

Variety of Neuronal Cholinergic Pathways of Hypoxic Preconditioning

Elena I Zakharova* and Alexander M Dudchenko

*Laboratory of General Pathology of Cardiorespiratory Systems, Institute of General Pathology and Pathophysiology, Moscow, Russia.

Received September 10, 2018; Accepted December 06, 2018; Published June 10, 2019

ABSTRACT

To date, it is known that short-term episodes of a moderate hypoxia (hypoxic preconditioning) can improve the tolerance to severe hypoxia or ischemia. In the problem of hypoxic preconditioning, the brain is central as the most sensitive organ to hypoxia and as the coordinator of the functions of all body organs and systems. Fundamental importance in the study of nervous tissue has the functional specificity and individual sensitivity to hypoxia of separate neuronal populations and the corresponding brain structures. On the model of single moderate hypobaric hypoxia in rats, using preparative technique and biochemical methods for determining the marker of cholinergic system of choline acetyltransferase in the sub-synaptic fractions of brain structures and also the influences of nicotinic antagonists on the preconditioning, we found: 1) the efficiency of hypoxic preconditioning does not depend on an innate resistance to severe hypoxia and prior severe hypoxic experience of rats; 2) the hypoxic preconditioning eliminates the differences in resistance to hypoxia between the groups of rats with different innate resistance to severe hypoxia and intact rats and 3) in the rats with different prior hypoxic experiences, the same preconditioning effect is achieved by different cholinergic pathways. The variety of neuronal pathways to achieve the same physiological affect demonstrates a great adaptive potential of brain. We have tried to identify some cholinergic neuronal populations or areas of their actions which may be involved in the hypoxic preconditioning mechanisms. The specific mechanisms of preconditioning may be the promising therapeutic targets. At the same time, for the study of innate mechanisms in intact rats, it is necessary to look for criteria for separation of animals in their sensitivity to hypoxic preconditioning. One such criterion (prepulse inhibition) is presented in this review.

Keywords: Severe hypoxia, Apne, Hypoxic preconditioning, Caudal brainstem, Cortex, Neuronal networks, Cholinergic system, Synaptic choline acetyltransferase, Alpha7 and non-alpha7 nicotinic receptors, Methyllycaonitine, Mecamylamine

Abbreviations: ACh: Acetylcholine; C1: Area of Premotor Sympathetic Neurons; ChAT: Choline Acetyltransferase; DFA: Dorsal Facial Area; HBH: Moderate Hypobaric Hypoxia; LDT: The Laterodorsal Tegmental Nucleus; mAChRs: muscarinic Cholinergic Receptors; MCVA: Medullary Cerebral Vasodilator Area; MEC: Mecamylamine; MLA: Methyllycaonitine; nAChRs: nicotinic Cholinergic Receptors; NTS: The Nucleus Tractus Solitary; PPI: prepulse inhibition of the acoustic startle reaction; PPT: the pedunculopontine tegmental nucleus; SHBH: severe Hypobaric Hypoxia; T: Survival Time under SHBH Conditions; VLM: The Ventrolateral Medulla

INTRODUCTION

The relevance of hypoxic preconditioning is due to its ability to increase the body's resistance to hypoxic/ischemic stress. Moreover, hypoxic component forms the pathogenesis of many diseases and an understanding of the preconditioning mechanisms is a high priority [1-7].

Special importance in the study of brain functions is to identify the functional neural networks [8-11]. In the hypoxic adaptation, the key role belongs to the autonomic respiratory and cardiovascular systems. Their central representation is located in the medulla oblongata and pons varolii (caudal brainstem) and closely interrelate with the "respiratory centre", groups of respiratory neurons, which

support respiratory rhythm [12-16]. The neuronal networks of central regulation of breathing and blood circulation in health and disease are in the focus of many researchers because of the basic value of this knowledge to maintain the

Corresponding author: Elena I Zakharova, Institute of General Pathology and Pathophysiology, Baltic Street 8, 125315 Moscow, Russia, Tel: +79199668657; Fax: +74956012366; E-mail: zakharova-ei@yandex.ru

Citation: Zakharova EI & Dudchenko AM. (2019) Variety of Neuronal Cholinergic Pathways of Hypoxic Preconditioning. *J Ageing Restor Med*, 2(2): 75-83.

Copyright: ©2019 Zakharova EI & Dudchenko AM. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

vitality of the body [14,15,17].

Data on the topography, functional significance and interaction of the central components of the autonomic cardiorespiratory system responsible for respiration are most fully presented in the review [14]. We have laid the remarkably laconic and optimal abstract of these authors as a basis for a summary of the anatomic basis of the center control of respiration. Cardiorespiratory activity is controlled by a networks of neurons located within the lower brainstem (the caudal brainstem in our paper). The basic rhythm of breathing is generated by neuronal circuits within the medullary pre-Bötzinger complex (preBötC), modulated by pontine and other inputs from cell groups within the medulla oblongata and then transmitted to bulbospinal pre-motor (reticulospinal) neurons neurons of area C1 of the rostral ventrolateral medulla (VLM) that relay the respiratory pattern to cranial and spinal motor neurons controlling respiratory muscles. Cardiovascular sympathetic and vagal activities have characteristic discharges that are patterned by respiratory activity. This patterning ensures ventilation-perfusion matching for optimal respiratory gas exchange within the lungs. Peripheral arterial chemoreceptors and central respiratory chemoreceptors are crucial for the maintenance of cardiorespiratory homeostasis. Inputs from these receptors ensure adaptive changes in the respiratory and cardiovascular motor outputs in various environmental and physiological conditions. Many of the connections in the reflex pathway that mediates the peripheral arterial chemoreceptor input network they have been established. The nucleus tractus solitarii (NTS), the respiratory network of VLM, pre-sympathetic circuitry and vagal pre-ganglionic neurons at the level of the medulla oblongata are integral components, although supramedullary structures also play a role in patterning autonomic outflows according to behavioral requirements.

This consensus should be supplemented with studies that the authors did not include in the review, or appeared later, about the neuronal components of the circuitry, especially significant for the maintenance of cardiorespiratory homeostasis under hypoxia and/or hypercapnia conditions: 1) the two centres and their outputs were identified for redistribution of blood flow towards the brain, dorsal facial area (DFA) [18] and medullary cerebral vasodilator area (MCVA) [19]; 2) new data has been obtained on functional features of the center of active expiratory, the pontine parafacial respiratory group (pFRG) of non-chemosensitive to CO₂ neurons and chemosensitive to CO₂ and H⁺ distinct populations of neurons in the the retrotrapezoid nucleus [20-25], and 3) some details of pFRG complex links with preBötC have been revealed [21,24], which led to the further development of physiological mechanisms within the cardiorespiratory circuitry. There is a concept of 'distributed' chemosensitivity that has been observed throughout the caudal brainstem [14], which is confirmed by the new data.

The stimulation of respiration, heart activity and blood circulation and redistribution of blood flow towards the brain, lungs and heart is the primary and immediate compensatory response to an environmental hypoxia that is always recorded in the autonomic respiratory and cardiovascular networks [26-30]. In general, this systemic response is a result of the wide cooperation of different functional groups of neurons of the caudal brainstem mentioned above. Under severe environmental hypoxia incompatible with survival, an initial augmentation of respiratory activity followed by secondary depression, which leads to central apnea, i.e., inactivation of the inspiratory muscles as a result of disturbances of the central respiratory rhythm (cat, rat) [29-31].

From the 'distributed' concept, the control mechanisms of chemosensory and respiratory neurons at different locations may be distinct. This applies equally to the norm and to the pathology and implies a multiplicity of central apnea mechanisms. The assumption is supported by data that, under conditions of severe hypobaric hypoxia (3% O₂), animals within the same species (cat, rat), despite the similar dynamics of the systemic reaction, clearly differ in the expressivity of reaction at its different stages and this is consistent with animal resistance to hypoxia (an exposure time of hypoxia before apnea): according to hemodynamic and respiratory indicators, the initial, compensatory stage of the systemic response is more pronounced in the more resistant to hypoxia individuals, and, conversely, the stage of depression is most pronounced in the least resistant individuals [29,30].

Under moderate hypoxia, the all compensatory reactions are maintained during the whole session of hypoxic training in cats or rats [28,30] and any of them can be the physiological basis of adaptation. Our interest lays in the study of the neurotransmitter synaptic mechanisms of hypoxic preconditioning in order to identify the neuronal populations involved in the adaptive respiratory pathways. The neurotransmitter pattern of local central respiratory networks of the cardiorespiratory system is far from complete. At the same time, data on the neurotransmitter specificity of neurons and their effects in the central regulation of respiration are accumulated. This allowed us to analyze the value of some neurotransmitter systems (opioid, serotonergic, GABAergic, glycinergic and adenosinergic) at the level of the caudal brain stem in the mechanisms of apnea [32]. The cholinergic contribution in the mechanisms of apnea and possible ways to prevent it were carefully analyzed in another article [33] and here we provide a mini-review of this publication.

Starting from Loeschcke studies [34,35], the central effects of acetylcholine (ACh) and its analogues on the respiration and blood circulation are intensively investigated. Cholinergic participation is detected in the majority of the functional sites of cardiorespiratory networks, as well as the

ambiguity of the cholinergic effects depending on drug application site, reception and dose. However, there were no data on the participation of cholinergic system in the cardiorespiratory networks under hypoxic preconditioning.

At the same time, it was shown in various organs including the brain that ACh simulates the effects of ischaemic/hypoxic preconditioning usually through nicotinic receptors (nAChRs) [35,37]. Homomeric alpha7 and heteromeric alpha4beta2, alpha3beta4 and alpha3beta2 are the most widespread nAChR subtypes in the mammalian brain [38] and they are expressed at all levels of autonomic regulation of respiration and blood circulation [33].

The most unstable to ischaemic/hypoxic impacts are the structures of forebrain [39], among which the cortex and hippocampus are of greatest interest as the higher brain structures responsible for cognitive functions. Both the cortex and hippocampus interact with the cardiorespiratory systems, participating in the regulation of voluntary breathing and supposedly adaptive reactions of the cardiovascular and respiratory systems [8-11,13,16].

In our investigations, single moderate hypobaric hypoxia (HBH) had a marked hypoxic preconditioning effect on rats, increasing a resistance to severe hypobaric hypoxia (SHBH) in intact rats and high- and low-resistant, pre-tested to SHBH rats [33,40]. Our data confirmed the obligatory participation of the brainstem autonomic systems in the HBH precondition mechanisms. The cholinergic synaptic pool of the caudal brainstem reacted to HBH in all groups of rats unlike those of the cortex and no reaction was shown in the hippocampus (biochemical data) [33,41].

Also, we found that, on the one hand, HBH equalized the resistance to SHBH in all rat groups [33, 40] and on the other hand, the same preconditioning effect was achieved by different synaptic plastic tools in different populations of pre-synapses [33,41]. It assumed the involvement of different neuronal networks in the preconditioning mechanisms in this rat groups.

The assumption was supported by our pharmacological data. We got the effects on HBH of antagonists of alpha7 and non-alpha7 subtypes of the nicotinic receptors (nAChRs) methyllycaconitine (MLA) and mecamlamine (MEC) in the low-resistant rat group and did not receive any effects in the high-resistant and intact rat groups. Moreover, in the low-resistant rat group, the effects of both antagonists on HBH were radically different from their direct effects on innate resistance to SHBH [33].

Thereby, HBH preconditioning is realized by the own mechanisms which eliminate the differences in innate resistance to SHBH between groups of rats independently of their prior hypoxic experiences.

Using the literature and own data, we have tried to identify some cholinergic neuronal populations or areas of their

actions which may be involved in the hypoxic preconditioning mechanisms to delay the time of apnea.

METHODICAL APPROACHES

Briefly, our experimental approaches on the study of cholinergic neuronal mechanisms of hypoxic preconditioning. For the details of experimental procedures [33,42].

Animals

The male outbred albino rats aged 2-2.5 months (200-250 g) at the beginning of the studies. All animal care and experimental procedures were conducted in accordance with the official regulations of the European Communities Council Directive on the use of laboratory animals of 24 November 1986 (86/609/EEC).

Hypoxic models

Hypoxic preconditioning, the continuous hypobaric hypoxia (HBH) at altitude of 5000 m (11% O₂), 60 min. Test for resistance to hypoxia, severe hypobaric hypoxia (SHBH) at critical altitude of 11500 m (4.5% O₂). In the latter case, resistance to hypoxia was recorded with respect to time (T) until agonal inspiration (apnea).

Preparative methods for biochemical investigations

From each brain structure (caudal brainstem, cortex and hippocampus), the sub-fractions of synaptoplasm and synaptic membranes were isolated from the fractions of "light" and "heavy" synaptosomes by routine preparative methods using discontinuous sucrose gradients.

Analytical methods

In the sub-synaptic fractions, the choline acetyltransferase activity (ChAT, EC 2.3.1.6, functional marker of cholinergic neurons) by radiometric method Fonnum and protein content by spectrophotometric method Lowry were assayed.

The sub-synaptic level of fractionation made it possible to study the water-soluble and membrane-bound indicators of two major functionally different pre-synaptic compartments and to disclose a response to exposure of the membrane-bound ChAT, whose activity is significantly less to that of the water-soluble ChAT [41,43,44].

Drug application

The rats received a single intraperitoneal injection of methyllycaconitine citrate (MLA, Tocris), a selective antagonist of alpha7 subtype of nAChRs, or mecamlamine hydrochloride (MEC, Sigma), a selective antagonist of non-alpha7 subtypes of nAChRs. Respectively, MLA (1.4 nmol/kg) or MEC (3.9 nmol/kg) was injected immediately (for MLA) or 30 min (for MEC) before SHBH or HBH. The control to drugs rat groups received physiological saline.

Statistics

The data were calculated using the non-parametric one-sided Fisher's Exact Test and the r-criterion of the Pearson's correlative test.

EXPERIMENTAL PROTOCOLS

In each lot of rats 4-5 weeks before the experiments, part of the animals were pre-tested under SHBH and divided into groups of high- and low-resistance to hypoxia with $T1 > 7$ min and $T1 < 3.5$ min, respectively. Then the rats in each pre-tested group and rats in not pre-tested group (intact rat group) were subdivided into experimental (HBH-SHBH) and control (SHBH) subgroups. The rats of each experimental group were subjected to the HBH session and, four min after the end of training, they were subjected to SHBH or taken in the biochemical experiment. The control groups underwent all procedures except for HBH simultaneously with the corresponding experimental groups.

In pharmacological experiments in each rat group, animals in the HBH-SHBH as well as in SHBH subgroup were subdivided into the drug (experimental) and saline (control) subgroups. After injection of MLA or MEC in the drug subgroups and saline in the control subgroups, animals were subjected to SHBH or to HBH-SHBH as described above.

IDENTIFICATION OF CHOLINERGIC NEURONAL POPULATIONS AND PARTWAYS OF CAUDAL BRAINSTEM AND CORTEX INVOLVED IN THE HBH PRECONDITIONING MECHANISMS AND/OR TARGETS OF THEIR SYNAPTIC ACTION

So, HBH removes the direct effects of SHBH. After HBH sessions, all rats in the HBH-SHBH groups show a similar range for resistance to SHBH with mean T values of 14.7 ± 1.7 , 14.9 ± 1.7 and 13.2 ± 1.8 min vs. mean T values under direct SHBH exposure 5.2 ± 0.9 , 10.3 ± 2.2 and 2.6 ± 0.5 min in the intact, high- and low-resistant rat groups, respectively. The T values of all groups formed the same variational series with the resistance to SHBH over a wide range from 4.5 to 24.5 min.

In accordance with the biochemical experiments, the reaction on HBH of synaptic pool of caudal brainstem and cortex in the studied groups of rats were found and no reaction was shown in the hippocampus. This was the basic experimental material for the identification of neuronal cholinergic pathways of hypoxic preconditioning. Additional material for analysis gave our pharmacological data [33,41].

Briefly, it was revealed the following.

Sources of cholinergic influences in the caudal brainstem and cortex

According to immunocytochemical data, the caudal brainstem includes several cholinergic sources: 1) projections from the reticular formation of the midbrain tegmentum; 2) afferents of the nodose ganglion sensory

neurons from the lung mechanoreceptors to NTS; and 3) neurons of the pons varolii and medulla oblongata, including neurons of reticular areas, NTS, and efferent parasympathetic preganglionic neurons of the motor cranial nerves nuclei.

The cortex (and hippocampus) has two main cholinergic sources, namely: a major source is the cholinergic projection neurons from the basal forebrain nuclei, and minor source, the cholinergic interneurons. We previously showed for the cortex and hippocampus that pre-synapses of the cholinergic projection neurons from the basal forebrain nuclei are mainly concentrated in the light synaptosomal fractions and pre-synapses of the cholinergic interneurons in the heavy synaptosomal fractions [44].

Low-resistant rats

In the caudal brainstem, the inhibition of water-soluble ChAT activity by 17% ($p < 0.025$) in the pre-synapses of heavy synaptosomal fraction was found in the HBH-SHBH subgroup. Conversely, from our previous study, in the fraction of heavy synaptosomes, isolated from NTS, pronounced activation of ChAT (represented mainly by water-soluble ChAT activity) was observed at the time of apnea under SHBH in the low-resistant rats [43]. Taken together, it seems that the reaction of the cholinergic subtype of lung barosensitive C-fibres conducting afferentation to NTS through the nodose ganglion was observed in this rat group [45,46]. The apnea is often preceded by the classic reflex of C-fibres (frequent shallow breathing, bradycardia and hypotension) [46-48]. The weakening of their influences under HBH led to the suppression of parasympathetic reflexes occurring in NTS and thereby to the augmentation of resistance to SHBH.

In our study, both nAChRs antagonists MLA and MEC potentiated the innate resistance to severe hypoxia: the T values in both SHBH drug subgroups were significantly higher compared to corresponding control SHBH subgroups (almost twice for MLA, $p < 0.025$ and more than three times for MEC, $p < 0.05$). In the HBH-SHBH drug subgroups, MLA only but not MEC had an influence on resistance to SHBH after HBH exposure and in this case MLA twice narrowed the preconditioning effect of HBH ($p < 0.025$).

Given the reaction of the cholinergic pool only in the caudal brainstem in the low-resistant rats, as well as the low doses of both antagonists providing their central effect [49], we assumed that the antagonists also act within the caudal brainstem region and that their direct action on the innate resistance to hypoxia in the SHBH drug subgroups of rats occurs specifically within the NTS. According to the literature data in NTS, the nAChRs, including $\alpha 7$ subtype, $\alpha 3$ and $\alpha 4$ subunits, are involved in parasympathetic functions only [49-54]. Therefore, MLA and MEC initiated the suppression of parasympathetic

reflexes occurring in NTS and thereby increased their resistance to SHBH in the SHBH drug subgroups.

In the same time, the cholinergic C-fibres could act on the same nAChRs as our antagonists directly and indirectly affecting theirs through secondary cholinergic barosensitive neurons [50,52,54-56]. In this way, the effects of MLA and MEC, potentiating resistance to SHBH, could not appear after HBH, against the background of the reduced cholinergic C-fibres influences. In other words, HBH preacted the protective effect of both antagonists.

Based on the above, it can be suggested that the suppression of resistance to hypoxia of the low-resistant rats under HBH by MLA took place in brainstem areas outside NTS, in which preconditioning effects could be stimulated through $\alpha 7$ nAChRs. That site may be 1) sympathoexcitatory pressor zone C1 in the rostral VLM [49,57] and it is assumed that the main way for cholinergic transmission is volume (non-synaptic) in this zone [58,59]; 2) DFA [60] and, possibly, the motoneurons in the hypoglossal nucleus [61,62].

High-resistant rats

In this rat group, HBH provoked inhibition of the water-soluble ChAT activity by 19% ($p < 0.05$) in the pre-synapses of light synaptosomal fraction of the caudal brainstem and membrane-bound ChAT by 33% ($p < 0.025$) in pre-synapses of cortical projection neurons (the cortical light synaptosomal sub-fraction). These HBH-induced changes in ChAT activity correlated with each other ($r = +0.911$, $p < 0.02$). In the high-resistant rats, there were no changes in the ChAT activity at the time of apnea under SHBH in the light and heavy fractions of synaptosomes, isolated from NTS [43]. From these data, it was necessary to seek either a direct connection between the cholinergic neurons of the caudal brainstem outside NTS and cortical projection neurons, or cholinergic neurons that connect the caudal brainstem and cortical projection neurons.

According to the literature, the second variant was validated. The tegmental nuclei of middle brain, including the laterodorsal (LDT) and/or pedunclopontine (PPT) nuclei, are the major switch between the caudal brainstem formations and basal forebrain nuclei and other higher brain structures [11,63,64]. Cholinergic neurons of LDT and PPT send plurality of the fibres to both the pons and medulla oblongata [59,63,65] and also to the cortical cholinergic projection neurons of the basal forebrain nuclei [63,65] and it is important that some of them send the projections in both directions [65].

Neurons of the PPT and LDT nuclei are projected into the many regions of caudal brainstem [59,65]. We did not have any influence of the antagonists of nAChRs on HBH-induced preconditioning in the high-resistant group of rats. Thus, we searched the sites of caudal brainstem, in which decrease of the cholinergic effects through muscarinic

cholinergic receptors (mAChRs) would contribute to the hypoxic preconditioning to delay the time of apnea.

We identified two such targets of the PPT and LDT projections: 1) the motoneurons of upper airway from the hypoglossal 12th cranial nerve nucleus that are pre-synaptically inhibited by the M2 subtype of mAChRs stimulation [66] and 2) the laryngeal motoneurons of the upper airway located in the loose formation of the nucleus ambiguus in the rostral VLM, the majority of which through the mAChRs stimulate the constriction of intrinsic laryngeal muscles conjugated with expiration [67,68].

Intact rats

In the intact rat group, in the caudal brainstem, HBH provoked an interconnected increase in the water-soluble ChAT activity and protein content by 27% ($p < 0.025$) and 22% ($p > 0.05$), respectively, in the light synaptosomal fraction ($r = +0.928$, $p < 0.02$) and decreased in the membrane-bound ChAT activity and protein content by 33% and 16% ($p < 0.025$), respectively, in the heavy fraction ($r = +0.933$, $p < 0.02$). The diminution of values of both indicators in the heavy fraction was inversely proportional to their growth in the light fraction (for ChAT activity, $r = -0.962$, $p < 0.02$; for protein content, $r = -0.921$, $p < 0.05$) and we believe that HBH initiated the transformation of cholinergic pre-synapses from the heavy fraction of synaptosomes which altered their density characteristics and during gradient fractionation the transformed presynaptic population appeared in the light fraction. Moreover, the water-soluble ChAT was activated in the transformed presynapses [41].

Several respiratory-related sites exist in VLM in which ACh stimulated breathing, maintained an inspiration through mAChRs and/or nAChRs [19,22,56]. Also, innervation of DFA by nicotine initiated the elevation of cerebral blood flow [60]. However, as in the group of the high-resistant rats, there was no effect of the nAChR antagonists for resistance to hypoxia and any information to identify the populations of cholinergic neurons of the caudal brainstem, involved in the hypoxic preconditioning mechanisms in the intact rats.

The water-soluble ChAT was activated by 28% ($p < 0.05$) in the cortical interneurons (the heavy fraction of synaptosomes). There was no correlation between the ChAT activity in the cortex and caudal brainstem in this rat group because of the absence of a direct link between the brain stem neurons and cortical cholinergic interneurons. The activation of ACh synthesis under HBH in the cortical interneurons could be related to their function of redistribution of the blood flow towards the brain. With respect to cerebral vessels, direct contacts with small cortical vessels and vasodilator effects of both the cholinergic projective neurons and interneurons were detected [69,70]. Thereby in intact brain, the cortical cholinergic interneurons

might be involved in the local mechanisms to maintain of cerebral blood flow.

CONCLUSION

Neuronal, mediator and receptor mechanisms of hypoxic preconditioning are poorly understood to date. The variety of neuronal pathways to achieve the same physiological affect demonstrates a great adaptive potential of brain.

However, the intact rats had a synaptic response to HBH, the opposite of that of pre-tested rats: the activation of cardiorespiratory functions dominated in the intact rats, while the inhibition of pathways initiating apnea appeared in the pre-tested rats. Evidently, that the single pre-testing under SHBH altered synaptic and neuronal preconditioning mechanisms [33,40]. Nonetheless, the specific preconditioning mechanisms of the pre-testing rats may be the therapeutic targets for individuals who survived severe hypoxia.

Cholinergic response, revealed in the caudal brainstem of the intact rats, did not provide any help for the identification of neuronal population reacted to HBH. At the same time, the wide range of HBH-initiated resistance to SHBH and some other evidence [40] convince us that reaction to HBH in the intact rats can be based on different preconditioning mechanisms. Also possible that the multiplicity of mechanisms in the regulation of vital functions such as breathing and blood circulation must be inherent to every organism as the ability to make a quick choice to maintain its viability. This is evidenced by the concept of 'distributed' chemosensitivity that provides 'the necessary level of redundancy within the system' [14].

To identify the innate preconditioning mechanisms, it is necessary to look for criteria for separation of animals in their sensitivity to adaptive hypoxia. One such criterion has recently been found. It was a prepulse inhibition (PPI) estimated in the model of acoustic startle reaction. It was found a correspondence between the values of PPI and T initiated by HBH [32,71]. There was no correlation between PPI and innate SHBH resistance [71] and between PPI and resistance initiated by HBH in the pre-testing to SHBH rats (unpublished data). So, the PPI model can be used to predict the innate efficiency of hypoxic preconditioning (RF patent no. 2571603).

Our first experiments with the pre-testing intact rats at the PPI model revealed the oppositely directed cholinergic effects on the HBH efficiency, separated at the border of PPI=36-40% and the $\alpha 7$ nAChRs participation in both of them [32,71]. The mechanisms of this phenomenon are unknown. We hope to clarify this problem somewhat in the neurochemical studies of synaptic pool in the intact rats pre-tested with PPI.

ACKNOWLEDGEMENT

We are grateful to the direction of Institute of General Pathology and Pathophysiology that supported and funded our research. And we would like to thank the team of the Proof-Reading-Service.com and personally Louise Bourdon for editing and proofreading the English of our manuscript.

RUNNING HEAD

The hypoxic preconditioning eliminates the innate differences in resistance to hypoxia and the same preconditioning effect is achieved by different neuronal pathways.

REFERENCES

1. Hawaleshka A, Jacobsohn E (1998) Ischemic preconditioning: Mechanisms and potential clinical applications. *Can J Anesth* 45: 670-682.
2. Lu GW, Yu S, Li RH, Cui XY, Gao CY (2005) Hypoxic preconditioning: A novel intrinsic cytoprotective strategy. *Mol Neurobiol* 31: 255-271.
3. Tapuria N, Kumar Y, Habib MM, Abu Amara M, Seifalian AM, et al. (2008) Remote ischemic preconditioning: A novel protective method from ischemia reperfusion injury - A review. *J Surg Res* 150: 304-330.
4. Lu GW, Shao G (2014) Hypoxic preconditioning: Effect, mechanism and clinical implication (Part 1). *Zhongguo Ying Yong Sheng Li Xue Za Zhi* 30: 489-501.
5. Speer R, Ratan RR (2016) Hypoxic adaptation in the nervous system: Promise for novel therapeutics for acute and chronic neurodegeneration. *Adv Exp Med Biol* 903: 221-243.
6. Li S, Hafeez A, Noorulla F, Geng X, Shao G, et al. (2017) Preconditioning in neuroprotection: From hypoxia to ischemia. *Progr Neurobiol* 157: 79-91.
7. Turovsky EA, Babaev AA, Tarabykin VS, Turovskaya MV (2017) Sip1 mutation suppresses the resistance of cerebral cortex neurons to hypoxia through the disturbance of mechanisms of hypoxic preconditioning. *Biochemistry (Moscow), Supplement Series A: Membrane and Cell Biology* 11: 330-337.
8. Mitchell RA, Berger AJ (1975) Neural regulation of respiration. *Am Rev Respir Dis* 111: 206-224.
9. Edlow BL, McNab JA, Witzel T, Kinney HC (2016) The structural connectome of the human central homeostatic network. *Brain Connect* 6: 187-200.
10. Arrigo A, Mormina E, Calamuneri A, Gaeta M, Marino S, et al. (2017) Amygdalar and hippocampal connections with brainstem and spinal cord: A diffusion MRI study in human brain. *Neuroscience* 343: 346-354.

11. Venkatraman A, Edlow BL, Immordino-Yang MH (2017) The brainstem in emotion: A review. *Front Neuroanatomy* 11: 15.
12. Saether K, Hilaire G, Monteau R (1987) Dorsal and ventral respiratory groups of neurons in the medulla of the rat. *Brain Res* 419: 87-96.
13. Tarakanov IA, Safonov VA (2003) Neurohumoral concept of central respiratory regulation. *Pathogenesis* 1: 11-24.
14. Spyer KM, Gourine AV (2009) Chemosensory pathways in the brainstem controlling cardiorespiratory activity. *Philos Trans R Soc Lond B Biol Sci* 364: 2603-2610.
15. Feldman JL (2011) Chapter 14 - Looking forward to breathing. *Prog Brain Res* 188: 213-218.
16. Richter DW, Smith JC (2014) Respiratory rhythm generation *in vivo*. *Physiology (Bethesda)* 29: 58-71.
17. Dick TE, Dutschmann M, Feldman JL, Fong AY, Hülsmann S, et al. (2018) Respiratory neurobiology consortium. Facts and challenges in respiratory neurobiology. *Resp Physiol Neurobiol* 258: 104-107.
18. Gong CL, Leung YM, Wang MR, Lin NN, Lee TJ, et al. (2013) Neurochemicals involved in medullary control of common carotid blood flow. *Curr Neuropharmacol* 11: 513-520.
19. Golanov EV, Ruggiero DA, Reis DJ (2000) A brainstem area mediating cerebrovascular and EEG responses to hypoxic excitation of rostral ventrolateral medulla in rat. *J Physiol* 529: 413-429.
20. Huckstepp RT, Henderson LE, Cardoza KP, Feldman JL (2016) Interactions between respiratory oscillators in adult rats. *Elife* 5 pii: e14203.
21. Li P, Janczewski WA, Yackle K, Kam K, Pagliardini S, et al. (2016) The peptidergic control circuit for sighing. *Nature* 530: 293-297.
22. Boutin RC, Alsahafi Z, Pagliardini S (2017) Cholinergic modulation of the parafacial respiratory group. *J Physiol* 595: 1377-1392.
23. de Britto AA, Moraes DJ (2017) Non-chemosensitive parafacial neurons simultaneously regulate active expiration and airway patency under hypercapnia in rats. *J Physiol* 595: 2043-2064.
24. Huckstepp RTR, Cardoza KP, Henderson LE, Feldman JL (2018) Distinct parafacial regions in control of breathing in adult rats. *PLoS One* 13: e0201485.
25. Pisanski A, Pagliardini S (2018) The parafacial respiratory group and the control of active expiration. *Respir Physiol Neurobiol*.
26. Sun MK, Reis DJ (1994) Central neural mechanisms mediating excitation of sympathetic neurons by hypoxia. *Prog Neurobiol* 44: 197-219.
27. Thomas T, Marshall JM (1994) Interdependence of respiratory and cardiovascular changes induced by systemic hypoxia in the rat: the roles of adenosine. *J Physiol* 480: 627-636.
28. Tabata M, Kurosawa H, Kikuchi Y, Hida W, Ogawa H, et al. (2001). Role of GABA within the nucleus tractus solitarius in the hypoxic ventilatory decline of awake rats. *Am J Physiol Regul Integr Comp Physiol* 281: R1411-R1419.
29. Sanotskaya NV, Matsievskii DD, Lebedeva MA (2004) Changes in hemodynamics and respiration in rats with different resistance to acute hypoxia. *Bull Exp Biol Med* 138: 18-22.
30. Sanotskaya NV, Matsievskii DD, Lebedeva MA (2012) Acute hypoxia influence on pulmonary and systemic blood circulation. *Patogenez* 10: 56-59.
31. Richter DW, Bischoff A, Anders KM, Windhorst U (1991) Response of the medullary respiratory network of the cat to hypoxia. *J Physiol* 443: 231-256.
32. Zakharova EI, Storozheva ZI, Proshin AT, Monakov MYu, Dudchenko AM (2018) Hypoxic preconditioning: The multiplicity of central neurotransmitter mechanisms and method of predicting its efficiency. Chapter 6 In: *Hypoxia and Anoxia*, Das KK, Biradar MS, (Eds), IntechOpen, London, UK, pp. 95-131.
33. Zakharova EI, Dudchenko AM (2016) Variety of neuronal pathways to achieve the same hypoxic preconditioning effect. *Biochem Physiol Open Access* 5: 212.
34. Feldberg W, Guertzenstein PG (1972) A vasodepressor effect of pentobarbitone sodium. *J Physiol* 224: 83-103.
35. Loeschcke HH (1982) Central chemosensitivity and the reaction theory. *J Physiol* 332: 1-24.
36. Takada-Takatori Y, Kume T, Izumi Y, Ohgi Y, Niidome T, et al. (2009) Roles of nicotinic receptors in acetylcholinesterase inhibitor-induced neuroprotection and nicotinic receptor up-regulation. *Biol Pharm Bull* 32: 318-324.
37. Liu AJ, Zang P, Guo JM, Wang W, Dong WZ, et al. (2012) Involvement of acetylcholine- $\alpha 7$ nAChR in the protective effects of arterial baroreflex against ischemic stroke. *CNS Neurosci Ther* 18: 918-926.
38. Dani JA, Bertrand D (2007) Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. *Annu Rev Pharmacol Toxicol* 47:699-729.

39. Araki T, Kato H, Fujiwara T, Kogure K, Itoyama Y (1995) Alteration of [3H]hemicholinium-3 binding in the post-ischemic gerbil brain. *NeuroReport* 6: 561-564.
40. Zakharova EI, Dudchenko AM (2016) Hypoxic preconditioning eliminates differences in the innate resistance of rats to severe hypoxia. *J Biomed Sci Eng* 9: 563-575.
41. Zakharova EI, Germanova EL, Kopaladze RA, Dudchenko AM (2013) Central cholinergic systems in the mechanisms of hypoxic preconditioning: Diverse pathways of synaptic reorganization *in vivo*. *Neurochem J* 7: 45-55.
42. Zakharova EI, Storozheva ZI, Dudchenko AM, Kubatiev AA (2010) Chronic cerebral ischemia forms new mechanisms of learning and memory. *Int J Alzheimers Dis*.
43. Zakharova EI, Dudchenko AM, Svinov MM, Fedorova MM, Germanova EL (2010) Cholinergic systems of the rat brain and neuronal reorganization under conditions of acute hypoxia. *Neurochem J* 4: 290-303.
44. Zakharova EI, Dudchenko AM (2014) Synaptic soluble and membrane-bound choline acetyltransferase as a marker of cholinergic function *in vitro* and *in vivo*. Chapter 5 In: *Neurochemistry*, Heinbockel T, (ed.), InTechOpen, Rijeka, Croatia, pp. 143-178.
45. Udem BJ, Chuaychoo B, Lee MG, Weinreich D, Myers AC, et al. (2004) Subtypes of vagal afferent C-fibres in guinea-pig lungs. *J Physiol* 556: 905-917.
46. Filippova LV, Nozdrachev AD (2013) An overview of the pulmonary sensory receptors. *Usp Fiziol Nauk* 44: 93-112.
47. Bonham AC, Joad JP (1991) Neurones in commissural nucleus tractus solitarii required for full expression of the pulmonary C fibre reflex in rat. *J Physiol* 441: 95-112.
48. Seifert E, Trippenbach T (1995) Baclofen attenuates cardiorespiratory effects of vagal C fiber stimulation in rats. *Can J Physiol Pharmacol* 73: 1485-1494.
49. Moore C, Wang Y, Ramage AG (2008) Cardiovascular effects of activation of central alpha7 and alpha4beta2 nAChRs: A role for vasopressin in anaesthetized rats. *Br J Pharmacol* 153: 1728-1738.
50. Ashworth-Preece M, Jarrott B, Lawrence AJ (1998) Nicotinic acetylcholine receptors in the rat and primate nucleus tractus solitarius and on rat and human inferior vagal (nodose) ganglia: Evidence from *in vivo* microdialysis and [125I] alpha-bungarotoxin autoradiography. *Neuroscience* 83: 1113-1122.
51. Dhar S, Nagy F, McIntosh JM, Sapru HN (2000) Receptor subtypes mediating depressor responses to microinjections of nicotine into medial NTS of the rat. *Am J Physiol Regul Integr Comp Physiol* 279: 132-140.
52. Haxhiu MA, Kc P, Moore CT, Acquah SS, Wilson CG, et al. (2005). Brain stem excitatory and inhibitory signaling pathways regulating broncho-constrictive responses. *J Appl Physiol* 98: 1961-1982.
53. Feng L, Uteshev VV (2014) Projection target-specific action of nicotine in the caudal nucleus of the solitary tract. *J Neurosci Res* 92: 1560-1572.
54. Furuya WI, Bassi M, Menani JV, Colombari E, Zoccal DB, et al. (2014) Differential modulation of sympathetic and respiratory activities by cholinergic mechanisms in the nucleus of the solitary tract in rats. *Exp Physiol* 99: 743-758.
55. Helke CJ, Handelmann GE, Jacobowitz DM (1983) Choline acetyltransferase activity in the nucleus tractus solitarius: Regulation by the afferent vagus nerve. *Brain Res Bull* 10: 433-436.
56. Ruggiero DA, Giuliano R, Anwar M, Stornetta R, Reis DJ (1990) Anatomical substrates of cholinergic-autonomic regulation in the rat. *J Comp Neurol* 292: 1-53.
57. O'Leary KT, Loughlin SE, Chen Y, Leslie FM (2008) Nicotinic acetylcholine receptor subunit mRNA expression in adult and developing rat medullary catecholamine neurons. *J Comp Neurol* 510: 655-672.
58. Milner TA, Pickel VM, Morrison SF, Reis DJ (1989) Adrenergic neurons in the rostral ventrolateral medulla: Ultrastructure and synaptic relations with other transmitter-identified neurons. *Prog Brain Res* 81: 29-47.
59. Jones BE (1990) Immunohistochemical study of choline acetyltransferase-immunoreactive processes and cells innervating the pontomedullary reticular formation in the rat. *J Comp Neurol* 295: 485-514.
60. Gong CL, Leung YM, Huang YP, Lin NN, Hung YW, et al. (2010) Nicotine activation of neuronal nitric oxide synthase and guanylyl cyclase in the medulla increases blood flow of the common carotid artery in cats. *Neurosci Lett* 486: 122-126.
61. Chamberlin NL, Bocchiaro CM, Greene RW, Feldman JL (2002) Nicotinic excitation of rat hypoglossal motoneurons. *Neuroscience* 115: 861-870.
62. Volgin DV, Rukhadze I, Kubin L (2008) Hypoglossal premotor neurons of the intermediate medullary reticular region express cholinergic markers. *J Appl Physiol* 105: 1576-1584.
63. Semba K, Reiner PB, McGeer EG, Fibiger HC (1988) Brainstem afferents to the magnocellular basalforebrain studied by axonal transport, immunohistochemistry and

- electrophysiology in the rat. J Comp Neurol 267: 433-453.
64. Semba K, Reiner PB, McGeer EG, Fibiger HC (1989) Brainstem projecting neurons in the rat basal forebrain: Neurochemical, topographical and physiological distinctions from cortically projecting cholinergic neurons. Brain Res Bull 22: 501-509.
 65. Woolf NJ, Butcher LL (1989) Cholinergic systems in the rat brain: IV. Descending projections of the pontomesencephalic tegmentum. Brain Res Bull 23: 519-540.
 66. Bellingham MC, Berger AJ (1996) Presynaptic depression of excitatory synaptic inputs to rat hypoglossal motoneurons by muscarinic M2 receptors. J Neurophysiol 76: 3758-3770.
 67. Haji A, Furuichi S, Takeda R (1996) Effects of iontophoretically applied acetylcholine on membrane potential and synaptic activity of bulbar respiratory neurones in decerebrate cats. Neuropharmacology 35: 195-203.
 68. Bautista TG, Sun QJ, Zhao WJ, Pilowsky PM (2010) Cholinergic inputs to laryngeal motoneurons functionally identified *in vivo* in rat: A combined electrophysiological and microscopic study. J Comp Neurol 518: 4903-4916.
 69. Dauphin F, Lacombe P, Sercombe R, Hamel E, Seylaz J (1991) Hypercapnia and stimulation of the substantia innominata increase rat frontal cortical blood flow by different cholinergic mechanisms. Brain Res 553: 75-83.
 70. Chédotal A, Cozzari C, Faure MP, Hartman B K, Hamel E (1994) Distinct choline acetyltransferase (ChAT) and vasoactive intestinal polypeptide (VIP) bipolar neurons project to local blood vessels in the rat cerebral cortex. Brain Res 646: 181-193.
 71. Zakharova EI, Storozheva ZI, Proshin AT, Monakov MYu, Dudchenko AM, et al. (2018) The prepulse inhibition of startle (PPI) predicts an efficiency of hypoxic preconditioning. J Biomed Sci Eng 11: 10-25.