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Differential expression of cardiac uncoupling proteins 2 and 3 in response to myocardial ischemia-reperfusion in rats

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ABSTRACT

Aims: We aimed to evaluate the transcription and translation of genes for uncoupling protein 2 (UCP2) & uncoupling protein 3 (UCP3) in rat heart mitochondria of both ventricles after myocardial ischemia followed by various periods of reperfusion.
Main methods: Seven groups of 8 male Wistar rats were evaluated for the effects of ischemia and also reperfusion, using Western blot of isolated mitochondrial proteins in addition to RNA extraction followed by real-time RT-PCR analysis.
Key findings: In rats with 30 min of reperfusion (R30) UCP2 protein was increased 213 ± 33%, which is meaningfully more than the control group ($P < 0.001$). Western blot showed increase in UCP2 protein level in groups receiving reperfusion for 60 min (R60), 120 min (R120) and 180 min (R180) as much as 152 ± 28% ($P < 0.001$ vs. control), 123 ± 19% ($P < 0.01$ vs. control) and 131 ± 30% ($P < 0.01$ vs. control), respectively. There was no statistically important difference in UCP2 mRNA between either right or left ventricles of ischemic and ischemia-reperfusion (IR) groups vs. control group. In the groups R180 and R240, UCP3 protein levels showed 131 ± 27% and 102 ± 18% increase, respectively (both $P < 0.001$ vs. control group). However, the change in UCP3 level in other groups was not significantly different from the control group.
Significance: UCP2 and UCP3 protein levels are considerably increased in the ischemic area early after acute myocardial IR. The right ventricular UCP2 protein expression does not change, that is, effect of IR on UCP2 protein is a local process. However, UCP3 protein level increased both in ischemic area of the left ventricle and in non-ischemic area of the right ventricle.

Introduction

Myocardial ischemia is associated with activation of many complex physiologic and pathologic processes. Numerous studies including our recently published data have shown that the levels of many proteins including anticoagulant factors, structural proteins, mediators of inflammation, proteins related to transcription and translation, and also proteins involved in regulation of metabolism are significantly increased after acute myocardial ischemia-reperfusion (IR) (Fert-Bober et al., 2008; De Celle et al., 2005; Safari et al., 2012, 2013). Obviously, accurate identification of these changes is quite necessary for development of more effective drugs for injuries induced by acute myocardial IR.

Uncoupling protein-2 (UCP2) and 3 (UCP3) are in the mitochondrial anion carrier family of proteins that catalyze translocation of protons across the inner mitochondrial membrane. It has been shown that decrease in proton motive force will reduce generation of mitochondrial reactive oxygen species (ROS), and will diminish production of adenosine triphosphate (ATP) by uncoupling of phosphorylation and oxidation pathways (Cioffi et al., 2009). It is also thought that UCPs are involved in free fatty acid (FFA) metabolism since they catalyze export of fatty acid peroxides to outside of the inner mitochondrial membrane (Himms-Hagen and Harper, 2001; Murray et al., 2005). Some studies have recently suggested the fundamental role of UCP2 and UCP3 in mitochondrial Ca^{2+} uniport, too (Trenker et al., 2007). So, these proteins can be regarded as multi-task cellular factors.

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There is some evidences that ROS (Brand et al., 2004; Azzu and Brand, 2010) and FFA (Murray et al., 2005; Cole et al. 2011) can induce expression of UCP2 and UCP3. The presence of UCP2 and UCP3 proteins in the rat heart has been shown in several studies including ours, but the cardiac regulation of their expression in ischemic myocardium remains unclear. Increased mitochondrial ROS production, FFA accumulation and calcium overload, and concomitantly decreased ATP have been implicated in myocardial damage induced by IR (Dorweiler et al., 2007; Asano et al., 2003). Acknowledging the crucial role of mitochondria in cardiac cells, assessment of changes in mitochondrial genes & proteins which affect physiological parameters such as free radical production, ATP generation, Ca^{2++} handling and FFA production in cardiomyocytes would be of great importance. Therefore, the aim of the current study was to evaluate the transcription of UCP2 & UCP3 in rat heart mitochondria after myocardial ischemia followed by various periods of reperfusion, and also assessment of the level of expression of their relevant proteins.

Materials and methods

Experimental groups

Seven groups ($n = 8$ in each group) of male Wistar rats were maintained on a reverse 12-h: 12-h light/dark cycle in a temperature-controlled room. All experimental procedures were done in accordance with the EU Directive 2010/63/EU for animal experiments and approved instructions of the Ethics Committee on Animal Experiments of Shahid Sadoughi University, Yazd, Iran.

Myocardial ischemia reperfusion was induced by ligation of the left anterior descending (LAD) branch of coronary artery. Briefly, the rats weighing 250–300 g were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal), intubated and ventilated artificially with room air at a frequency of 80 inflations/min and a tidal volume of 1 mL/100 g. Lead II of electrocardiogram (ECG) was recorded using cutaneous needle electrodes. Mean arterial blood pressure in the left carotid artery was monitored by a blood pressure transducer. A left thoracotomy was performed in the 4th intercostal space, the pericardium incised, and the heart exposed. In ischemic group (I) the LAD branch was occluded for 30 min by lifting a 5/0 silk thread which was passed below the LAD through a piece of polyethylene tube. The criteria for confirmation of successful coronary occlusion were development of a pale color in the distal myocardium, ST elevation on ECG, and reduction of the mean arterial pressure. In five ischemia-reperfusion groups (IR groups) after 30 min of ischemia, we allowed reperfusion of ischemic myocardium for 30, 60, 120, 180 and 240 min by loosening the silk thread. In order to recognize the ischemic area at the end of the reperfusion, the LAD was occluded again and the pale ischemic area from the left ventricle was cut immediately. At the end of the reperfusion the heart was immediately removed and washed in cold isotonic saline. Since UCP measurement in right ventricle was one of the main goals of this study, Evans blue staining was not used for determining ischemic area. In the I and IR groups, the ischemic area from the left ventricle was biopsied, but in the sham-operated and the control animals corresponding normal left ventricular region was cut out. In all groups a right ventricle sample was also collected. Liquid nitrogen was used for rapid freezing of the tissue samples at −80 °C for further studies. The same surgical procedures were performed in sham-operated rats, except that the suture around the coronary artery was not tied. The seventh group included intact animals which served as control.

Cardiac mitochondrial isolation

According to the modified method of Butz et al., rat heart mitochondria were isolated using differential centrifugation (Butz et al., 2004). In this way, tissue was placed in ice-cold cardiac homogenization and isolation buffer A (2 mM EGTA, 40 mM NaCl, 210 mM sucrose, and 30 mM HEPES, pH 7.4) supplemented with protease inhibitors. Tissue was disrupted by the use of a Teflon-glass homogenizer. To eliminate nuclei and cell debris, the homogenate was centrifuged at 600 ×*g* for 10 min at 4 °C. One mL of supernatant was centrifuged at 10,000 g at 4 °C for 20 min. The pellet from this step was washed in 1 mL of isolation medium B which contained 10 mM Tris and 1 mM EDTA, pH 7.4. The final pellet containing mitochondria was resuspended in 66 μL of 16% SDS and 200 μL of buffer B, and centrifuged at 1100 *g* for 20 min at room temperature to remove insoluble materials. The supernatants which contained mitochondria were stored at −80 °C.

Western blot analysis

By the use of the Bradford method, protein concentrations of homogenates were determined (Bradford, 1976). The sample buffer contained 10% (v/v) glycerol, 5% (w/v) SDS, 0.25% (w/v) bromophenol blue, 5% (v/v) 2-mercaptoethanol, and 0.0625 M Tris–HCl, pH 6.8, and the protein extract was incubated for 7 min at 100 °C in it before being loaded onto the wells. The homogenate proteins were loaded in equal amounts on a 12.5% SDS-polyacrylamide gel electrophoresis mini-gel in each lane (Bio-Rad laboratories, Hercules CA, USA). Electrophoresis was performed for about 1.5 h at 110 V in running buffer (0.025 M Tris–HCl, 0.2 M glycine, 1 mM EDTA, and 3.5 mM SDS). Using a blot apparatus (Bio-Rad, USA), proteins were transferred from the gels to polyvinylidene fluoride membrane (Amersham Bioscience Co., UK). The membranes were blocked with 2% ECL Advance Blocking Milk (Amersham Bioscience Co., UK) and 1% bovine serum albumin in TBST (0.01 M Tris–HCl, pH 7.6, 1.5 mM NaCl, 0.1% (v/v) Tween-20) for 1 h. Western blotting was performed with polyclonal goat anti-UCP2 and anti-UCP3 primary antibodies (Santa Cruz Biotechnology, Inc., USA) and with donkey anti-goat horseradish peroxidase-conjugated secondary antibody (from the same manufacturer) at a dilution of 1:500 and 1:10,000, respectively. After this step, chemiluminescent substrate (ECL Advance reagents, Amersham Bioscience Co., UK) was added, and blots were exposed on film. Bands were scanned, and their densities determined using ImageJ software (version 1.43). Data were normalized to control bands.

RNA extraction and real-time RT-PCR analysis

Expression levels of the genes were determined by real-time RT-PCR. The RNeasy fibrous tissue mini kit from QIAGEN, USA was used for total RNA extraction according to the manufacturer's instructions. Concentrations of RNA were determined by measuring the absorbance at 260 nm, and its purity was assessed by 260/280 nm absorbance ratio (Eppendorf, Hamburg, Germany). For synthesis of first strand cDNA, we used 1 μg of total RNAs using random hexamers, dNTP and Moloney murine leukemia virus reverse transcriptase (Fermentas), in a total volume of 20 μL. The RT-PCR was performed using the Rotor Gene system (Corbett Research 2004, Australia) and SYBR Green I. Relative quantity of gene expression was analyzed according to the Pfaffl method. The nucleotide sequences of the PCR primers were as follows: UCP2: forward 5′-GCCC GGGCTGGTGGTGGTC-3′ and reverse 5′-CCCCGAAGGCAGAAGTGAAG TGG-3′; UCP3: forward 5′-CGTCTCGGTACATCCTGACTA-3′ and reverse 5′-TTCTTCCCTGGCGTGGTTCTGTA-3′; β-actin: forward 5′-GAACCCTAAG GCCAACCGTGAAAAGAT-3′ and reverse 5′-ACCGCTCGTTGCCAATAGTG ATG-3′.

Statistical analysis

For comparison of the differences between control, sham and IR groups, one-way analysis of variance (ANOVA) was used. Post hoc analysis was performed using the Tukey multiple comparison test. To compare hemodynamic parameters in each group, we used paired *t*-test.

Statistical significance level was set at $P < 0.05$. The Prism software was used for statistical analysis.

Results

Hemodynamic parameters

After anesthesia and initiation of ventilator-assisted ventilation, the carotid artery was canulated, and the canula connected to pressure transducer. Electrocardiography leads were attached to limbs. Therefore, blood pressure and heart rate were continuously measured. Blood pressure and heart rate of rats before ligation of LAD and initiation of ischemia were regarded as baseline values. LAD ligation suddenly decreases mean arterial pressure, which lasts for 10–15 min, followed by increase but not to initial level, so that the mean arterial pressure at the end of ischemic period is usually less than normal. Reperfusion causes rise in mean arterial pressure, but again not to the baseline level. In Table 1 changes in mean arterial pressure and heart rate in various groups before arterial ligation, 5 min after ligation, and at the end of test (just before myocardial tissue sampling) are shown. Paired *t*-test revealed that the mean arterial pressure during ischemia was significantly decreased in comparison with the baseline level in some groups. However, one-way ANOVA did not show any significant difference in mean arterial pressure and heart rate at sampling time between various groups.

Cardiac UCP2 transcription and expression in response to myocardial ischemia reperfusion

Our first objective was evaluation of changes in UCP2 transcription (mRNA) as a response to myocardial ischemia without reperfusion. As is shown in Fig. 1A, there was no statistically important difference between either right or left ventricle of ischemic and IR groups as compared to the control group.

For assessment of UCP2 protein, at first in those rats receiving only ischemia for 30 min (Isc group), mitochondria were isolated from the ischemic tissue, and measurement of UCP2 protein was performed. As is seen in Fig. 1B, ischemia alone increased mitochondrial content of UCP2 protein in ischemic part of the left ventricle for 25 ± 12%, which is not meaningfully different from the left ventricle in the control group. Also, in non-ischemic area of the right ventricle there was no statistically significant increase in mitochondrial content of UCP2 protein as compared with the right ventricle in the control group.

Then we assessed the changes in UCP2 protein in those groups who received ischemia for 30 min followed by different periods of reperfusion. As shown in Fig. 1B, in rats with 30 min of reperfusion (R30) the mitochondrial content of UCP2 protein was increased 213 ± 33% which is meaningfully more than the control group ($P < 0.001$). Results of Western blot showed increase in UCP2 protein level in groups subjected to reperfusion for 60 min (R60), 120 min (R120) and 180 min (R180) for 152 ± 28% ($P < 0.001$ *vs.* control), 123 ± 19% ($P < 0.01$ *vs.* control) and 131 ± 30% ($P < 0.01$ *vs.* control), respectively. The increase in UCP2 protein level in right ventricle of no group was significantly different from the right ventricle of the control group.

Cardiac UCP3 transcription and expression in response to myocardial ischemia reperfusion

Changes in UCP3 gene transcription in various groups showed that the level of UCP3 mRNA in right ventricle and ischemic part of the left ventricle do not significantly differ from those in the left and right ventricles of the control group (Fig. 2A). Measurement of mRNA level in the ischemic area of left ventricle in IR groups demonstrated its significant rise in IR60 (83 ± 20%, $P < 0.05$), IR120 (129 ± 24%, $P < 0.001$) and IR180 (112 ± 16%, $P < 0.001$) groups, respectively.

Evaluation of UCP3 gene transcription in the non-ischemic area of the right ventricle in IR groups showed that the amount of increase in mRNA was significant in IR120 (96 ± 18%, $P < 0.01$), IR180 (104 ± 28%, $P < 0.01$) and IR240 (73 ± 15%, $P < 0.05$), respectively. UCP2 and UCP3 mRNA and protein levels didn't change significantly in sham-operated groups, therefore the data of sham group are not shown in the figures.

Changes in UCP3 protein level in the left and right ventricles of groups were also compared. It showed that as a response to myocardial ischemia without perfusion (group Isc) in ischemic area of the left ventricle UCP3 protein content was increased 9 ± 10.1%, and in the non ischemic area of the right ventricle it increased 9.5 ± 7.4%, neither of them showing a significant difference when compared with the left and right ventricles of control group (Fig. 2B).

Assessment of UCP3 protein level in groups under ischemia followed by different durations of reperfusion revealed that in the group R30 the mitochondrial protein UCP3 was increased 19 ± 10.1%. UCP3 protein levels in groups R60 and R120 were 23 ± 16.6% and 31 ± 14.9%, respectively. The increase in UCP3 protein levels of groups R30, R60 and R120 was not significant in comparison with the control group, just like the situation for aforementioned groups. However, in the groups R180 and R240, UCP3 protein levels showed 131 ± 27% and 102 ± 18% increase, respectively, both of them were significantly more than the control group ($P < 0.001$).

In the right ventricle of IR groups, it showed that UCP3 protein was increased 90 ± 21% and 111 ± 22% in groups IR180 and IR240, respectively, which is significantly more than the amounts in right ventricle of the control group ($P < 0.001$). Other IR groups had no significant change in UCP3 protein when compared with the right ventricle of control group.

Discussion

The results of this study indicated that UCP2 and UCP3 protein levels are considerably increased in the ischemic area after acute myocardial IR, i.e., as an "early" response to acute myocardial IR. The right ventricular UCP2 protein expression does not change, that is, effect of IR on UCP2 protein is a local process. However, UCP3 protein level increased both in ischemic area of the left ventricle and in non-ischemic area of the right

Table 1
Mean arterial pressure (MAP) and heart rate (HR) of the study groups.

BP at the sampling time		BP after LAD occlusion		Baseline		Groups
HR	MAP	HR	MAP	HR	MAP	
402	97 ± 10.36	416	81.40 ± 28.19*	380	118 ± 14.83	**Isc**
419	101.56 ± 15.46	431	93.11 ± 26.21	385	107.78 ± 18.04	**R30**
394	85 ± 21.79	423	92.86 ± 25.62	387	107.14 ± 12.19	**R60**
393	83.5 ± 22.4	395	80.5 ± 20.29**	370	116 ± 11	**R120**
389	96.25 ± 23.1	404	79.75 ± 24.31*	381	105 ± 16.11	**R180**
414	93.57 ± 11.07	425	110.71 ± 16.16	369	115.71 ± 9.75	**R240**

Hemodynamic parameters in various experimental groups are shown at baseline (before occlusion of the left anterior descending branch of coronary artery), at ischemia (after arterial occlusion), and at the end of test (i.e., at the sampling time), all in comparison with the basal condition of each individual group. *$p < 0.05$; **$p < 0.01$ (paired *t*-test). There was no significant difference in mean arterial pressure and heart rate between various groups at sampling time. Data are shown as Mean ± SD.

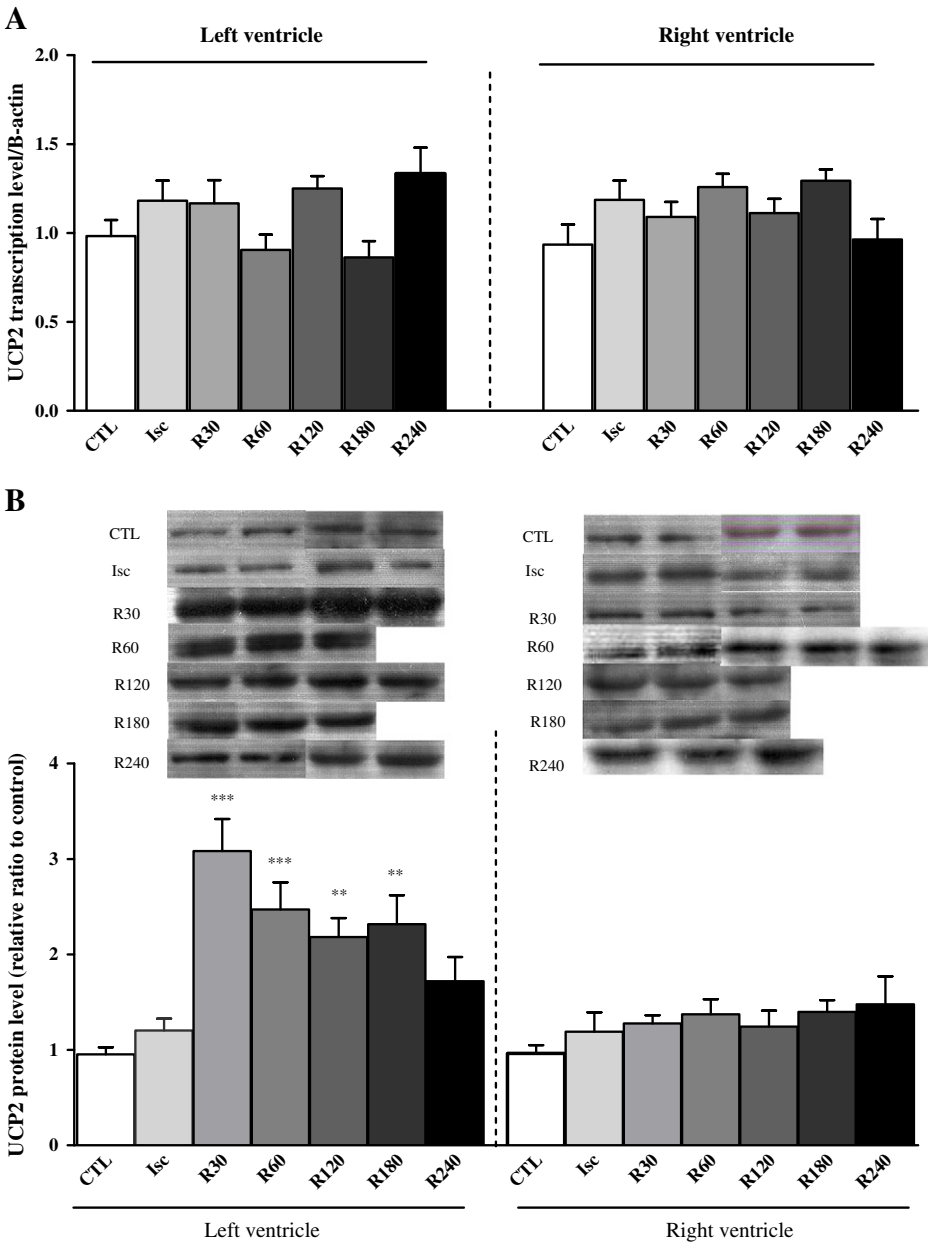

Fig. 1. UCP2 transcription and expression levels following myocardial ischemia reperfuson. Panel A: UCP2 transcription in the ventricles of different experimental groups. Panel B: Expression of UCP2 protein in the ventricles of different experimental groups. Representative Western blot analyses of mitochondrial UCP2 content are shown at the top of the panel. CTL: The control group; Isc: The ischemia group (without reperfusion); R30: 30 min of reperfusion; R60: 60 min of reperfusion; R120: 120 min of reperfusion; R180: 180 min of reperfusion; R240: 240 min of reperfusion. Values are mean ± S.E.M, $n = 8$; Tests were duplicated or in some cases, triplicated; $^{**}p < 0.01$;$^{***}p < 0.001$ vs. control.

ventricle, which denotes a global response in the heart, in sharp contrast with the limited local response of UCP2 protein.

In our previous study which measured only UCP2 protein after 30 min ischemia and 180 min reperfusion, the mitochondrial UCP2 was increased dramatically, being limited to only ischemic myocardium but not non-ischemic tissue of the right ventricle (Safari et al., 2013). The current study supports our past results. The present work has added measurements of UCP3 protein and has also improved our understanding of effects of ischemia alone in addition to its effects when followed by various durations of reperfusion.

Our study also considered changes in mRNA and protein in the sham group, which showed that the operation itself cannot induce the involved genes, so the observed changes are solely attributed to IR phenomenon. Table 1 shows that there is no significant difference between various experimental groups regarding their mean arterial pressure and heart rate at the time of sampling. Therefore, the changes in proteins are independent of hemodynamic changes.

The results of our study are in accordance with other studies. For example, Almsherqi et al. showed that with 6 and 24 h of ischemia in the left ventricle, the level of UCP3 protein in the non-ischemic wall of right ventricle will increase up to 70% and 340%, respectively (Almsherqi et al, 2006). Increased UCP2 protein level in the ischemic area of left ventricle after chronic myocardial ischemia, induced by LAD ligation for 10 weeks, was also shown (McFalls et al., 2006).

In our study, acute myocardial IR led to considerable increase in both UCP2 and UCP3 proteins, which may be due to different mechanisms. Past studies have indicated that ROS is the most important regulating factor in activity and expression of UCP2 and UCP3 (Brand et al., 2004; Ishizawa et al., 2006). Production of oxygen free radicals in cardiac myocytes is rapidly increased due to damage to anti-oxidant systems

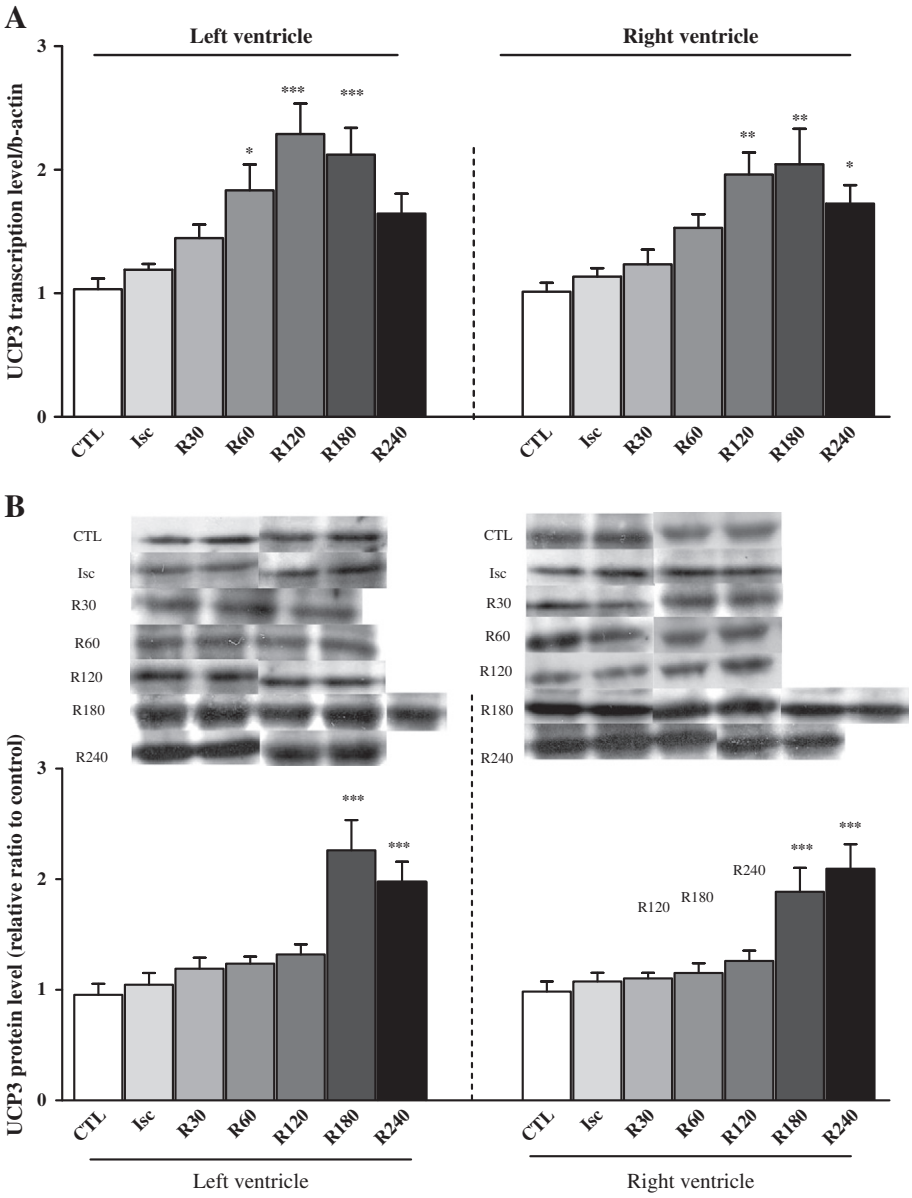

Fig. 2. Results of the measurement of UCP3 mRNA and UCP3 protein. Panel A: UCP2 transcription in the ventricles of different experimental groups. Panel B: Expression of UCP2 protein in the ventricles of different experimental groups. Representative Western blot analyses of mitochondrial UCP3 content are shown at the top of the panel. CTL: The control group; Isc: The ischemia group (without reperfusion); R30: 30 min of reperfusion; R60: 60 min of reperfusion; R120: 120 min of reperfusion; R180: 180 min of reperfusion; R240: 240 min of reperfusion. Values are mean ± S.E.M, $n = 8$; Tests were duplicated or in some cases, triplicated; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ vs. control.

during myocardial ischemia and also due to blood rush and delivery of large amounts of oxygen to ischemic area cells during reperfusion (Gross and Auchampach, 2007; Misra et al., 2009). Therefore, one can attribute increased level of those proteins mainly to raised amounts of ROS during myocardial IR. Some in vivo and in vitro studies also have shown that expression of UCP2 and UCP3 proteins is increased during oxidative stress, as a feedback response aimed at decreasing ROS production. In other words, increase in UCPs can act as a protective mechanism toward lowering the ROS production (Lee et al., 2005; Collins et al., 2005; Xie et al., 2008). It can be said that mitochondria are not merely succumbed to ischemia, but they respond to ischemia so actively and dynamically that will increase cardiac cell resistance to ischemia. Increased expression of UCPs can protect cardiac cells against injury as an adaptive response, just like what is seen in ischemic preconditioning. After short periods of ischemia and induction of rat heart resistance, cardiac mitochondria show more hydrogen leak, less membrane potential, less ATP content, more oxygen consumption and considerably lowered production of ROS. The interesting fact is that the levels of mRNA as well as UCP2 & UCP3 proteins in the protected heart are increased in comparison with non-protected hearts (McLeod et al., 2005). Mitochondria isolated from ischemic area of chronically ischemic myocardium which have higher levels of UCP2 will produce less ROS when under anoxia and reperfusion. It means that increase in UCP2 level acts as an adaptive mechanism of mitochondria, and decreases production of ROS during ischemia (McFalls et al., 2006).

Increase in ROS production, apoptotic cell death, infarct size and incidence of arrhythmias as well as impairment of cardiac function in $UCP3^{-/-}$ hearts confirm the cardioprotective effects of UCPs in ischemic heart (Ozcan et al., 2013; Perrino et al., 2013).

So, in our study it is possible that increased ROS generation during acute myocardial IR is one cause of raised level of UCP2 and UCP3 proteins. Past studies have detected a sequence before the promoter of UCP2 and UCP3 genes, which contains a binding site for ROS-sensitive factors. Therefore, under oxidative stress, UCPs can also be increased at transcriptional level (Brand et al., 2004; Azzu and Brand, 2010). In our study, the amount of transcription of UCP2 did not show significant

change in response to either ischemia or reperfusion. So, we can rule-out at least the effect of oxidative stress on transcription of UCP2 in response to myocardial IR. However, the role of this important regulator of UCPs' expression on UCP3 cannot be denied because, as mentioned above, the level of UCP3 mRNA was increased in both ventricles as a response to myocardial IR. It is also probable that oxidative stress has increased mitochondrial content of UCP2 as a post-translational effect without affecting transcription, since the role of ROS on UCP2 post-translation has been shown previously (Chan et al., 2004).

On the other hand, UCPs separate the phosphorylation pathway from oxidation by increasing leak of hydrogen, so part of the energy will be lost as heat instead of being used for production of ATP, hence decreasing cellular ATP (Noma et al., 2001; Murakami et al., 2002; Laskowski and Russell, 2008). Some researchers believe that acute increase in UCP protects heart against oxidative stress by effectively decreasing ROS generation, but its chronic increase will lower energy efficiency of the heart by lowering production of ATP, thus threatening viability of cardiac cells.

Free fatty acid anions are one of the other (beside ROS) known regulating factors of expression of UCPs (especially UCP3). One of the physiologic functions of UCP3 is prevention of excessive accumulation of fatty acids in mitochondria by catalyzing the export of fatty acid peroxides outside the inner leaflet of the mitochondrial membrane (Himms-Hagen and Harper, 2001; Hoeks et al., 2006). Studies have shown that impairment of the energetic activity of the heart is associated with increased level of circulating free fatty acids during myocardial ischemia, including the period after cardiac surgery and after angina (see review: Lopaschuk, 2004). Murray et al. demonstrated that serum free fatty acids are elevated in patients candidate for coronary artery bypass graft surgery, which is associated with increased level of UCP2 (Murray et al., 2004). Higher level of UCP3 concurrent with increased circulatory free fatty acids is also shown in rats with heart failure (Murray et al., 2008).

So, it might be that regulation of UCP3 expression by circulating fatty acids is one of the reasons of steadiness of UCP2 in the right ventricle in spite of significant increase in the level of UCP3 protein in non-ischemic area of right ventricle.

The other finding in this study was lack of parallel change in level of UCP2 mRNA (i.e., unaffected transcription) and level of mitochondrial UCP2 protein (i.e., increased translation) during IR. Our past study also showed such disparity between UCP2 mRNA and protein (Safari et al., 2013). There are other studies that support this, too. Although the mechanism of this separation is not yet understood, some studies have proposed post-translational regulation of those proteins (Pecqueur et al., 2001; Rodriguez et al., 2002; Ealey et al., 2002).

UCPs are coded by genomic DNA, and enter the space between two layers of mitochondrial membrane. One of the reasons for disparity of changes in UCP2 protein and its mRNA in our study may be regulation at the level of protein trafficking from synthesis site to mitochondrial localization. Another possible reason for that disparity is the prexsence of various regulatory factors at transcriptional level, for example, heterogeneous nuclear ribonucleoprotein K. It is now evident that increase in expression of K protein mediates increase in UCP2 level by insulin. It means that insulin increases level of UCP2 protein without altering mRNA level, but it raises level of UCP2 protein through increasing expression and phosphorylation of protein K (Ostrowski et al., 2004).

Since we assessed the changes after about 210 min (30 min ischemia and 180 min reperfusion) in our previous study, we were unaware of probable changes in either mRNA level or protein level during that time interval. Considering the fact that ischemia influences complex processes inside the cell even after a few minutes, it seems probable that the level of UCP2 mRNA has increased after ischemia, and then returned to its basal level due to being degraded by unknown mechanisms. Therefore, in the current study changes in mRNA and protein were assessed after ischemia and in different times after reperfusion, which showed no significant increase in UCP2 protein after ischemia or reperfusion. So, we disregard our suggested mechanism in the previous study, and will seek other possible mechanisms that explain this disparity. We did not find any disparity in UCP3. In fact, the increase in UCP3 level as a response to ischemia was due to increased transcription. In other words, the level of UCP3 protein parallels changes in its mRNA. So, it can be concluded that the ways UCP2 and UCP3 proteins are regulated, at least in response to myocardial ischemia, are different.

In this study there was little mortality, too low to be statistically compared with alive animals. Technical mortalities included one rat after anesthesia, 2 animal deaths during carotid canulation due to rupture of carotid artery, and one rat due to myocardial rupture during LAD occlusion. Arrhythmia-induced deaths were 2 fatalities at 6 and 11 min after start of reperfusion due to irreversible ventricular fibrillation, the latter showing no increase in either UCP2 or UCP3 protein.

Conclusion

To summarize, the results of this study showed dynamic and different changes in mitochondrial content of UCP2 and UCP3 proteins in response to acute myocardial ischemia reperfusion, and this alteration, which is most likely a compensation directed at reduction of cardiomyocyte injuries, is part of the complex system of changing level of genes and proteins in cardiac tissue. It is obvious that understanding these changes is quite necessary for acknowledging the cellular and molecular mechanisms responsible for myocardial ischemic injury. However, the ways by which these two proteins are regulated in myocardium in response to acute pathologic circumstances such as IR are probably different.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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