5. Kodama T., Shimizu N., Yoshikawa N. et al. Role of the glucocorticoid receptor for regulation of hypoxia-dependent gene expression // J. Biol. Chem. – 2003. – Vol. 278, № 35. – P. 33384–33391.

6. Krzywinska E., Stockmann C. Hypoxia, metabolism and immune cell function // Biomedicines. – 2018. – Vol. 6, № 2. – P. E56.

7. Kurhaluk N., Zaitseva O.V., Sliuta A. et al. Melatonin diminishes oxidative stress in plasma, retains erythrocyte resistance and restores white blood cell count after low dose lipopolysaccharide exposure in mice // Gen. Physiol. Biophys. – 2018. – Vol. 37, № 5. – P. 571–580.

8. Levine R.L., Garland D., Oliver C.N. et al. Determination of carbonyl content in oxidatively modified proteins // Methods Enzymol. – 1990. – Vol. 186. – P. 464–478.

9. Lukyanova L.D., Kirova Y.I. Mitochondria-controlled signaling mechanisms of brain protection in hypoxia // Front. Neurosci. – 2015. – Vol. 9. – P. 320.

10. Lukyanova L.D., Kirova Y.I., Germanova E.L. The Role of Succinate in Regulation of Immediate HIF-1α Expression in Hypoxia // Bull. Exp. Biol. Med. – 2018. – Vol. 164, № 3. – P. 298–303.

11. Masukawa T., Tochino Y. Circadian rhythm in the cerebral resistance to hypoxia in mice // Jpn. J. Pharmacol. – 1993. – Vol. 61, № 3. – P. 197–201.

12. Serebrovskaya T.V., Xi L. Individualized intermittent hypoxia training: principles and practices. In: Intermittent Hypoxia and Human Diseases, L. Xi and T. Serebrovskaya, Eds., Springer: London, UK, 2012.

13. Strauss E., Waliszewski K., Oszkinis G., et al. Polymorphisms of genes involved in the hypoxia signalling pathway and the development of abdominal aortic aneurysms or large-artery atherosclerosis // J. Vasc. Surg. – 2015. – Vol. 61, № 5. – P. 1105–1113.

14. Veloso M.A. Ecological, biological and genetic research amongst the vertebrate and human populations of the altiplano (Chile). In: L'homme et son environnement a haute altitude (Environmental and human population problems at high altitude), Editions du Centre national de la recherche scientifique, 1981. – P. 37–43.

15. Verrastro I, Pasha S, Jensen KT, et al. Mass spectrometry-based methods for identifying oxidized proteins in disease: advances and challenges // Biomolecules. – 2015. – Vol. 5, № 2. – P. 378–411.

16. Zar J.H. Biostatistical Analysis. 4th ed., Prentice-Hall Inc., Englewood Cliffs, New Jersey, 1999.

STRESS-INDUCED MITOCHONDRIAL FUNCTIONING IN RAT CARDIAC TISSUE: ROLE OF K_{ATP} CHANNEL MODULATORS AND INTERMITTENT HYPOXIC TRAINING

Halyna Tkachenko, Natalia Kurhaluk

Institute of Biology and Earth Sciences, Pomeranian University in Słupsk, Poland

Mitochondrial dysfunction and oxidative stress are largely involved in metabolic disorders, aging, cancer, age-related neurodegenerative syndrome, etc. (Bhatti et al., 2017). ATP-sensitive potassium channels (K_{ATP} channels) are a major contributor to cardiac protection against ischemia (Wang and Ashraf, 2005). Opening of the myocardial K_{ATP} channel may serve a cardioprotective function against various stressors, including hypoxia, ischemia, heat shock, etc. K_{ATP} openers as a class protect ischemic/reperfused myocardium and appear to do so by conservation of energy. The reduced rate of ATP hydrolysis during ischemia exerted by these openers is not due to a cardioplegic effect and is independent of action potential shortening (Grover and Garlid, 2001). Opening of the mitochondrial K_{ATP} channel was found to preserve mitochondrial function and the normal mitochondrial oxygen consumption rate, and lowering Ca^{2+} overloading of mitochondria during hypoxia in ischemic rat heart (Iwai et al., 2000).

Adaptation to intermittent hypoxia has been demonstrated to confer cardiovascular protection against more severe and sustained hypoxia, and, moreover, to protect against other stresses, including ischemia. On the other hand, the physiological activating of the NO-dependent protective mechanisms provides the effective reduction of negative effects of oxidative tissue damage and promotes the adaptive possibilities of organisms (Manukhina et al., 2006). Intermittent hypoxia improves the energy support produced by increasing the formation of mitochondria, activating electron flux through mitochondrial respiratory complex I, and increasing the efficiency of oxidative phosphorylation (Kurhaliuk and Tkachenko, 2004). The protective effects of NO have been linked directly to the activation of mitochondrial K_{ATP} channels (Ockaili et al., 1999). Thus, an ideal treatment for the stress-induced conditions should encompass both openers of the K_{ATP} channel and intermittent hypoxia actions. Therefore, it was of particular interest to ascertain whether pinacidil, the opener of the K_{ATP} channel, and intermittent hypoxia training also possess protective properties and whether their co-administration could offer additional beneficial effects on mitochondrial respiration processes in stress-induced states.

Accordingly, the goals of the present study were 1) to show that the pharmacological opening of the K_{ATP} channel with pinacidil induces cardioprotection in stressed conditions, 2) to investigate the protective effect of adaptation to intermittent hypoxic training (IHT) in mitochondrial function, 3) to determine whether the protective role of pinacidil is dependent on the effect of adaptation to intermittent hypoxia.

Experiments were carried out on adult male Wistar rats weighing 200-220 g. The role of K_{ATP} channel opener pinacidil, inhibitor glibenclamide, and IHT on the mitochondrial function in stress-induced condition was evaluated in six groups of rats: 1) a control group; 2) a stress-induced group; 3) a group that was treated with IHT before stress session; 4) a group that was stress session and injected intraperitoneally with 0.06 mg pinacidil/kg b.w. before stress session; 5) a group that was treated with IHT and injected intraperitoneally with 0.06 mg pinacidil/kg b.w. before stress session; 6) a group that was treated with IHT and injected intraperitoneally with 1 mg glibenclamide/kg b.w. before stress session. Each experimental group consisted of 6 rats.

Experimental rats were subjected to acute stress by swimming for 30 minutes in ambient temperature water (22°C) in a cylindrical container with a net (Bondarenko et al., 1999). Rats were exposed to intermittent normobaric hypoxia, i.e. one session every day for 14 consecutive days. For this training, the rats were placed in a Plexiglas chamber, and O_2 was reduced in the chamber to 10%. Chamber O_2 was monitored with an O_2 analyzer. During this period, rats were exposed to 15 min of hypoxia (10% O_2) followed by 15 min of air. This cycle was repeated 5 times.

Mitochondria were isolated from rat hearts by differential centrifugation as described by Kondrashova (2001). Mitochondria respiration and oxidative phosphorylation were studied with polarographic methods using a Clark oxygen electrode (Chance and Williams, 1955). Succinate (0.35 mM final concentration) and α-ketoglutarate (1 mM) were used as oxidative substrates. ADP (phosphate acceptor) was administered in concentration 0.2 mM. The respiratory control ratio and ADP/O ratio were calculated by the method of Chance and Williams (1955).

Stress conditions significantly increased mitochondrial respiration in the presence of succinate (Table 1). The addition of 200 μM ADP increased the rate of oxidative phosphorylation to (134.92 ± 10.15) μM ADP/min per mg protein and the ADP/O ratio from (1.36 ± 0.01) to (1.84 ± 0.09) μM ADP/ng at O ($p < 0.05$). Respiration in the presence of NADH-generating substrate (1 mM α-ketoglutarate) is presented in Table 2. The stress model induced an increase in the ADP-induced state 3 respiration – from (36.24 ± 3.39) to (60.75 ± 4.57) ng at O/min per mg protein without changing the total amount of the ADP-stimulated O2 consumption. The stress model markedly decreased the efficiency of phosphorylation (ADP/O) by 26.3% ($p < 0.05$) in cardiac mitochondria.

Table 1. – Mitochondrial respiration data of heart mitochondria under influence of pinacidil or glibenclamide and stress in rats with intermittent hypoxia training. Substrate of oxidation – 0.35 mM succinate

Groups of animals	V_3, ng at O/min·mg protein	Respiratory control ratio, V_3/V_4	ADP/O, μM ADP/ng at O	V_{ph}, μM ADP/min·mg protein
Control	61.33±5.12	2.53±0.16	1.36±0.01	83.41±7.96
Stress	72.19±6.37	2.94±0.18	1.84±0.09*	134.92±10.15*
IHT and stress	73.06±5.53	3.17±0.09	1.83±0.07	133.70±10.39
Pinacidil and stress	84.70±6.81	3.94±0.24**	2.04±0.08	174.36±12.35**
IHT, pinacidil and stress	58.08±4.80	3.68±0.12$^{\#}$	1.92±0.16	113.38±9.68
IHT, glibenclamide and stress	39.14±4.57$^{\#}$	2.83±0.20	1.48±0.10$^{\#}$	57.94±4.72$^{\#}$

* P<0.05 changes were statistically significant between the control and stress group, ** P<0.05 between the IHT or pinacidil in stress group and stress groups; # P<0.05 between the IHT, pinacidil, or glibenclamide in stress groups and IHT and stress groups

Table 1 shows that mitochondrial respiration data were not changed by the presence of 0.35 mM succinate. As shown in Table 2, the respiratory ratio by Chance in cardiac mitochondria subjected to IHT (4.65 ± 0.21) was increased by 62% compared to the rate measured in rats exposed by stress (2.87 ± 0.25). IHT significantly improved the rate of oxidative phosphorylation to (144.26 ±12.61) μM ADP/min per mg protein (36.4% of the rate measured in rats exposed by stress).

Table 2. – Mitochondrial respiration data of heart mitochondria under influence of pinacidil or glibenclamide and stress in rats with intermittent hypoxia training. Substrate of oxidation – 1 mM α-ketoglutarate

Groups of animals	V_3, ng at O/min·mg protein	Respiratory control ratio, V_3/V_4	ADP/O, μM ADP/ng at O	V_{ph}, μM ADP/min·mg protein
Control	36.24±3.39	3.22±0.21	2.36±0.10	85.54±7.06
Stress	60.75±4.57*	2.87±0.25	1.74±0.19*	105.74±8.25
IHT and stress	69.69±6.98	4.65±0.21**	2.07±0.09	144.26±12.61**
Pinacidil and stress	79.18±5.31**	3.84±0.20**	2.38±0.12**	190.10±14.82**
IHT, pinacidil and stress	44.73±2.63$^{\#}$	4.40±0.24	2.12±0.15	95.21±6.38$^{\#}$
IHT, glibenclamide and stress	41.01±4.07$^{\#}$	3.97±0.28	1.96±0.08	81.87±7.89$^{\#}$

* P<0.05 changes were statistically significant between the control and stress group, ** P<0.05 between the IHT or pinacidil in stress group and stress groups; # P<0.05 between the IHT, pinacidil, or glibenclamide in stress groups and IHT and stress groups

We also studied the effects of the K_{ATP} channel opener pinacidil on the functional activity of the cardiac mitochondria in the stress model. Respiration in rat hearts in the stress group was taken as control. The ADP/O ratio was not changed in the presence of 0.35 mM succinate. However, the rate of oxidative phosphorylation and RC were enhanced – from (2.94 ± 0.18) to (3.94 ± 0.24) and (134.92 ± 10.15) to (174.36 ± 12.35) μM ADP/min per mg protein, respectively (both $p < 0.05$). However, the effects of pinacidil on mitochondrial oxygen consumption data were more significant at α-ketoglutarate oxidation. The results are presented in Table 2. Treatment by pinacidil at stress model induced a maximal respiration rate that was considerably augmented in the presence of NADH-generating substrates, i.e. α-ketoglutarate. The increase of rate ADP-induced mitochondrial respiration of state 3 deals with markedly augmentation of the efficiency of phosphorylation by 36.8% ($p < 0.05$), the respiratory control ratio by 33.8% ($p < 0.05$), and the rate of oxidative phosphorylation by 79.8% ($p < 0.05$).

Treatment with pinacidil, an opener of the K_{ATP} channel, before the stress model resulted in decreasing the rate of respiration in state 3 by 35.8% ($p<0.05$), and the rate of phosphorylation by 34% ($p<0.05$) without changing the RCR and the efficiency of phosphorylation in heart mitochondria of rats after IHT protocol at α-ketoglutarate oxidation. To test if the opener of K_{ATP} channels could affect mitochondrial function when the respiratory chain used other reduced equivalents, this function was studied in the presence of 0.35 mM succinate. Oxidation of FAD-generated substrate (succinate) in mitochondria was correlated with increasing of RCR by 16% ($p<0.05$). Mitochondrial respiration in the hearts of rats in a group of IHT and stress was taken as control. Glibenclamide markedly decreased the rate of respiration in state 3 and the rate of phosphorylation in the presence of FAD- and NAD-generated substrates.

Our results demonstrate that both intermittent hypoxia and pinacidil improve the energy support produced by activating electron flux through mitochondrial respiratory complex I and increasing the efficiency of oxidative phosphorylation at α-ketoglutarate oxidation. The treatment of stress-exposed animals with a combination of IHT and pinacidil was significantly more effective than either of them alone in reversing the mitochondrial respiration data in rat hearts. The decrease in mitochondrial respiration data in glibenclamide-exposed animals seems to be the result in inhibition of the rate but not in efficiency of phosphorylation and respiratory control ratio as an adaptive mechanism of the IHT method.

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REFERENCES

1. Bhatti J. S., Bhatti G. K., Reddy P. H. Mitochondrial dysfunction and oxidative stress in metabolic disorders – A step towards mitochondria based therapeutic strategies // Biochim. Biophys. Acta Mol. Basis Dis. – 2017. – Vol. 1863, № 5. – P. 1066–1077.
2. Bondarenko O. N., Bondarenko N. A., Manukhina E. B. Effects of various stress induction methods and adaptation on behavioral and somatic parameters in rats // Biull. Eksp. Biol. Med. – 1999. – Vol. 127, № 8. – P. 157–160.
3. Chance B., Williams G.R. The respiratory chain and oxidative phosphorylation // Adv. Enzymol. Relat. Subj. Biochem. – 1956. – Vol. 17. – P. 65–134.
4. Grover G. J., Garlid K. D. ATP-Sensitive potassium channels: a review of their cardioprotective pharmacology // J. Mol. Cell Cardiol. – 2000. – Vol. 32, № 4. – P. 677–695.
5. Iwai T., Tanonaka K., Koshimizu M., Takeo S. Preservation of mitochondrial function by diazoxide during sustained ischaemia in the rat heart // Br. J. Pharmacol. – 2000. – Vol. 129, № 6. – P. 1219–1227.
6. Kondrashova M. N., Fedotcheva N. I., Saakyan I. R., et al. Preservation of native properties of mitochondria in rat liver homogenate // Mitochondrion. – 2001. – Vol. 1, № 3. – P. 249–267.
7. Kurhaliuk N. M., Tkachenko H. M. Myocardium mitochondria functional state during adaptation to intermittent hypoxia and treatment with L-arginine // Ukr. Biokhim. Zh. (1999). – 2004. – Vol. 76, № 3. – P. 79–84.
8. Manukhina E. B., Downey H. F., Mallet R. T. Role of nitric oxide in cardiovascular adaptation to intermittent hypoxia // Exp. Biol. Med. (Maywood). – 2006. – Vol. 231, № 4. – P. 343–365.
9. Ockaili R., Emani V.R., Okubo S., et al. Opening of mitochondrial K_{ATP} channel induces early and delayed cardioprotective effect: role of nitric oxide // Am. J. Physiol. – 1999. – Vol. 277, № 6. – P. H2425–2434.
10. Wang Y., Ashraf M. Role of protein kinase C in mitochondrial K_{ATP} channel-mediated protection against Ca^{2+} overload injury in rat myocardium // Circ. Res. – 1999. – Vol. 84, № 10. – P. 1156–1165.