. In general, venlafaxine showed potential to worsen rather than improve mild effects of pre-gestational stress on CVS of rat offspring, probably related to lower heart weight.

Both stress and venlafaxine are known to affect the function of CVS, but in spite of that, their common effects during pregnancy and the impact on the fetus and the adult offspring is not properly described. We focused on the effects of pre-gestational stress and prenatal administration of venlafaxine on BP and selected ECG parameters in both genders monitored in "*in vivo*" conditions.

Our study showed that differences in blood pressure due to pre-gestational stress and/or prenatal venlafaxine administration were more evident in the females. Venlafaxine alone had a tendency to increase systolic and diastolic BP. This effect was potentiated by stress and could be characterized as a pre-hypertension.

Some studies (Capello et al., 2011; Bourke et al., 2013) showed, that chronic mild unpredictable stress during gestation leads to chronic activation of hypothalamic-pituitary-adrenal (HPA) axis because of increased basal plasma corticosterone levels in offspring. According to Szuran et al. (2000), plasma levels of corticosterone were higher only in females, not males, after prenatal exposure to stress. Venlafaxine administration is known to have a dose-dependent hypertensive effect (Feighner, 1995), and similarly, the other AD from the group of SSRIs could lead to gestational hypertension (Toh et al., 2009). Mild electrophysiological disturbances were observed in the duration of QT interval and QRS complex. Prolongation of QT interval was more evident in females. Venlafaxine alone was able to non-significantly prolong the QT interval, and this change was potentiated by pre-gestational stress. We also found venlafaxine-induced prolongation of QRS complex duration in males and, by contrast, shortening of QRS complex in females in combination of pre-gestational stress and venlafaxine. In experimental and clinical practice, the usage of venlafaxine was previously connected with QT interval prolongation (Bavle, 2015; Vicen et al., 2016), but this effect was never studied in children or adults prenatally exposed to venlafaxine. Sari & Zhou (2003) presented, that prenatal administration of SSRIs can affect the heart development, by decreasing of proliferation of cardiomyocytes and, in a lower rate, also non-muscular cardiac cells. Therefore, prenatal

exposure to SSRIs and possibly SNRIs may lead to conduction changes in the heart of offspring, demonstrated as changes in selected ECG parameters.

Biometrical analysis showed that pre-gestational stress and venlafaxine caused a decrease in the heart weight of male and female offspring. As the venlafaxine belongs to the group of SNRIs, it affects the levels of serotonin in the mother and her unborn child. Serotonin is a neurotransmitter involved in heart development; therefore, serotonergic manipulation could induce morphological changes through two mechanisms: damage of heart cells (reduction of proliferation, not apoptosis) and reduction in their size (insufficient growth in postnatal development). Experiments with mice lacking serotonin 5-HT_{2B} receptor, that is expressed in embryotic and adult heart, showed that this receptor is required for cardiovascular regulation. 5-HT_{2B} receptor mutant mice suffered from gross heart deficit, while the CNS was not evidently affected (Nebigil et al., 2000; Nebigil et al., 2001). Toscano et al. (2008) found lower heart weight compared to control, due to a decrease in myocyte size, after fluoxetine treatment (SSRI).

In conclusion, the exposure to pre-gestational chronic mild unpredictable stress and/or prenatal venlafaxine administration lead to changes in the cardiovascular system of rat offspring of both genders. Even though these changes are mild, still they are statistically significant and represent evidence that more experiments are needed to confirm the safety or risk of venlafaxine use in pregnancy. This study brings the original results, transferring the characteristic of animal depression-like status of mother induced by repeated stress conditions to simulate the maternal depression observed in clinical practice and the effect on the children in early and later life.

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SDF-1 and its receptor in the ventricles of rat with monocrotaline-induced pulmonary hypertension

Original research article/Review

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Abstract Aim: Chemokine stromal cell derived factor-1 (SDF-1) plays an important role in many processes such as apoptosis, proliferation, migration and angiogenesis, and these effects are mediated mostly by the receptor CXCR4. The aim of this study was to determine the expression of SDF-1 and CXCR4 in the ventricles of rats with monocrotaline-induced pulmonary hypertension.
Methods: 10–12 weeks old male Wistar rats were injected with monocrotaline (s. c., 60mg/kg; MON) or vehicle (CON). Rats were sacrificed 1 week (1W-MON, 1W-CON), 2 weeks (2W-MON, 2W-CON) and 4 weeks after monocrotaline administration (4W-MON, 4W-CON). Gene expression of SDF-1 and CXCR4 was determined by qRT-PCR.
Results: We observed a decrease in the SDF-1 expression on mRNA level in the right ventricle in 2W-MON and 4W-MON rats without any changes in the left ventricles and a decrease in CXCR4 expression in 1W-MON in both ventricles with an increase of CXCR4 expression in 4W-MON in the left ventricle (*P < 0.05).
Conclusion: SDF-1/CXCR4 axis is affected in both ventricles of rats with monocrotaline model of pulmonary hypertension.
Keywords: Pulmonary hypertension, monocrotaline, stromal cell-derived factor 1, ventricles

Keywords *Pulmonary hypertension – monocrotaline – stromal cell derived factor-1 – ventricles*

INTRODUCTION

Pulmonary hypertension (PH) is defined as an increase in the mean pulmonary arterial pressure over 25 mmHg at rest measured by the right heart catheterization (Hoeper et al., 2013). PH is an orphan disease (European PH prevalence is 15–60 subjects per million population) (Galiè et al., 2015); however, its development is quite progressive and fatal (almost 50% mortality rate in 3 years without treatment) (Lai et al., 2014). Elevation of pulmonary arterial pressure along with remodelling of the pulmonary arterial vessels increase the right ventricular (RV) afterload, thus contributing to the development of RV dysfunction and failure (Bogaard et al., 2009). Current therapeutic approach for PH has evolved with increase in complexity, which arises from the complex molecular mechanism of PH development (Galie et al., 2015). Recently, it has been noticed that stromal cell derived factor-1 (SDF-1) concentration were elevated in the plasma of patients with pulmonary arterial hypertension compared to healthy controls. Furthermore, the elevated circulating SDF-1 seems to be an independent risk factor for reduced survival in these patients (McCullagh et al., 2015). SDF-1 was originally identified as a molecule secreted in the bone marrow stromal cell lines attracting and stimulating the growth of B-cells (Bleul et al., 1996). As a ligand for chemokine (C – X – C motif) receptor 4 (CXCR4), SDF-1 plays a role in the recruitment of stem cells to areas of tissue injury in multiple organ system. CXCR4 receptor has been described as an essential chemokine receptor for development, haematopoiesis, organogenesis and vascularization (Wang et al., 2014). Increase in expression of CXCR4 and SDF-1 in lungs of hypoxia-induced PH mice (Gambaryan et al., 2011) or pulmonary arterial pressure reduction, attenuation of right ventricle hypertrophy and wall thickness of pulmonary arteries with CXCR4 inhibition in hypoxic-induced PH rats (Yu et al., 2011) indicate that of SDF-1/CXCR4 axis also takes part in hypoxic model of PH. Hypoxia stimulates the expression of CXCR4 in endothelial cells and also potentiates migratory response of endothelial cells to exogenous SDF-1 (Schioppa et al., 2003). Increased SDF-1 levels were also demonstrated in the decompensated right ventricle of monocrotaline-induced PH in rats (Sutendra et al., 2013).

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In our study, we aim to determine the involvement of SDF-1 and CXCR4 receptor in the ventricles of monocrotaline-induced PH rats in development of the disease.

METHODS

Experimental Design

10–12 weeks old male Wistar rats were administered a single dose of monocrotaline (Sigma Aldrich, USA) injection (s. c., 60mg/kg, MON) or vehicle (CON). Animals were handled under standard conditions with free access to food and drinking water. All experimental procedures involving experimental animals were approved by a local Ethical Committee and the State Veterinary and Food Administration of the Slovak Republic. Rats were sacrificed 1, 2 or 4 weeks after monocrotaline administration (1W-CON, 1W-MON; 2W-CON, 2W-MON;4W-CON, 4W-MON) and tissue samples from right and left ventricle were isolated.

Gene Expression

Total RNA was isolated from the samples by phenol/chloroform extraction using Tri-Reagent (Sigma Aldrich, USA). Quality of isolated RNA was verified by agarose gel electrophoresis and spectrophotometric analysis (NanoDropND-1000, Thermo Scientific, USA). Total RNA were reverse-transcribed to cDNA (High capacity cDNA Reverse Transcription Kit, Applied Biosystems, USA) and real-time PCR (StepOne Plus System, Applied Biosystem, USA) was performed using SYBR green PCR Master Mix kit (Applied Biosystems, USA). Expression of SDF-1 and CXCR4 were determined using gene specific primers (Srankova et al., 2016). All primers were verified to yield a single PCR product with the correct molecular weight. Beta-2-microglobulin was used as endogenous reference gene and results were calibrated to the control groups.

Statistical Analysis

Results are expressed as average ± standard error of the mean. Mean PCR efficiency estimates per amplicon and quantification cycle (Cq) values per sample were determined with LinRegPCR software (version 2015.0) and relative expression were calculated (Doka et al., 2017). Statistical significance was determined by a non-parametric Mann-Whitney test or parametric t-test after Shapiro-Wilk´s test of normality. Differences were considered significant at $P < 0.05$. Results were analysed by GraphPad Prism 4.0 (GraphPad Software, California).

RESULTS

We observed a decrease in the relative expression of SDF-1 on mRNA level in the right ventricle in 2W-MON and 4W-MON rats, however, without any changes in the left ventricle in all observed weeks (Figure 1). A small, but significant decrease of expression of receptor CXCR4 were determined in 1W-MON rats in both ventricles. Elevated expression of CXCR4 was observed in 4W-MON animals in the left ventricle (Figure 2).

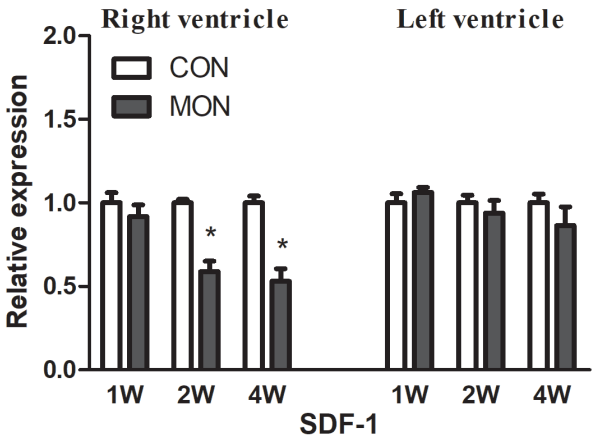

*Figure 1. Relative expression of SDF-1 determined by qRT-PCR in the right and the left ventricle in 1, 2 and 4 weeks after monocrotaline administration. Results are expressed as average ± SEM. *P < 0.05 vs. CON.*

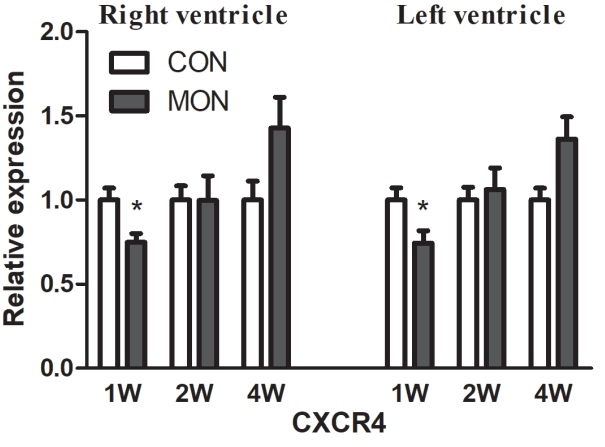

*Figure 2. Relative expression of CXCR4 receptor determined by qRT-PCR in the right and the left ventricle in 1, 2 and 4 weeks after monocrotaline administration. Results are expressed as average ± SEM. *P < 0.05 vs. CON.*

DISCUSSION

We aimed to determine the expression of SDF-1 during the development of monocrotaline-induced PH. In this model, the severe PH stage with elevated RV pressure, RV hypertrophy and clinical signs of PH (dyspnoea, cachexia, loss of social interactions) develops approximately 4 weeks after monocrotaline administration. In the early stages of the model (1 or 2 weeks after monocrotaline administration), none of these features occur (data not shown), despite the supposed endothelial cell injury (Gomez-Arroyo et al., 2012).

We found that SDF-1 expression on mRNA level was decreased 2 and 4 weeks after monocrotaline administration. Sutendra et al. linked SDF-1 expression in the developed stages of monocrotaline PH to RV function as they recognised the elevation of SDF-1 expression in the compensated RV failure and SDF-1 decrease in decompensated right ventricular failure with high mortality rate and severe PH symptoms (Sutendra et al., 2013).

Influence of SDF-1 on heart tissue has been seen in the models of systemic hypertension or cell culture systems. Increase of SDF-1 was described in spontaneously hypertensive rats, which further increased with aging (Shao et al., 2015). SDF-1 on cultures of myofibroblast increased the migration of myofibroblasts and wound healing (Shao et al., 2015), treatment with exogenous SDF-1 has concentration-dependent effects on proliferation, hypertrophy and collagen production in activated cardiac fibroblasts from normotensive and hypertensive rats (Jackson et al., 2017). All these effects were almost completely blocked with antagonist of CXCR4 receptor AMD3100 (Shao et al., 2015; Jackson et al., 2017). Whether SDF-1 is associated with improvement of cardiac function or further drives pathological changes in the hearts in PH models remains to be elucidated.

Data from the models of PH are almost completely from work on hypoxic models and effects of SDF-1 on lung vasculature. Not only SDF-1, but also CXCR4 was elevated in lungs of hypoxic-induced PH mice and antagonism with AMD3100 partially improved right ventricular pressure (Gambaryan et al., 2011). In hypoxic rat model of PAH, an increase in SDF-1 preceded the development of complex inflammatory microenvironment in lungs and pulmonary artery and an increase in the expression of SDF-1 was parallel to an increase in the CXCR4 expression (Burke et al., 2009). In our study, CXCR4 expression seems to be independent on the SDF-1 mRNA level as we have seen a decrease in the CXCR4 expression in 1W-MON animas in both ventricles, where SDF-1 was not changed. Treatment with CXCR4 inhibitor in hypoxia-induced PH rats improved RV pressure, RV hypertrophy and prevented increased wall thickness of pulmonary arteries. Also, electroporation of bone marrow cells with the CXCR4 shRNA inhibited the development of hypoxia-induced PH and lung vasculature remodelling (Yu et al., 2011).

SDF-1 can act beside CXCR4 receptor also on CXCR7 receptor, but data about CXCR7 in PH models are to this date limited to one study from Gambaryan et al., where they noticed the elevation of CXCR7 in lungs of hypoxic-induced PH mice and antagonism of this receptor only partially improved RV pressure. However, in combination with CXCR4 antagonism significant pressure and RV hypertrophy reduction were determined (Gambaryan et al., 2011).

CONCLUSION

In conclusion, we determined a decrease in SDF-1 in right ventricle in monocrotaline model of PH and also changes in CXCR4 expression. Involvement of SDF-1/CXCR4 axis in the development of PH is undeniable as we can see not only from our study, but also from elevation of SDF-1 in plasma of patients with pulmonary arterial hypertension. Since SDF-1/CXCR4 axis is pharmacologically modifiable, it remains to be further studied in PH.

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