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SHORT COMMUNICATION

Cardioprotective Effect of Chronic Hypoxia Involves Inhibition of Mitochondrial Permeability Transition Pore Opening

Petra ALANOVA[1], Lukas ALAN[2,3], Jan NECKAR[1], Bohuslav OSTADAL[1], Frantisek KOLAR[1]

[1]Laboratory of Developmental Cardiology, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic, [2]Laboratory of Bioenergetics, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic, [3]Department of Biology, University of Padova, Italy

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Summary

The aim of the study was to examine the potential role of mitochondrial permeability transition pore (mPTP) in the cardioprotective effect of chronic continuous hypoxia (CH) against acute myocardial ischemia/reperfusion (I/R) injury. Adult male Wistar rats were adapted to CH for 3 weeks, while their controls were kept under normoxic conditions. Subsequently, they were subjected to I/R insult while being administered with mPTP inhibitor, cyclosporin A (CsA). Infarct size and incidence of ischemic and reperfusion arrhythmias were determined. Our results showed that adaptation to CH as well as CsA administration reduced myocardial infarct size in comparison to the corresponding control groups. However, administration of CsA did not amplify the beneficial effect of CH, suggesting that inhibition of mPTP opening contributes to the protective character of CH.

Key words

Cardioprotection • Chronic hypoxia • Mitochondria • Permeability transition pore • Myocardial infarction

Corresponding author

P. Alanova, Laboratory of Developmental Cardiology, Institute of Physiology of the Czech Academy of Sciences, Videnska 1083, Prague, Czech Republic. E-mail: Petra.Alanova@fgu.cas.cz

According to the World Health Organization, ischemic heart disease is the leading cause of death and disability worldwide. During ischemia, severely reduced or interrupted blood flow to the heart causes disproportion between oxygen demand and supply, resulting in damage to the cardiac tissue. Early and fast restoration of blood supply is essential for the salvage of ischemic myocardium. However, it is well established that reperfusion itself causes further cardiomyocyte death [1].

Mitochondria play a fundamental role in cardiac physiology and metabolism to meet the high energy demand of the beating heart [2,3]. Mitochondrial dysfunction, particularly opening of the mitochondrial permeability transition pore (mPTP), is responsible for cardiomyocyte damage and necrotic cell death under many conditions such as ischemia/reperfusion (I/R) injury [4]. mPTP is a voltage- and Ca^{2+}-dependent, nonselective channel of the inner mitochondrial membrane [5]. During acute myocardial ischemia, the absence of oxygen switches cellular metabolism to anaerobic glycolysis, resulting in the accumulation of lactate and reduction in intracellular pH. The acidic conditions during ischemia prevent mPTP opening. During reperfusion, the washout of lactate and rapid restoration of physiological pH releases the inhibitory effect on mPTP. This event, together with an increased matrix Ca^{2+} and ROS, results in mPTP opening followed by mitochondrial swelling, rupture and induction of cell death [6]. Despite the fact that the molecular identity of the mPTP is still a matter of debate, longstanding data show that pore opening is facilitated by binding of

a soluble matrix protein cyclophilin D (CypD) to the inner mitochondrial membrane [7]. The mPTP inhibition was shown to afford a significant cardioprotection [4], so experimental studies challenge the clinical use of drugs interacting with CypD such as CsA and emphasize the importance of further studies to clarify whether CypD is a feasible target for inhibition that can protect the heart from I/R injury.

Adaptation to chronic hypoxia (CH) is a natural stimulus conferring cardioprotection [8]. In line with the human epidemiological surveys [9], we have demonstrated that CH activates the protective phenotype [10,11]. Despite the fact that CH-induced cardio-protection has been known for many decades, the underlying mechanism is still unclear. We have previously observed that mitochondrial channels located also on the inner mitochondrial membrane contribute to the cardioprotective effect of CH [12,13]. The purpose of the present study was; therefore, to explore the role of mitochondria, and mPTP in particular, in the cardioprotection induced by CH.

Adult male Wistar rats were adapted to CH (continuous normobaric hypoxia, inspired O_2 fraction 0.1) in a hypoxic chamber for 3 weeks without any reoxygenation [14]. At the end of adaptation, all rats underwent surgery according to the open-chest model of I/R injury as described previously [15] with minor modifications. Briefly, in anesthetized rats 20-min regional ischemia was performed via left anterior descending coronary artery ligation. CsA (Merck, 30024; 2.5 mg/kg body weight, i.p.) or corresponding volume of saline was administered 5 min before the onset of reperfusion. At the end of 3-h reperfusion, the heart was excised, and infarct size (IS) and area at risk were histochemically determined and analyzed. A single-lead electrocardiogram (ECG) was registered during ischemia and the first 3 min of reperfusion. The incidence and severity of ischemic and reperfusion ventricular arrhythmias were evaluated from ECG records according to Lambeth Conventions [16].

Statistical analyses were performed using GraphPad Prism 8 software (Graph Pad Inc., CA, USA). Normally distributed variables are expressed as mean ± SD. Two-way analysis of variance (ANOVA; with phenotype and treatment as categories) were carried out to determine significant interactions, followed by Bonferroni´s test for multiple comparisons. Not normally distributed data (arrhythmias) are expressed as median ± interquartile range.

To assess whether mPTP inhibition affects the increased ischemic tolerance induced by adaptation to CH, we subjected the normoxic and chronically hypoxic rats administered with CsA to acute I/R injury. The mean area at risk normalized to left ventricle did not significantly differ among the groups, which allowed us to compare the average values of the IS (Fig. 1A, C). Our results confirmed the cardioprotective effect of CH, as chronically hypoxic rats demonstrated reduced IS compared to their normoxic counterparts. Acute CsA administration reduced IS in normoxic rats; however, it did not provide any additive protection to increased ischemic tolerance induced by adaptation to CH (Fig. 1B, C). The total number of ischemic (Fig. 1D) and reperfusion (Fig. 1E) ventricular arrhythmias did not differ among the experimental groups. However, animals adapted to CH demonstrated a tendency to decrease the number of arrhythmias occurring during reperfusion.

Adaptation to CH serves as a useful tool for studying molecular identity underlying cardioprotective pathways. The most important feature of this cardioprotective phenomenon is than the changes induced by CH persist much longer that any form of conditioning [17]. The present study confirmed the IS-limiting effect elicited by adaptation to CH. The novelty lies in the fact that not only adaptation to CH, but also administration of CsA significantly reduced the IS; the combination of adaptation to CH and mPTP inhibition by CsA had no additive effect as it led to the same degree of protection as CH alone. This result suggests that CypD-dependent inhibition of the mPTP is likely involved in the signaling pathway activated by adaptation to CH. The IS reduction induced by CH is significantly higher than by CsA in normoxic group suggesting that additional mechanism also plays a role in protection by CH. Other cardioprotective strategies such as ischemic preconditioning and postconditioning were also showed to inhibit mPTP opening [18,19], the mechanism is, however, currently unresolved. Experimental evidence suggests that both pharmacological and genetic treatments designed to prevent mPTP opening at the onset of myocardial reperfusion are able to reduce myocardial IS [20]. It is necessary to mention that a large multicenter clinical trial (CIRCUS) revealed no protective effect of CsA on clinical outcome in patients with myocardial infarction [21]. Several factors, such as the severity of infarction, a quite narrow window of protection, route of application, timing of administration as well as comorbidities may be responsible for the lack

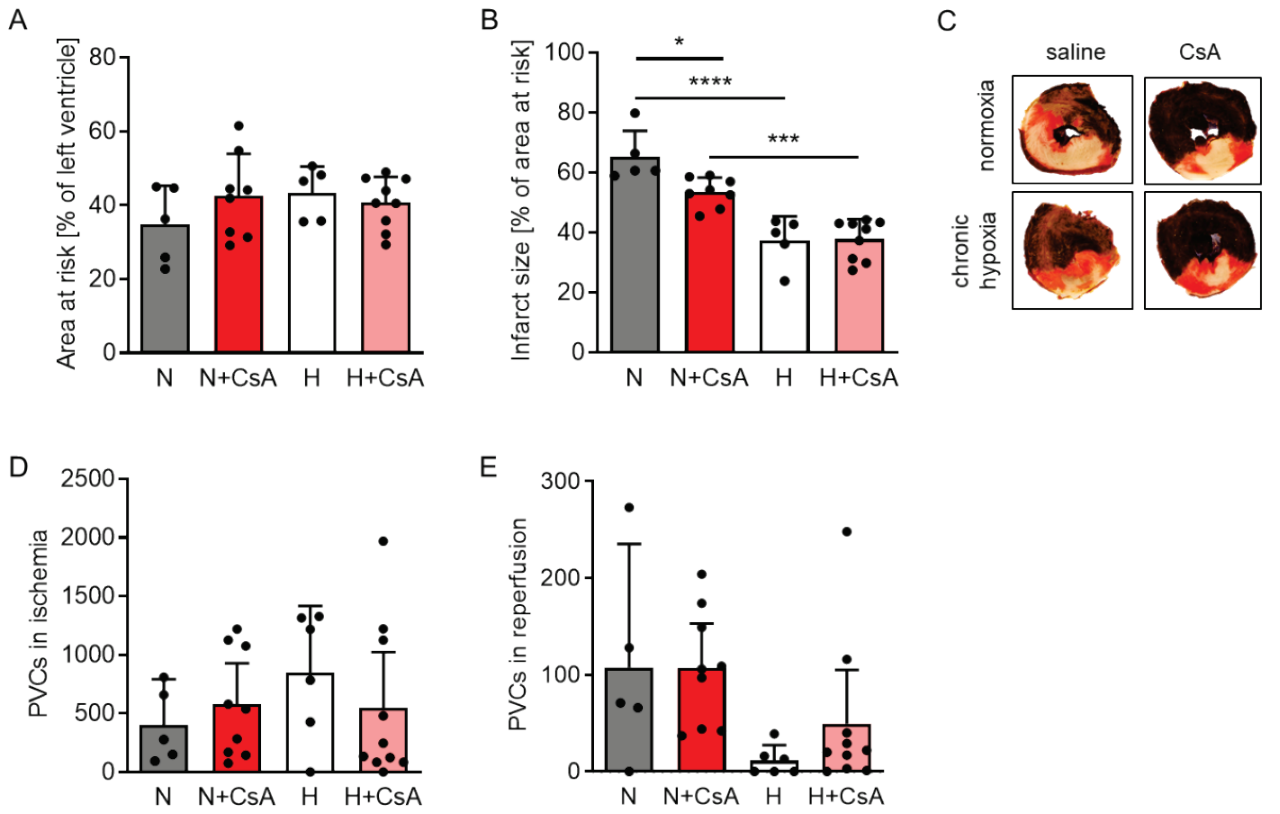

Fig. 1. Myocardial infarction induced by ischemia/reperfusion injury in normoxic (N) and chronically hypoxic (H) rats administered either with saline or CsA. (**A**) The size of area at risk expressed as the percentage of the left ventricle, and (**B**) infarct size expressed as the percentage of the area at risk. Values are mean ± SD; n=5-9 rats per group; * P≤0.05, *** P≤0.001, **** P≤0.0001, two-way ANOVA with Bonferroni´s multiple comparisons test. (**C**) Representative image of heart cross sections used for the infarct size analysis. Brown color represents normally perfused tissue stained by potassium permanganate. Red area, tetrazolium positive, represents surviving tissue, and white area, tetrazolium negative, is infarcted tissue. Total number of premature ventricular complexes (PVCs) during (**D**) ischemia and (**E**) reperfusion. Values are expressed as median ± interquartile range; n=5-10 rats per group.

of cardioprotection in the CIRCUS trial. Clinical data remain equivocal, also due to lack of suitable mPTP inhibitors without side effects. This limitation might contribute to controversial findings in clinical studies. In conclusion, experimental evidence pinpoints a central role of mPTP opening in the I/R injury, so targeting mPTP holds a promise for the new treatment strategies.

Conflict of Interest

There is no conflict of interest.

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References

1. Jennings RB, Sommers HM, Smyth GA, Flack HA, Linn H. Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. Arch Pathol 1960;70:68-78.

2. Semenzato M, Kohr MJ, Quirin C, Menabò R, Alanova P, Alan L, Pelattiero A, ET AL. Oxidization of optic atrophy 1 cysteines occurs during heart ischemia-reperfusion and amplifies cell death by oxidative stress. Redox Biol 2023;63:102755. https://doi.org/10.1016/j.redox.2023.102755
3. Alan L, Scorrano L. Shaping fuel utilization by mitochondria. Curr Biol 2022;32:R618-R623. https://doi.org/10.1016/j.cub.2022.05.006
4. Griffiths EJ, Halestrap AP. Protection by Cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts. J Mol Cell Cardiol 1993;25:1461-1469. https://doi.org/10.1006/jmcc.1993.1162
5. Bernardi, P, Di Lisa F. The mitochondrial permeability transition pore: molecular nature and role as a target in cardioprotection. J Mol Cell Cardiol 2015;78:100-106. https://doi.org/10.1016/j.yjmcc.2014.09.023
6. Griffiths EJ, Halestrap AP. Mitochondrial non-specific pores remain closed during cardiac ischaemia, but open upon reperfusion. Biochem J 1995;307:93-98. https://doi.org/10.1042/bj3070093
7. Di Lisa F, Bernardi P. A CaPful of mechanisms regulating the mitochondrial permeability transition. J Mol Cell Cardiol 2009;46:775-780. https://doi.org/10.1016/j.yjmcc.2009.03.006
8. Ostadal B, Kolar F. Cardiac adaptation to chronic high-altitude hypoxia: beneficial and adverse effects. Respir Physiol Neurobiol 2007;158:224-236. https://doi.org/10.1016/j.resp.2007.03.005
9. Hurtado A. Some clinical aspects of life at high altitudes. Ann Intern Med 1960;53:247-258. https://doi.org/10.7326/0003-4819-53-2-247
10. Alanova P, Kolar F, Ostadal B, Neckar J. Role of NO/cGMP signaling pathway in cardiac ischemic tolerance of chronically hypoxic rats. Physiol Res 2015;64:783-787. https://doi.org/10.33549/physiolres.932939
11. Hrdlicka J, Neckar J, Papousek F, Vasinova J, Alanova P, Kolar F. Beneficial effect of continuous normobaric hypoxia on ventricular dilatation in rats with post-infarction heart failure. Physiol Res 2016;65:867-870. https://doi.org/10.33549/physiolres.933308
12. Neckar J, Szarszoi O, Koten L, Papousek F, Ostadal B, Grover GJ, Kolar F. Effects of mitochondrial K(ATP) modulators on cardioprotection induced by chronic high altitude hypoxia in rats. Cardiovasc Res 2002;55:567-575. https://doi.org/10.1016/S0008-6363(02)00456-X
13. Borchert GH, Yang C, Kolar F. Mitochondrial BKCa channels contribute to protection of cardiomyocytes isolated from chronically hypoxic rats. Am J Physiol Heart Circ Physiol 2011;300:H507-H513. https://doi.org/10.1152/ajpheart.00594.2010
14. Hahnova K, Kasparova D, Zurmanova J, Neckar J, Kolar F, Novotny J. β-Adrenergic signaling in rat heart is similarly affected by continuous and intermittent normobaric hypoxia. Gen Physiol Biophys 2016;35:165-173. https://doi.org/10.4149/gpb_2015053
15. Alanova P, Chytilova A, Neckar J, Hrdlicka J, Micova P, Holzerova K, Hlavackova M, ET AL. Myocardial ischemic tolerance in rats subjected to endurance exercise training during adaptation to chronic hypoxia. J Appl Physiol (1985) 2017;122:1452-1461. https://doi.org/10.1152/japplphysiol.00671.2016
16. Curtis MJ, Hancox JC, Farkas A, Wainwright CL, Stables CL, Saint DA, Clements-Jewery H, ET AL. The Lambeth Conventions (II): guidelines for the study of animal and human ventricular and supraventricular arrhythmias. Pharmacol Ther 2013;139:213-248. https://doi.org/10.1016/j.pharmthera.2013.04.008
17. Neckar J, Ostadal B, Kolar F. Myocardial infarct size-limiting effect of chronic hypoxia persists for five weeks of normoxic recovery. Physiol Res 2004;53:621-628. https://doi.org/10.33549/physiolres.930710
18. Javadov SA, Clarke S, Das M, Griffiths EJ, Lim KHH, Halestrap AP. Ischaemic preconditioning inhibits opening of mitochondrial permeability transition pores in the reperfused rat heart. J Physiol 2003;549:513-524. https://doi.org/10.1113/jphysiol.2003.034231
19. Hausenloy D, Wynne A, Duchen M, Yellon D. Transient mitochondrial permeability transition pore opening mediates preconditioning-induced protection. Circulation 2004;109:1714-1717. https://doi.org/10.1161/01.CIR.0000126294.81407.7D
20. Hausenloy DJ, Duchen MR, Yellon DM. Inhibiting mitochondrial permeability transition pore opening at reperfusion protects against ischaemia-reperfusion injury. Cardiovasc Res 2003;60:617-625. https://doi.org/10.1016/j.cardiores.2003.09.025

21. Cung TT, Morel E, Cayla G, Rioufol G, Garcia-Dorado D, Angoulvant D, Bonnefoy-Cudraz E, ET AL. Cyclosporine before PCI in Patients with Acute Myocardial Infarction. N Engl J Med 2015;373:1021-1031. https://doi.org/10.1056/NEJMoa1505489