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Short Communication

Mitochondrial function in heart and kidney of spontaneously hypertensive rats: influence of captopril treatment

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Abstract. Effect of captopril treatment on capability of heart and kidney mitochondria to produce ATP was investigated in spontaneously hypertensive rats (SHR). Heart mitochondria from SHR responded to hypertension with tendency to compensate the elevated energy demands of cardiac cells by moderate increase in mitochondrial Mg^{2+}-ATPase activity, membrane fluidity (MF) and in majority of functional parameters of the mitochondria ($p > 0.05$). Significant increase exhibited only the oxygen consumption (QO_2; $p < 0.01$–0.001) and oxidative phosphorylation rate (OPR; $p < 0.003$) with glutamate + malate (GLUT+MAL) as substrates. Lowering the blood pressure ($p < 0.02$) captopril also eliminated the above compensatory response and impaired the oxidative ATP production by decreasing OPR ($p < 0.001$). Kidney mitochondria of SHR experienced serious disarrangement in parameters of oxidative ATP production: increase in Mg^{2+}-ATPase activity ($p < 0.05$) but, also scattered QO_2 values ($p < 0.03$–0.01) leading to decrease in OPR and the ADP:O ($p < 0.05$–0.01) values with both GLUT+MAL and succinate as substrates. Captopril treatment does not alleviated but even worsened the above alterations. Mg^{2+}-ATPase became also decreased and the depression of ADP:O became aggravated ($p < 0.0001$).

Key words: Spontaneous hypertensive rats — Heart — Kidney — Mitochondria — Captopril

Abbreviations: SHR, spontaneously hypertensive rats; ACE, angiotensin converting enzyme; QO_2, oxygen consumption; S3, state 3 respiration; S4, state 4 respiration; RCI, respiratory control index; OPR, oxidative phosphorylation rate; ADP:O, ADP : oxygen ratio; MF, membrane fluidity; DPH, 1,6-diphenyl-1,3,5,-hexatriene; SUC, succinate; GLUT, glutamate; MAL, malate; DNP, 2,4-dinitrophenol; Mg^{2+}-ATPase, Mg^{2+}-dependent DNP-stimulated ATPase; r_s, fluorescence anisotropy.

In spite of the fact that already very much knowledge have been accumulated about pathogenesis and the treatment of hypertension, this topic is still in high interest of investigators and clinicians (Kuneš and Zicha 2006; Rydén et al. 2009; Šimko and Pecháňová 2009). However, surprisingly, relative little interest was focused to hypertension-induced alterations in cellular energetics (Seppet et al. 2007), particularly to changes in function of the mitochondria that occur parallel in the heart and kidney (De Cavanagh et al. 2005). But, unfortunately, even the few available studies often present opposite results or conclusions (Roman et al. 1992, Postnova et al. 2007).

Several classes of drugs, such as diuretics, angiotensin II receptor blockers – type AT1, inhibitors of angiotensin con-

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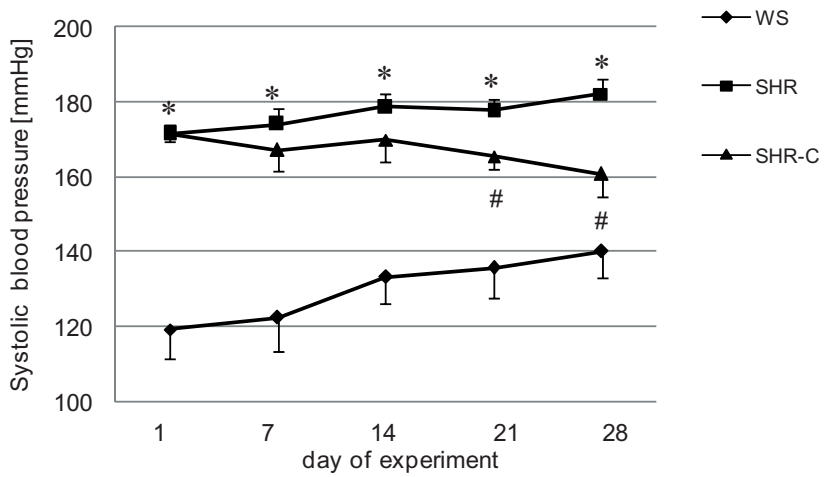

Figure 1. Systolic blood pressure in normotensive and spontaneously hypertensive rats untreated and treated with captopril. WS, normotensive Wistar rats; SHR, spontaneously hypertensive rats; SHR-C, spontaneously hypertensive rats treated with captopril. Results are means ± S.E.M. of 9 experiments. Significances: * $p < 0.01$, WS *vs.* SHR; $^{\#}$ $p < 0.02$, SHR *vs.* SHR-C.

verting enzyme (ACE), β-receptor-blockers, calcium entry blockers, etc., are wide-spread applied in mono- therapy and also in combined therapy of the hypertension. Nevertheless, which between the last two types of therapy is more advantageous is not yet from all sides elucidated. Aim of the present study is to investigate the effect of spontaneous hypertension and its treatment with ACE inhibitor captopril on the capability of rat heart and kidney mitochondria to supply the cells with ATP adequately.

Sixteen weeks old (n = 30) spontaneously hypertensive rats (SHR), (Okamoto Kyoto, Charles River) were treated daily with ACE inhibitor captopril (Sigma-Aldrich, 80 mg *per* kg body weight, perorally) for 4 weeks. Parallel running untreated SHR and similarly old healthy Wistar rats (n = 30 each) were used as controls. Animals were housed under 12 h light/12 h dark regimen at 22 ± 2°C. They were fed a standard pellet diet and had free access to water. All experiments were performed in accordance with the rules issued by the State Veterinary and Alimentary Administration of the Slovak Republic based on paragraph 37 (6), legislation No. 488/2002 of the Slovak Parliament. Systolic blood pressure of rats was monitored at the beginning, on each 7th day and before termination of the experiment using the tail cuff technique (AD Instruments, PowerLab, Germany). At the end of experiment the animals were killed by cervical dislocation. Mitochondria from heart were isolated according to Ferko et al. (2006) and the kidney mitochondria according to De Cavanagh et al. (2003). Purity of the mitochondrial preparation was tested by estimation of marker ATPases (Máleková et al. 2007). The method for estimation of the mitochondrial Mg^{2+}-ATPase activity and determination of the proper season for carrying out the experiment were described in our earlier paper Mujkošová et al. (2008). Fluidity of mitochondrial membranes was estimated according to Waczulíková et al. (2007), mitochondria oxygen consumption (QO_2) and oxidative phosphorylation according to Ziegelhöffer et al. (2009) and protein concentration in the mitochondrial fraction according to Lowry et al. (1953). If not indicated differently in the text, all organic chemicals were purchased from Sigma-Aldrich and the inorganic ones from Merck or Lachema (Czech Republic) and were of analytical grade.

At the beginning of experiment the systolic blood pressure amounted to 119 ± 8 mmHg in healthy controls and 171.50 ± 2.18 mmHg in SHR and it increased spontaneously during the 4 weeks experiment to 140 ± 7.07 mmHg (by 17.48%) in healthy controls and to 182.06 ± 5.74 mmHg (by 6.16%) in SHR (Fig. 1). Treatment with captopril decreased the blood pressure of SHR significantly from 182.06 ± 5.74 to 160.63 ± 3.89 mmHg i.e., by 11.77% ($p < 0.02$).

Hypertension exerted strong influence on parameters of the cell energetics (Table 1). In 20-week-old SHR, heart mitochondria exhibited a 33.77% increase in QO_2 of respiration state 3 (S3) ($p < 0.001$) as well as a 20.17% elevation of oxidative phosphorylation rate (OPR) values in comparison with heart mitochondria of healthy animals (Table 1, upper part), but only with glutamate + malate (GLUT+MAL) as substrates. The values of QO_2 of respiration state S4 (S4), respiratory control index (RCI) and ADP : oxygen ratio (ADP:O) showed only a moderate increase which, however, neither reached statistical significance ($p > 0.05$) with GLUT+MAL nor with succinate (SUC) as substrates. These results point to a spontaneous, although weak tendency for elevation of oxidative ATP production in heart mitochondria

Table 1. Parameters of oxidative ATP production in heart and kidney mitochondria in normotensive and spontaneously hypertensive rats with and without treatment with captopril

Oxidative parameters	Normotension		Hypertension		Hypertension + Captopril
	16 week	20 week	16 week	20 week	
HEART					
QO_2(S3) glutamate +malate	265.27 ± 10.27	228.71 ± 11.13°	295.41 ± 5.25	305.94 ± 16.12***	230.27 ± 14.20$^{\#\#\#}$
QO_2(S4) glutamate+malate	58.09 ± 4.55	54.25 ± 0.93	64.45 ± 0.93^{+}	62.62 ± 1.65^{++}	56.89 ± 4.87
RCI glutamate+malate	4.58 ± 0.20	4.30 ± 0.20	4.58 ± 0.06	4.75 ± 0.29	4.16 ± 0.33$^{\#}$
OPR glutamate+malate	717.88 ± 10.69	667.55 ± 27.41	804.39 ± 18.78*	802.18 ± 44.62††	629.82 ± 27.87$^{\#\#}$
ADP:O glutamate+malate	2.87 ± 0.03	2.92 ± 0.02	2.76 ± 0.04°	2.75 ± 0.06$^{\circ\circ}$	2.77 ± 0.06
QO_2(S3) succinate	237.73 ± 4.06	223.62 ± 10.22	252.92 ± 3.85	254.50 ± 14.48	237.17 ± 10.27
QO_2(S4) succinate	131.93 ± 1.55	122.95 ± 7.12	123.10 ± 1.38	130.30 ± 7.08	126.86 ± 6.96
RCI succinate	1.80 ± 0.04	1.83 ± 0.07	2.06 ± 0.05°	2.10 ± 0.15	1.87 ± 0.12
OPR succinate	350.15 ± 12.74	331.19 ± 36.52	438.34 ± 14.16†	448.15 ± 41.00	345.44 ± 53.25
ADP:O succinate	1.51 ± 0.05	1.44 ± 0.07	1.55 ± 0.05	1.54 ± 0.12	1.48 ± 0.06
KIDNEY					
QO_2(S3) glutamate +malate	127.38 ± 2.86	122.24 ± 4.26	124.52 ± 1.26	116.87 ± 1.73*	96.44 ± 2.28$^{\#\#\ \#\#\#}$
QO_2(S4) glutamate+malate	44.68 ± 0.83	41.81 ± 1.22	47.16 ± 0.99	45.12 ± 1.02†	37.87 ± 2.33$^{\#\#\#}$
RCI glutamate+malate	2.85 ± 0.05	2.95 ± 0.09	2.65 ± 0.07	2.69 ± 0.03°	2.54 ± 0.04
OPR glutamate+malate	358.95 ± 3.48	349.80 ± 9.90	338.50 ± 6.00	321.13 ± 10.95°	296.65 ± 5.54$^{\Delta}$
ADP:O glutamate+malate	2.90 ± 0.02	2.85 ± 0.02	2.88 ± 0.03	2.71 ± 0.06	2.79 ± 0.02
QO_2(S3) succinate	135.80 ± 1.21	135.48 ± 8.17	148.17 ± 1.79††	152.49 ± 6.45*	99.56 ± 8.41$^{+\ \#\#\#}$
QO_2(S4) succinate	93.29 ± 0.81	99.09 ± 3.40	107.26 ± 2.28***	107.71 ± 3.61	78.11 ± 4.53$^{\#\#\#}$
RCI succinate	1.36 ± 0.02	1.37 ± 0.20	1.39 ± 0.04	1.42 ± 0.05	1.27 ± 0.03*
OPR succinate	349.49 ± 5.32	347.58 ± 14.61	312.98 ± 4.84*	309.37 ± 7.57*	241.41 ± 13.34$^{\#\#\#}$
ADP:O succinate	1.72 ± 0.03	1.65 ± 0.01	1.61 ± 0.03	1.50 ± 0.05†	1.58 ± 0.03

QO_2(S3), rate of oxygen consumption by mitochondria in presence of exogenous ADP (state 3) (nmol of oxygen atoms·mg protein^{-1}·min^{-1}); QO_2(S4), rate of basal oxygen consumption by mitochondria in absence of exogenous ADP (state 4) (nmol of oxygen atoms·mg protein^{-1}·min^{-1}); RCI, respiration control index (S3·S4^{-1}); OPR, oxidative phosphorylation rate (nmol ATP·mg protein^{-1}·min^{-1}); ADP:O, coefficient of oxidative phosphorylation (nmol ADP·nAtO^{-1}). Values are means ± S.E.M. Statistical significance was evaluated by means of Kruskal-Wallis test. **Heart:** $^{\circ}$ $p < 0.05$, W-16 *vs.* W-20; *** $p < 0.001$, W-20 *vs.* SHR-20; $^{\#\#\#}$ $p < 0.0001$, SHR-16 *vs.* SHR-C, SHR-20 *vs.* SHR-C; $^{+}$ $p < 0.02$, W-16 *vs.* SHR-16; $^{++}$ $p < 0.002$, W-20 *vs.* SHR-20; $^{\#}$ $p < 0.01$, SHR-20 *vs.* SHR-C; $^{\circ}$ $p < 0.05$, W-16 *vs.* SHR-16; * $p < 0.01$, W-16 *vs.* SHR-16; † $p < 0.03$, W-16 *vs.* SHR-16; †† $p < 0.003$, W-20 *vs.* SHR-20; $^{\#\#}$ $p < 0.001$, SHR-20 *vs.* SHR-C; $^{\circ\circ}$ $p < 0.005$, W-20 *vs.* SHR-20. **Kidney:** * $p < 0.01$, SHR-16 *vs.* SHR-20, W-20 vs. SHR-20; $^{\#\#}$ $p < 0.001$, SHR-20 *vs.* SHR-C; $^{+}$ $p < 0.03$, W-16 *vs* SHR-C; †† $p < 0.003$, W-16 *vs.* SHR-16; † $p < 0.03$, W-20 *vs.* SHR-20; *** $p < 0.0001$, W-16 *vs.* SHR-16; $^{\#\#\#}$ $p < 0.0001$, W-16 *vs.* SHR-C, SHR-20 *vs.* SHR-C; $^{\circ}$ $p < 0.05$, W-20 *vs.* SHR-20; * $p < 0.01$, SHR-20 *vs.* SHR-C; $^{\circ}$ $p < 0.05$, W-20 *vs.* SHR-20; $^{\Delta}$ $p < 0.05$, SHR-20 *vs.* SHR-C; * $p < 0.01$, W-16 *vs.* SHR-16, W-20 *vs.* SHR-20; $^{\#\#\#}$ $p < 0.0001$, SHR-16 *vs.* SHR-C, SHR-20 *vs.* SHR-C.

as a compensatory response to enhanced energy demands of heart cells caused by hypertension. Captopril treatment do not supported the above development. It counteracted the original moderate tendency to elevation of ATP production and in addition even decreased the RCI (12.42%) and OPR (21.49%) values ($p < 0.01$), but again with GLUT+MAL as substrates only. In respect to the latter findings it may be assumed that the action of captopril is in some way interfering with function of the complex 1 in the respiratory chain.

In contrast to the heart, in kidney mitochondria (Table 1, lower part, results with GLUT+MAL), spontaneous hypertension induced an 8.20% decrease in OPR and an 8.83% lowering in RCI values, both $p < 0.05$, but in addition to changes in the heart it also induced a 4.91% decrease in the value of ADP:O ($p < 0.05$). During the followed period of hypertension (between the 16–20 weeks) we also observed a decrease in values of QO_2 S3 and QO_2 S4 which, however, remained without any influence on the value of RCI. Findings in kidney mitochondria of SHR testify for serious perturbations in oxidative ATP production including an uncoupling of oxidation from phosphorylation. The latter could be confirmed also with SUC ($p < 0.03$) as substrate. Captopril treatment made these disturbances even more pronounced. It decreased significantly the values of QO_2 in the states S3 and S4 ($p < (0.001–0.0001)$) both with GLUT+MAL and SUC as well as the value of OPR with GLUT+MAL ($p < 0.05$) and

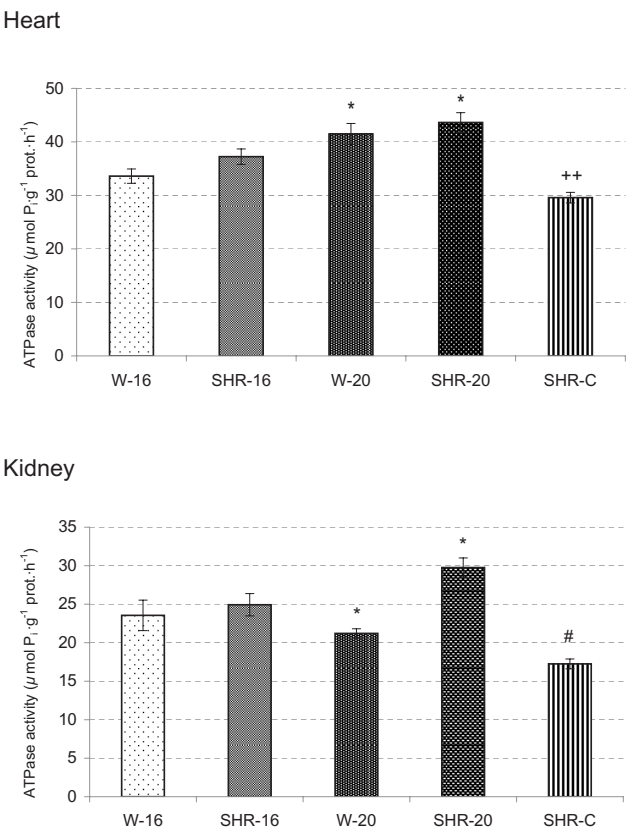

Figure 2. Activity of Mg^{2+}-ATPase in heart and kidney mitochondria from normotensive and spontaneously hypertensive rats untreated and treated with captopril. W-16, 16-weeks old normotensive Wistar rats; SHR-16, 16-weeks old spontaneously hypertensive rats; W-20, 20-weeks old normotensive Wistar rats; SHR-20, 20-weeks old spontaneously hypertensive rats; SHR-C, 20-weeks old spontaneously hypertensive rats treated with captopril. Results are means ± S.E.M. of 9 experiments. Statistical significance was evaluated by means of Kruskal-Wallis test. Heart: * $p < 0.05$, W-16 *vs.* W-20, SHR-16 *vs.* SHR-20; $^{++}$ $p < 0.0003$, SHR-20 *vs.* SHR-C. Kidneys: * $p < 0.05$, SHR-16 *vs.* SHR-20, W-20 *vs.* SHR-20; $^{\#}$ $p < 0.001$, SHR-20 *vs.* SHR-C.

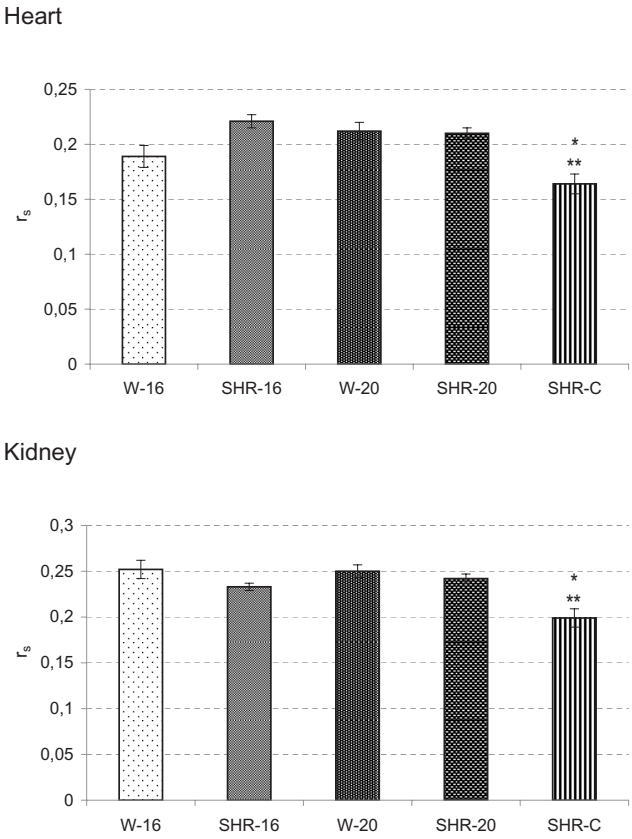

Figure 3. Fluorescence anisotropy of DPH in heart and kidney mitochondria from normotensive and spontaneously hypertensive rats untreated and treated with captopril. r_s, fluorescence anisotropy of DPH, the reciprocal value of membrane fluidity; W-16, 16-weeks old normotensive Wistar rats; SHR-16, 16-weeks old spontaneously hypertensive rats; W-20, 20-weeks old normotensive Wistar rats; SHR-20, 20-weeks old spontaneously hypertensive rats; SHR-C, 20-weeks old spontaneously hypertensive rats treated with captopril. Results are means ± S.E.M. of 9 experiments. Statistical significance was evaluated by means of Kruskal-Wallis test. Heart: * $p < 0.05$, W-16 *vs.* SHR-C; ** $p < 0.02$, SHR-20 *vs.* SHR-C. Kidneys: * $p < 0.05$, SHR-20 *vs.* SHR-C; ** $p < 0.02$, W-16 *vs.* SHR-C.

with SUC ($p < 0.0001$). All these results indicate that in spite of its potency to decrease blood pressure captopril exerts negative influence on oxidative production of ATP in the heart and especially in the kidney mitochondria of SHR.

Specific activity of the heart mitochondrial Mg^{2+}-ATPase (the reverse estimated ATP synthase) in SHR (Fig. 2, upper panel) increased during the duration of experiment significantly ($p < 0.05$). Further increase in Mg^{2+}-ATPase activity amounting to 16.55% was, however, completely eliminated by captopril ($p < 0.003$). Our findings point to involvement of this enzyme in the process of compensation for increased energy demands of tissues which are lowered by the antihypertensive action of captopril. In respect to Mg^{2+}-ATPase activity kidney mitochondria behaved similarly to the heart mitochondria but the captopril-induced decrease in enzyme activity was more evident (Fig. 2, lower panel) and amounting to 42.62% it reached a value significantly ($p < 0.001$) below that in healthy controls.

In addition to alterations in oxygen consumption and ATP production, hypertension induced also changes in membrane fluidity (MF) of the mitochondria (Fig. 3). In comparison with healthy hearts the mitochondria of SHR (Fig. 3, upper panel) exhibited, but slightly ($p > 0.05$) higher values of MF during the whole experiment. The observed moderate elevation in MF is in concert with the majority of changes in QO_2 and RCI. Fluidization of heart mito-

chondrial membranes may be caused by various factors. Nevertheless, it can´t be excluded that an increase in the amount of trans-membrane substrate and energy transition pores associated with a decrease in mitochondrial membrane potential (Waczulíková et al. 2007; Ferko et al. 2008) is also participating in this phenomenon. Such an increase in substrate and energy transition pores formation in mitochondrial membranes is a feature common for diabetes as well as for hypertension and represents a protective process triggered by strong calcium signaling (Ziegelhöffer et al. 2009). In heart mitochondria captopril treatment induced a significant 21.74% ($p < 0.02$) decrease in fluorescence anisotropy of DPH (i.e., increase in MF) in comparison with mitochondria from untreated SHR and it reached a degree lying by 14.74% ($p < 0.05$) even over the MF value in healthy controls. Kidney mitochondria (Fig. 3, lower panel) of SHR exhibited slightly decreased fluorescence anisotropy i.e., increased MF values ($p > 0.05$) during the whole experiment. Captopril aggravated the depression in fluorescence anisotropy of DPH (i.e., increase in MF) significantly even below the value in healthy controls ($p < 0.02$). For the mechanism of MF elevating effect of captopril, which may represent a side effect of the drug, we don't have at present any explanation.

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