ongata and influences of unidentified cholinergic neurons through the alpha7 nicoti
caudal brainstem areas outside the nucleus tractus solitary. In intact rats, cortical choli
unidentified cholinergic neurons of the brainstem structures. In conclusion, the variety o
off apnea indicates a great adaptive potential of brain, and the specific mechanisms
promising therapeutic targets.

Keywords: Severe hypoxia; Apnea; Hypoxic preconditioning; Caudal brainstem; Cortex; Neuronal networks; Cholinergic system; Alpha7 and non-alpha7 nicotinic receptors; Synaptic choline acetyltransferase

Abbreviations: ACh: Acetylcholine; C1: Area of premotor sympathetic neurons; ChAT: Choline Acetyltransferase; DFA: Dorsal Facial Area; HBH: Moderate Hypobaric Hypoxia; IC: Intracisternal; IP: Intraperitoneal; LDT: Laterodorsal Tegmental Nucleus; mAChRs: Muscarinic Cholinergic Receptors; MCVA: Medullary Cerebral Vasodilator Area; MEC: Mecamylamine; MLA: Methyllycaconitine; nAChRs: Nicotinic Cholinergic Receptors; NTS: Nucleus Tractus Solitary; PPT: Pedunculopontine Tegmental Nucleus; SHBH: Severe Hypobaric Hypoxia; T: Survival time under SHBH conditions; VLM: Ventrolateral Medulla.

Introduction

The relevance of hypoxic preconditioning is due to its ability to increase the body's resistance to hypoxic/ischemic stress. Hypoxic factor is the main in ischemic preconditioning effect. It is likely that hypoxic component forms the pathogenesis of many diseases, and a study of the preconditioning mechanisms is a high priority [1-4].

In the problem of hypoxic adaptation, the brain is central, not only as the most sensitive organ to hypoxia, but also as the coordinator of functions of all body organs and systems. In the nervous tissue, the functional specificity and individual sensitivity to hypoxia of separate neuronal populations and the corresponding brain structures is of fundamental importance. The structures of forebrain are most unstable to ischaemic/hypoxic injuries [5]. The most interesting are the cortex and hippocampus, as these are the higher brain structures responsible for cognitive functions and complex behaviour.

Under moderate hypoxia conditions (10-12% O2 for rat) which are parts if hypoxic training, the key role in the adaptive transformations of the body functions belongs to the autonomic respiratory and cardiovascular systems. Their central representation is located in the medulla oblongata and pons varolii (caudal brainstem). Autonomic systems are functionally closely interrelated by the “respiratory centre”, groups of respiratory neurons which support respiratory rhythm [6-8]. 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 [7-10].

The study of neuronal networks of the central autonomic regulation of breathing and blood circulation, and the mediator and functional specificity of their components in health and disease is the focus of many researchers because of the basic value of this knowledge to maintain the vitality of the body. Starting from Loeschcke studies [11,12], 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 [13-23].

It was later shown in various organs including the brain that ACh simulates the effects of ischaemic/hypoxic preconditioning which are usually realised through nicotinic receptors (nAChRs) [24-27]. Homomeric alpha7 and heteromeric alpha4beta2, alpha3beta4 and alpha3beta2 are the most widespread nAChR subtypes in the mammalian brain [28]. NAChRs are expressed at all levels of autonomic regulation of respiration and blood circulation from peripheral organs [25,27,29-31] to the central sensory, respiratory and motor neurons [17,20,22,30,32-34].

In our investigations, single moderate hypobaric hypoxia (HBH) significantly increases a resistance to severe hypobaric hypoxia (SHBH) in intact rats and high- and low-resistant, pre-tested to SHBH rats [35,36]. Our data confirm the obligatory participation of the brain stem autonomic systems in the HBH precondition mechanisms. The synaptic pool of the caudal brainstem reacts to HBH in all groups of rats unlike those of the cortex and hippocampus (biochemical data)

All data were generated in a double blind manner which has been achieved thanks to the technical assistance of our colleague.

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 in Microsoft Excel with the formula being adjusted for a small number of observations (n=4-15) [43]. Differences were considered to be statistically significant if $p<0.05$. According to the Bonferroni correction, differences were considered to be statistically significant if $p<0.025$ for four selections and if $p<0.02$ for more than four selections. In last cases, the significance level $p<0.05$ was considered a tendency to the validity of events and was taken into account in the presence of other ways of comparisons.

For the statistical analysis were also used previously published biochemical data on choline acetyltransferase (ChAT, EC 2.3.1.6) activity in synaptic membrane and synaptoplasm sub-fractions (membrane-bound and water-soluble ChAT, respectively) of "light" and "heavy" synaptosomes isolated from the caudal brainstem (medulla oblongata and pons varolii) and cortex of the intact, high-resistant and low-resistant HBH rat groups. Synaptic tools of cholinergic reaction in these brain structures on HBH were analysed in detail [37]. However, the correlation between the response of the synaptic pool in the caudal brainstem and cortex were not analysed.

Results and Discussion

Action of MLA and MEC on direct and HBH-mediated resistance to SHBH in the intact, high and low resistant rats

Pharmacological data

In the intact and high-resistant SHBH drug rat groups, both MLA and MEC had no effect on the direct SHBH action (Figures 1 and 2). In the low-resistant rats, both MLA and MEC potentiated the direct resistance to hypoxia and T2 values in the SHBH drug groups significantly different from the T2 values in the SHBH control groups (Figures 1C and 2C).

HBH removes the direct efects of SHBH. After HBH sessions, all HBH-SHBH control rat groups show a similar range of values 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.

Rat group	Caudal brainstem		Cortex	
	Light synaptosomal fraction %; sub-	Heavy synaptosomal fraction	Light synaptosomal fraction	Heavy synaptosomal fraction

presynaptic glutamate release [34]. These data bring us to the above report of the glutamate-mediated stimulation of the

from laterodorsal (LDT) and/or pedunculopontine (PPT) tegmental cholinergic nuclei of the middle brain. LDT and more intensively PPT send plurality of the fbres to both the pontine and medulla oblongata nuclei and also to the higher brain structures, including the basal forebrain nuclei [56,57,95]. T e density of the projection fbres of these nuclei is considerably greater than the fbre network of the cholinergic interneurons in the reticular formation of the medulla oblongata [49,56,57]. At the same time, representation of these projections in the NTS is minimal or absent [55].

T e cholinergic neurons of the same two tegmental nuclei are functionally closely associated with the cortical cholinergic projection neurons of the basal forebrain nuclei. T e brainstem send most of their projections to the forebrain nuclei from LDT and PPT and very few from the medulla oblongata [95]. T e authors estimated that 21% of the identifed cholinergic neurons of the basal ganglia, which project their fbres to the cortex, are activated or inhibited following stimulation of the cholinergic neurons from LDT and PPT.

Also, some neurons in these tegmental nuclei send the projections to the medulla oblongata as well as to the basal ganglia [56]. T e cholinergic neurons of LDT and PPT are the main switch between the cortical cholinergic projective neurons and brainstem formations. It is important to note that the middle brain projective neurons of the basal forebrain are diferent from the cortical projective neurons topographically, by their electrophysiological properties and they are non-cholinergic. Only a few cholinergic projective cortical neurons send their collaterals to the brainstem too [96]. For this reason, the reaction of the direct cholinergic projections from the forebrain among the total cholinergic synaptic pool of the brainstem is not possible to identify easily by our biochemical methods.

From the above data, it also follows that in the conjunction tegmentum-cortex, the inhibition of membrane-bound ChAT activity (and ACh quantum releasing) in the cortical projective cholinergic neurons was a consequence of the aferent infuences from the tegmental PPT/LDT nuclei, and not vice versa, whereas feedback between these neuronal groups has, at least, one relay neuron. Such indirect connections, as a rule, do not achieve statistical signifcance in biological systems. T erefore, we believe that this adaptive inhibition of the cortical neuronal projections was caused by neuronal infuences and not by tissue oxygen defciency in the cortex. Also, a coordinated response to HBH of these two cholinergic neuronal populations might refect the repair processes, because HBH restores the resistance of the high-resistant rats which is chronically reduced in this group afer the initial pre-testing SHBH exposure [36].

Neurons of the PPT and LDT nuclei are projected into many areas of the caudal brainstem [56]. T e targets of some tegmental cholinergic projections are known. We shall describe the cholinergic efects on the identifed sites, in which the functional inhibition would contribute to the hypoxic preconditioning to put of apnea.

Already mentioned motoneurons of the upper airway from the hypoglossal 12th cranial nerve nucleus are located in the dorsal part of the medulla oblongata. T e hypoglossal motoneurons receive permanent excitation drive from the inspiratory, mainly glutamatergic, neurons from the medullary caudal intermediate reticular region. Intensifcation of inspiratory activation, for example, in OSA patients (obstructive apnea during sleep), increases the innervation of these motoneurons and, respectively, of the upper airway ġ z piu e

13. 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.
14. Haji A, Furuichi S, Takeda R (1996) Efects of iontophoretically applied acetylcholine on membrane potential and synaptic activity of bulbar respiratory neurones in decerebrate cats. Neuropharmacology 35: 195–203.

reorganization under conditions of acute hypoxia. Neurochemical J 4: 290–303.

54. https: //www.hindawi.com/journals/ijad/2010/954589/

55. Rye DB, Lee HJ, Saper CB, Wainer BH (1988) Medullary and spinal efferents of the pedunculopontine tegmental nucleus and adjacent mesopontine tegmentum in the rat. J Comp Neurol 269: 315–341.

56. Woolf NJ, Butcher LL (1989) Cholinergic systems in the rat brain: IV. Descending projections of the pontomesencephalic tegmentum. Brain Res Bull 23: 519-540.

57. 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.

58. Reynolds DJ, Lowenstein PR, Moorman JM, Grahame-Smith DG, Leslie RA (1994) Evidence for cholinergic vagal afferents and vagal presynaptic M1 receptors in the ferret. Neurochem Int 25: 455-464.

59. Schäfer MK, Eiden LE, Weihe E (1998) Cholinergic neurons and terminal fields revealed by immunohistochemistry for the vesicular acetylcholine transporter. I. Central nervous system. Neuroscience 84: 331-359.

60. Armstrong DM, Rotler A, Hersh LB, and Pickel VM (1988) Localization of choline acetyltransferase in perikarya and dendrites within the nuclei of the solitary tracts. J Neurosci Res 20: 279–290.

61. Kc P, Mayer CA, Haxhiu MA (2004) Chemical profle of vagal preganglionic motor cells innervating the airways in ferrets: The absence of non-cholinergic neurons. J Appl Physiol 97: 1508-1517.

62. Reis DJ, Ruggiero DA, Morrison SF (1989) The C1 area of the rostral ventrolateral medulla oblongata: A critical brainstem region for control of resting and refex integration of arterial pressure. Am J Hypertens 2:

96. Semba K, Reiner PB, McGeer EG, Fibiger HC (1989) Brainstem projecting neurons in the rat basal forebrain: Neurochemical,