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Comparative analysis of various modes of preconditioning to increase high altitude tolerance

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Abstract. *Hypoxic preconditioning* (HPC) represents an effective tool to increase high altitude tolerance but requires rather severe conditions of hypoxic interventions. We aim to investigate the possibility to reduce the intensity of the HPC factor without losing its effectiveness. Adult male rats were divided as follows: one group was subjected to severe hypoxia (SH); other groups before SH were treated with: three trials of HPC at 5,000 m "altitude" for 2 hours daily (originally proven effective mode); HPC with three trials at 5,000 m "altitude" for 1 hour; HPC with three trials at 3,500 m for 2 hours; HPC with one trial at 5,000 m for 2 hours; HPC with one trial at 5,000 m "altitude" for 2 hours on the background of glucocorticoid administration. In addition, the effects of the non-hypoxic preconditioning with two injections of sodium valproate (a histone deacetylase inhibitor) were studied in a separate group. The survival rate of animals, neuronal loss, neurological status, and behavioral and hormonal reactions were assessed. It was found that all tested modes improved the survival of the rats and their neurological status to a varying degree, but only one trial of HPC in combination with glucocorticoid injection was comparable in efficacy with the original mode of preconditioning proposed by us earlier and produced no side effects. Based on the experimental findings, we suggest a new effective mode of HPC based on a single exposure to the altitude of 5,000 m combined with an injection of dexamethasone.

Keywords: glucocorticoids, high altitude, hypobaric hypoxia, hypoxic tolerance, preconditioning, Wistar rats

Introduction

Preconditioning is a pre-exposure to brief or mild repetitive episodes of hypoxia or ischemia to increase brain and body resistance to more severe hypoxic or ischemic insults (Murry et al. 1986). Numerous techniques of preconditioning have been elaborated, including different protocols of subliminal ischemic episodes produced by transient artery occlusions interrupting the blood flow (Gempp, Blatteau 2010; Salvador et al. 2016; Vinciguerra et al. 2018), normobaric hypoxic treatments by inhalation of reduced oxygen gas mixtures (Hobbins et al. 2017; Serebrovskaya et al. 2013) or remote ischemia (Heusch et al. 2015). Application of ischemic preconditioning techniques in humans has stalled due to considerable risk factors. In particular, ischemic preconditioning by artery occlusion requires surgery and is potentially risky itself, whereas a relatively safe remote ischemic preconditioning by compression of a limb has comparatively low efficacy (Berger et al. 2015; Hausenloy et al. 2015). The hypoxic preconditioning (HPC) techniques lack these disadvantages, but still rather severe hypoxia (SH) is needed to achieve notable and sustained protective effects, which might provoke undesirable pathological processes or be accompanied by subjective feelings of ill health. Thus, both the efficacy and safety depend on the dose of hypoxia, and a very important practical challenge is to decrease the dose without reducing the benefits (Navarrete-Opazo, Mitchell 2014; Serebrovska et al. 2016).

In our studies, we developed a highly effective mode of HPC by three 2 h episodes of hypobaric hypoxia corresponding to the altitude of 5,000 m simulated in a barochamber. Such exposure improved survival of rats in conditions of SH (5% of oxygen), prevented neuronal injury and loss as well as behavioral and hormonal abnormalities in surviving animals (Rybnikova et al. 2006; 2008). In addition, such HPC induced development of cross-tolerance to psychoemotional and traumatic stress (Rybnikova et al. 2007; 2008).

Although the exposure of humans to the altitude of 5,000 m is relatively safe, it can be tolerated poorly and produce unwanted side-effects including headache, chest pain associated with decreased blood flow to the heart, palpitations, and dizziness (Farinelli et al. 1994). For these reasons we continue to search for milder but effective preconditioning techniques. The aim of the present study was to decrease a dose of hypoxia by reducing the "altitude", duration, or a number of HPC sessions in our model of hypobaric hypoxia. We also considered administration of a histone deacetylase inhibitor sodium valproate as a preconditioning factor since epigenetic regulation processes are involved in the processes of adaptation to high altitude (Julian 2017). We also revealed that activation of histone acetylation processes represents a key mechanism of neuroprotective HPC action on vulnerable brain neurons (Samoilov et al. 2016). In the present study, we test whether activation of histone acetylation is sufficient to confer neuronal protection from the SH. Another hypothesis is based on our earlier study which demonstrated that the effective three-trial HPC compared to the non-effective one trial HPC had significantly higher peak amplitude of the corticosterone response to SH (Rybnikova et al. 2008). Taking this into account, we hypothesise that addition of exogenous glucocorticoids to the one-trial HPC in order to reach peak levels of three-trial HPC could increase the efficacy of the one-trial HPC mode. To test this hypothesis, we combine the one-trial HPC with injection of glucocorticoid in a dose calculated as a subtraction of the peak concentrations observed in the effective and non-effective modes of HPC. Summarising the above, the aim of the present study was to examine several preconditioning modes including milder hypoxic exposures, or administration of pharmacological agents, or combining both approaches.

Materials and methods

The study was performed on Wistar male rats weighting 220–250 g. The rats were divided into eight groups, including the control group and seven experimental ones (Fig. 1).

The animals of Group 1 were subjected to severe hypobaric hypoxia (SH) for 3 hours in a flow-type pressure chamber (180 mmHg, 3 hours, equivalent to altitude of 11,000 m). Such parameters were used successfully in our previous studies as a valid rat model of SH (Rybnikova et al. 2006). The rats of Group 2 were preconditioned by pre-exposure to three episodes of moderate hypobaric hypoxia (2 hours at 5,000 m, 360 mmHg, spaced at 24 hours) (the effective protocol). The new HPC modes for testing were designed to decrease the dose of the hypoxic factor by reducing the "altitude", duration, or the number of HPC trials in our model of hypobaric hypoxia. Consequently, they included HPC with three trials at 5,000 m "altitude" lasting for 1 hour (Group 3, shorter duration), HPC with three trials at 3,500 m "altitude" for 2 hours (Group 4, lower "altitude"), HPC with one trial at 5,000 m "altitude" for 2 hours (Group 5, fewer trials), HPC with one trial at 5,000 m "altitude" for 2 hours in combination with administration of glucocorticoid (dexamethasone, 4 mg/kg, i. p.) immediately

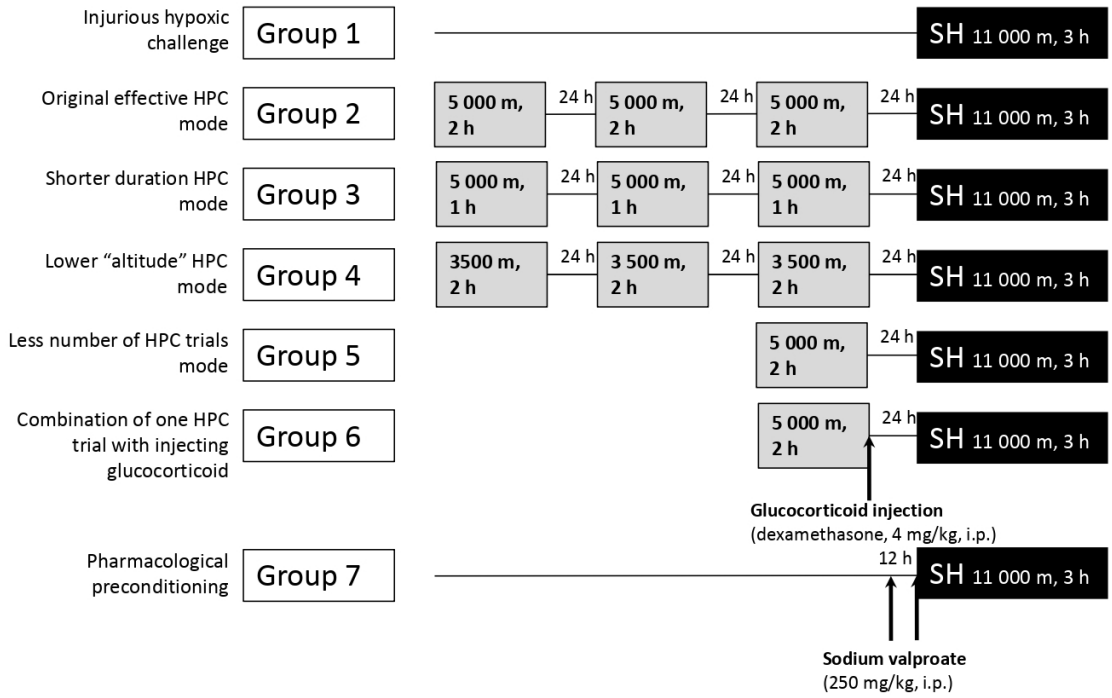

Fig. 1. The experimental design of the study. Groups 1–7, the experimental groups of rats subjected to different treatments. Grey boxes represent preconditioning hypoxic trials with parameters of hypobaric hypoxia (altitude and duration). Black boxes represent severe injurious hypoxia (SH), applied to test the level of tolerance. The arrows indicate timing of the injections. Control group with no treatments is not shown

after the HPC trial (Group 6). In addition, in Group 7 we applied pharmacological preconditioning which consisted of two injections of sodium valproate (250 mg/kg, one in 12 hours and another just before SH). The control group included the rats placed in the barochamber for 3 hours without hypoxia. In all experimental groups the survival rates after SH, post-SH neurological status of rats, their behavioral reactions as well as blood levels of corticosterone were analysed. Each experimental group consisted of 12 animals.

Neurological test

The neurological status was evaluated using the Julon-Courvoisier pull-up test that makes it possible to assess the ability of an animal to hold on a horizontal rope with its forepaws and pull the hanging hind limbs onto the rope (Boissier et al. 1960), which reflects muscle strength and coordination. A rope with a diameter of 0.5 cm was placed horizontally at a height of 70 cm from a cloth hammock. The front legs of an animal were placed on the rope and its hind limbs were released. The number of successful pull-ups out of three attempts when the animal pulled both hind limbs onto the rope was counted.

Behavioral tests

Testing in the elevated plus maze was applied to assess the level of anxiety of animals (Pellow et al. 1985). On the fifth day after SH the rats were tested one at a time for 5 min in the maze apparatus, located at a height of 75 cm above the floor, and consisting of two open illuminated and two enclosed arms with exits. The time spent by an animal inside and outside the closed arms, the number of transitions between the arms, and the dynamics of the "peeping" behavior were evaluated. The anxiety behavior of the animal is characterised by the preference for the closed arms over the open ones and over the centre of the maze (Pellow et al. 1985; Walf, Frye 2007).

The open-field test, as a classical method of assessing the level of locomotor activity of rodents in an unfamiliar environment (Hall 1936), was carried out in a 90 × 90 × 45 cm chamber without a roof whose floor was divided into 15 × 15 cm squares and lit from above with a 60 W lamp. On the sixth day after the final trial with SH in each tested mode a rat was placed in the centre of the open field, and the latent period before the start of its movement, the number of crossed lateral and

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central squares, and the duration of rearing, grooming, and freezing were recorded during 5 minutes.

Blood corticosterone assay

Evaluation of the levels of circulating glucocorticoid hormones (corticosterone as a rat analogue of cortisol in humans) in the blood was performed on days 1 and 7 after SH. The corticosterone levels were determined by enzyme-linked immunosorbent assay with Corticosterone-ELISA reagent kits (Hema, Russia) in two parallel samples.

Histology

To assess the extent of neuronal damage and loss, animals were decapitated on the seventh day after SH and their brains were quickly removed. The hippocampal regions with the adjacent areas were isolated, fixed in 4% buffered paraformaldehyde, and embedded in paraffin. Brain sections were made and stained according to the classical Nissl protocol. The cresyl violet stained paraffin sections were analysed using an image analysis system (VideoTest Master Morphology, VideoTest Ltd., Russia). The number of pyramidal neurons with clear undamaged morphology in the CA1 hippocampal field (area of 350 μm in length) was counted.

Statistics

Statistical processing was performed using the ANOVA one-way analysis of variance (Statistica 7.0) with a posterior comparison using the Fisher method if the distribution of the sample was normal and the group variances were equal. Otherwise, the non-parametric Kraskel-Wallis ANOVA test was used. Differences between groups were considered significant at $p \leq 0.05$. The results for the experimental subgroups are expressed as a percentage of the average value of the control group, taken as 100% ± standard error of the mean.

Results

Average data on survival of rats in the experimental groups were, respectively, 8% in the SH group, 83% in Group 2 (the initial mode), 92% in Group 6, 50% in Group 5 and Group 3, 17% in Group 4 and 42% in Group 7 (Table 1). Thus, judging by the survival rate, only the HPC mode of Group 6 (the combination of a single HPC trial with the dexamethasone injection) was comparable in efficacy with the effective protocol (Group 2). All other tested modes of preconditioning, including the pharmacological one (Group 7), improved rat survival after SH to some extent compared to the non-preconditioned animals. Lowering the altitude was the least protective mode.

The histological analysis revealed that SH resulted in injury and loss of more than 30% of the CA1 neurons, which was a statistically significant difference to control (Fig. 2, Fig. 3). All the preconditioning modes improved neuronal survival except for the mode in Group 4, which showed the same statistically significant neuronal loss as the non-preconditioned Group 1. However, only two modes prevented the neuronal death completely—the original effective mode (Group 2) and exposure to one HPC trial combined with glucocorticoid administration (Group 6). In these two experimental groups the injurious effects of SH were prevented totally and statistically significantly.

Table 1. Survival rates for the groups exposed to SH

Experimental groups	% of survived rats	Number of survived rats
SH	8%	1
3PC + SH	83%	10
3PC(1 h) + SH	50%	6
3PC(3.5 km) + SH	17%	2
1PC + SH	50%	6
1PC + GC + SH	92%	11
2Valp + SH	42%	5

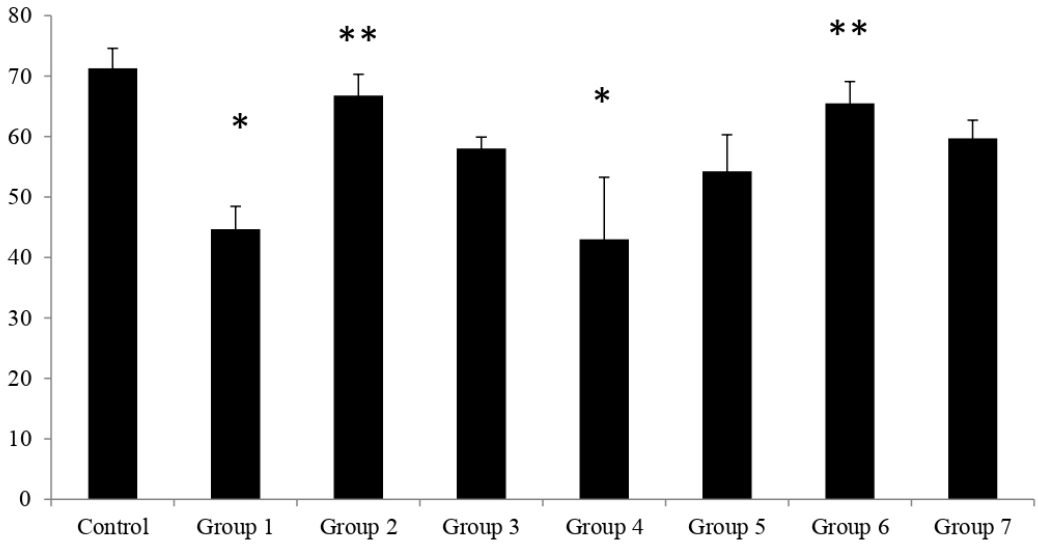

Fig. 2. The number of neurons in the CA1 field of the hippocampus seven days after SH. OY axis indicates the number of pyramidal neurons with unaffected morphology in a randomly selected area of 350 μm. Data presented as mean ± SEM; *—changes are significant compared to control, $p \leq 0.05$; **—changes are significant compared to the non-preconditioned SH group, $p \leq 0.05$

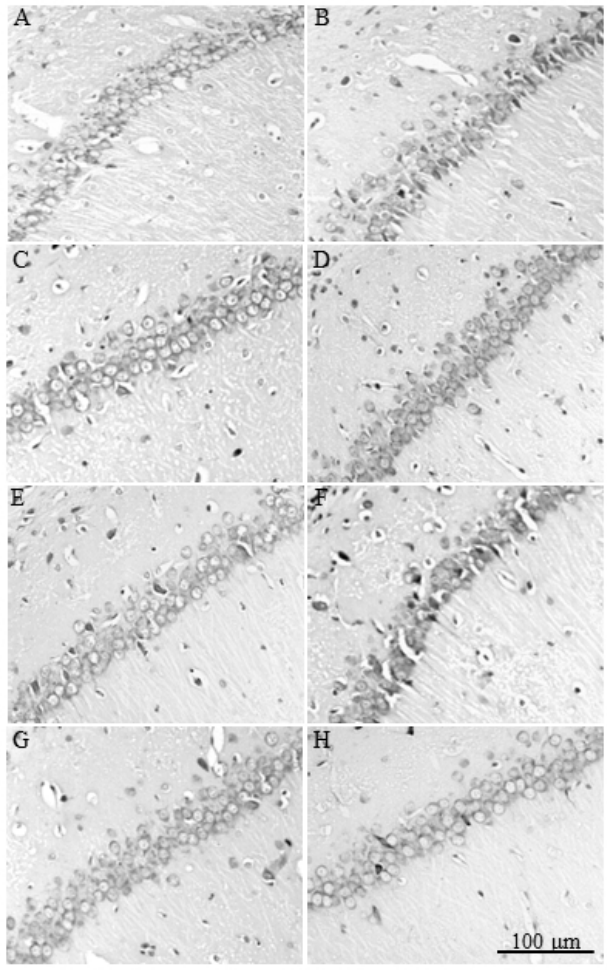

Fig. 3. Microphotographs illustrating the injurious effect of SH on vulnerable neurons of the hippocampal CA1 field in non-preconditioned (B) and preconditioned (C, D, E, F, G, H) rats. A—control; B—Group 1; C—Group 2; D—Group 3; E—Group 4; F—Group 5; G—Group 6; H—Group 7. Scale bar, 100 μm

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In rats which were treated with SH only, a serious neurological deficit was detected. It was observed that total and statistically significant exposure to SH impaired the performance of successful pull-ups. HPC in the effective mode (Group 2) reduced the percentage of errors in the neurological test to the control level. In other tested HPC modes, the rats showed a level of successful attempts to pull-up in the range of 30–56%, which indicated their improved neurological scores compared to the non-preconditioned animals in the SH group, although they were lower than in Group 2 (Table 2).

Table 2. Neurological scores of the rats

Experimental groups	Successful pull- ups from total attempts, %	Successful pull-ups from 3 attempts
Control	0.61%	1.7 ± 0.5
SH	0%	0.0 ± 0
3PC + SH	77%	2.3 ± 0.4
3PC(1 h) + SH	56%	1.7 ± 0.5
3PC(3.5 km) + SH	33%	1.7 ± 1.4
1PC + SH	50%	1.5 ± 0.7
1PC + GC + SH	56%	1.7 ± 0.7
2Valp + SH	47%	1.4 ± 0.7

By monitoring the horizontal activity in the open field, we revealed that lowering the "altitude" in the HPC trials (Group 4) worsened rat behavior after SH and statistically significantly reduced the number of squares crossed compared to control (Fig. 4, A). Similar but non-significant changes were observed in the groups with reduced number of HPC exposures (Group 3) and injected with valproate (Group 7). Similar results were also obtained in the elevated plus maze test where statistically significant changes were observed only in Group 3 and Group 7, suggesting some anxiogenic effects (Fig. 4, B).

To study the hormonal status of the animals and the activity of the pituitary-adrenal system (HPA), we measured the levels of glucocorticoid hormones (corticosterone) in the peripheral blood in the early (one day) and delayed (seven days) periods after SH. Selection of these specific time-points for the analysis was aimed at the assessment of such important parameters of HPA functioning as its feedback regulation in the acute period and baseline activity in the delayed period after SH. It was found that, 24 hours after the exposure to SH, the levels of corticosterone in the blood of the non-preconditioned animals remained statistically significantly elevated above the baseline (Fig. 5). This finding apparently indicates a weakening of the glucocorticoid feedback and correlates with our earlier published data (Rybnikova et al. 2008). On the seventh day after SH, serum corticosterone levels in the SH animals (Group 1) did not statistically differ from the baseline. The animals preconditioned in the initial HPC mode (Group 2) showed a similar statistically significant elevation of corticosterone levels on the first day which, nevertheless, persisted for seven days. These data confirm that application of the original mode of HPC used in our studies persistently up-regulates the baseline activity of HPA, which is also consistent with our previous report (Rybnikova et al. 2008). All other modes of HPC tested in this study did not result in the statistically significant elevation of corticosterone levels in the early period after SH. Hence, it might be suggested that these modes preserve normal functioning of the HPA feedback mechanisms by switching off the acute response. However, the modes of HPC applied in Group 3 and Group 4 caused an abrupt HPA up-regulation in the remote period (on the seventh day after SH) when corticosterone levels increased over 300–500% of the control. This might be considered as a manifestation of impaired glucocorticoid feedback observed

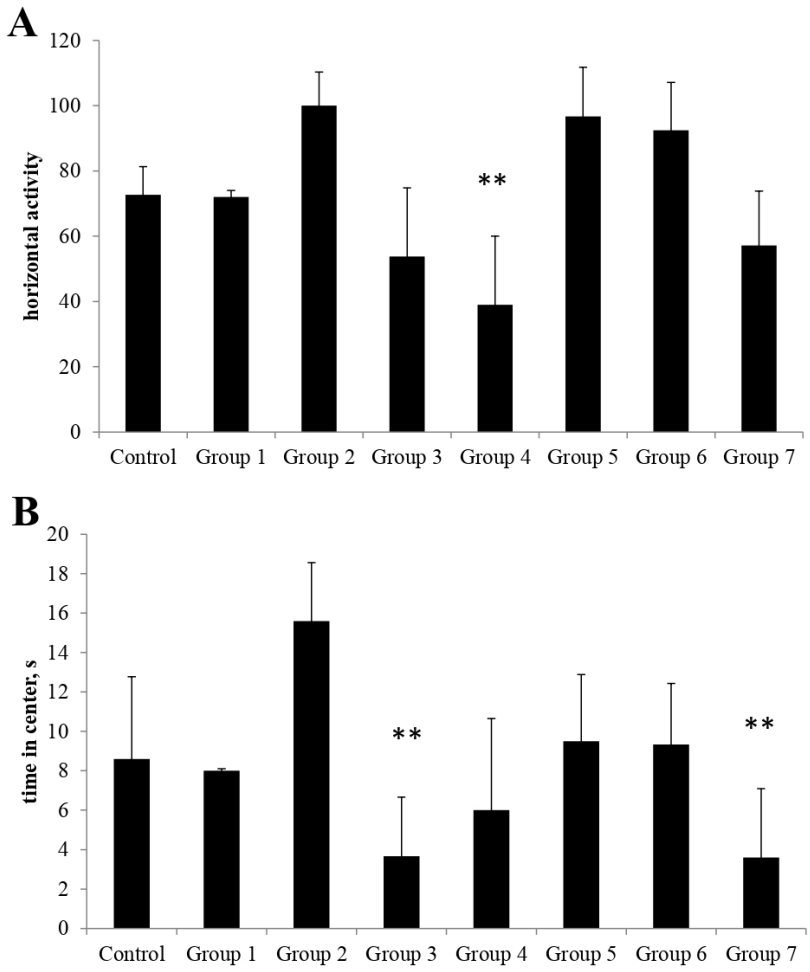

Fig. 4. Behavior of rats in the open field (A) and elevated plus maze (B) tests. A—locomotor activity (ambulances, number of squares crossed for 5 min); B—time spent in the central square of the maze (sec). Data presented as mean ± SEM; **— changes are significant compared to the control group, $p \leq 0.05$

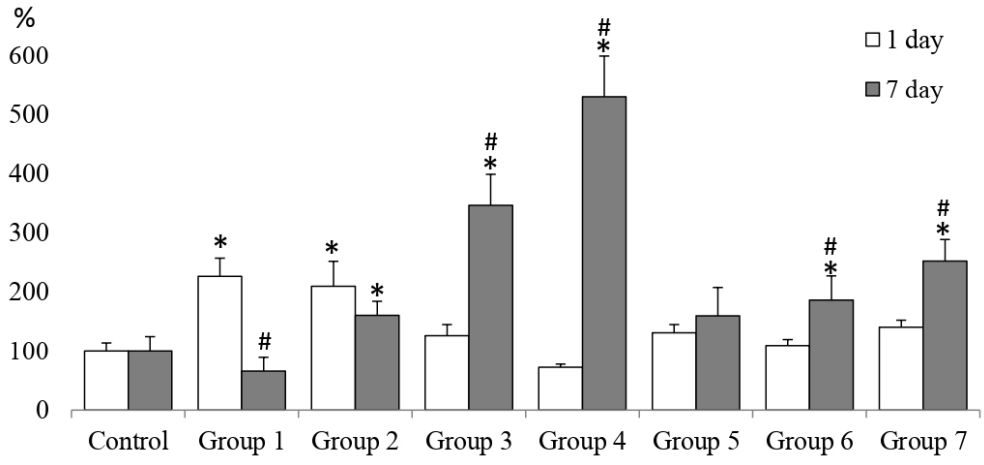

Fig. 5. Corticosterone levels in blood plasma on the first (white bars) and seventh days (grey bars) after SH. Data presented as mean ± SEM and expressed as a percent of the control group level taken as 100%. *—changes are significant compared to baseline, $p \leq 0.05$; #, *—hormonal levels on the seventh day after SH significantly differ from the levels on the first day in the same experimental group, $p \leq 0.05$

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in depression (Pariante, Lightman 2008) or other disruption of the HPA regulation. Comparatively much lower but still statistically significant increase in the levels of corticosterone (220–320%) on the seventh day after SH was also observed in Group 6 and Group 7, suggesting some enhancement of the HPA baseline activity similar to that induced by our original HPC mode.

The data obtained by this comparative analysis demonstrate that amongst all tested modes of HPC only a single HPC exposure to the altitude of 5,000 m for 2 hours combined with the glucocorticoid injection was as effective as the original mode of the three-trial HPC at 5,000 m for 2 hours and produced no side-effects.

Discussion

Although hypoxic training of pilots in a barochamber was suggested by Holdein as early as in 1919, the increase of high altitude tolerance produced by physical exercise and high altitude acclimatisation was convincingly described later, in the middle of the 20th century (Balke, Wells 1958). Since that time intensive research has been performed on the phenomenon of cross-adaptation between various training techniques, mainly hypoxic ones, and increased resistance to high altitude (Meerson 1984; Meerson et al. 1994). For a long time, such research was in line with the development of various types and modes of the hypoxic training, including the normobaric intermittent hypoxic training, the hypoxia-hyperoxia training, and the barochamber training (Karash et al. 1988; Meerson et al. 1994; Serebrovska et al. 2016; Serebrovskaya 2002; Serebrovskaya et al. 2003; 2013; Strelkov, Chizhov 2001).

Upon the discovery of the phenomenon of HPC, it became clear that it provides a different but perhaps more promising approach to increase high altitude tolerance. At first glance, it may seem that the phenomena of the hypoxic training and HPC have a lot in common, since in both cases pre-exposure to non-injurious doses of the harmful factors is used. However, over the years basic research has accumulated evidence that the underlying mechanisms of enhanced tolerance involved in these two approaches differ significantly. Hypoxic training methods are based on a so-called "training reaction" triggered under the influence of a wide variety of active factors in a small, weak, threshold dose (Garkavi 2015). The key stage of the training reaction—the stage of passive resistance—develops not due to an increase in the activity of the protective subsystems of the body but to a decrease in their sensitivity, so that the stimulus is perceived as less intensive, below the threshold. The training-induced resistance to hypoxia is associated with increased efficacy of oxygen delivery and utilisation allowing cells and tissues to function under the conditions of hypoxia and accompanied by enhanced antioxidant defences, remodelling of mitochondrial processes with increased efficiency of ATP production (Levine 2002; Serebrovskaya et al. 2003; 2013). These changes are similar to the changes underlying genetic adaptation or acclimation to high altitude in the populations of residents or newcomers living at such altitude (Moore 2017).

In contrast to the training protocols, HPC requires a more intensive stimulus which induces the "reaction of activation" that represents acute mobilisation of the adaptive resources. The reaction of activation significantly and quickly increases the resistance of the body by increasing the activity of all subsystems of the body up to the intracellular structures and primarily the activity of the protective subsystems of the body (Garkavi 2015). When such an activation reaction develops, changes in the central nervous system, endocrine and immune systems, plasticity and energy metabolism indicate a high functional activity within the upper half of the comfort zone to its upper limit. At the molecular level, the reaction of activation in HPC starts from the immediate expression of transcription factor HIF-1α induced by succinate that reflects a key role of succinate-dependent signalling for immediate and delayed molecular adaptation and increased body resistance to oxygen deficiency (Lukyanova, Kirova 2015; Lukyanova et al. 2018). After termination of the activating stimulus, the reaction of activation transits into the stage of persistent activation and is maintained there for a rather long time. The evidence described above indicates that HPC-based therapeutic approaches could be successfully implemented as a mode of activation therapy, but as already mentioned, it requires a rather high intensity of the preconditioning factor that creates additional risks and/or is worse tolerated. For this reason, we set a task to reduce the intensity of the HPC factor by applying such stimuli which are targeted at additional stimulation of the endogenous mechanisms of tolerance.

In our previous work we studied the mechanisms mediating the development of high altitude tolerance induced by three trials of hypobaric HPC (our effective protocol of HPC) and revealed persistent activation of the pro-adaptive resources, including stabilisation of HIF-1α, activation of MAP kinases, transcription factors CREB, NF-kB, c-Fos, NGFI-A, increased acetylation of histones, and neuronal expression of neuroprotective proteins and antioxidants (Rybnikova, Samoilov 2015; Samoilov

et al. 2016). In addition, the wave-like rhythm of moderate activation of the HPA in response to each of three HPC trials allowed the multi-level rearrangement of HPA regulation resulting in its enhanced reactivity to the hypoxic or other stresses (Rybnikova et al. 2008). Having analysed our own experimental data, we suggested that in the complex coordinated response induced by HPC the key roles belonged to three critical steps: activation of HIF-1 followed by up-regulation of its target genes, enhanced HPA reaction to high altitude stress, and relaxation of chromatin due to histone acetylation. In the present study, we designed more experimental paradigms to examine the importance of each step individually. Milder hypoxic exposures with shorter duration (Group 3) or lower altitude (Group 4) were supposed to be enough to stabilise HIF-1α, as in response to any hypoxic exposure. The earlier reported ineffective mode of a single HPC trial was also taken as a group for comparison for the new mode of single HPC combined with a supplement of exogenous glucocorticoids imitating thereby the enhanced glucocorticoid response to the altitude stress. The final experimental group (Group 7) was designed to examine the role of chromatin relaxation due to increased histone acetylation induced pharmacologically by injecting a histone deacetylase inhibitor, sodium valproate.

The results of this study demonstrate that the combined effect of a single HPC with the injection of exogenous corticosteroids was the most effective in terms of the sum of beneficial outcomes, while the least protective effect accompanied by manifestation of the side effects was observed in the three-trial HPC with a reduced "altitude" (3,500 m). It is interesting that the changes in the HPA functioning differed between the original and the new effective modes in such a way that the animals from Group 6 showed better feedback regulation in the early post-hypoxic period (24 h) since their levels of circulating corticosterone did not differ from the baseline. Regarding the effects of other new modes of preconditioning studied, although they did not improve the survival of rats after SH significantly, their application notably reduced the severity of the post-hypoxic pathology.

These data appear to support the key role of HIF-1 in the activating mechanisms of HPC, since all the modes with decreased hypoxic dose (reduced altitude, duration or number of trials) were less effective. Earlier the dose-dependent activation of HIF-1 was demonstrated in the brain neurons (Sidorova et al. 2013). It was shown that neuroprotection is conferred by using HPC exposures which induce more than 60–70% up-regulation of HIF-1α levels, whereas no protection was seen after HPC exposures which up-regulated HIF-1α levels only by 20–30%. Accordingly, the present data suggest that all the modes with reduced altitude, duration, or the number of hypoxic trials did not activate HIF-1 sufficiently to increase the body resistance. Nevertheless, even slight activation of HIF-1 by lower doses of hypoxia might still contribute to better recovery of the animals after SH observed in those HPC modes.

The highest efficacy observed in the combination of one-trial HPC with glucocorticoid injection (Group 6) allow us to suggest that the interaction between HIF-1 and glucocorticoids might be extremely important for mediating the protection induced by HPC, even compensating presumably low activation of HIF-1. This suggestion is strongly supported by the data reporting that glucocorticoids limit the expression of Von Hippel Lindau protein (pVHL), a negative regulator of HIF-1, and thereby can stabilise HIF-1α and activate HIF-associated transcriptional responses (Vettori et al. 2017). It was also shown that HIF-1 and glucocorticoid receptors are co-assembled on the promoters of some genes in response to either hypoxia or dexamethasone (Anderson et al. 2016). Hence, HIF-1 and glucocorticoids can functionally interact at multiple levels, from stabilisation of HIF-1α to joint regulation of their target genes.

The experiments on animals injected with sodium valproate (Group 7) showed that simple activation of the histone acetylation processes without any external hypoxic factor was not sufficient to achieve hypoxic tolerance. A possible explanation of this observation might be that de-compaction of chromatin caused by enhanced histone acetylation facilitates access to multiple gene promoters but lacks specific transcriptional activators, such as HIF-1, not allowing up-regulation of desired target genes.

In summary, possible ways to reduce the severity of the HPC factor without reducing its effectiveness as well as the possibility of pharmacologic preconditioning by administration of sodium valproate were studied. As a result, it has been demonstrated that only one of the introduced new modes, namely a single hypoxic trial at 5,000 m for 2 hours combined with dexamethasone administration, is as effective as the original mode of three trials of HPC at 5,000 m for 2 hours. The findings open new perspectives for development of effective and safe HPC techniques based on a combination of the hypoxic factors and hormonal therapy.

Conflict of Interest

The authors declare no conflicts of interest, either existing or potential.

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Ethics Approval

The experiments were approved by the Ethical Committee for the Use of Animal Subjects at the Pavlov Institute of Physiology (Saint Petersburg, Russia).

Author Contributions

a. Elena A. Rybnikova—general management of the experiment, writing the article;

b. Ksenia A. Baranova—setting up experiments, histological and hormonal studies, analysis and presentation of data;

c. Mikhail Yu. Zenko—setting up experiments, behavioral testing, analysis and presentation of data;

d. Anna V. Churilova—assistance in setting up experiments and taking samples;

e. Konstantin N. Stupin—assistance in setting up experiments and taking samples.

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