ORIGINAL SCIENTIFIC ARTICLE

THE ROLE OF PHOSPHOCREATINE IN THE PERCONDITIONING AND POSTCONDITIONING OF ISOLATED RAT HEART

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ABSTRACT

The present study strives to assess the cardioprotective role of phosphocreatine as an agent for postconditioning and perconditioning of isolated rat heart.
Rat hearts (n=30) were perfused with a Langendorff apparatus and randomly assigned to three groups subjected to 20 minutes of global ischemia and 30 minutes of reperfusion: control group (untreated rat hearts), postconditioning group (hearts treated with 0.2 mmol/l of phosphocreatine during the first 5 minutes of reperfusion), and perconditioning group (hearts treated with 0.2 mmol/l of phosphocreatine during the first 5 minutes of ischemia). During the experimental protocol, cardiodynamic parameters were evaluated, while oxidative stress parameters such as superoxide anion radical, hydrogen peroxide, nitrites and index of lipid peroxidation were determined in coronary venous effluent.
Postconditioning and perconditioning with phosphocreatine improved contractile function, heart rate and coronary flow, while the examined oxidative stress parameters in coronary venous effluent were significantly reduced in groups of treated rat hearts.
The results of this study indicate that phosphocreatine has the potential as a therapeutic agent for perconditioning and postconditioning the heart in ischemia reperfusion injury.

Keywords: *Langendorff apparatus, creatin phosphate, cardioprotection, oxidative stress.*

INTRODUCTION

A major problem of the health system around the world is the high prevalence and poor prognosis of patients with heart failure. In order to start discovering new therapeutic strategies, it is crucial to fully elucidate all mechanisms involved in the development of cardiac dysfunction. Given that cardiomyocytes' energy needs are high, it is extremely important to have an adequate balance between energy produced and expended by continuous resynthesis in order to maintain an optimal contractile response of the heart muscle (1, 2). Chemical energy in the form of adenosine triphosphate (ATP) is created in mitochondria during the process of oxidative phosphorylation, and most of the substrate for energy production are fatty acids, while less than 10% is obtained from glucose, lactate, and ketone bodies (3, 4). The phosphagen system should provide the energy necessary for myocardial function through the interaction of creatine and ATP in the presence of creatine kinase. In addition to numerous studies, the precise role of high-energy phosphate metabolism in cardiovascular disease has not yet been fully elucidated. There is thought to be an association between phosphocreatine levels and the development and progression of heart disease, and this claim is supported by the results of studies conducted on both animal models and the human population (5).

During ischemia, the process of oxidative phosphorylation is disturbed, the production of ATP and the energy supply of the heart muscle are reduced. Damaged membrane potential on mitochondria and inability to undergo oxidative phosphorylation lead to deeper myocardial damage. However, available data suggest that significant cardioprotection can be achieved by accelerating the creatine kinase system in the heart (6), while the loss of creatine kinase system components can be extremely harmful in the conditions of ischemia/reperfusion (I/R). Given that, it is believed that phosphocreatine can reduce the release of heart enzymes and the part of the heart affected by a heart attack. On the other hand, exogenous administration of phosphocreatine can prevent the occurrence of potentially fatal arrhythmias after ischemia and reperfusion, which together with the aforementioned benefits can greatly improve heart recovery after ischemia (7).

Given that the research focuses on novel modalities of conditioning, it would be very interesting to examine the effects of phosphocreatine as a post- and perconditioning agent. By definition, perconditioning is the administration of a specific conditioning agent during ischemia, while postconditioning is the administration of an agent immediately after ischemia. According to the literature, the application of a protective agent during ischemia will lead to a reduction in the necrotic area and the infarct size of the affected tissue, however, the mechanisms leading to protection are different from the mechanisms involved in preconditioning and postconditioning (8).

Since there is a lack of data regarding the role of phosphocreatine in the therapy of ischemic/reperfusion injury, the aim of this study was to examine the role of phosphocreatine as an agent for postconditioning and perconditioning of isolated rat heart.

MATERIALS AND METHODS

This is an experimental study on *ex vivo* animal material (isolated rat heart). During the experimental work, the provisions of the prescribed acts (EU Directive for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes 86/609 / EES) and the principles of ethics were respected.

Animals and experimental protocol

In order to investigate the effects of phosphocreatine on I/R heart damage, healthy *Wistar albino* male rats, 8 weeks old, weighing 200-250 g, were used. Rats were raised in the vivarium of the Center for Clinical and Functional Research, Faculty of Medical Sciences, University of Kragujevac in standard laboratory conditions - air temperature 23 ± 1° C, relative humidity 50%, 12:12 hours cycle light:darkness (with at the beginning of the light period at 9:00 am) and with free (*ad libitum*) access to water and food.

Ex vivo examination of cardiac function was performed using the Langendorff model of retrograde perfusion of an isolated heart (Langendorff apparatus, Experimetria Ltd, 1062 Budapest, Hungary). Hearts of all animals (30 in total) were isolated and placed on cannula of Langendorff apparatus. After placing the heart on the device, stable cardiac work was confirmed by detecting unchanged values of coronary flow and heart function parameters after several consecutive measurements. All hearts were subjected to 20 minutes of global ischemia, followed with 30 minutes of reperfusion.

Isolated hearts were randomly divided into control (n = 10, hearts of rats with acute I/R injury) and two experimental (n = 20) groups depending on the period of application phosphocreatin; postconditioning group - phosphocreatine at a dose of 0.2 mmol/l was applied in the heart during the first 5 minutes of reperfusion, perconditioning group - phosphocreatine at the same dose was administered during first five minutes of reperfusion.

Examination of the effect of phosphocreatine on cardiodynamic parameters and coronary flow

Using the Langendorf apparatus and software followed cardiodynamic parameters were continuously monitored: dp/dt max - maximum rate of change of pressure in the left ventricle, which is expressed in mmHg/s; dp/dt min - minimum rate of pressure change in the left ventricle expressed in mmHg/s; SLVP - systolic pressure in the left ventricle expressed in mmHg; DLVP - diastolic pressure in the left ventricle expressed in mmHg; HR - heart rate expressed as beats per minute (bpm). Coronary flow (CF) was measured flowmetrically and expressed in mL of coronary venous effluent per minute. Values of cardiodynamic

parameters as well as coronary venous effluent were collected at: stabilization points (S), first (1), fifth (5), tenth (10), fifteenth (15), twentieth (20), twenty-fifth (25) and in the thirtieth (30) minute of reperfusion.

Examination of the effect of phosphocreatine on cardiac redox status

In order to examine the effects of phosphocreatine on cardiac production of prooxidans in coronary venous effluent samples, oxidative stress parameters were determined. All biochemical analyzes were performed spectrophotometrically (Specord S-600 Analytik Jena, UK).

The lipid peroxidation index was determined indirectly by measuring the products of the lipid peroxidation reaction with thiobarbituric acid, ie the levels of TBARS (Thiobarbituric Acid Reactive Substances). The spectrophotometric method is based on the determination of lipid peroxide levels based on the reaction of malonyldialdehyde with thiobarbituric acid (9).

Measurement of nitrite released levels in coronary venous effluent is a suitable method for indirect assessment of the functionality of the endothelial L-arginine, based on the use of Griess reagent (10).

The determination of the amount of superoxide anion radical (O_2^-) in coronary venous effluent and plasma is based on the reaction of O_2^- with nitro tetrazolium blue (NBT) to form nitroformasan blue (11).

The determination of H_2O_2 is based on the oxidation of phenol red by a hydrogen peroxide reaction catalyzed by the enzyme Horse Radish Peroxidase. With this method, it is possible to determine the formation and release of H_2O_2 during a time interval of 5-60 minutes (12).

Statistics

During the experimental protocol, the parameters of interest were monitored at the time of stabilization, as well as every five minutes during the thirty-minute reperfusion (points marked with 1, 5, 10, 15, 20, 25, 30). All obtained results are presented as mean ± standard deviation and are presented using graphs created in Microsoft Excel. In order to perform adequate comparison between groups and subgroups, all points of interest were also presented as percentage values in relation to the stabilization point, which was 100%.

RESULTS

The effects of different phosphocreatine conditioning modalities on cardiodynamic parameters and coronary flow of isolated heart of rats subjected to acute ischemic-reperfusion injury

Figure 1 shows the absolute values of cardiodynamic parameters (dp / dt max, dp / dt min, SLVP, DLVP, HR), as well as CF of the control and experimental groups recorded every five minutes during thirty-minute reperfusion period (points 1, 5, 10, 15, 20, 25, 30). Based on the obtained results, it is clear that the values of most of the monitored parameters were statistically significantly higher in experimental than in the control group. Only the value of DLVP in the control group was significantly higher in the first and the fifth minute of reperfusion compared to the experimental groups. In the group of hearts postconditioned with phosphocreatine, most of the monitored parameters were almost constant during the reperfusion period, except for SLVP where a decrease was observed. Similar results were obtained in perconditioning group except in HR where significant decrease during the first 5 minutes of reperfusion is observed, but during the remaining 25 minutes of reperusion the HR values gradually increased. To compare the effects of different models of phosphocreatine conditioning on cardiodynamic parameters and CF of an isolated rat heart, results were presented also as a percentage relative to stabilization (Figure 2). In perconditioning group most of the monitored parameters after thirty minutes of reperfusion have values of about 90% of the values recorded before ischemia, except for diastolic pressure (73.59%) at which a decrease was observed. More importantly, all values in the perconditioning group were significantly higher than the values obtained in the control group.

The effects of different phosphocreatine conditioning modalities on oxidative stress parameters of isolated heart of rats subjected to acute ischemic-reperfusion injury

Concentrations of prooxidants such as O_2^-, NO_2^-, H_2O_2 and TBARS were determined from coronary venous effluent collected at stabilization time (S), as well as every five minutes during thirty-minute reperfusion (points marked 1, 5, 10, 15, 20, 25, 30). However, three points of interest were considered for data analysis: the 1st, 5th, and 30th minute of reperfusion. Figure 3 shows the absolute values of these prooxidants during the ex vivo experiments. Based on these results it can be noticed that O_2^- and TBARS values were significantly higher, while NO_2^- was significantly lower in the control group, compared to experimental groups during the first minutes of reperfusion. On the other hand, at the end of reperfusion only TBARS remained higher in the control compared to the examined groups. Also, on this graph it is clear that the use of 0.2 mmol/l phosphocreatine after ischemia significantly increased the values of O_2^- and H_2O_2, compared to the group in which this agent was used during ischemia (perconditioning). Furthermore, Figure 4 shows the values in all points of interest expressed as a percentage through stabilization values of 100%. Both tested phosphocreatine conditioning maneuvers at a concentration of 0.2 mmol/l showed potential in reducing prooxidation parameters, especially O_2^-, H_2O_2, and NO_2^-. Namely, these graphs show that postconditioning with phosphocreatine led to significant fluctuations in O_2^- levels during reperfusion (67.08% in the first minute and 114.43% in the last minute of reperfusion), while the values of other measured prooxidants remained unchanged during the whole experimental protocol. On the other hand, use of phosphocreatine during the first five minutes of ischemia

significantly prevented fluctuations in monitored prooxidants, except for O_2^- levels which, after thirty minutes of reperfusion, were 27.47% higher than those measured at the time of stabilization.

Figure 1. Cardiodynamic parameters and coronary flow of the control (CTRL), postconditioning (Post) and perconditioning (Per) groups of rats, presented as absolute values (mean ± standard deviation) recorded every five minutes during the 30-minute reperfusion period. Interrupted line – control group, gray line – postconditioning group, black line – perconditioning group.

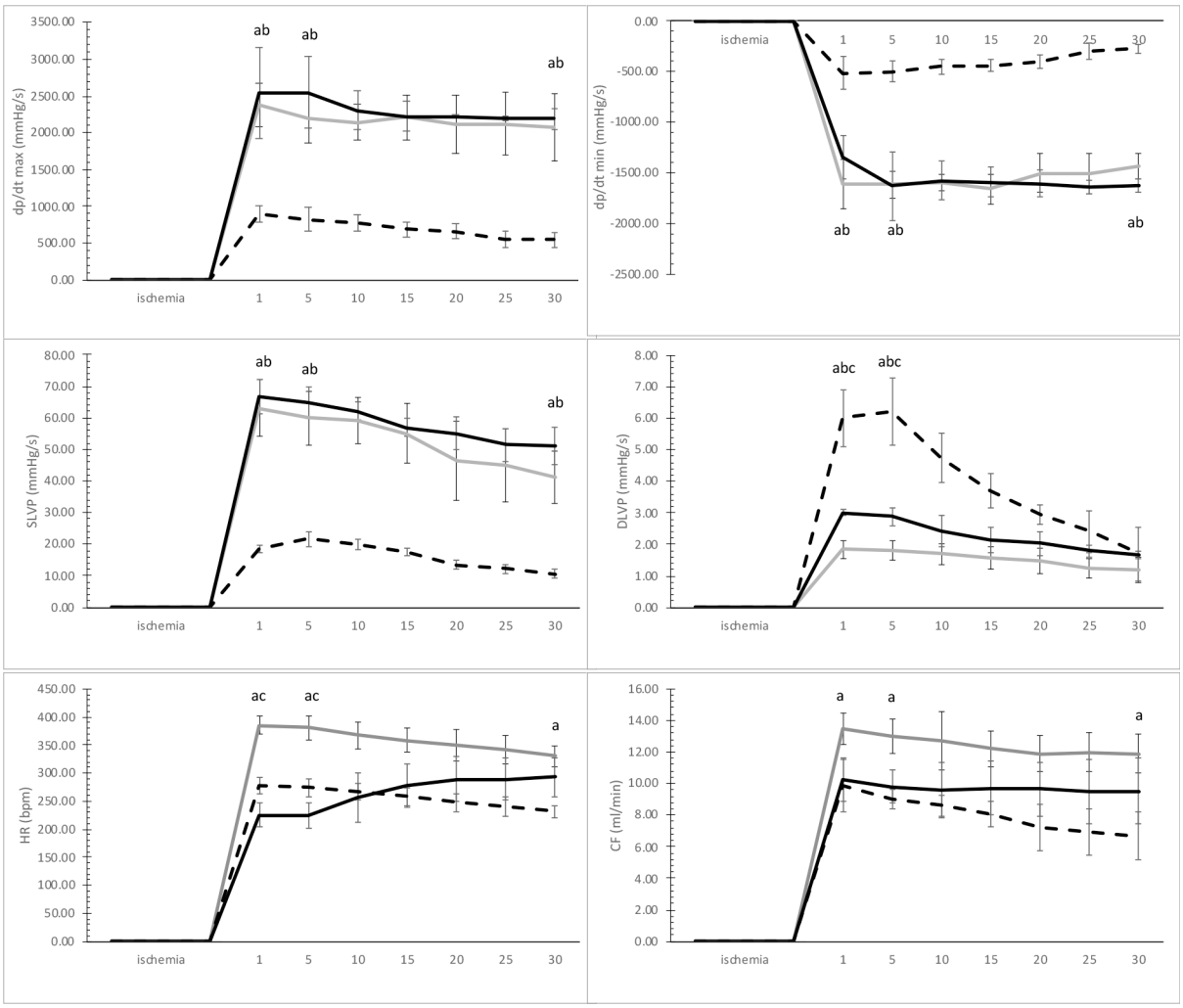


Figure 2. Values of cardiodynamic parameters and coronary flow of the control group and groups whose isolated hearts were subjected to different modalities of phosphocreatine conditioning expressed as a percentage of the stabilization point of 100%.

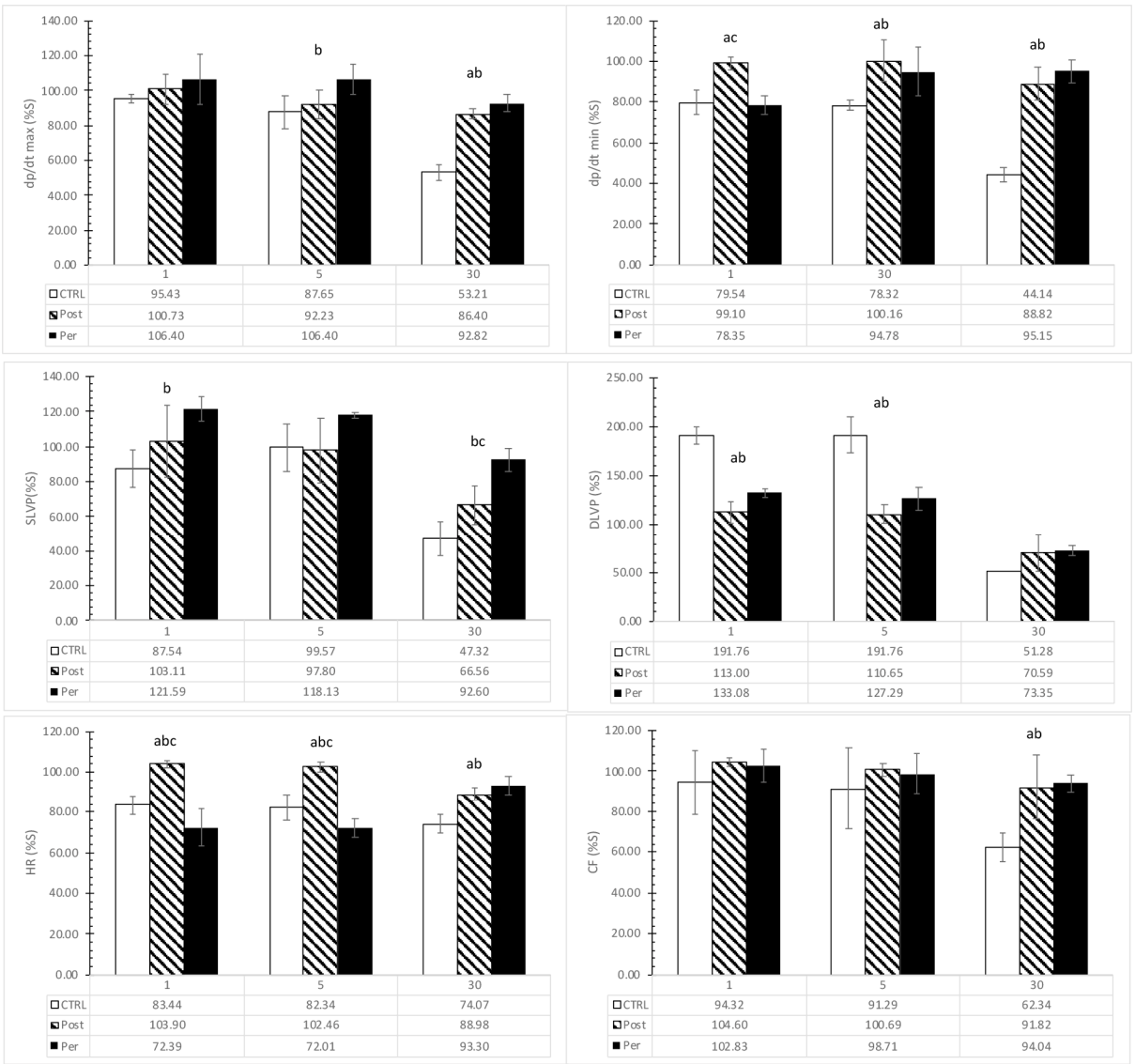


Figure 3. Oxidative stress parameters of the control (CTRL), postconditioning (Post) and perconditioning (Per) groups of rats, presented as absolute values (mean ± standard deviation) recorded every five minutes during the 30-minute reperfusion period. Interrupted line – control group, gray line – postconditioning group, black line – perconditioning group.

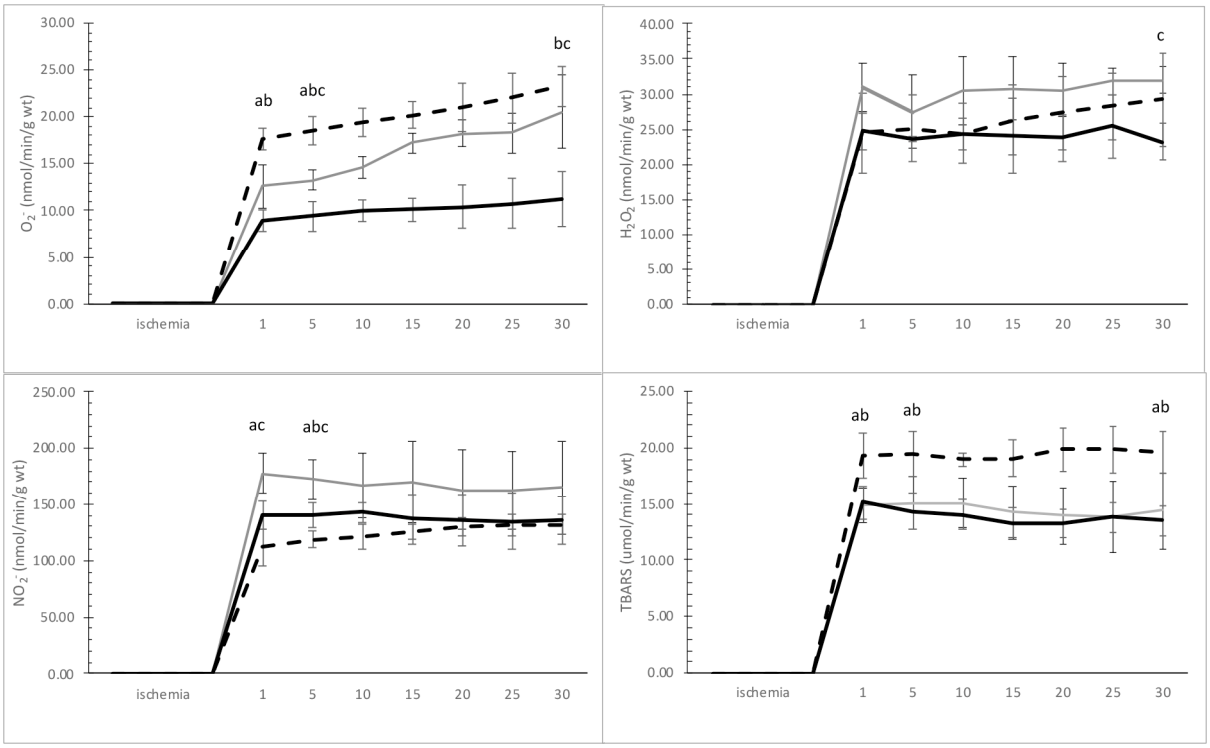
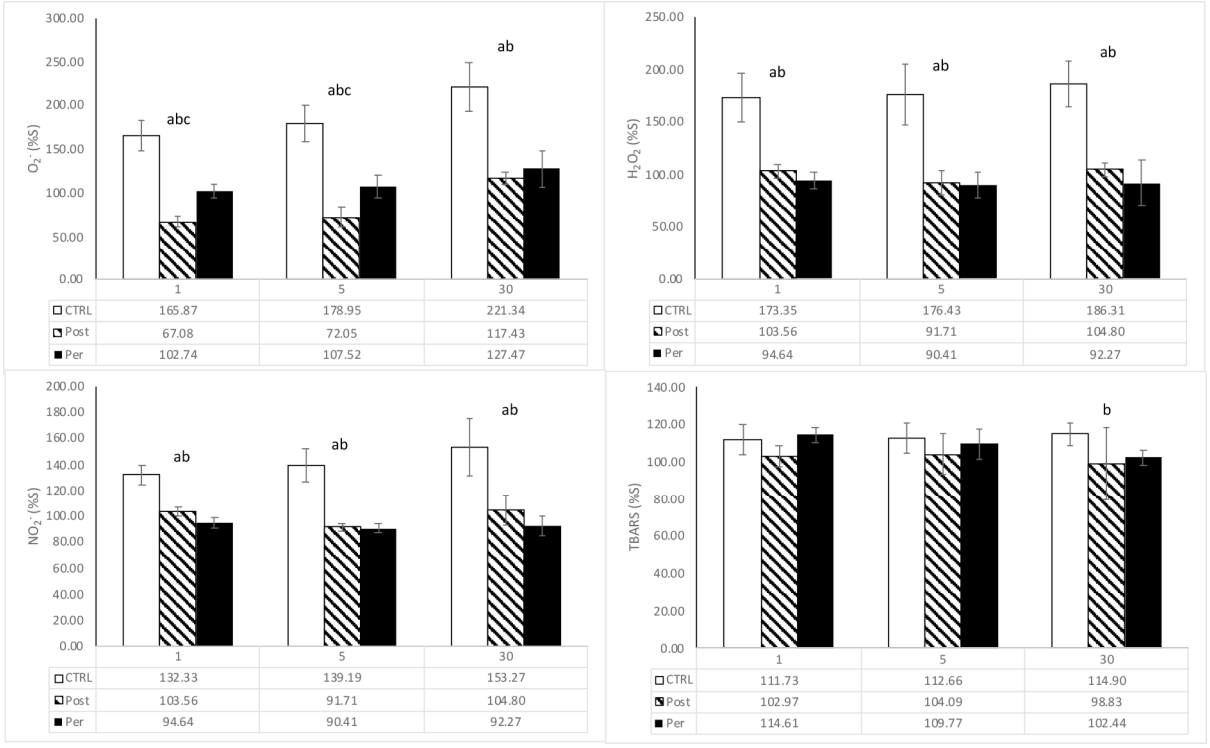


Figure 4. Values of oxidative stress parameters of the control group and groups whose isolated hearts were subjected to different modalities of phosphocreatine conditioning expressed as a percentage of the stabilization point of 100%.



DISSCUSSION

In the period of ischemia, anaerobic metabolism is of a crucial importance in energy delivery to the heart and preservation of myocardial viability, while rapid energy depletion worsens I/R injury (13-16). Therefore, we hypothesized that the use of phosphocreatine after, or during ischemia itself could be a crucial step in alleviating I/R-induced heart damage, especially because phosphocreatine is first consumed in ischemia.

Postconditioning attracts a lot of attention from scientists who have done a lot over the years to prove that the model used in preconditioning can be applied to postconditioning (17). Time stands out as a very important factor because only agents applied in early reperfusion have the potential to protect tissue while a more significant effect cannot be achieved 5-10 minutes after. These allegations are completely logical because the applied agents at this moment prevent the occurrence of further damage, but without affecting the already caused damage (18-20). Our results showed that phosphocreatine administered immediately after ischemia decreased fluctuations of cardiodynamic parameters during reperfusion. Similar results were obtained in a previous study, which examined the postconditioning effect of phosphocreatine (200 mg/kg through the femoral artery) on a model of ischemia induced by left anterior descending artery blockage for 30 minutes, followed by reperfusion for 120 minutes. The results showed that the use of this agent can reduce the serum level of creatine kinase and lactate dehydrogenase, which is an indicator of the protective role of phosphocreatine on damaged myocardium (7).

During the years, researchers have been trying to find out whether changes in the myocardium occur predominantly under the influence of ischemia or during the reperfusion. Since numerous pathophysiological processes occur in ischemia, it is hypothesized that better effects would be expected if management starts before or during ischemia (21, 22). Having in mind the pathological events that occurred in ischemia, we wanted to examine whether the exogenous use of phosphocreatine as a high-energy compound can prevent heart damage and whether its use during ischemia is more beneficial than in reperfusion, after ischemia. In our study, it was observed that acute application of phosphocreatine in ischemia shows a tendency to keep the values of cardiodynamic parameters unchanged during the entire reperfusion period. The changes observed were mild and reversible and did not affect cardiac output. Although both tested modalities of conditioning the isolated heart of rats with phosphocreatine at a concentration of 0.2 mmol/l showed that this agent preserves heart function during ischemic-reperfusion injury, it is undeniable that phosphocreatine showed better effect if used during ischemia, as a perconditioning agent.

The creatine kinase system acts as an energy buffer for the rapid regeneration of ATP when energy demand becomes greater than supply as occurs during ischemia (23). Our study showed that acute application of phosphocreatine, i.e. direct delivery of energy sources significantly contributes to the functional recovery of the myocardium in ischemic conditions. Since reperfusion leads to the return of phosphocreatine and gradual recovery of the heart, it is of great importance to restore phosphocreatine reserves and to establish ionic homeostasis. In this way, myocardial damage will be prevented (6). Phosphocreatine, due to its extreme polarity, cannot pass through the membrane by passive transport, so the potential to stabilize the membrane stands out as one of its key beneficial effects that is very important for I/R injury (24, 25).

It is also important to note that one of the crucial factors contributing to heart damage in reperfusion is oxidative stress (14). The danger of prooxidant accumulation arises not only from the potential to cause direct damage to cellular structures, but also from the ability to induce secondary ROS release by activating immune cells and disrupting mitochondrial function (26-32). It is also very important that cardiomyocyte death during I/R injury occurs due to changes in the extent of mitochondrial metabolism, which is also associated with excessive ROS production (29). For this reason, the question arises as to whether the use this agent during reperfusion can protect the myocardium and reduce the rate of damage. In this regard, we also wanted to examine the effects of phosphocreatine in a model of perconditioning and postconditioning on cardiac status by measuring oxidative stress parameters in coronary venous effluent.

Postconditioning with phosphocreatine in our study prevented an increase in the measured prooxidants, which significantly protects the heart from oxidative damage in I/R. Namely, under the influence of NADPH oxidase-NOX present in heart cells, a large amount of prooxidants forms. During the reperfusion process, neutrophils in which the NOX enzyme is present are activated. This enzyme catalyzes the reduction of O_2 with NADPH as an electron source, resulting in toxic reactive oxygen species $O_2^{\cdot-}$. The formation of ROS seriously contributes to heart damage, as well as loss of functional and structural characteristics of the heart. These findings have been confirmed in both animal and human I/R myocardial models and are consistent with our results in the control group of animals (14, 30). Also, perconditioning with phosphocreatine prevented an increase in most of the measured prooxidants in reperfusion and our results have shown that phosphocreatine has potential to improve redox signaling after ischemia.

CONCLUSION

Acute application of phosphocreatine during the period of ischemia or during period of reperfusion, leads to the maintenance of cardiodynamic parameters, which clearly shows the beneficial effect of this agent in conditioning models. Also, perconditioning and postconditioning with phosphocreatine prevents the rise of most of the measured proxidants during the reperfusion period. Given all the results obtained in this study as well as the previously mentioned studies indicating the potential of phosphocreatine to maintain ATP levels in

ischemia, this substance should be nominated as potentially cardioprotective and possibly with a better effect in perconditioning than preconditioning.

ETHICS APPROVAL

All research procedures were carried out in strict accordance with the European Union Directive for the welfare of laboratory animals (No. 2010/63/EU) and approved by the Ethics Committee for welfare of experimental animals, Faculty of Medical Sciences University of Kragujevac.

COMPETING INTERESTS

There are no conflicts of interest.

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REFERENCES

1. Gaddi AV, Galuppo P, Yang J. Creatine Phosphate Administration in Cell Energy Impairment Conditions: A Summary of Past and Present Research. Heart Lung Circ. 2017; 26(10):1026-35.
2. Weiss R, Gerstenblith G, Bottomley P. ATP flux through creatine kinase in the normal, stressed, and failing human heart. Proc Natl Acad Sci U S A. 2005; 102(3):808-13.
3. Pascual F, Coleman R. Fuel Availability and Fate in Cardiac Metabolism: A Tale of Two Substrates. Biochim Biophys Acta. 2016; 1861(10):1425-33.
4. Goldberg IJ, Trent CM, Schulze PC. Lipid metabolism and toxicity in the heart. Cell Metab. 2012; 15(6):805-12.
5. Lopez R, Marzban B, Gao X, Lauinger E, Van den Bergh F, Whitesall SE, Converso-Baran K, Burant CF, Michele DE, Beard DA. Impaired Myocardial Energetics Causes Mechanical Dysfunction in Decompensated Failing Hearts. Function (Oxf). 2020;1(2):zqaa018. doi: 10.1093/function/zqaa018. Epub 2020 Sep 22.
6. Cao F, Zervou S, Lygate C. The creatine kinase system as a therapeutic target for myocardial ischaemia–reperfusion injury. Biochem Soc Trans. 2018; 46(5):1119-27.
7. Zhang W, Zhang H, Xing Y. Protective Effects of Phosphocreatine Administered Post-Treatment Combined With Ischemic Post-Conditioning on Rat Hearts With Myocardial Ischemia/Reperfusion Injury. J Clin Med Res. 2015; 7(4):242-7.
8. Vinten-Johansen J, Shi W. Perconditioning and postconditioning: current knowledge, knowledge gaps, barriers to adoption, and future directions. J Cardiovasc Pharmacol Ther. 2011 Sep-Dec;16(3-4):260-6.
9. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95(2):351-58.
10. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 1982; 126(1):131-8.
11. Auclair C, Voisin E (1985). Nitroblue tetrazolium reduction. In: Greenvvald RA (ed) Handbook of methods for oxygen radical research. CRC Press Une, Boca Raton, pp 123-132.
12. Pick E, Keisari Y. A simple colorimetric method for the measurement of hydrogen peroxide produced by cells in culture. J Immunol Methods. 1980; 38(1-2): 161-70.
13. Klabunde, Richard E. Cardiovascular Physiology Concepts. Philadelphia, PA: Lippincott Williams & Wilkins/Wolters Kluwer, 2012.
14. González-Montero J, Brito R, Gajardo AIJ, Rodrigo R. Myocardial reperfusion injury and oxidative stress: Therapeutic opportunities. World J Cardiol. 2018; 10(9): 74–86.
15. Kryzhanovskii SA, Kandelaki IN, Sharov VG, Kaverina NV, Sakset VA. Effect of exogenous phosphocreatine on the size of experimental myocardial infarction. Kardiologiia. 1988; 28:88–91.
16. Prabhakar G, Vona-Davis L, Murray D, Lakhani P, Murray G. Phosphocreatine Restores High-Energy Phosphates in Ischemic Myocardium: Implication for Off-Pump Cardiac Revascularization. J Am Coll Surg. 2003; 197(5):786-91.
17. Zhao ZQ, Corvera JS, Halkos ME, et al. Inhibition of myocardialinjury by ischemic postconditioning during reperfusion: comparison with ischemic preconditioning. Am J Physiol Heart Circ Physiol. 2003; 285(2):H579-H588.
18. Granfeldt A, Lefer D, Vinten-Johansen J. Protective Ischaemia in Patients: Preconditioning and Postconditioning. Cardiovasc Res. 2009; 83(2):234-46.
19. Vander Heide RS, Steenbergen C. Cardioprotection and myocardial reperfusion: pitfalls to clinical application. Circ Res. 2013; 113(4):464-77.
20. Yeh RW, Sidney S, Chandra M, Sorel M, Selby JV, Go AS. Population trends in the incidence and outcomes of acute myocardial infarction. N Engl J Med. 2010; 362(23):2155-65.
21. Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. Physiol Rev. 2008; 88(2):581-609.
22. Stephanou A, Brar B, Liao Z, Scarabelli T, Knight RA, Latchman DS. Distinct initiator caspases are required for the induction of apoptosis in cardiac myocytes during ischaemia versus reperfusion injury. Cell Death Differ. 2001; 8(4):434-5.
23. Spindler M, Meyer K, Stromer H, Leupold A, Boehm E, Wagner H, et al. (2004) Creatine kinase-deficient hearts exhibit increased susceptibility to ischemia-reperfusion injury and impaired calcium homeostasis. Am J Physiol Heart Circ Physiol 287: H1039–1045.
24. Tokarska-Schlattner M, Epand RF, Meiler F, Zandomeneghi G, Neumann D, Widmer HR, et al. Phosphocreatine interacts with phospholipids, affects membrane

properties and exerts membrane-protective effects. PLoS One. 2012; 7(8):e43178.
25. Bolli R, Becker L, Gross G, Mentzer R Jr, Balshaw D, Lathrop DA. Myocardial protection at acrossroads: the need for translation into clinical therapy. Circ Res. 2004; 95:125–134.
26. Zuo L, Zhou T, Pannell BK, Ziegler AC, Best TM. Biological and physiological role of reactive oxygen species—The good, the bad and the ugly. Acta Physiol. 2015; 214:329–348.
27. Tann AW, Boldogh I, Meiss G, Qian W, van Houten B, Mitra S, et al. Apoptosis induced by persistent single-strand breaks in mitochondrial genome: Critical role of EXOG (5′-EXO/endonuclease) in their repair. J. Biol. Chem. 2011; 286:31975–31983.
28. Fleury C, Mignotte B, Vayssiere JL. Mitochondrial reactive oxygen species in cell death signaling. Biochimie. 2002; 84:131–141.
29. Lesnefsky EJ, Chen Q, Tandler B, Hoppel CL. Mitochondrial Dysfunction and Myocardial Ischemia-Reperfusion: Implications for Novel Therapies. Annu Rev Pharmacol Toxicol. 2017; 57: 535-565.
30. Brandes RP, Kreuzer J. Vascular NADPH oxidases: molecular mechanisms of activation. Cardiovasc Res. 2005; 65(1):16-27.
31. Fernandez J, Perez-Alvarez JA, Fernandez-Lopez JA. Thiobarbituric acid test for monitoring lipid oxidation in meat. Food Chemistry. 1997;59(3):345-353.
32. Cunha MP, Martín-de-Saavedra MD, Romero A, Egea J, Ludka FK, Tasca CI, et al. Both creatine and its product phosphocreatine reduce oxidative stress and afford neuroprotection in an in vitro Parkinson's model. ASN Neuro. 2014; 6(6):1759091414554945.