THE EFFECTS OF VALSARTAN ON CARDIAC FUNCTION AND PRO-OXIDATIVE PARAMETERS IN THE STREPTOZOTOCIN-INDUCED DIABETIC RAT HEART

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EFEKTI VALSARTANA NA FUNKCIJU SRCA I PRO-OKSIDACIONE PARAMETRE KOD PACOVA SA STREPTOZOTOCINOM-IZAZVANIM DIJABETESOM

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ABSTRACT

Diabetes mellitus is a major risk factor for cardiovascular diseases, while cardiovascular diseases are a leading cause of morbidity and mortality worldwide. The renin–angiotensin–aldosterone system controls renal, cardiovascular, adrenal function and regulates fluid and electrolyte balance as well as blood pressure. Because of his role, inhibition of renin-angiotensin-aldosteron system is another therapy approach that reduces the risk of diabetes and cardiovascular disease. In this study, our goal was to evaluate effect of valsartan,as inhibitor of angiotensin II receptor type 1, on cardiac tissue and function, with focus on cardiodynamic and oxidative stress. The present study was carried out on 20 adult male Wistar albino rats (8 week old and with body masses of 180-200 g). Rats were divided randomly into 2 groups (10 animals per group). Healthy animals treated with 1 μM of valsartan and streptozotocin-induced diabetic animals perfused with 1 μM of valsartan 4 weeks after the induction of diabetes. Our results demonstrated that acute application of valsartan has different effect on cardiodynamics in rat heart of diabetic and healthy animals but did not improve cardiac function in hyperglycemia-induced changes. A challenge for further investigations are studies with chronic or acute administration, alone or in combination with other angiotensin-converting-enzyme inhibitor in various models of diabetes.

Keywords: *cardiodynamics, redox status, renin-angiotensin-aldosteron system, diabetes, valsartan.*

SAŽETAK

Dijabetes melitus je glavni faktor rizika za nastanak kardiovaskularnih bolesti, dok su kardiovaskularne bolesti vodeći uzrok morbiditeta i mortaliteta širom sveta. Sistem renin-angiotenzin-aldosteron kontroliše bubrežnu, kardiovaskularnu, i nadbubrežnu funkciju i reguliše ravnotežu tečnosti i elektrolita, kao i krvni pritisak. Zbog svoje uloge, inhibicija renin-angiotenzin-aldosteron sistema predstavlja još jedan terapijski pristup koji smanjuje rizik od nastanka dijabetesa i kardiovaskularnih bolesti. Cilj naše studije je bio da ispita akutni efekat valsartana, kao inhibitora angiotenzin II receptora (podtipa 1) na srčano tkivo i funkciju, sa fokusom na kardiodinamiku i oksidacioni stres. Ova studija je sprovedena na 20 odraslih mužjaka Wistar albino pacova (starosti 8 nedelja, telesne mase 180-200 g). Pacovi su svrstani nasumično u 2 grupe (10 životinja po grupi): zdrave životinje tretirane sa 1 μM valsartana i dijabetične životinje tretirane sa 1 μM valsartana, 4 nedelje nakon indukcije dijabetesa streptozotocinom. Naši rezultati pokazuju da akutna primena valsartana ima različiti efekat na kardiodinamiku srca pacova dijabetičnih i zdravih životinja, ali bez pozitivnog uticaja na promene srčane funkcije koje su izazvane hiperglikemijom. Izazov za dalja istraživanja su studije sa hroničnom ili akutnom primenom, samostalno ili u kombinaciji sa drugim inhibitorima angiotenzin konvertujućeg enzima u različitim modelima dijabetesa.

Ključne reči: *kardiodinamika, redoks status, renin-angiotenzin-aldosteron sistem, dijabetes, valsartan.*

ABBREVIATIONS

ACE - Angiotensin-converting-enzyme
ARBs - Angiotensin-receptor blockers
AT1 - Angiotensin type 1
AT2 - Angiotensin type 2
ATII - Angiotensin II
CPP - coronary perfusion pressure
CVD - Cardiovascular diseases
CVS - cardiovascular system
DM - diabetes mellitus
eNOS - endothelial nitric oxide synthases
RAAS - Renin–angiotensin–aldosterone system
STZ - Streptozotocin

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INTRODUCTION

The renin–angiotensin–aldosterone system (RAAS) is a synchronized hormonal cascade that controls renal, cardiovascular and adrenal function and regulates fluid and electrolyte balance as well as arterial pressure (1). RAAS contribute in pathogenesis and development of hypertension, atherosclerosis and cardiac disease. It provokes vasoconstriction, inflammation, cardiac remodeling and sodium retention and other possible harmful effects (2). Recent study showed that beside global RAAS activation, the cellular RAAS have an important role in physiology responses in cardiovascular diseases (CVD) (3).

Angiotensin II (ATII) is the major bioactive peptide of the RAAS that has a great influence in the functioning of many cells. As a pleiotropic hormone this peptide controls many organ systems principally through redox-sensitive processes (4). It is powerful vasoconstrictor that also indicates hypertrophy, inflammation and fibrosis that leads to vascular tissue damage and remodeling in cardiovascular diseases (5). There are two different AT II receptors: Angiotensin type 1 (AT1) and angiotensin type 2 (AT2) but AT1 receptor is more important for the cardiovascular system due to the fact that it plays predominant and mediate role in harmful effects of ATII (6).

According to previous research data, it has been shown that patients with diabetes mellitus (DM) have 3.4 times higher intracellular ATII levels than those without diabetes and in diabetic patients who also have hypertension this difference can be double (7). In addition to the standard therapy in the prevention of diabetes and lifestyle modification inhibition of the RAAS is another approach that reduces the risk of diabetes and cardiovascular disease (8).

Angiotensin-converting-enzyme (ACE) inhibitors and angiotensin-receptor blockers (ARBs) may reduce the incidence of diabetes mellitus and the risk of cardiovascular events (9, 10) compared to other anti-hypertensive drugs (11, 12) in patients with hypertension, heart failure, stroke as well as myocardial infarction (13).

An important role in the pathogenesis and progression of diabetic complications plays oxidative stress. Mitochondrial superoxide overproduction in endothelial of vessels as well as in the myocardium can be involved in the pathogenesis of these complications. In addition to the above-mentioned ATII is also known as an inducer of oxidative stress in cardiovascular tissue (14). Previously, it has been reported that RAAS antagonists such as ACE inhibitors or ARBs can reduce oxidative stress and inflammation (15, 16).

Valsartan is a potent, non-peptide tetrazole derivative that selectively inhibits ATII receptor type 1. Inhibition of AT1 receptor has a plenty of beneficial effects, as antiinflamatory, antioxidative, cardioprotective and antiatherosclerosis effect (17, 18). As a ARB valsartan prevents ATII-mediated adverse effects on the cardiovascular system (CVS) and it widely used in treatment of hypertension and organ damage that hypertension can lead (19). Recent study showed that valsartan has a protective effect on myocardial injury with a reduction in myocardial enzymes and proinflammatory mediators (6) as well as beneficial effects on DM (9).

Considering the role of ATII and AT1 receptors in cardiac tissue especially in hyperglycemic conditions as well as beneficial effects of valsartan in CVD and DM, aim of our study was to estimate effect of diabetes and valsartan on cardiac tissue and function.

MATERIALS AND METHODS

Animals and experimental design

The present study was carried out on 20 adult male *Wistar* albino rats (8 weeks old and body mass of 180–200 g). They were housed under controlled environmental conditions: temperature 25°C with an established photoperiod of 12 h light/day. The rats had free access to food and tap water - *ad libitum*. Rats were divided randomly into 2 groups (10 animals per group): Non-diabetic animals perfused with 1 μM of valsartan and streptozotocin-treated diabetic animals perfused with 1 μM of valsartan four weeks after the induction of diabetes (20).

All experimental procedures were done in accordance with prescribed legislation (EU Directive for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes 86/609/EES) and the principles of ethics. Animals were cared in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 1996). The experimental protocol was approved by Ethics committee for experimental animal well being of the Faculty of Medical Sciences of the University of Kragujevac.

Diabetic Rat Model

Before induction of DM two weeks of adaptation were provided. To develop a rat model of experimentally-induced type 1 DM, which resembles that occurring in human population, overnight fasting rats were injected with a single intraperitoneal dose of Streptozotocin (STZ) (60 mg/kg) (Sigma, St. Louis, MO, USA) dissolved in cold fresh 0.01 M citrate buffer fresh or frozen in 1 mL aliquots at 20°C (0.1 mol/L citric acid, 0.1 mol/L sodium citrate), pH 4.5. DM was confirmed 72 hours later when blood glucose were > 11.1 mmol/l (21). Animals that developed DM were held in the same conditions and followed for the next 4 weeks.

Isolated heart perfusion

On the 29th day from beginning of experimental protocol, animals were euthanized by cervical dislocation and the chest was opened via midline thoracotomy. The hearts were immediately removed and immersed in cold saline, then mounted on a stainless steel cannula of the Langendorff perfusion apparatus to provide retrograde perfusion under gradually increasing coronary perfusion pressure (CPP) (CPP from 40 cmH_2O to 120 cmH_2O). Krebs-Henseleit buffer was used for retrograde perfusion (in mmol/l: NaCl 118, KCl 4.7, $CaCl_2$ x $2H_2O$ 2.5, $MgSO_4$ x $7H_2O$ 1.7, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, and pyruvate 2). The buffer was bal-

anced with 95% O_2 and 5% CO_2, with a pH value of 7.4 and temperature of 37°C. Following the establishment of heart perfusion, the preparations were stabilised within 30 minutes with a basal CPP of 70 cmH_2O. Following the stabilisation period, the perfusion pressure was reduced to 50 and 40 cmH_2O and then gradually increased to 60, 80, 100 and 120 cmH_2O to establish coronary autoregulation. Testing started immediately after the control experiment to avoid unwanted time-dependent consequences. The administration of 1μM valsartan lasted until the achievement of a stable flow but not under 5 minutes for each value of perfusion pressure.

After placing the sensor (transducer BS4 73-0184, Experimetria Ltd., Budapest, Hungary) into the left ventricle (LV) following cardio-dynamic parameters were continuously registered: maximum and minimum rate of pressure development in left ventricle (dp/dt max, dp/dt min), systolic and diastolic left ventricle pressure (SLVP, DLVP), and heart rate (HR) on each of predetermined values of perfusion pressure (40, 60, 80, 100 and 120 cmH_2O). CF was measured by flowmetry. Each heart was its own control.

Biochemical analysis

Markers of oxidative stress were measured spectrophotometrically in the collected samples of coronary venous effluent. Samples were collected after stabilization of the coronary flow and after drug administration for each perfusion pressure. We performed quantification of nitrites (the amount of NO_2^- released), superoxide anion radical (O_2^-), and hydrogen peroxide (H_2O_2) and an indirect quantification of the index of lipid peroxidation via reactive thiobarbituric substances (TBARS), for all samples.

Determination of superoxide anion radicals (O_2^-)

Superoxide anion radical concentrations were measured using the NTB (Nitro Blue Tetrazolium) reagent in TRIS buffer (assay mixture) with coronary venous effluent. The measurement was performed at a wavelength of 550 nm. Distilled water was used as a blank probe (22).

Determination of nitrites (NO_2^-)

Nitric oxide quickly decomposes into stable metabolite nitrites/nitrates. Nitrites can therefore be used as an index of NO_2^- production via a spectrophotometric method using the Griess reagent. Briefly, 0.5 ml of the perfusate was precipitated with 200 μl of 30% sulfosalicylic acid, vortexed for 30 min and centrifuged at 3000 x g. Equal volumes of the supernatant and Griess reagent containing 1 % sulphanilamide in 5 % phosphoric acid/0.1 % naphthalene ethylenediamine dihydrochloride was added and stabilized for 10 min in the dark and measured spectrophotometrically at a wavelength of 550 nm. The nitrite concentrations were determined using sodium nitrite as the standard (23).

Determination of hydrogen peroxide (H_2O_2)

A measurement of hydrogen peroxide was based on the oxidation of phenol red by hydrogen peroxide in a reaction catalysed by horseradish peroxidase (HRPO). A total of 200 μl of perfusate was precipitated with 800 ml of freshly prepared phenol red solution, followed by the addition of 10 μl of (1:20) HRPO (made ex tempore). Distilled water was used as a blank probe (instead of coronary venous effluent). The level of H_2O_2 was measured at 610 nm (24).

Determination of the index of lipid peroxidation measured as TBARS

The index of lipid peroxidation was determined indirectly by measuring the products of the reaction with thiobarbituric acid (TBARS or **T**hiobarbituric **A**cid **R**eactive **S**ubstances). Briefly, 1% thiobarbituric acid (TBA) in 0.05 M NaOH was incubated with coronary venous effluent at 100˚C for 15 min and then measured at 530 nm of wavelength, spectrophotometrically. Distilled water was used as a blank probe (25).

Drugs

Streptozotocin and valsartan were purchased from Sigma–Aldrich Chemie GmbH Eschenstr. 5, 82024 Taufkirchen, Germany.

Statistics

Statistical analysis of experimental data included the following basic descriptive statistics: the mean value (X) ± standard deviation (SD). The following statistical tests were used to test the statistical significance of the results and to confirm the hypothesis: Paired – Samples *T* test and Independent *T* test. A database analysis of the results was performed using software package SPSS 18th (SPSS Inc., Chicago, IL, USA). *P* values lower than 0.05 ($p<0.05$) were considered to be significant while *P* values lower than 0.01 ($p<0.01$) were considered to be high significant.

RESULTS

Cardiodynamic parameters

Ventricular contractility assessment (dp/dt max and dp/ dt min)

The maximum rate of pressure development in LV wall (dp/dt max) was non-significantly higher in healthy relative to diabetic rats at all CPP pressures (40–120 cmH_2O) (Fig. 1a). Perfusion with valsartan in healthy animals led to decreased dp/dt max with the statistical significance at CPP 80 and 120 cmH_2O (Fig. 2a). In diabetic animals, the change in dp/dt max is absent for most CPPs. Nevertheless, after valsartan perfusion a statistically significantly lower value at CPP 100 cmH_2O was observed (Fig. 3a). The minimum rate of pressure development in LV wall (dp/dt min) was not significantly different between the healthy and diabetic animals; values of this parameter were quite similar (Fig. 1b). Perfusion with valsartan increased dp/dt min in the healthy animals with statistical significance at CPP at 80-120 cmH_2O (Fig. 2b). As well as in the healthy rats, in diabetic animals, perfusion with valsartan increased values

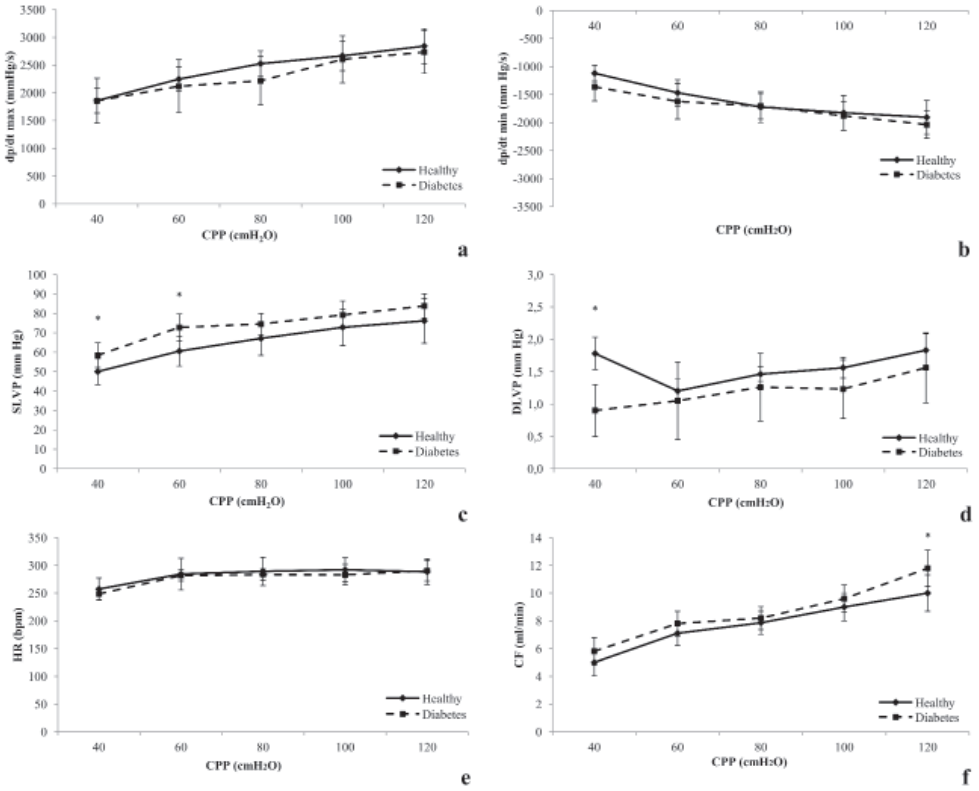

Figure 1. Cardiodynamic parameters in hearts of healthy rats compared to hearts of diabetic rats.
Data are presented as following: a – Maximum rate of development pressure in left ventricle (dp/dt max); b – Minimum rate of development pressure in left ventricle (dp/dt min); c – Systolic left ventricle pressure (SLVP); d - Diastolic left ventricle pressure (DLVP); e – Heart rate (HR); f - Coronary flow (CF). Data are presented as mean ± SD (*p<0.05; **p<0.01).

of dp/dt min with statistically significance at CPP 80-120 cmH_2O (Fig. 3b).

Left ventricular pressures (SLVP, DLVP)

Systolic left ventricle pressure was increased in diabetic group relative to healthy rats at all CPPs with statistically significance at 40 and 60 cmH_2O (Fig. 1c). SLVP was decreased after perfusion with valsartan in healthy rats at all CPPs (40–120 cmH_2O) and statistical significance was

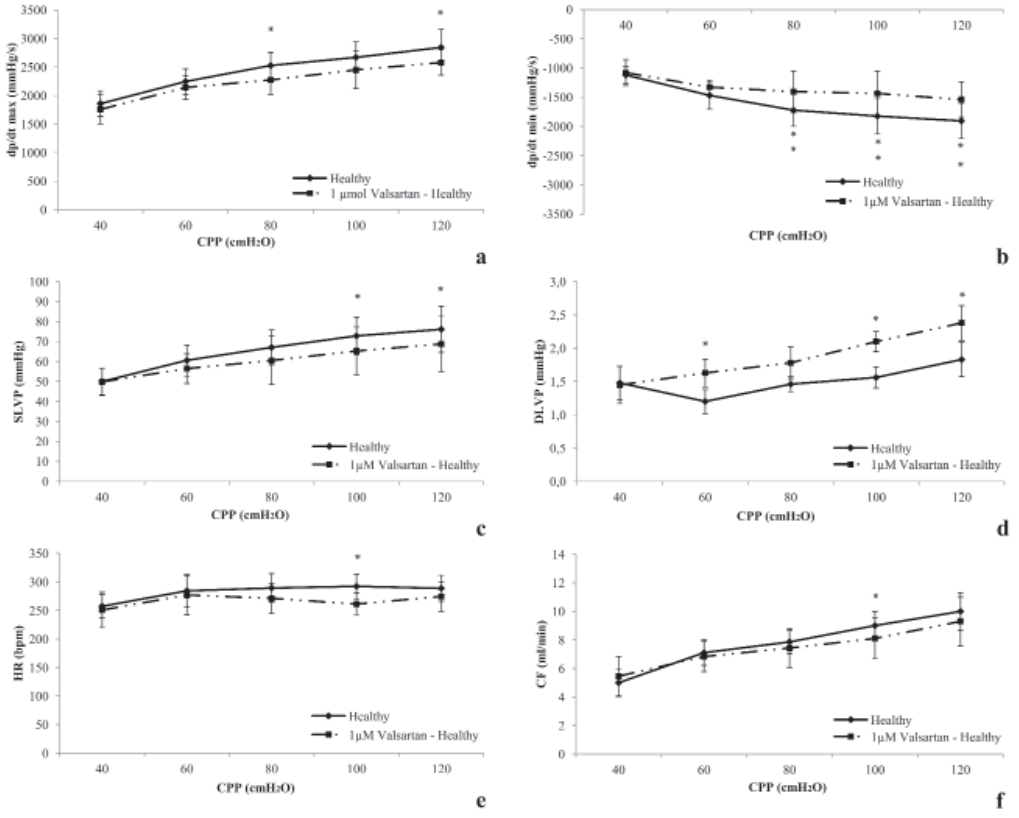

Figure 2. Cardiodynamic parameters in hearts of healthy rats compared to hearts of healthy rats perfused with 1 µM of valsartan.
Data are presented as following: a – Maximum rate of development pressure in left ventricle (dp/dt max); b – Minimum rate of development pressure in left ventricle (dp/dt min); c – Systolic left ventricle pressure (SLVP); d - Diastolic left ventricle pressure (DLVP); e – Heart rate (HR); f - Coronary flow (CF). Data are presented as mean ± SD (*p<0.05; **p<0.01).

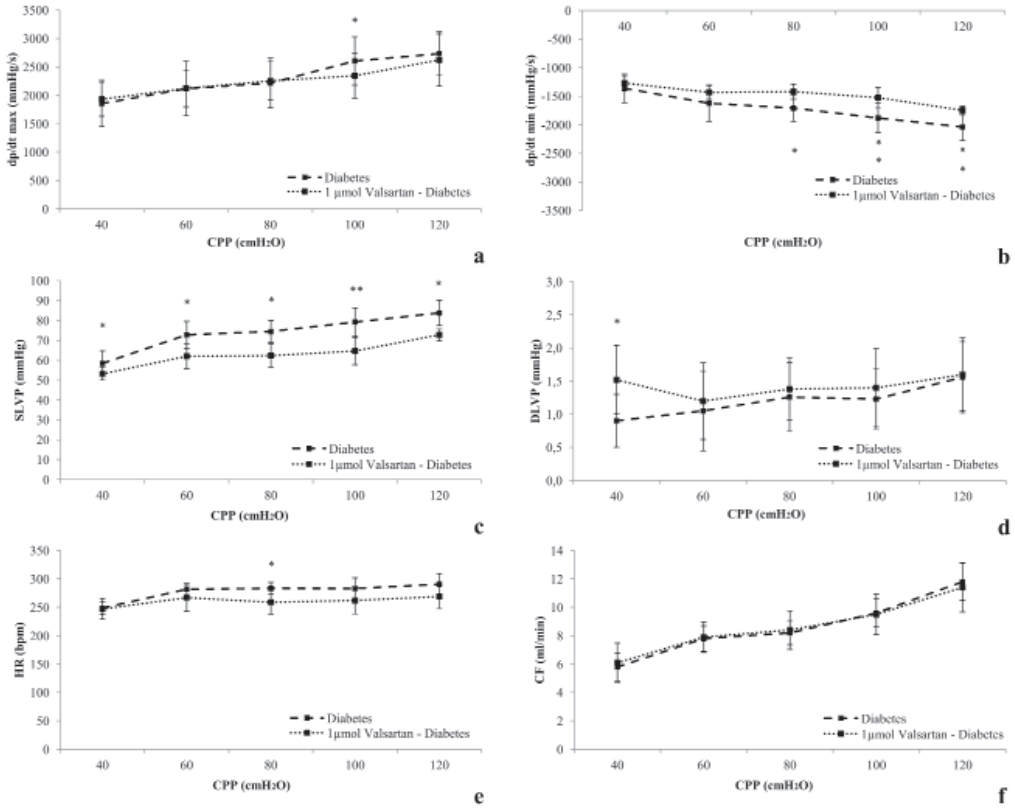

Figure 3. Cardiodynamic parameters in hearts of diabetic rats compared to hearts of diabetic rats perfused with 1 µM of valsartan.
Data are presented as following: a – Maximum rate of development pressure in left ventricle (dp/dt max); b – Minimum rate of development pressure in left ventricle (dp/dt min); c – Systolic left ventricle pressure (SLVP); d - Diastolic left ventricle pressure (DLVP); e – Heart rate (HR); f - Coronary flow (CF). Data are presented as mean ± SD (*p<0.05; **p<0.01)

noticed at CPP 100 and 120 cmH_2O (Fig. 2c). The same trend as in healthy animals was noted in diabetic group but the effect of valsartan on the SLVP reduction is even more pronounced it these animals. All values after valsartan perfusion are statistically significantly lower and at CPP 100 cmH_2O the difference is highly significant (p<0.01) (Fig. 3c). Diastolic left ventricle pressure was decreased in diabetic relative to healthy group with statistical significance at 40 cmH_2O (Fig. 1d). Additionally, in the healthy animals DLVP is statistically higher after valsartan perfusion at CPP 60, 100 and 120 cmH_2O (Fig. 2d). In the group of diabetic animals DLVP is also increased after valsartan perfusion and statistical significance is achieved only at CPP 40 cmH_2O (Fig. 3d).

Heart rate (HR) and coronary flow *(CF)*

Heart rate displayed a non-significant decrease in the diabetic group compared to healthy animals at all CPP values (Fig. 1e). Valsartan affects the heart rate by decreasing its values in both groups, healthy and diabetic rats. The values are non-significant lower at all CPP values, except at CPP 100 cmH_2O in healthy rats group (Fig. 2e) and at 80 cmH_2O in diabetic group (Fig. 3e) after valsartan perfusion. Coronary flow was increased in the diabetic animals

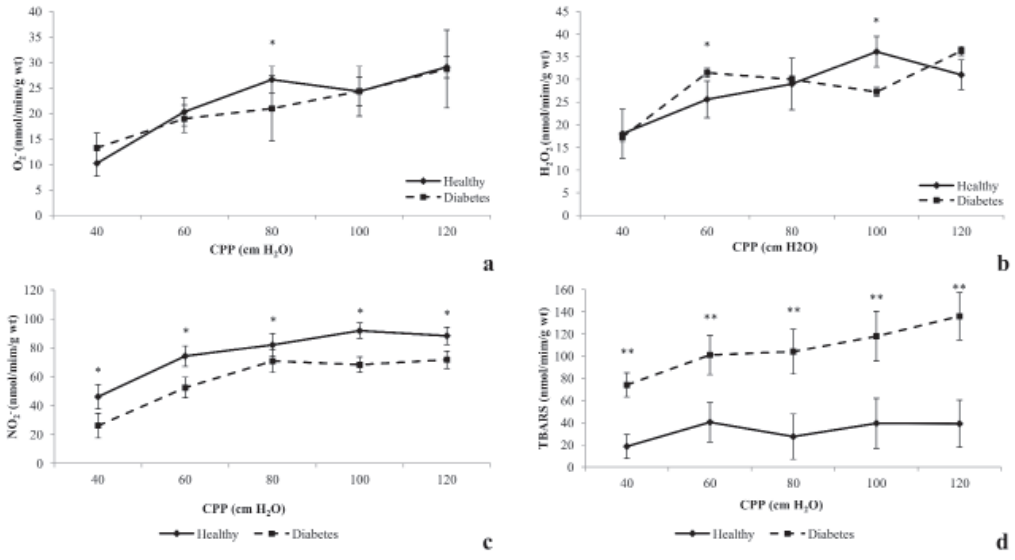

Figure 4 - Oxidative stress parameters in healthy rats compared to diabetes rats.
Data are presented as following: a - Level of superoxide anion radical (O_2-); b – Level of hydrogen peroxide (H_2O_2); c - Level of nitric oxide (NO) measured in the form of nitrite (NO_2-); d - level of index of lipid peroxidation measured as thiobarbituric acid reactive substances (TBARS). Data are presented as mean ± SD (*p<0.05; **p<0.01)

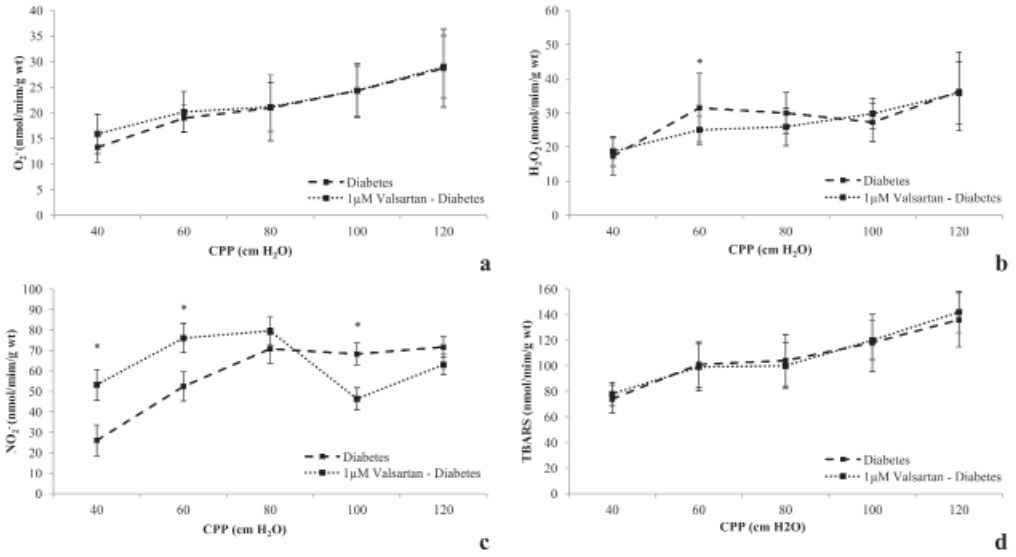

Figure 5 - Oxidative stress parameters in healthy rats compared to healthy rats with perfusion of 1 μM of valsartan.
Data are presented as following: a - Level of superoxide anion radical (O_2-); b – Level of hydrogen peroxide (H_2O_2); c - Level of nitric oxide (NO) measured in the form of nitrite (NO_2-); d - level of index of lipid peroxidation measured as thiobarbituric acid reactive substances (TBARS). Data are presented as mean ± SD (*p<0.05; **p<0.01)

at all CPP values compared to healthy rats, significantly at CPP 120 cmH_2O (Fig. 1f). After valsartan perfusion CF was discretely increased at CPP 40 cmH_2O and after that CF values began to decrease in healthy animals. Statistically significant lower CF after valsartan perfusion was detected at CPP 100 cmH_2O (Fig. 2e). In diabetic rats group there was no statistical significance after valsartan perfusion at all CPP (Fig. 3e).

Oxidative stress parameters

Values of O_2-

The level of superoxide anion radical in healthy relative to diabetic animals was significantly higher at CPP 80 cmH_2O while at the other CPPs these values were similar (Fig. 4a). In healthy rats valsartan firstly significantly increased and thereafter highly significantly decreased level of O_2^- at CPPs 60 – 120 cmH_2O (Fig. 5a). In diabetic animals valsartan didn't induces any statistical significance, values between groups were quite similar (Fig. 6a).

Values of H_2O_2

Between healthy and diabetic groups of animals there was difference in level of H_2O_2 at CPP 60 and 100 cmH_2O. In diabetic animals at CPP 60 cmH_2O level of H_2O_2 was significantly higher relative to healthy group while opposite to this result at CPP 100 cmH_2O in group of healthy animals level of H_2O_2 was statistically increased (Fig. 4b). Valsartan perfusion statistically reduced level of H_2O_2 in healthy rats at CPP 100 cmH_2O while at the other CPPs level of this parameter was quite similar (Fig. 5b). On the other hand, valsartan statistically decreased level of H_2O_2 at 60 cmH_2O while at the other CPPs values were slightly different (Fig. 6b).

Values of NO_2^-

NO_2^- activity was measured as nitrite. The nitrite level was significantly decreased in diabetic group relative to the healthy animals at all CPPs (Fig. 4c). In healthy animals after valsartan perfusion, nitrite level wasn't changed significantly and the values were slightly similar in both groups (Fig. 5c). In diabetic animals valsartan increased nitrite

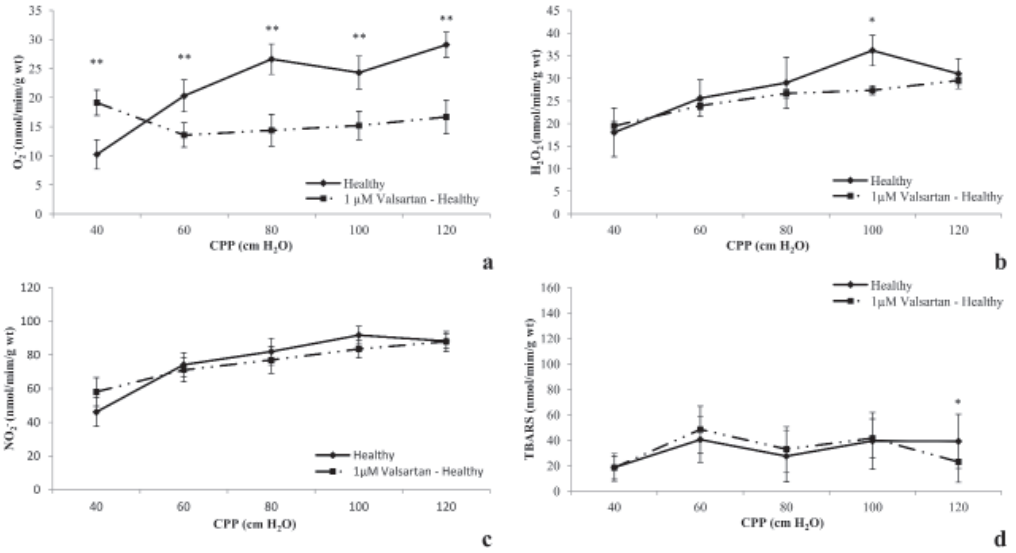

Figure 6 - Oxidative stress parameters in diabetic rats compared to diabetic rats with perfusion of 1 μM of valsartan.
Data are presented as following: a - Level of superoxide anion radical (O_2-); b – Level of hydrogen peroxide (H_2O_2); c - Level of nitric oxide (NO) measured in the form of nitrite (NO_2-); d - level of index of lipid peroxidation measured as thiobarbituric acid reactive substances (TBARS). Data are presented as mean ± SD (*p<0.05; **p<0.01)

level with statistically difference at CPP 40 and 60 cmH_2O but opposite to these results, valsartan decreased NO level at CPP 100 and 120 cmH_2O with statistical significance at CPP 100 cmH_2O (Fig. 6c).

Values of TBARS

The TBARS values were highly significant decreased in healthy animals relative to diabetic rats at all CPPs (Fig. 4d). After valsartan perfusion in healthy animals, level of TBARS was statistically decreased at CPP 120 cmH_2O (Fig. 5d) while in diabetic animals there is no notably difference, values were quite similar after valsartan perfusion (Fig. 6d).

DISCUSSION

The aim of this study was to determine acute effects of valsartan on heart function as well as on oxidative stress in STZ-induced DM isolated rat hearts. Additionally, we would like to investigate whether STZ-induced DM had influence on cardiac function or whether valsartan can reduce effects of STZ-induced DM on cardiovascular parameters and oxidative stress isolated rat heart.

There are different models developed to investigate pathophysiological mechanisms of diabetic cardiomyopathy in rats whereby STZ-induces DM is most extensively and the most similar to human DM (26). The injection of streptozotocin leads to the development of a clinical syndrome characterized by hyperglycemia, excessive diuresis and loss of weight (7).

It is interesting that although the myocardial concentrations of renin is 1–4% of those in plasma, the cardiac interstitial fluid concentrations of angiotensinogen I and angiotensinogen II are over 100-fold those of plasma. There is evidence that the cardiac interstitial fluid represents a separate compartment from the systemic circulation and that interstitial ATII derives exclusively from *de novo* biosynthesis in the heart (1).

As previously mentioned, valsartan is a potent, orally active, non-peptide antihypertensive drug that is highly selective antagonist of the ATII AT1 receptor with no significant affinity for other receptors (27, 28). It has been shown that in patients with diabetes blockade of AT1 receptors reduces cardiovascular mortality and morbidity. Previous clinical trials with ATII receptor blockers in addition to reducing cardiovascular morbidity and mortality likewise results in the consistently reduces of the incidence of new onset DM (29, 30)

Although a large number of studies investigated metabolic disorders in the heart of diabetic rats there is almost no data regarding acute influence of valsartan on cardiodynamics and functional status of the diabetic heart.

In our study, dp/dt max and dp/dt min were decreased in diabetic rats but in physiological range while systolic pressure was increased in hypoxia condition opposite to diastolic pressure which was decreased compared to healthy heart also in hypoxia condition (Figs. 1a-d). We have recently showed that myocardial contractile function is impaired in STZ- induced diabetic rats (7). Depressed cardiac function in diabetic rats is due to gross changes with increased extracellular type 1 collagen synthesis (31) and impaired Ca^{2+} release that leads to reduced contractility and slower Ca^{2+} uptake by SERCA which results in impaired relaxation of cardiac muscle (32). Increasing of systolic pressure may indicate a lower tolerance to increase tensions in the system. However, present results showed diminished cardiac function in diabetic rats mostly in condition of hypoxia which can be explained by the fact that there is no damage of the heart muscle during short-time perfusion in normoxy condition and that diabetic heart is more vulnerable in hypoxic conditions than healthy one.

On the other hand, valsartan did not significantly changed cardiac contractility and relaxation in normoglycemic conditions. Nonetheless, valsartan decreased systolic pressure and increased diastolic pressure on the higher CPPs where the oxygenation was over the normal range (Figs. 2c-d). Heart rate and coronary flow were decreased after valsartan perfusion but in physiology range. Heart rate was significantly decreased after valsartan perfusion at CPP 100 cmH_2O but at CPP 120 cmH_2O values were similar to control conditions. Withal, increase of pressure value leads to slightly flow increase (Figs. 2e-f).

In the diabetic rats systolic pressure in LV was statistically significantly lower at all CPP that can be explained by chronic hyperglycemia which affect the further reaction of the tissue after perfusion with valsartan (Fig. 3c). This reduction in value after valsartan perfusion on SLVP indicates a change in heart tissue in diabetes despite seemingly approximate values of the cardiodynamic parameters in previous comparisons of healthy and diabetic rats. Our results are in accordance with Chan and coworkers (27) who showed that valsartan applied as intravenous injection in STZ-diabetic rats also reduces systolic blood pressure. Diastolic pressure at diabetic rats was increased but less than in healthy rats after valsartan perfusion whereby that can indicate on rigidity of the chamber (Fig. 3d) as a consequence of hyperglycemia for a period of 4 weeks before valsartan perfusion. As well as in healthy rats there is no significant difference in value of HR but the changes are less in diabetic rats. Literature data suggested that ARBs have positive effects in the prevention of chronic hyperglycemia influence on tissues (33) but there is no precise mechanism that explains how acute valsartan administration affects heart ratio. With all, previous studies have found that streptozotocin-induced diabetes is characterized by downregulated endothelial nitric oxide synthases (eNOS) that localizes at the caveolae where it controls heart rate, contraction, diastolic relaxation and oxygen utilization (34). It has been snown that eNOS plays a protective role in cardiovascular system which is confirmed by Janssens et al (35). They observed that cardiomyocyte-restricted eNOS overexpression was protective against adverse LV remodeling after coronary artery ligation in transgenic mice that correlate with other studies (35-37).

It may be possible that during long-time of hyperglycemia in diabetic rats, downlegulation of eNOS induced increase diastolic pressure in LV and slightly but not significant decreased heart rate (Fig. 3d; Fig. 3f).

As the effects are more pronounced at dp/dt min than on dp/dt max (Fig. 2a-b; Fig. 3a-b) we can assume that in valsartan group the effect is more pronounced on the relaxation capability. This is indicated by changes in the diastole (Fig. 2d; Fig. 3d). Taken together, it seems that valsartan improved LV properties of the heart during hyperglycemia and thus have positive influence on diastolic function of the STZ-induced diabetic heart.

In the second part of our study we examined the oxidative status of heart in normoglycemic and hyperglycemic conditions as well as effect of valsartan on redox status. In control conditions we can notice that TBARS values were statistically higher at all CPPs in diabetic compared to healthy rats (Fig. 4d) which indicates muscle damage in diabetic heart. Opposite trend was noticed in NO_2^-, values were statistically higher in healthy group while O_2^- was statistically higher only at CPP 80 cmH_2O also in healthy group. These results may indicate that DM led to myocardial damage. One of the possible explanations for this could be excessive NO_2^- produced by iNOS which can be activated by prolonged hyperglycemia and its interaction with O_2^- to create peroxynitrite ($ONOO^-$) and to reduce O_2^- levels. O_2^- has a half-life of 10^{-6} of a second, and NO molecules last for few seconds, both of which support this possibility (7).

Based on previous data it was expected that the highest activity of reactive oxygen species would be observed in diabetic rats. Result is little bit different compared to the stated assumption. In healthy animals, at the lowest, hypoxic CPP, O_2^- statistically significantly increased than it was significantly lower in other CPPs after valsartan perfusion (Fig. 5a). Compared to these results in diabetic animals there wasn't significant increase of O_2^- (Fig. 6a) like it was expected that can be consequence of reaction with NO produced by pathological activation of iNOS due to enhancement of inducible iNOS activity because the metabolism of the L-arginine NO system is altered in diabetes (38, 39). O_2^- results indicate that valsartan has a lower impact on O_2^- reduction in diabetes than in the control group. This result is difficult to explain, and can be consequence of either higher O_2^- in normoglycemic group or modified AT1 receptors in hyperglycemic conditions, however exact mechanisms for this effect is still unknown.

Oxidative stress reducing by using the AT II AT1 receptor antagonist is expected in the pathological conditions of diabetes (40) but from some unexpected reason the effect is absent that can be explained by increased concentration of NO (Fig. 6c) that reacted with O_2^- and led to the formation of peroxynitrite, which unfortunately we did not measured. Peroxynitrite contributes to contractile dysfunction and even apoptosis. According to previously mentioned half-life of O_2^- and NO_2^-, that can explain non-significant difference in concentration of O_2^- between control and diabetic rats (Fig. 6a) (34). Continuously increasing of NO_2^- in diabetic rats may be result of inducible enzyme activation (iNOS) whereby its expression during pathological states continuously produces NO until the enzyme is degraded, and this leads to excessive reactive nitrogen species (RNS) production as well as peroxynitrite, which as previously mentioned contributes to contractile dysfunction. On the other hand, another explanation is that prolonged and significantly elevated hyperglycemia and consequent activation of the PKCβ2–iNOS pathway leading to loss of O_2^- in the reaction with NO (34) that we noticed in our results (Fig. 6a; Fig. 6c). As already mentioned, it is possible that uncontrolled iNOS activity in diabetes caused such a NO_2^- oscillations. TBARS level is expectedly higher in diabetic rats, because, normally, a diabetic heart makes greater use of free fatty acids for metabolism. Movement of values in control groups are relatively, until highest CPP, apropos in condition of hypertension and overloaded tissue. In this conditions valsartan decreases TBARS level which can be significant in hypertension without diabetes, because it represent additional antioxidative effect of valsartan.

CONCLUSION

In summary, it can be concluded that heart from STZ-induced diabetic animals were not functionally different. Acute application of valsartan failed to improve these hyperglycemia-induced changes of cardiac function. Finally, it seems that oxidative stress does not have role in these effects of valsartan. A challenge for future investigations will be to identify the effects either of acute or chronic valsartan treatment alone or in combination with other ACE inhibitors in various models of diabetes.

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CONFLICT OF INTERESTS

The authors declare that there are no competing interests associated with the manuscript.

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