ELSEVIER

Pathophysiology 20 (2013) 165–170

ISP PATHOPHYSIOLOGY

www.elsevier.com/locate/pathophys

Tolerance to acute hypoxia maximally increases in case of joint effect of normobaric hypoxia and permissive hypercapnia in rats

Pavel Tregub *, Vladimir Kulikov, Andrey Bespalov

Department of Pathophysiology, Altai State Medical University, Barnaul, Russia

Received 24 January 2013; received in revised form 26 March 2013; accepted 7 September 2013

Abstract

Introduction: We studied the comparative efficacy of independent and combined effects of normobaric hypoxia (90 mmHg) and permissive hypercapnia (50 mmHg) in increasing the tolerance of rats to acute hypobaric hypoxia. **Methods:** We determined the time to loss of pose and life duration as a measure to assess the degree of tolerance of animals to hypobaric hypoxia by exposing them to an altitude of 11,500 m (barometric = 180 mmHg). **Results:** Exposure to hypercapnic hypoxia increased the tolerance to acute hypobaric hypoxia compared to exposure to normobaric hypoxia or permissive hypercapnia alone. **Discussion:** The positive effects of hypercapnia and hypercapnic hypoxia occurred after one exposure, and increasing the number of exposures proportionally increased the tolerance to acute hypobaric hypoxia. The effect of permissive hypercapnia on increasing the tolerance to acute hypobaric hypoxia was found to be significantly greater than that of exposure to normobaric hypoxia. Therefore, we propose that hypercapnia is the dominant factor in increasing tolerance to acute hypobaric hypoxia. **Conclusion:** Tolerance to acute hypoxia maximally increases in case of joint effect of normobaric hypoxia and permissive hypercapnia.

Keywords: Hypoxia; Hypercapnia; Hypercapnic hypoxia; Hypoxic tolerance; Adaptation

1. Introduction

Hypoxia is an effective means to increase the tolerance of organs and tissues to acute oxygen deficiency [1,14]. Many researches in this field have dealt with intermittent hypoxic effects [9,18,21]. In addition, the comparative effectiveness of intermittent hypoxia with different numbers of exposures has also been described [13]. However, a disadvantage of hypoxia is the necessity of long-term exposure and a great number of sessions (at least seven) [4,13,18,26]. Therefore, the search for and optimization of alternative variants of hypoxia application to increase human tolerance to stressors are topical.

We presume that the joint effect of normobaric hypoxia (NbH) with permissive hypercapnia (PH) may be the most efficient way to increase the tolerance to acute hypoxia. There are some data confirming the protective potential of the hypercapnic effect. Carbon dioxide has been proven to be effective in providing neuroprotection in case of hypoxic-ischemic cerebral damage [23]. In 2010, the therapeutic effect of permissive hypercapnia after transient global cerebral ischemia–reperfusion injury was reported [30]. However, studies showing the effectiveness of hypercapnia as a factor in increasing the tolerance to acute hypoxia have not been conducted.

Previously, we observed a significant protective effect of hypercapnic hypoxia (HcH) in cerebral ischemia [25]. However, we did not find any research concerning the comparative efficacy of isolated and joint effects of hypercapnia and hypoxia in increasing the tolerance of an organism to acute hypoxia.

Thus, the aims of the present study were to compare the efficacy of isolated and joint effects of hypercapnia and hypoxia in increasing the tolerance of an organism to acute hypoxia, and to study the dynamics of tolerance formation with different numbers of exposures.

* Corresponding author at: Department of Pathophysiology, Altai State Medical University, 40 Lenin Prospekt, Barnaul 656038, Altai Region, Russia. Tel.: +7906 942 64 67.

E-mail addresses: pfiza_asmu@mail.ru (P. Tregub), kulikov57@mail.ru (V. Kulikov), bespalov@asmu.ru (A. Bespalov).

0928-4680/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.pathophys.2013.09.001

2. Materials, methods and techniques

All experimental procedures used in this study were approved by the local Animal Care Committee of the Altai State Medical University in Barnaul. The experiments were carried out on 240 adult male Wistar rats (240–320 g, about 8–9 months old; the Institute of Cytology and Genetics of the Russian Academy of Sciences, Novosibirsk, Russia). The animals in each series were randomized by SPSS 11.5 (SPSS Inc., Chicago, IL). The rats were kept in cages under controlled room temperature (~22 °C) and natural lighting. They had ad libitum access to food and water. The animals were weighed before and after the experiments.

2.1. Animal groups

The research included four series of experiments with equal numbers of groups and animals. In the first series, the rats were placed in the chamber for respiratory exposure once for 20 min. In the second series, the rats were placed in the chamber for respiratory exposure three times for 20 min, with a 24-h interval between exposures. In the third series, the rats were placed in the chamber for respiratory exposure seven times for 20 min, with a 24-h interval between exposures. In the fourth series, the rats were placed in the chamber for respiratory exposure 15 times for 20 min, with a 24-h interval between exposures. The initial level of tolerance to acute hypoxia was evaluated in all animals 14 days before the experiments. A reevaluation of the tolerance level in rats was made 24 h after the last respiratory exposure.

There were four groups of 15 rats each in every experimental series. The groups differed in oxygen partial pressure (PO_2) and carbon dioxide partial pressure (PCO_2). The volume of general sampling and the volume of sampling for each group were estimated on the basis of our previous researches using a similar model and method of quantitative scale [8].

NbH group (normobaric hypoxia: PO_2 90 mmHg; $PCO_2 \sim 1$ mmHg): In this group, the rats were allowed to breathe the gas mixture for 20 min such that PO_2 remained within 90 mmHg.

PH group (permissive hypercapnia: PO_2 150 mmHg; $PCO_2 \sim 50$ mmHg): In this group, the rats were allowed to breathe the gas mixture for 20 min, but PO_2 remained within 150 mmHg and PCO_2 within 50 mmHg.

HcH group (hypercapnic hypoxia: PO_2 90 mmHg; PCO_2 50 mmHg): In this group, the rats were allowed to breathe the gas mixture for 20 min, but PO_2 remained within 90 mmHg and PCO_2 within 50 mmHg.

C group (control group: PO_2 150 mmHg; PCO_2 2 mmHg): In this group, the rats underwent all experimental procedures except the respiratory exposure.

2.2. Realization of respiratory exposures

For the respiratory exposures, a flowing chamber with a total volume of 60 l was used. The gas mixture was infused into the chamber by a compressor; the gas flow was 15 l/min. To maintain uniform pressure inside, the chamber had a discharge outlet connected to a reservoir that was filled with water through a tube coil, which prevented reverse gas diffusion into the chamber. The experimental groups of rats breathed the gas mixture, the composition of which depended on the purpose of the group. The control group was placed in the chamber under the same conditions, but atmospheric air was pumped in instead of the gas mixture. The gas composition was monitored with a MIKON gas analyzer (Laspek Inc., Novosibirsk, Russia).

2.3. Evaluation of tolerance to acute hypoxia

Resistance was determined by the tolerance to acute hypobaric hypoxia [22]. Acute hypobaric hypoxia was simulated in a pressure chamber with a volume of 7 l. The air from the pressure chamber was evacuated with a vacuum pump for 1 min. The level of atmospheric pressure was checked by an altimeter during the whole experiment. The atmospheric pressure in the pressure chamber was equal to the pressure at the altitude 11,500 m (180 mmHg). To restore the initial level of atmospheric pressure, the pressure chamber had an intake valve. Pressure recovery occurred within 1 min after the vacuum pump was switched off. After the experiment, all rats were found to be alive and to have resumed normal activity without any evident sign of pathology. The experiment was carried out at an outdoor temperature of 20–22 °C and humidity of 40–50%. The pressure in the chamber was set to 180 mmHg, and parameters such as time to loss of pose (TtLoP, in s) and life duration (LD, in s) were registered.

The registration of the parameters was performed by three experimenters, two of whom were blinded to the group purpose. The time was fixed with the use of an electronic stopwatch. TtLoP is the period from the moment the pressure of 180 mmHg was set until the rat took the recumbent position with pathological types of respiration. This parameter characterizes the tolerance of the organism to extreme conditions (the condition of motor activity and respiration). LD is the period from the time the pressure of 180 mmHg was set until the agony (the pathological type of respiration up to the second agonal inhalation), which defines the utmost protective functions of the body (the viability of the organism under acute oxygen-limited conditions).

2.4. Statistical analysis

Statistical analysis was carried out using the SPSS 11.5 software. The hypothesis of normalcy of distribution was confirmed with the help of the Shapiro–Wilk test. Some data did not follow the normal distribution law; thus, the groups were compared by a density-free Mann–Whitney U test. The data are presented as median ±25th/75th percentiles. Values of $p < 0.05$ were considered statistically significant.

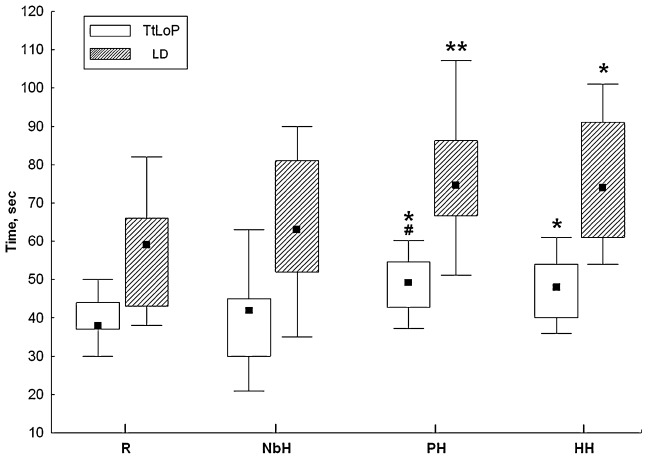

Fig. 1. Tolerance to acute hypobaric hypoxia in rats after 1-time respiratory exposure. The data are presented as median ±25th/75th percentiles. *$p < 0.05$ = Differences with Group R. **$p < 0.01$ = Differences with Group R. $p < 0.01$ = Differences with Group R. #$p < 0.05$ = Differences with Group NbH. TtLoP = time to loss of pose, LD = life duration, R = control group, NbH = normobaric hypoxia, PH = permissive hypercapnia, and HcH = hypercapnic hypoxia.

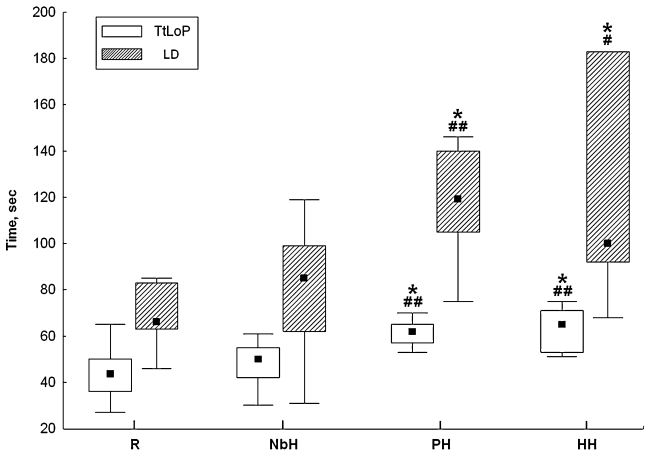

Fig. 2. Tolerance to acute hypobaric hypoxia in rats after 3-time respiratory exposure. The data are presented as median ±25th/75th percentiles. *$p < 0.01$ = Differences with Group R. #$p < 0.05$ = Differences with Group NbH. ##$p < 0.01$ = Differences with Group NbH. TtLoP = time to loss of pose, LD = life duration, R = control group, NbH = normobaric hypoxia, PH = permissive hypercapnia, and HcH = hypercapnic hypoxia.

3. Results

The body mass and initial parameters of TtLoP and LD did not differ in all the rat groups (data not shown). Initial values of TtLoP and LD made up 41.2 ± 5.7 s and 61.9 ± 12.7 s, respectively (the data are given as mean value ± standard deviation). In the control groups of all series, the tolerance to acute hypoxia did not increase.

In the first series (one-time breathing of the gas mixture for 20 min), the TtLoP in the HcH and PH groups was higher than in the control group at 26% ($p < 0.05$) and 31% ($p < 0.01$), respectively; the LD was 25% ($p < 0.05$) and 28% ($p < 0.01$), respectively (Fig. 1). In the NbH group, the TtLoP and the LD were similar to those in the control group. It should be noted that the LD was significantly higher in the PH group compared to the NbH group ($p < 0.05$). Therefore, one-time exposure to permissive hypercapnia and hypercapnic hypoxia increases the animal tolerance to acute hypoxia.

In the second series (three-time breathing of the gas mixture for 20 min at 24-h intervals), the TtLoP in the HcH and PH groups was higher than in control group at 49% ($p < 0.05$) and 42.5% ($p < 0.01$), respectively; the LD was 51% ($p < 0.05$) and 80% ($p < 0.01$), respectively (Fig. 2). In the NbH group, TtLoP and LD were the same as in the control group but were significantly lower than those in the PH and HcH groups ($p < 0.05$). Therefore, three-time 20-min respiratory exposure to permissive hypercapnia and hypercapnic hypoxia increases the animal tolerance to acute hypoxia.

In the third series (seven-time breathing of the gas mixture for 20 min at 24-h intervals), the TtLoP in the HcH, PH, and NbH groups was higher than in the control group at 104% ($p < 0.01$), 96% ($p < 0.01$), and 61% ($p < 0.01$), respectively;

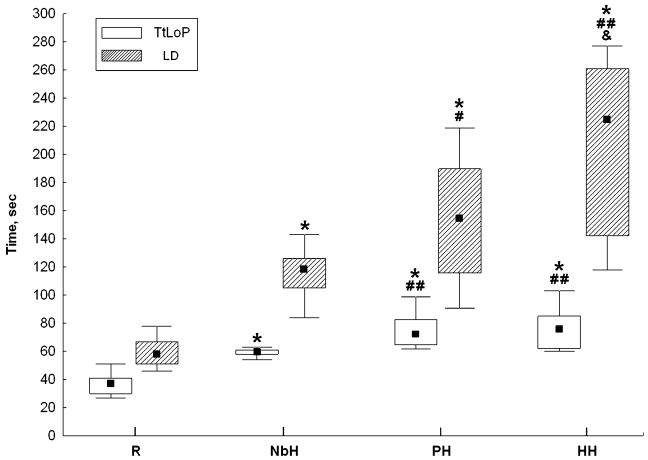

Fig. 3. Tolerance to acute hypobaric hypoxia in rats after 7-time respiratory exposure. The data are presented as median ±25th/75th percentiles. *$p < 0.01$ = Differences with Group R. #$p < 0.05$ = Differences with Group NbH. ##$p < 0.01$ = Differences with Group NbH. &$p < 0.05$ = Differences with Group PH. TtLoP = time to loss of pose, LD = life duration, R = control group, NbH = normobaric hypoxia, PH = permissive hypercapnia, and HcH = hypercapnic hypoxia.

the LD was 278% ($p < 0.01$), 166% ($p < 0.01$), and 103% ($p < 0.01$), respectively (Fig. 3). It should be noted that the tolerance was significantly higher in the PH and HcH groups than in the NbH group, and differed by 21% ($p < 0.01$) and 26% ($p < 0.01$) for TtLoP and by 31% ($p < 0.05$) and 91% ($p < 0.01$) for LD, respectively. The highest tolerance increase was observed after exposure of hypercapnic hypoxia, when compared with the PH group ($p < 0.05$).

In the fourth series, TtLoP and LD were significantly increased (Fig. 4). The lowest increase in TtLoP and LD, when compared with the control group, was observed in the

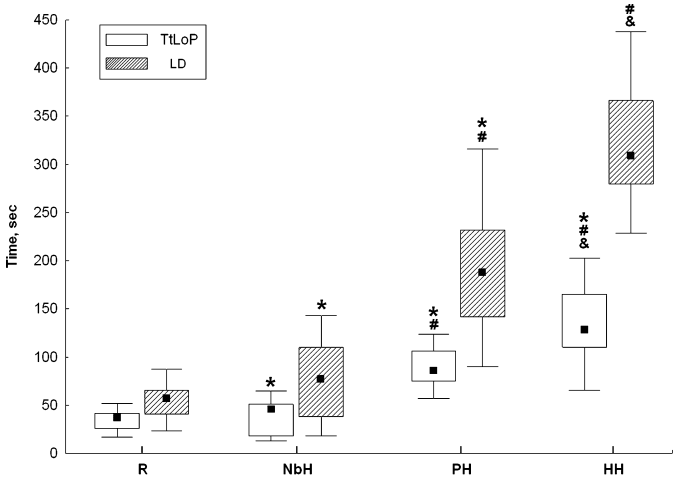

Fig. 4. Tolerance to acute hypobaric hypoxia in rats after 15-time respiratory exposure. The data are presented as median ±25th/75th percentiles. *$p < 0.01$ = Differences with Group R. #$p < 0.01$ = Differences with Group NbH. &$p < 0.01$ = Differences with Group PH. TtLoP = time to loss of pose, LD = life duration, R = control group, NbH = normobaric hypoxia, PH = permissive hypercapnia, and HcH = hypercapnic hypoxia.

NbH group; there was a 30% increase in TtLoP, and LD was half as high ($p < 0.01$) in the NbH group. In the PH group, TtLoP was two-and-a-half times higher and LD was three-and-a-half times higher ($p < 0.01$) than those observed in the control group. The respiratory exposure to hypercapnic hypoxia showed the highest increase in tolerance among the experimental groups. The TtLoP in this group was three-and-a-half times higher and LD was five-and-a-half times higher ($p < 0.01$) than those observed in the control group. All the results of the experimental groups significantly differed among themselves ($p < 0.01$).

The dynamics of the formation of tolerance to acute hypoxia differed among the experimental groups, as was shown more obviously by LD. In the HcH and PH groups, even one-time exposure for 20 min was found to significantly increase the rats' tolerance to acute hypobaric hypoxia when compared with the control group ($p < 0.01$). In the NbH group, the tolerance was significantly increased only within 3–7 days of exposure ($p < 0.01$). Furthermore, during the period from 7 to 15 days of exposure, there was a decrease in tolerance in the NbH group, which was not noticed in the HcH and PH groups. A significant increase in tolerance occurred in the HcH and PH groups when compared with the NbH group ($p < 0.01$), and in the HcH group when compared with the PH group ($p < 0.01$).

4. Discussion

The present research showed the comparative effectiveness of hypoxic, hypercapnic, and hypercapnic hypoxic exposure on rat tolerance to acute hypoxia with different numbers of exposures. The study was carried out to test a hypothesis about the possible increase in the effectiveness of interval hypoxia when combined with permissive hypercapnia.

Many experimental studies have examined the tolerance to acute hypoxia. Different authors have used subjective evaluation criteria, which are rather informative. Shrivastava et al. [22] developed a model of acute hypobaric hypoxia in rats with a simulated altitude of 10,668 m. During the experiment, the agonal breathing time and LD in hypoxia were recorded. The same method was also applied to mice, which were exposed to an altitude of 10,000 m and hypoxia simulation at a rate of 50 m/s for 15 min; the absolute apnea and death time were registered [28]. Lukyanova et al. [13] and Mironova et al. [16] modeled acute hypobaric hypoxia at a simulated altitude of 11,500 m and registered the onset of agonal breathing time.

In the current study, acute hypobaric hypoxia at an altitude of 11,500 m was simulated to assess the tolerance. This altitude was used because it is the threshold of survival for most animals [22].

During hypoxia, first, the function of the CA1 region neurons of the hippocampus is impaired, and the ability to sustain normal motor activity is lost [29]. Therefore, TtLoP allows determining the threshold of sensitivity to severe hypoxia. The appearance of pathological breathing in acute hypoxia exposure is known to indicate serious damage to the functions of the respiratory system and exhaustion of compensatory resources of the organism [19,20]. Furthermore, LD shows the maximal tolerance of the body to severe hypoxia.

According to the published data, different numbers of hypoxic exposures have been used in research. Numerous studies have been done on one-time use of hypoxic preconditioning [2,3,15,24], and it has been reported that the preconditioning effect develops after hypoxic exposure when breathing air with PO_2 of 57–71 mmHg for 1–6 h.

In our present study, the absence of a similar effect after one-time exposure to normobaric hypoxia can be explained by the short duration of exposure (20 min) and higher oxygen concentration (PO_2 of 90 mmHg). Such parameters of hypoxic exposure were chosen because of their high effectiveness in combination with permissive hypercapnia (PCO_2 of 50 mmHg) in preventing experimental stroke in rats [25].

In a study carried out by Lukyanova et al. [13], 3-, 7-, 15-, and 21-fold exposures of normobaric hypoxia were used. Furthermore, there are reports about the use of 30-fold [18,26], 45-fold [14], and 56-fold courses of hypoxic exposures [4]. In our present study, the results concerning 3-, 7-, and 15-fold hypoxic exposures are practically similar to the data on course exposure of hypoxia described by Lukianova et al. [13]. As in the above-mentioned study, the tolerance maximally increased after the 7th exposure with a further decreasing.

The use of permissive hypercapnia and hypercapnic hypoxia as a method of increasing tolerance to acute hypoxia has not been presented in any other studies except our previous research, which revealed an increase in the collateral

cerebral circulation reserve over the course of hypercapnic hypoxic exposure [10]. However, carbon dioxide was found to produce a cerebral protective effect in case of hypoxic/ischemic damage [23]. Some studies have examined the positive effect of hypercapnia on the potassium channel [11], antioxidant system [27], and blood circulation in the lungs [5]. In addition, there are also data indicating that apoptosis inhibition is affected by permissive hypercapnia [30]. Such data may support our hypothesis about the potentiating hypoxic effects caused by hypercapnia on increasing the tolerance to acute hypoxic exposure. The main mechanisms of these hypoxia effects are probably the increase in mRNA expression in early gene families [6], the activation of the K^+ channel by proteinkinase C [17], the activation of succinatoxidase oxidation [7], and genome reprogramming by HIF-1 redox-sensitive protein [12]. However, toxic effects of hypercapnia should be kept in mind; it consists of an acid–base balance shift toward acidosis development, metabolism depression, intracellular homeostasis, and mineral metabolism disorder, as well as in respiratory center work depression [1,5,10,23]. The negative effects of carbon dioxide described above manifest themselves at PCO_2 above 100 mmHg (30). In this research, safe permissive hypercapnia was applied and PCO_2 was kept below 50 mmHg.

In our study, the earliest effect on increasing the tolerance to acute hypoxia was achieved after one-time exposure to permissive hypercapnia. Furthermore, the tolerance was found to increase proportionally to the increase in the number of exposures. The most obvious increase was observed after the seventh and 15th exposures, which significantly increased the effectiveness of normobaric hypoxia.

The use of 1- and 3-fold exposures to hypercapnic hypoxia to increase the tolerance showed dynamics similar to permissive hypercapnia. However, by the seventh exposure to hypercapnic hypoxia, the tolerance had significantly exceeded the results observed in other experimental groups. This phenomenon can prove the potentiation of the protective effects of hypoxia by hypercapnia. The dominant factor is hypercapnia because its effectiveness was higher than that of normobaric hypoxia at any number of exposures.

References

[1] N.A. Agadzhanyan, L.Kh. Bragin, G.A. Davydov, Yu.A. Spasskii, Dynamics of external respiration and gas exchange during combined exposure to hypoxia and hypercapnia, Hum. Physiol. 10 (1984) 282–288.

[2] P.C. Beguin, M. Joyeux-Faure, D. Godin-Ribuot, P. Livy, C. Ribuot, Acute intermittent hypoxia improves rat myocardium tolerance to ischemia, J. Appl. Physiol. 99 (2005) 1064–1069.

[3] M. Bemaudin, F.R. Sharp, Methods to detect hypoxia-induced ischemic tolerance in the brain, Methods Enzymol. 381 (2004) 399–416.

[4] W.J. Chen, H.W. Chen, S.L. Yu, C.H. Huang, T.D. Wang, J.J. Chen, C.T. Chien, H.Y. Chen, P.C. Yang, Y.T. Lee, Gene expression profiles in hypoxic preconditioning using cDNA microarray analysis: altered expression of an angiogenic factor, carcinoembryonic antigen-related cell adhesion molecule 1, Shock 24 (2005) 124–131.

[5] I.C. Chuang, H.P. Dong, R.C. Yang, T.H. Wang, J.H. Tsai, P.H. Yang, M.S. Huang, Effect of carbon dioxide on pulmonary vascular tone at various pulmonary arterial pressure levels induced by endothelin-1, Lung 188 (2010) 199–207.

[6] D.R. Das, N. Maulink, Cardiac genomic response following preconditioning stimulus, Cardiovasc. Res. 70 (2006) 254–263.

[7] M.M. Da Silva, A. Sartori, E. Belisle, A.J. Kowaltowski, Ischemic preconditioning inhibits mitochondrial respiration, increase H_2O_2 release, and enhances K^+ transport, Am. J. Physiol. Heart. Cric. Physiol. 285 (2003) 1154–1162.

[8] R.B. Dell, S. Holleran, R. Ramakrishnan, Sample size determination, ILAR J. 4 (2002) 207–213.

[9] A.V. Goryacheva, S.V. Kruglov, M.G. Pshennikova, B.V. Smirin, I.Y. Malyshev, I.V. Barskov, I.V. Viktorov, H.F. Downey, E.B. Manukhina, Adaptation to intermittent hypoxia restricts nitric oxide overproduction and prevents beta-amyloid toxicity in rat brain, Nitric Oxide 23 (2010) 289–299.

[10] V.P. Kulikov, A.G. Bespalov, N.N. Yakushev, The state of cerebral hemodynamics in conditions of prolonged adaptation to hypercapnic hypoxia, Neurosci. Behav. Physiol. 39 (2009) 269–273.

[11] U. Lindauer, J. Vogt, S. Schuh-Hofer, J.P. Dreier, U. Dirnagl, Cerebrovascular vasodilation to extraluminal acidosis occurs via combined activation of ATP-sensitive and Ca^{2+}-activated potassium channels, J. Cereb. Blood Flow Metab. 23 (2003) 1227–1238.

[12] L.D. Lukyanova, Current issues of adaptation to hypoxia. Signal mechanisms and their role in system regulation, Patol. Fiziol. Eksp. Ter. 1 (2011) 3–19.

[13] L.D. Lukyanova, E.L. Germanova, R.A. Kopaladze, Development of resistance of an organism under various conditions of hypoxic preconditioning: role of the hypoxic period and reoxygenation, Bull. Exp. Biol. Med. 147 (2009) 400–404.

[14] F.Z. Meerson, E.E. Ustinova, E.H. Orlova, Prevention and elimination of heart arrhythmias by adaptation to intermittent high altitude hypoxia, Clin. Cardiol. 10 (1987) 783–789.

[15] B.A. Miller, R.S. Perez, A.R. Shah, E.R. Gonzales, T.S. Park, J.M. Gidday, Cerebral protection by hypoxic preconditioning in a murine model of focal ischemia–reperfusion, Neuroreport 12 (2001) 1663–1669.

[16] G.D. Mironova, M.I. Shigaeva, E.N. Gritsenko, S.V. Murzaeva, O.S. Gorbacheva, E.L. Germanova, L.D. Lukyanova, Functioning of the mitochondrial ATP-dependent potassium channel in rats varying in their resistance to hypoxia. Involvement of the channel in the process of animal's adaptation to hypoxia, J. Bioenerg. Biomembr. 42 (2010) 473–481.

[17] E. Murphy, Primary and secondary signaling pathways in early preconditioning that converge on the mitochondria to produce cardioprotection, Circ. Res. 94 (2004) 7–16.

[18] J. Neckár, F. Papousek, O. Nováková, B. Ost'ádal, F. Kolár, Cardioprotective effects of chronic hypoxia and ischaemic preconditioning are not additive, Basic. Res. Cardiol. 97 (2002) 161–167.

[19] W.M. St John, Medullary regions for neurogenesis of gasping: noeud vital or noeuds vitals, J. Appl. Physiol. 81 (1996) 1865–1877.

[20] W.M. St John, K.V. Knuth, A characterization of respiratory pattern of gasping, J. Appl. Physiol. 50 (1981) 984–993.

[21] V.B. Shatilo, O.V. Korkushko, V.A. Ischuk, H.F. Downey, T.V. Serebrovskaya, Effects of intermittent hypoxia training on exercise performance, hemodynamics, and ventilation in healthy senior men, High Alt. Med. Biol. 9 (2008) 43–52.

[22] K. Shrivastava, M.S. Ram, A. Bansal, S.S. Singh, G. Ilavazhagan, Cobalt supplementation promotes hypoxic tolerance and facilitates acclimatization to hypobaric hypoxia in rat brain, High Alt. Med. Biol. 9 (2008) 63–75.

[23] R.C. Vannucci, J. Towfighi, D.F. Heitjan, R.M. Brucklacher, Carbon dioxide protects the perinatal brain from hypoxic-ischemic damage: an experimental study in the immature rat, Pediatrics 95 (1995) 868–874.

[24] L. Xi, D. Tekin, E. Gursoy, F. Salloum, J.E. Levasseur, R.C. Korea, Evidence that NOS2 acts as a trigger and mediator of late preconditioning

induced by acute systemic hypoxia, Am. J. Physiol. Heart. Circ. Physiol. 283 (2002) 145–152.
[25] N.N. Yakushev, A.G. Bespalov, V.P. Kulikov, Prevention of experimental stroke by hypercapnic–hypoxic preconditioning, Bull. Exp. Biol. Med. 146 (2008) 289–290.
[26] C.C. Yang, L.C. Lin, M.S. Wu, C.T. Chien, M.K. Lai, Repetitive hypoxic preconditioning attenuates renal ischemia/reperfusion induced oxidative injury via up regulating HIF-1 alpha-dependent bcl-2 signaling, Transplantation 88 (2009) 1251–1260.
[27] S. Zakynthinos, P. Katsaounou, M.H. Karatza, C. Roussos, T. Vassilakopoulos, Antioxidants increase the ventilatory response to hyperoxic hypercapnia, Am. J. Respir. Crit. Care Med. 175 (2007) 62–68.
[28] K. Zhang, T. Zhao, X. Huang, Z.H. Liu, L. Xiong, M.M. Li, L.Y. Wu, Y.Q. Zhao, L.L. Zhu, M. Fan, Preinduction of HSP70 promotes hypoxic tolerance and facilitates acclimatization to acute hypobaric hypoxia in mouse brain, Cell Stress Chaperones 14 (2009) 407–415.
[29] Y.D. Zhao, S.Y. Cheng, S. Ou, Z. Xiao, W.J. He, H.Z. Jian-Cui Ruan, Effect of hypobaric hypoxia on the P2X receptors of pyramidal cells in the immature rat hippocampus CA1 sub-field, Brain Inj. 26 (2012) 282–290.
[30] Q. Zhou, B. Cao, L. Niu, X. Cui, H. Yu, J. Liu, H. Li, W. Li, Effects of permissive hypercapnia on transient global cerebral ischemia–reperfusion injury in rats, Anesthesiology 112 (2010) 288–297.