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Hypoxic Preconditioning: The Multiplicity of Central Neurotransmitter Mechanisms and Method of Predicting Its Efficiency

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<http://dx.doi.org/10.5772/intechopen.80333>

Abstract

In rats, a single moderate hypobaric hypoxia (HBH) increased the resistance to severe hypoxia (SHBH). The HBH efficiency and neurotransmitter mechanisms of its preconditioning action were investigated by biochemical and pharmacological methods. It will be substantiated in the chapter: (1) HBH preconditioning has its own mechanisms that do not depend on an innate resistance to SHBH and prior hypoxic experience of rats; (2) the same preconditioning effect can be achieved by diverse neuronal pathways and synaptic plasticity means; (3) cholinergic and, presumably, serotonergic, GABAergic and/or glutamatergic systems of the caudal brainstem, cortex and some other brain structures are involved in HBH realisation; (4) the rate of sensorimotor gating estimated in the model of acoustic startle pre-pulse inhibition (PPI) predicts the efficiency of hypoxic preconditioning and (5) the cholinergic system, including $\alpha 7$ nicotinic receptors, is involved in the mechanisms of HBH-PPI-dependent preconditioning effects.

Keywords: hypoxic preconditioning, resistance to severe hypoxia, apnoea, adaptation to hypoxia, mechanisms of hypoxic preconditioning, brainstem, cortex, central neurotransmitter systems, pre-pulse inhibition in acoustic sensorimotor startle reaction, cholinergic system, nicotinic receptors

1. Introduction

1.1. Protective action of moderate hypoxia

It is well known that pathological factors (many poisons, pathogenic viruses) in low doses can initiate protective mechanisms and increase resistance to the corresponding pathologies. Similarly, it is possible to increase resistance to severe hypoxia or ischaemia by exposing the organism or organ to adaptation in conditions of moderate hypoxia [1].

Short-term hypoxic adaptation is a single 1- or 3-h moderate hypoxic continuous or intermittent exposure in which hypoxia alternates with normoxia or hyperoxia [2–5]. Such hypoxic adaptation is characterized by the mobilization of available cellular reserves and its effect may be manifested within 24 hours [2, 5–7]. In experimental practice, Murry et al. were the first to describe the protective effect of the short ischaemic exposure against ischaemic stroke, suggested a preconditioning term for this phenomenon [8]. Later, the same authors identified the reperfusion (re-oxygenation) as the second most important adaptive component [9].

Hypoxic factor is the main in ischaemic preconditioning. Therapeutic potential of the hypoxic and ischaemic preconditioning are closely related. Protection from hypoxic damage is also very relevant because the hypoxic component is involved in pathogenesis of many diseases. *In vivo*, protective action of the hypoxic or ischaemic preconditioning was identified in the diseases of brain [3, 10–15], heart [8, 16–18], liver [19–21] and kidneys [22, 23]. Direct hypoxic preconditioning or drugs that mimic the hypoxic protective response could reveal promising therapeutic targets. Today, understanding of the hypoxic or ischaemic preconditioning mechanisms is a high priority [15].

The brain is central in the problem of hypoxic adaptation not only as the most sensitive organ to hypoxia but also as the coordinator of the 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 are of fundamental importance. However, in search of key targets of hypoxic preconditioning, the neuronal mechanisms remain the least studied. It is the aspect of hypoxic preconditioning that is in the centre of our attention. On the other hand, the neuronal autonomous mechanisms of respiration and blood circulation in the norm and under severe hypoxic conditions are intensively studied. These data serve as a serious help in the analysis of neuronal mechanisms of hypoxic preconditioning.

Another important problem that we have tried to solve is the methodology for studying the mechanisms of hypoxic preconditioning.

2. Systemic reaction of autonomic systems to hypoxia

The primary and immediate response to hypoxia is always recorded in the autonomic respiratory and cardiovascular systems. Central representation, the neurons of both systems, is

located in the medulla oblongata and pons Varolii (caudal brainstem) and spinal cord. Autonomic systems are functionally closely interrelated by the “respiratory centre”, groups of respiratory neurons, which support respiratory rhythm [24–26].

It has been shown that the “hypoxic” response involves activation of the autonomic sympathoexcitatory reticulospinal pathways, primarily from the peripheral or central chemoreceptors and the stimulation of respiration, heart activity and blood circulation aimed at restoring the blood level of O₂ and CO₂ exchange and pH [27–32].

The sequence and development of hypoxia-induced events are carefully researched [30, 31]. In general, this systemic response is a result of the wide cooperation of different functional groups of neurons of the central autonomic respiratory and cardiovascular systems: sensory, primary and secondary chemo and baroreceptors of the nucleus tractus solitarius (NTS) and ventrolateral medulla (VLM); relay neurons; reticulospinal neurons (area C1 of the rostral VLM); efferent neurons of the preganglionic parasympathetic nuclei of cranial nerves and spinal motoneurons.

Another important adaptive express response to hypoxia is non-sympathetic activation of the cerebral blood flow that is based on the redistribution of blood flow towards the brain [31, 33–36]. Two centres have been detected: the “parasympathetic cerebrovasodilator centre” or in another way “dorsal facial area” (DFA) and the “medullary cerebral vasodilator area” (MCVA). Both centres are located in the rostralateral part of medulla oblongata (and DFA also partially covers the pons Varolii), and their innervation, including hypoxia, initiates elevation of the cerebral blood flow by parasympathetic [33, 37, 38] or relay cerebripetal pathways [34, 39]. Also, the connection of MCVA with the sympathetic vasomotor mechanisms is shown: in the sympathoexcitatory zone C1, the presence of the O₂-sensitive neurons of which activation excites the cerebripetal pathway; dependence of the cerebrovascular MCVA efficiency of safety of the reticulospinal pathway and existence of collaterals from the sympathoexcitatory neurons in the cerebripetal direction [34, 39].

All of these systemic reactions are revealed in cats [40] and respiratory reactions from those in rats [36, 41, 42] with high, intermediate and low resistance to very severe hypoxia (3% O₂). Differences between these groups were mainly in expression and duration of the responses before apnoea. Under moderate hypoxia, all the compensatory reactions, including cerebral blood flow elevation, are maintained during the whole session of hypoxic training in rats [36, 43, 44], and all of them are the physiological basis of hypoxic preconditioning [44–46].

It should also be noted that the structures of the forebrain are the most unstable to ischaemic/hypoxic injuries [47], and the most interesting have been shown to be the cortex and hippocampus, as these are the higher brain structures responsible for cognitive functions. Both the cortex and hippocampus interact with the cardiorespiratory systems, participating in the regulation of voluntary respiration and hypothetically adaptive reactions of the respiratory and cardiovascular systems [25, 26, 48, 49].

But we assumed that under moderate hypoxia conditions (10–12% O₂ for rat), the key role in the preconditioning belongs to the autonomic systems.

3. Autonomous regulation of respiration: overview of neuronal populations responsible for generation of apnoea

In our experiments on rats, the preconditioning effect of moderate hypoxia was evaluated under conditions of severe hypoxia by the time (T) until agonal inspiration (apnoea).

Thus, among the key neurotransmitters of caudal brainstem are of interest, involved in the generation of apnoea. The most studied in this respect are inhibitory neuromediators or neuromodulators of the opioid, serotonergic, GABAergic, glycinergic and adenosinergic systems. And we will certainly touch upon the cholinergic system as an object of our research, which, according to our data, occupies not the last place in the preconditioning mechanisms.

In the overview, special attention was paid to the synaptic transmission; since in our studies, we evaluated the response of the synaptic pool of the caudal brainstem and some other brain structures.

3.1. Opioid system

Opioids cause apnoea selectively through μ -receptors. The action of the majority of opioid analgesics is associated with the stimulation of μ -receptor type. However, μ -receptor agonists cause side effects, among them respiratory depression. It was shown in cats and rats that μ -receptor agonists morphine and/or fentanyl depressed respiration, initiated central apnoea or apneusis breathing [50–54]. μ -Receptors are widely distributed in the brain, but their mechanisms of action and targets are still poorly understood. According to some data, Böttinger Complex (BötC) and especially pre-Böttinger Complex (preBötC) are responsible for opioid-initiated destruction of respiration [51, 52]. According to other data, such opioid-sensitive sites are numerous in the brainstem [53]. An endogenous ligand of μ -receptors is β -endorphin. However, endorphinergic fibres or terminals in the caudal brainstem have not been described to date. Hormonal mode of distribution of endorphins through the blood is well known. In consideration of the chemical stability of the ligands of the opiate receptors, it is assumed that they penetrate into the respiratory centre through the cerebrospinal fluid [25].

3.2. Serotonergic system

I.v. administration of serotonin (5-hydroxytryptamine, 5-HT) or 5-HT₃ receptor agonist phenylbiguanide provoked “von Bezold-Jarisch” or C-fibres reflex (bradycardia, drop in blood pressure, apnoea) passing, the authors believe, through 5-HT₃ receptors in the nucleus tractus solitarius NTS [55]. Really, bradycardia from the triad of Bezold-Jarisch reflex was potentiated by the i.c. administration of phenylbiguanide and was dose-dependently weakened by the i.c. administration of receptor antagonist granisetron [55]. Granisetron microinjected into NTS significantly attenuated both bradycardia and hypotension [55].

Under normoxic conditions (cat), both i.v. administration and microinjection into preBötC of 5-HT_{1A} receptor agonist 8-OH-DPAT produced apnoea and arrested respiratory neuronal activity [32]. Previously, it has been found that stimulation of the nucleus raphe obscurus provoked

that apnoea and 5-HT_{1A} receptors, which are abundantly expressed in the respiratory neurons of ventral respiratory group, were involved in this mechanism [56].

Under hypoxic conditions (cat), using microdialysis and registration of the phrenic nerve and respiratory neurons of the ventral respiratory group activity, it was shown that elevation of 5-HT levels in the extracellular space of the ventral respiratory group clearly coincided with the beginning of hypoxic depression and apnoea (5–10% O₂) [32]. The authors revealed that such high correlation with hypoxic depression was selective for 5-HT in this respiratory region because it was absent with the levels of other investigated mediators or modulators (GABA, glutamate and adenosine). In the same study, microinjection of 8-OH-DPAT into preBötC on the apneustic patterns background, initiated by prolonged moderate hypoxia (cat, 15% O₂), resulted in normal respiratory parameters. In contrast to the 8-OH-DPAT effects, blockade of 5-HT_{1A} receptors during hypoxia by antagonist NAN-190 resulted in dramatic enhancement of apneustic inspiratory activity patterns.

The molecular signalling pathway was later traced through these receptors on the glycinergic respiratory neurons of preBötC and, possibly, neighbouring regions of the ventral respiratory group [51]. The activation of 5-HT_{1A} potentiated glycinergic currents in all postsynaptic neurons receiving glycinergic inputs through glycine alpha₃ receptors (GlyR_{alpha3}) that not only excitatory (glutamatergic) but also inhibitory (glycinergic) neurons and enhanced their inhibition. It is proved that the 5-HT_{1A}-GlyR_{alpha3} signalling pathway can restore the respiratory circuitry and disturbed by hypoxia or some other factors (opioid intoxication) [26, 51].

In the rat, the long deep apnoea occurs when stimulation of special neurons within cluster of the serotonergic neurons in the medullary raphe nuclei (the raphe pallidus, magnus and obscurus) [57]. The point of these neurons is called the “midline apneic site” (MAS) and suggested their 5-HT_{1A} nature. By the morphoimmunological methods, the same researchers proved the relationship of MAS with many higher brain regions and some areas of the medulla oblongata, including the ventral respiratory group [58].

It was also revealed the action of 5-HT on the cerebral circulation. Intravenously (i.v.) injections of 5-HT (cat, rat) may have different regional actions on the brain blood vessels of various categories, but its decisive value was the development of cerebrovascular constriction, a decrease in the rate of cerebral blood flow and a drop in blood pressure [59], which was enhanced by ischaemic exposure [60]. All cerebrovascular reactions appeared similar or were more pronounced at i.c.v. injections (cat) or the application of 5-HT on the brain (rat) indicating the central nature of its actions [59]. It has been proven the central action of 5-HT in the DFA on the cerebral circulation. Injected into DFA, 5-HT or alaproclate, a 5-HT reuptake inhibitor, synaptically inhibited the glutamatergic activation of the parasympathetic preganglionic cholinergic motoneurons and thus reduced the rate of blood flow in the common carotid arteries [38]. Also, by i.v. administration of 5-HT, a significant drop in the rate of the cerebral circulation was revealed in the cortical parietal area as well as in the frequency and depth of breathing [61]. The drop in the rate of the cerebral circulation coincided with the accumulation of CO₂ in the blood. It should be noted that some serotonergic neurons in the **dorsal** raphe nucleus (in the midbrain), connected with MAS [58], have CO₂/pH chemoreception and deep hypercapnia (9% CO₂) produced an increase in their firing rate [62].

These data suggest that the reduction of the serotonergic influences on, presumably, any site of the autonomous cardiorespiratory regulation, except the ventral respiratory group facilitates breathing and/or conduces to a delay of generation of apnoea.

3.3. GABAergic system

GABA side by side with glutamate is the most widespread mediator in the central nervous system and is involved virtually in all nervous processes. Central GABAergic effects on the cardiorespiratory functions are not unidirectional. GABA is a principal inhibitory neurotransmitter of the sympathoexcitatory and baroreflex sympathoinhibitory glutamatergic pathways from peripheral chemo- and baroreceptors through the second-order sensitive neurons of NTS [63, 64]. The GABAergic neurons of caudal VLM are interneurons in the baroreceptor reflex arc and directly inhibit the sympathoexcitatory C1 zone neurons of rostral VLM [64].

In the NTS (rat), the agonist of GABA_B receptor baclofen attenuated the cardiorespiratory reflexes of C-fibres, provoked by phenylbiguanide, bradycardia and decrease in frequency of breathing with no effect on hypotension and apnoea when microinjected into any point of dorsomedial NTS in dose of 60 pmol [65]. When it was injected into the inhibitory zone of the dorsal respiratory group of NTS only (0.5–0.6 mm caudal to the obex [66]), baclofen removed C-fibre-provoked apnoea and the antagonist of GABA_B receptor CGP 35348 (2.8 nmol) newly restored it. Similar effect was obtained by the systemic administration of high doses of the GABA_B receptor agonists hydroxybutyrate and phenibut (6.9 mmol/kg, i.v., and 2.3 mmol/kg, I.P., respectively, rat) [67, 68]. Both agonists attenuated the decrease in the frequency of breathing and abolished or shortened the duration of apnoea of C-fibres reflex, which are provoked by 5-HT. Hydroxybutyrate (i.v.) and phenibut (I.P.) caused a complete loss of sensitivity of the respiratory system to vagotomy are supposed by central blocking transmission of afferent impulses from the baroreceptors of lungs and airways to the second-order barosensitive neurons in NTS [54, 67].

Under the normoxic conditions, baclofen also prolonged the inspiration and increased the heart rates injected into NTS in the same dose of 60 pmol in rats [65]. In the high doses in cats, in hundreds nmoles for baclofen and micromoles for GABA and sodium hydroxybutyrate, the decrease in respiratory frequency and apneusis breathing arose in the majority of intact animals when GABA or the GABA_B receptor agonist was microinjected into the dorsal respiratory group region (ventrolateral NTS) [69]. The same respiratory reactions were obtained after the i. v. administration of hydroxybutyrate in cats and rats [25, 69] and after the I.P. administration of phenibut in rats [54].

Under hypoxic conditions, similar to our HBH (10% O₂, 45–50 minutes, rat), participation of GABA in respiratory mechanisms of hypoxia was investigated [43]. Moderate hypoxia initiated a primary pronounced ventilatory increase (minute respiratory volume) followed by a gradually decline to a second-stable level above pre-hypoxic level and a sustained increase in respiratory frequency during the hypoxic exposure. Under these conditions, GABA had a depressant effect on the ventilation. By *in vivo* microdialysis, the elevation of GABA concentration in NTS coincided with the ventilatory decline. By microinjections into sensitive non-apnoeic

region of NTS of agonists and antagonists of GABA_A and GABA_B receptors, both the agonists muscimol (150 pmol) and baclofen (400 pmol) were injected 10 minutes before the hypoxic exposure significantly attenuated the early increase of ventilation, and on the contrary, the antagonists of GABA_A receptor bicuculline and of GABA_B receptors saclofen (400 pmol) and CGP-35348 (2.5 nmol) in the 40-minute hypoxic exposure abolished the late ventilatory decline and reduced the GABA elevation [43]. The authors have shown that for GABA activation in NTS, peripheral chemoreceptor stimulation is essential because it is the normoxia or under denervation of the carotid body, GABA level in the NTS did not change and the effects of GABA antagonists did not appear.

Another study also showed that endogenous GABA in the NTS inhibits the carotid chemoreflex (rat) [70]. Microinjection of the selective GABA uptake inhibitor nipecotic acid into the commissural sub-nucleus of NTS attenuated the increases in respiration and elevation in arterial blood pressure elicited by carotid chemoreceptor stimulation. These effects were completely antagonised by the GABA_A antagonist bicuculline (20 pmol) but not by the GABA_B antagonist saclofen (400 pmol), injected into the same site.

I.v. administration of GABA_A receptor antagonist picrotoxin enhanced ventilation through an increase in respiratory frequency and minute tidal volume (rat) [42]. A severe hypoxia (3% O₂, rat) in the first few minutes, like in the moderate hypoxia, initiated the same dynamics in the increase of minute tidal volume and respiratory frequency [40–42], and picrotoxin (i.v.) significantly potentiated the activation of both respiratory functions (cat and rat) [42]. Also, the authors observed that these effects of picrotoxin were most pronounced in the high resistance rats compared with low and intermediate resistance rats. Moreover, it was found that under these hypoxic conditions, picrotoxin greatly extended the time before apnoea in all resistance rat groups [42].

Note that these data indicate that systemic administration of the GABA receptor agonists and antagonists reflects the central action of these drugs within NTS.

In the ventral respiratory group of VLM, GABAergic neurons have other effects and can have a protective action on respiration under hypoxic conditions. By *in vivo* microdialysis, the level of GABA (and glutamate) in the respiratory region of VLM increased transiently during early periods of severe hypoxia (5–10% O₂, cat), coinciding with augmented phrenic nerve activity and fell below the control levels during central apnoea [32]. The authors suggest that GABA may be important for regulation of level of enhanced respiratory network activity at the onset of hypoxia. In addition, in BötC and preBötC of VLM, the pacemaker nature of some GABAergic respiratory types of neurons is assumed [26, 71]. Microinjections of GABA into BötC facilitated respiration (increased the tidal volume) and into preBötC significantly inhibited respiration (reduced the tidal volume) [72]. The same lack of uniformity was observed under the action on GABA receptors of B and A subtypes at BötC/preBötC.

Participation of GABA_B receptors in preBötC and BötC respirator functions was revealed using agonist baclofen. Under the normoxic conditions, the influences through GABA_B were directed towards the respiratory depression when baclofen was administered into both BötC and preBötC [73] or BötC only and, on the contrary, towards the weak respiratory stimulation

when it was microinjected into preBötC [72]. These two studies were performed on rabbits (first) and rats (second). However, we believe that the main difference was in doses. It seems that baclofen is more selective at 15–25 times smaller dose (2 pmol) [72] and therefore caused the opposite effects and influenced the breathing of GABA_B receptors in BötC, which was more expressed than in preBötC. At the same time, it should be noted that GABA_B receptor stimulation by baclofen at BötC suppressed breathing in both doses.

The blockade of GABA_A receptors within these respiratory complexes by the antagonist bicuculline or gabazine disturbed respiration until apnoea [71, 73, 74]. Blocking GABA_A receptors by picrotoxin also disturbed respiratory rhythm and provoked apneusis breathing when it was injected into the fourth ventricle [42]. At the same time, microinjections of bicuculline into preBötC recovered the respiration against the background of apnoea caused by the blockade of GABA_A receptors in BötC [73].

Thus, the natural reduction of GABAergic synapses would help the delay of generation of apnoea by cutting back the impacts of GABA in the sympathoexcitatory C1 zone neurons of rostral VLM, through both GABA_A and GABA_B receptors in the sympathoexcitatory conductive sensory pathways and dorsal respiratory group within NTS and through at least GABA_B receptors in BötC and possibly GABA_A receptors in preBötC within VLM.

3.4. Glycinergic system

Glycinergic neurons of the medulla oblongata were identified in the ventral respiratory group. In BötC and especially preBötC, glycinergic neurons are more than half of all respiratory neurons [74]. It was shown *in vivo* that many of them generate the respiratory activity, that is, pacemakers [71]. Both in preBötC and in BötC, the blockade of glycine receptor by the antagonist strychnine resulted in the suppression of respiratory activity until apnoea [26, 51, 73] or disturbance of the respiratory cycle, the modification of activity of the post-inspiratory neurons and, as a consequence, the transfer of the normal three-phase cycle into the pathological biphasic [26, 75]. These data indicate that the inhibitory effects via glycine receptors are required for normal respiratory function.

By other data [76], glycine is also required for normal respiratory function in the dorsal respiratory group. In the intermediate sub-nucleus of NTS, the secondary barosensitive neurons receive glutamatergic afferent inputs from the pulmonary rapidly adapting stretch receptors and have inhibitory influences on respiration. The authors found that these secondary neurons receive the phasically acting inputs from glycinergic neurons, which inhibit their activity in the inspiratory phase.

In another networks of the medulla oblongata, participation of glycine was identified as a sympathoexcitatory or sympathoinhibitory modulator. Sympathoinhibitory influence on glutamate pathway from caudal to rostral VLM was mediated by glycine in a manner independent of GABA_A and GABA_B receptors [77]. Microinjections of glycine into NTS decreased arterial pressure and heart rate [78], inhibited the pressor but not bradycardic responses produced by L-glutamate microinjection in the same site [79] and, at the same time, inhibited the depressor and bradycardic responses to L-glutamate [78].

Thus, on the certain sites of the glutamatergic pathways of VLM and NTS, attenuation of the glycinergic influences may contribute to the delay of apnoea generation.

3.5. Adenosinergic system

Adenosine is present in the CNS at pharmacologically active concentrations [80–82], and it is now recognised as a neuromodulator. The extracellular concentration of adenosine in the brain increases dramatically during hypoxia or ischaemia [32, 80, 83]. Adenosine has a depressor effect on the neuronal activity through A1 and A3 receptor types and antagonistic effect through A2 receptors [83, 84]. Autoradiographic and immunohistochemical studies illustrate the presence of A1 and A2 (mainly A2a) binding sites/receptors in NTS, VLM and other brainstem regions that are important in cardiorespiratory control [81, 85].

The presence of the enzyme 5'-nucleotidase, which converts AMP to adenosine in the fractions of synaptosomes (cortex and hippocampus, rat) [86] and adenosine in the fraction of the synaptic vesicles (rat brain) [87], indicates the existence of adenosinergic pre-synapses in the brain. In addition, a high-affinity transport system for adenosine and adenosine deaminase, an enzyme of adenosine cleavage in the synaptic cleft, was revealed in the crude synaptosomal fraction of NTS (rat brain) [88, 89].

In the caudal brainstem, adenosine and as a rule, the following selective agonists and antagonists of A1 and A2a adenosine receptors used to study the role of adenosine in the regulation of cardiorespiratory functions: the agonist N6-cyclopentyladenosine (CPA) and antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) of A1 receptors; the agonist 2-[p-(2-carboxyethyl)-phenethylamino]-5'-N-ethylcarboxamide (CGS 21680) and antagonists 9-chloro-2-(2-furanyl)-5,6-dihydro-1,2,4-triazolo(1,5-c)quinazoline-5-imine (CGS 15943A) and 4-(2-[7-amino-2-[2-furyl][3,2,4]triazolol[2,3-a][1,3,5]triazin-5-yl-amino]ethyl)phenol (ZM 241385) of A2a receptors. Also, the non-selective adenosine receptor antagonist 8-phenyltheophylline (8-PT) and A1/A2 receptor antagonist 8-(p-sulphophenyl)theophylline (8-SPT) with higher affinity for A1 receptors [85] were applied.

In the chemo- and baroreceptive sites of the NTS, opposite effects on cardiovascular parameters were revealed under the action of these two types of receptors in the intact rat brain. Microinjection into NTS of A1 receptor agonist CPA stimulated and of A2a receptor agonist CGS21680 decreased the blood pressure, and both agonists provoked bradycardia [82, 85, 90–92]. It was found that the opposite effects of A1 and A2a receptor agonists on blood pressure are due to the action on the glutamatergic afferent nerve fibres from arterial and pulmonary baroreceptors. Accordingly, the stimulation of A1 receptors inhibited glutamate release, and the stimulation of A2a receptors activated it [91, 92]. The effects of A2a receptors considerably dominated over A1 receptors in NTS [82, 90, 91], and the decrease of blood pressure in all of these studies was removed by the antagonists of A2a receptor CGS15943A or ZM241385 and of A1 receptors agonist DPCPX, while bradycardia was selectively antagonised by CGS15943A but not by DPCPX. The same agonist of A1 receptors CPA had the hypotensive action followed by microinjections into NTS at doses far exceeding those used in the cited studies and was antagonised by i.v. infusions of A1 receptor antagonist DPCPX [93].

From the above data, under certain conditions of the disturbance of cardiorespiratory functions, the NTS A2a receptors in concert with A1 receptors are involved in the activation of the sympathetic functions. At the same time, the decrease of synaptic influences on the A2a receptors and possibly on the A1 receptors of NTS could contribute to the delay of apnoea generation.

Stimulation of 5HT₃ receptors by agonist phenylbiguanide (i.v. injection) initiated "cardiopulmonary chemoreflex" (hypotension, bradycardia and apnoea) [82, 94], aka reflex of C-fibres or von Bezold-Jarisch in other sources. This reflex was attenuated/blockaded or conversely potentiated by microinjections into NTS of the A2a receptor agonist CGS21680 or antagonist ZM241385, respectively [82]. The same reflex, when developed under a severe haemorrhage model, was inhibited by endogenous adenosine; this inhibition was removed by microinjections into NTS of the antagonist 8-SPT [82]. The authors suggested that A2a receptors were responsible for the activation of inhibitory influence on the "cardiopulmonary chemoreflex" pathway and later proved a participation of GABA_A and much weaker GABA_B receptors in this mechanism within NTS [94].

The role of A1 receptors in the cardiorespiratory functions was revealed in the VLM, where this receptor type had the highest density [85]. In the rostral VLM (rat), microinjections of adenosine into the pressor zone C1 augmented the sympathoexcitatory reflex of increase in blood pressure evoked by electrical stimulation of the "hypothalamic defence area" and, on the contrary, the microinjections of 8-SPT into C1 or both peripheral and central injections of the selective A1 receptor antagonist DPCPX this reflex reduced [80]. Vice versa, microinjections of adenosine into the ventral respiratory group (cat), acting on pre- and postsynaptic A1 receptors, led to the depression of spontaneous and stimulus-evoked synaptic activity of the respiratory neurons and to the fall of mean respiratory drive potentials. The depressive effects of adenosine were abolished after the i.v. administration of the antagonist DPCPX [95].

Under severe hypoxia (5–10% O₂, cat), i.v. DPCPX administration retained the same direction of action on respiration. This A1 antagonist showed marked protective properties, namely preventing the early hypoxic depression of stimulus-evoked activity of the respiratory neurons in the ventral respiratory group, significantly delayed the onset of apnoea and reduced the recovery time [95]. In the same hypoxic conditions in the ventral respiratory group using microdialysis, an intensified increase of a level of the endogenous extracellular adenosine was identified, and this reaction was developed in the background of the apnoea after it began [32]. The authors noted that the hypoxic release of adenosine was occurred surprisingly late than hypoxic depression of respiration and cannot be responsible for the onset of the hypoxic depression of respiratory neurons, and that it is noteworthy that increases in adenosine levels outlasted hypoxic periods, which were not associated with pronounced depression of phrenic nerve activity.

However, endogenous adenosine may be involved in the mechanism of a secondary suppression of the hypoxic activation of the cerebral blood flow. As mentioned above, the fall in the rate of cerebral blood flow under the von Bezold-Jarisch reflex conditions also occurred after the apnoea beginning in the background of its end and coincided with the accumulation of CO₂ in the blood [61]. Under severe hypoxia (8% O₂, rat), i.v. administration of the non-selective antagonist 8-PT, penetrating through the blood-brain barrier unlike not penetrating 8-SPT, potentiated hypoxic alkalosis and hypocapnoea that arise from the initial hyperventilatory

response, extended the increase in tidal volume and heart rate, reduced the decrease in arterial pressure, stopped the progressive increase of the carotid vascular conductance and, at the same time, showed a pronounced tendency for cerebral blood flow to be better maintained during hypoxia [31, 96]. The authors proved that all effects of 8-PT were central and were a consequence of the influence of the antagonist on the secondary fall in ventilation.

Taken together, under hypoxia, endogenous adenosine of the ventral respiratory group can contribute to the fall in the ventilation and, as a consequence, initiate hypercapnia. Accordingly, attenuation of these effects of adenosine can contribute to maintaining the cerebral blood flow.

3.6. Glutamatergic system

It should also be added that apnoea can be also caused by microinjection of the main excitatory neurotransmitter glutamate into certain sites of the medulla. Therefore, the neurons of the raphe nuclei were stimulated by glutamate for apnoea in MAS [57]. Also, apnoea occurred when glutamate was microinjected into the inhibitory site of the respiratory neurons of the dorsal group of NTS (behind the obex) [66]. Apparently, it is the action of the described above glutamate afferents triggering baroreflex [64, 78]. In NTS, these afferents switched on bulbar interneurons, mainly glutamatergic, which, as shown, transfer drive signals to the parasympathetic motor nucleus ambiguous and stimulate bronchoconstrictor reflex [97] and also through the caudal VLM, having an inhibitory action on the sympathoexcitatory reflex [64].

3.7. Cholinergic system

Starting from Loeschcke studies [27, 98], the central effects of ACh and its analogues on the respiration and blood circulation are intensively investigated. As for many other neurotransmitters, the cholinergic participation is detected in the majority of functional sites of cardiorespiratory networks as well as the ambiguity of the cholinergic effects depending on drug dose, application site and reception [39, 99].

Caudal brainstem structures include several cholinergic sources: (1) projections from the reticular formation of the midbrain tegmentum [100–103]; (2) afferents of the nodose ganglion sensory neurons from the lung mechanoreceptors to the NTS [97, 104, 105] and (3) the neurons of pons Varolii and medulla oblongata, including reticular areas, NTS and efferent parasympathetic preganglionic neurons of the motor cranial nerves nuclei [99, 102, 103, 105–107].

Concerning the connections and functional effects of the cholinergic system of the caudal brainstem in response to hypoxia, we recently published an overview similar to the above [108, 109].

4. Preconditioning effects on resistance to severe hypoxia and synaptic pool of caudal brainstem, cortex and hippocampus

The sub-chapter briefly describes our experimental approaches on the study of neuronal mechanisms of hypoxic preconditioning. During the planning of experiments, we were guided

by the data that the short-term adaptation, especially after continuous hypobaric hypoxia, had the pronounced and rapid preconditioning effect in the first minute of re-oxygenation [2, 6, 7].

The experiments were carried out according to two protocols.

4.1. General experimental conditions and procedures

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 November 24, 1986 (86/609/EEC).

Hypoxic models. Hypoxic preconditioning, the continuous hypobaric hypoxia (HBH): an altitude of 5000 m (11% O₂), 60 minutes. Test for resistance to hypoxia, severe hypobaric hypoxia (SHBH): the critical altitude of 11,500 m (4.5% O₂). In the latter case, resistance to hypoxia was recorded with respect to time (T) until agonal inspiration (apnoea) in combination with a loss of voluntary control of body tone. Apnoea was a defining attribute.

Re-oxygenation after HBH. Four minutes.

Brain structures for biochemical investigations. The caudal brainstem, cortex and hippocampus.

Preparative methods for biochemical investigations. From each brain structure, the sub-fractions of synaptic membrane and synaptoplasm were isolated from the fractions of “light” and “heavy” synaptosomes by routine preparative methods using discontinuous sucrose gradients.

The sub-synaptic level of fractionation made it possible to study the largest functionally different pre-synaptic compartments, and it was very informative. Moreover, synaptic membrane sub-fractions were considerably cleaned from glial, mitochondrial and free (not docked) synaptic vesicle contaminations.

Analytical methods. In the sub-synaptic fractions, the choline acetyltransferase (ChAT, functional marker of cholinergic neurons) activity by radiometric method [110] and protein content by spectrophotometric method [111] were assayed.

For details of the experimental procedures, see [112–114].

4.2. Experimental protocol number 1

It is important to bear in mind that animals (and humans) are very different in their resistance to severe hypoxia, and this implied different mechanisms. Because of this, since the publication of Purshottam and Ghosh [115], animals were divided into resistance to severe hypoxia, using pre-testing them under the same hypoxic conditions [116–118]. Later, the pre-testing under severe hypoxia was applied to rats for the investigation of mechanisms of hypoxic preconditioning [2, 7] and in our experiments [108, 109, 113, 119].

So, in our experiments (**Figure 1**) in each sample, most of the rats were pre-tested under SHBH and divided into groups of low, high and intermediate resistance to hypoxia with T₁ < 3.5 minutes, T₁ > 7 minutes and between them, respectively. For the following 4–5 weeks, all pre-testing rats

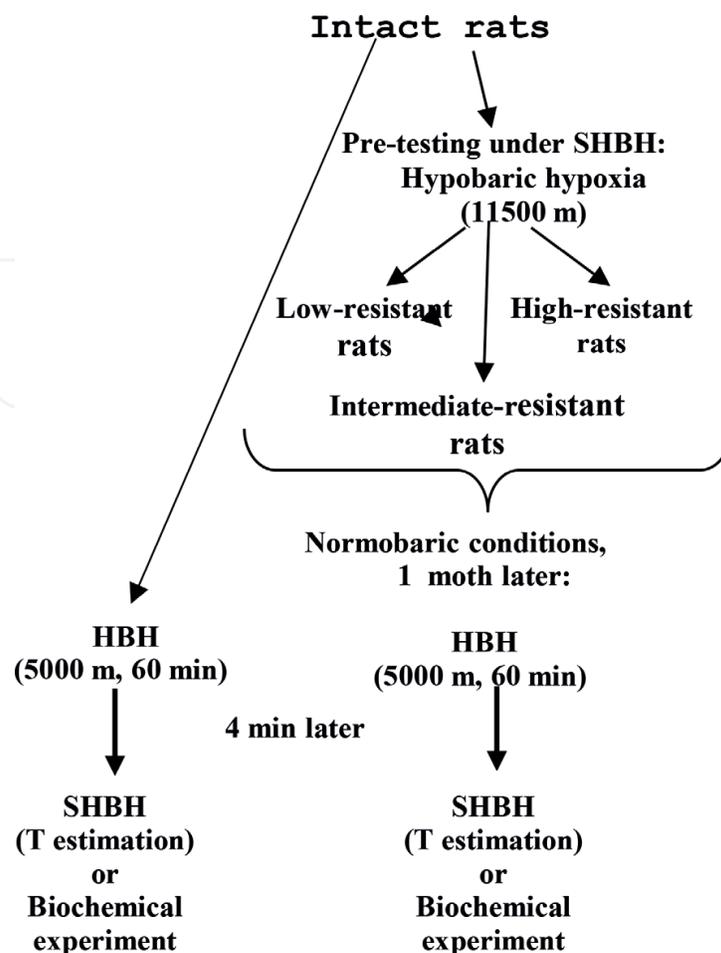


Figure 1. Scheme of experimental protocol number 1.

were kept under standard vivarium conditions after which the rats in each pre-tested group and the rats in not pre-tested group (intact group) were sub-divided into experimental (HBH) and control groups, and the rats of all experimental groups were subjected to a single HBH session. Four minutes after the end of HBH, the rats from each experimental group, which were subjected to SHBH and T2 (or T1 in intact group), were estimated or taken in the biochemical experiment. The control groups underwent all the procedures after HBH simultaneously with the corresponding experimental groups.

4.3. Experimental protocol number 2

In these experiments, the pre-testing under SHBH was excluded. Instead, all rats were pre-tested in the model of acoustic sensorimotor startle reaction, and the magnitude of pre-pulse inhibition (PPI) was estimated [120]. Two to four days after pre-testing, the experimental rats were subjected to a single HBH session, and 4 minutes after the end of HBH were subjected to SHBH (as in Scheme number 1). The control rats underwent all the same procedures except HBH.

Using this experimental scheme, pharmacological experiments were also carried out (Figure 2). The cholinergic nicotinic mechanisms of HBH preconditioning were investigated using the selective agonists of nicotinic receptors (nAChRs) $\alpha 4\beta 2$ type metanicotine RJR 2304 (RJR) and $\alpha 7$ type

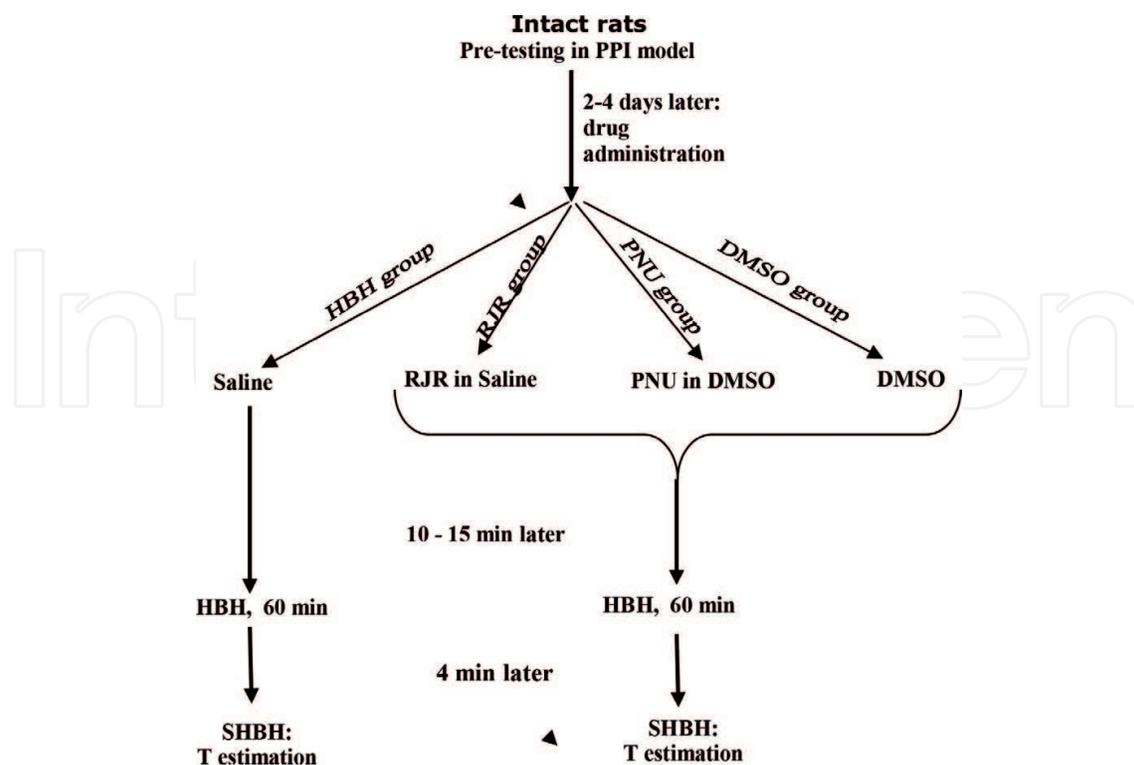


Figure 2. Scheme of experimental protocol number 2.

PNU-282,987 (PNU, Tocris Bioscience, Bristol, UK for both agonists) and a bipolar aprotic solvent for PNU dimethyl sulfoxide (DMSO, LLC “Tula Pharmaceutical Factory”, Tula, RF).

Rats in the RJR group received a single I.P. injection of RJR (26 nmol/kg, $n = 8$) in the physiological saline. Rats in the PNU group received a single I.P. injection of PNU (26 nmol/kg, $n = 23$, or 260 nmol/kg, $n = 12$) in 3% DMSO. Rats in the DMSO group received a single I.P. injection of 3% DMSO ($n = 16$). Rats in the HBH group received a single I.P. injection of the saline ($n = 23$). Both drugs, DMSO and saline, were injected 10–15 minutes before HBH session.

5. Experiments on the protocol number 1

5.1. The effect of HBH on the resistance of rats to SHBH

HBH markedly increased the mean values of the resistance of rats to SHBH in all the investigated groups (Figure 3). After HBH session, all rat groups showed a similar range of values for resistance to SHBH. In fact, the T values of these groups formed the same variational series (Figure 4) [113].

In biochemical experiments, the reaction on HBH of synaptic pool of caudal brainstem and cortex (no reaction was shown in the hippocampus) in the low- and high-resistant rats and intact rats showed that the same preconditioning hypoxic effect can be achieved by various neuronal pathways and plastic synaptic tools. For a detailed analysis, see [108, 109, 112]. Briefly, it was revealed in the following.

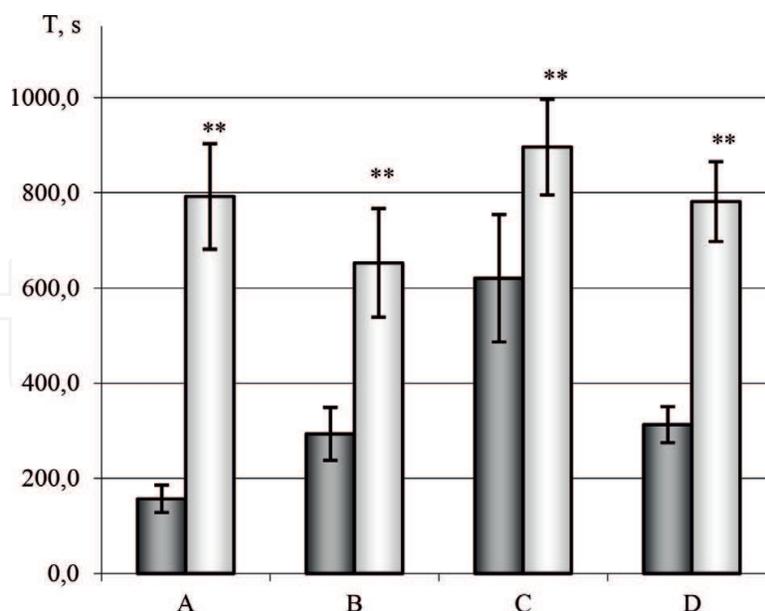


Figure 3. Preconditioning effects of HBH on the resistance to SHBH of the low-resistant (A), intermediate-resistant (B), high-resistant (C) and intact (D) rats. *T* values, a time before apnoea, are expressed as means \pm SE. For each group of bars: grey bars, T2 values in the control pre-tested rat groups (A, B, C; $n = 12, 9, 14$, respectively) and T1 value in the control intact rat group (D; $n = 18$); light bars, T2 (A, B, C) or T1 (D) values in the corresponding HBH groups ($n = 11, 8, 10$ and 19 in A, B, C and D, respectively). ** $p < 0.025$ compared to the respective control, Fisher's exact test.

5.1.1. In the low-resistant rats

In the low-resistant rats, in the caudal brainstem (**Figure 5a**), the inhibition of water-soluble ChAT activity in the pre-synapses of heavy synaptosomal fraction corresponded to the functional characterisation of subtypes of the lung barosensitive C-fibres conducting afferentation to NTS through the nodose ganglion [121, 122]. It is known that apnoea is often preceded by the classic reflex of C-fibres (frequent shallow breathing, bradycardia and hypotension) [65, 122, 123]. We substantiated that the cholinergic C-fibres could act on nAChRs affecting theirs through secondary cholinergic barosensitive neurons and the weakening of their influences led to the suppression of parasympathetic reflexes occurring in NTS and thereby to the augmentation of resistance to SHBH.

5.1.2. In the high-resistant rats

In the high-resistant rats, in the caudal brainstem (**Figure 5b**), HBH provoked inhibition of the water-soluble ChAT activity in the pre-synapses of light synaptosomal fraction. We substantiated that the nerve endings of this rat group may be outside NTS. Additionally, a correlation was found between the HBH-induced changes in activity of water-soluble ChAT in caudal brainstem and membrane-bound ChAT in pre-synapses of cortical projection neurons (**Figure 6b**, the cortical light synaptosomal fraction [124, 125]) ($r = +0.911, p < 0.02, n = 6$, Pearson's correlative test). This allowed us to assume that the inhibition of the water-soluble ChAT activity under HBH conditions in this group of rats occurred in pre-synapses of the projection neurons from laterodorsal (LDT) and/or pedunclopontine (PPT) tegmental cholinergic nuclei of the middle brain. LDT and more intensively PPT send plurality of the fibres to both the pontine and the

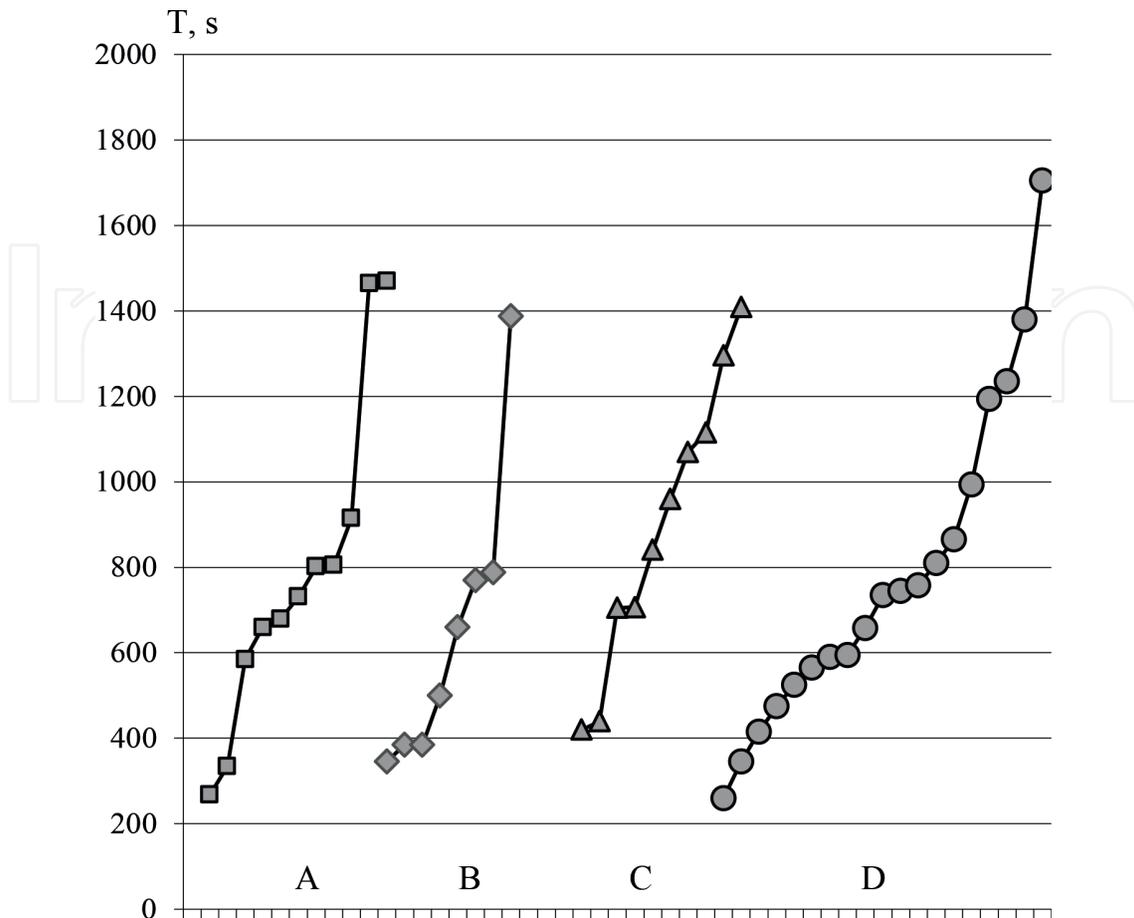


Figure 4. Individual T values of the rat resistance to SHBH after HBH in the low-resistant (A), intermediate-resistant (B), high-resistant (C) and intact (D) rat groups. Resistance to SHBH demonstrates that T values of all HBH groups formed the same variational series. $n = 11, 8, 10$ and 19 in A, B, C and D as in the corresponding HBH groups in **Figure 3**.

medulla oblongata nuclei [101, 102, 126] and also to the cortical cholinergic projection neurons of the basal forebrain nuclei [126].

In the high-resistant rats, in the caudal brainstem (**Figure 5b**), a simultaneous decrease in the content of synaptic c- and m-proteins ($r = +0.871, p < 0.05, n = 6$) in the heavy fraction in non-cholinergic pre-synapses (correlation between cChAT activity and c-protein content is absent) suggests the possibility of reduction of the number of synapses in non-cholinergic neurons.

According to the literary data in sub-chapter 3, in the first place, the serotonergic system is reported to be involved in the provocation of apnoea in all of the studied key areas of the autonomic regulation of the cardiorespiratory functions. At the same time, the reduction of the influences of any analysed neurotransmitter systems at corresponding sites may be involved in the mechanisms of the hypoxic preconditioning. It was therefore necessary to analyse the presence of pre-synapses of these neurotransmitter systems in the heavy fraction of synaptosomes, and their representation in the fraction must be enough to identify their reduction by means of such non-specific parameters as protein content.

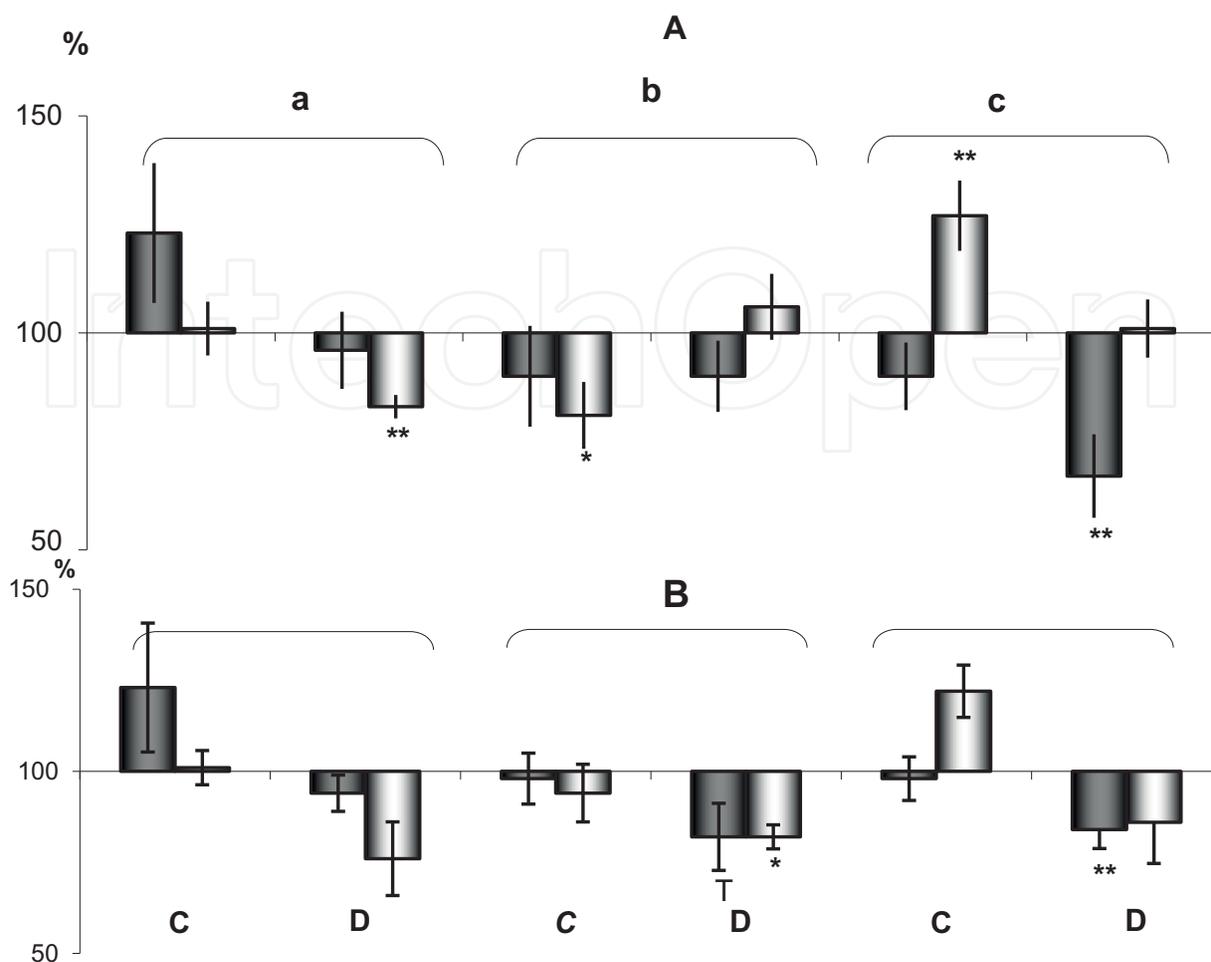


Figure 5. The effect of a single HBH session on the ChAT activity (A) and protein content (B) in the sub-synaptic fractions of the caudal brainstem in the pre-tested low-resistant (a) and high-resistant (b) rat groups and in the intact rat group (c). The values of ChAT activity and protein content are expressed as means \pm SE. (C) Sub-fractions of light synaptosomes; (D) sub-fractions of heavy synaptosomes. In each pair of bars: left (dark) bar, sub-fraction of synaptic membranes; right (light) bar, sub-fraction of synaptoplasm. The data are shown as percentages as compared to the control, which was taken as 100%. * $p < 0.05$; ** $p < 0.025$, Fisher's exact test..

5.1.2.1. Mediator composition of the heavy fraction of synaptosomes

Analysis of the synaptosomes with the above mediator specificity showed that, as expected, synaptosomes with any mediatory specificity have a wide range of density and sizes. This is shown for glycine, glutamate, serotonin and GABAergic pre-synapses under fractionation in continuous sucrose density gradient [127–129]. Also, in discontinuous sucrose density gradient, serotonin, glutamate and GABAergic pre-synapses were revealed in the light and heavy fractions of synaptosomes [130–135].

In sucrose-percoll gradient, the adenosinergic pre-synapses were isolated in the percoll interlayer 10–16% [86]. This percoll fraction apparently corresponds to the heavy fraction of synaptosomes in the sucrose gradient. This is indicated by a similarity in size of synaptosomes ([136, 137] compared with our data [124]) and a significant percentage of the free mitochondria

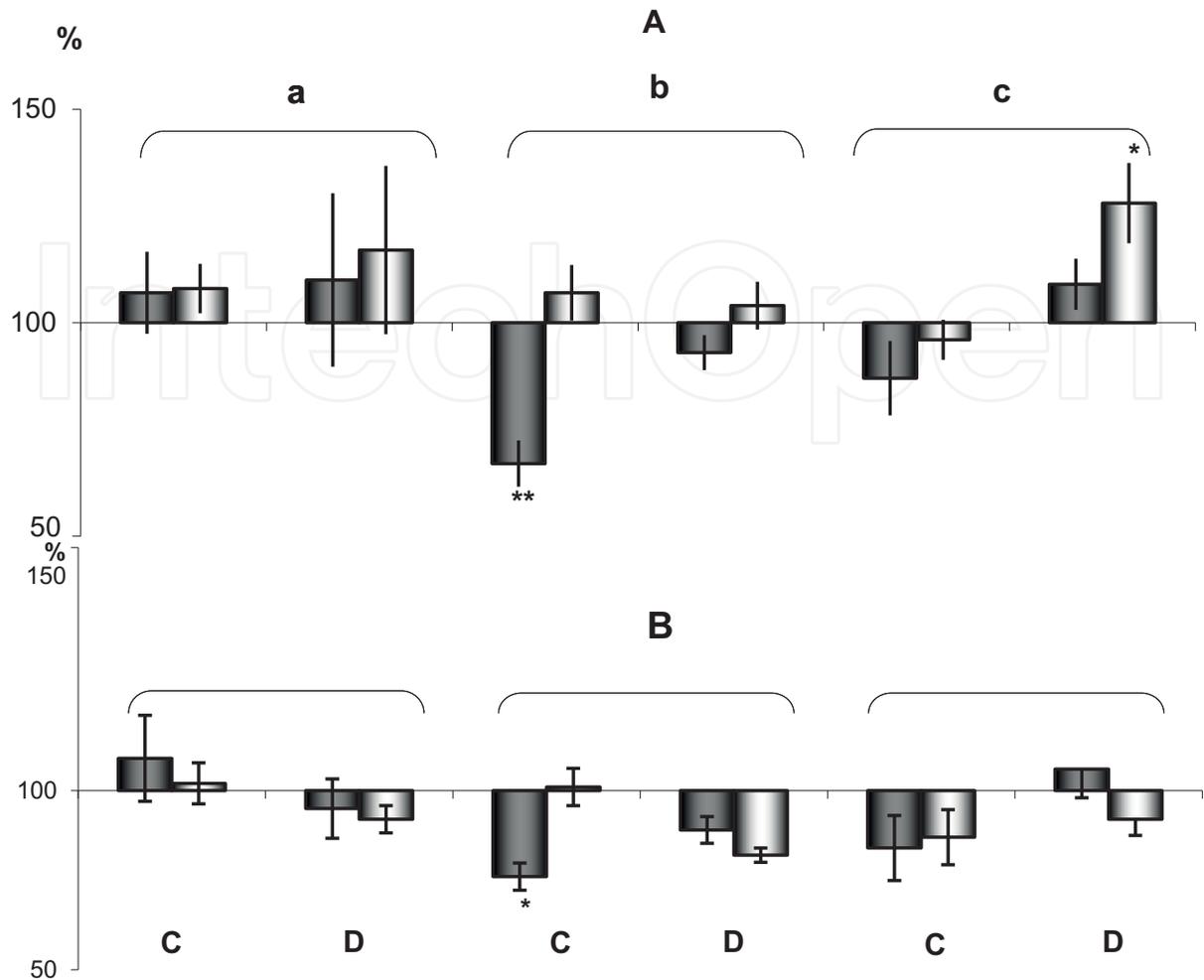


Figure 6. The effect of a single HBH session on the ChAT activity (A) and protein content (B) in the sub-synaptic fractions of the cortex in the pre-tested low-resistant (a) and high-resistant (b) rat groups and in the intact rat group (c). Designations are as shown in Figure 5.

in the fractions. It is known that the density of a substantial part of the free mitochondria coincides with the density of synaptosomes with an expressed vector of the concentration of the mitochondrial organelles towards denser layers of sucrose [128]. As a result, the mitochondria are present in large numbers in the heavy fraction of synaptosomes (20–40% and more) [124, 134], while they were revealed only as the single irregular inclusions in the light fraction [124, 134, 138]. The similar pattern is observed in the percoll gradient [137].

Therefore, the pre-synapses of all listed mediatory systems are present in the heavy fraction of synaptosomes with the exception of the opioid system, which synaptic transmission is absent in the caudal brainstem.

In accordance with the above literature, any of them dominate the heavy fraction or constitute at least half of the quantity/activity of the corresponding mediatory marker in the light fraction of synaptosomes. However, it is turned out in our case that the ratios between mediators within the light and heavy fractions are not as important when compared with their ratios

within the heavy fraction. It is well known that glutamate and GABA are the prevailing neurotransmitters in any brain formation. This is manifested in synaptosomal fractions. For example, according to a comparative study of Johnson and Roberts [134], in the heavy fraction of the whole mouse brain, the content of glutamate-GABA-glycine-5-HT in the percentage distribution was 52-36-4-9%.

The relationships in the fraction between the protein content of the pre-synapses with different mediators would be similar. Therefore, the percentage of the sought-for mediator in the heavy fraction should be more than 18%, that is, above the fall in the protein content in our research. This requirement is consistent only with glutamate and GABA. According to the strictest calculation, they will dominate and be more than 33–23% in the heavy synaptosomal fraction when you consider that the entire brain glycine level is concentrated in the caudal brain stem [129] and that the heavy fraction also includes pre-synapses with some other “small “mediators (adenosinergic, cholinergic, etc.).

However, if GABA is the main inhibitory neurotransmitter of the brain, glutamate is the major excitatory neurotransmitter, including their physiological effects. Therefore, in apnoea, mechanisms may be involved only in a small part of a total pool of glutamate neurotransmission. Nevertheless, it is reasonable to assume that the pre-synapses of the powerful glutamatergic afferents in NTS are concentrated in the heavy fraction of synaptosomes by similarity with the pre-synapses of the cholinergic C-fibres, and thus, their concentration may be sufficiently representative in the heavy fraction of the caudal brainstem.

Taken together, it seems that GABAergic or glutamatergic neurons are the principal candidates in the hypoxic preconditioning mechanism of the reduction of the pre-synapses in the corresponding apnoeic sites of the caudal brainstem of the high-resistant rats. Perhaps HBH initiates signalling to pre-synapse reduction in the multiple mediatory neuronal populations (and in this case, the greatest interest represents the 5-HTergic system) but with the obligatory participation of GABAergic or glutamatergic neurons.

5.1.3. In the intact rats

In the intact rats, in the caudal brainstem (**Figure 5c**), HBH provoked an interconnected increase in the cChAT activity and c-protein content in the light synaptosomal fraction ($r = +0.928$, $p < 0.02$, $n = 6$) and a decrease in the mChAT activity and m-protein content ($r = +0.933$, $p < 0.02$, $n = 6$) in the heavy fraction. Changes in the activity of mChAT and content of m-protein in the heavy fraction were inversely proportional to the changes in cChAT activity and c-protein content in the light fraction ($n = 6$: mChAT-cChAT, $r = -0.962$, $p < 0.02$; m-protein-c-protein, $r = -0.921$, $p < 0.05$). Note that significant interfraction correlations between the light and heavy synaptosomal fractions were not found after HBH in the pre-testing rat groups.

We believe that in the intact rats, 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, cChAT activated in the transformed pre-synapses [112].

Activation of acetylcholine synthesis and non-quantum leakage in response to HBH points to the direct involvement of the relevant neurons in the preconditioning mechanisms in the intact caudal brainstem. Several respiratory-related sites exist in the VLM in which acetylcholine stimulated breathing and maintained an inspiration through mChR and/or nAChRs. Also, innervation of DFA by acetylcholine through nAChRs initiated the elevation of cerebral blood flow [108, 109].

In the intact rats, cChAT was activated in the cortical interneurons (**Figure 6c**, the heavy fraction of synaptosomes [124, 125]). There was no correlation between cChAT activity in the caudal brainstem and cortex in this rat group because of the absence of a direct link between the brain stem neurons and the cortical cholinergic interneurons.

Acetylcholine synthesis activation 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 [139–141]. Thereby in intact brain, the cortical cholinergic interneurons might be involved in the local mechanisms to maintain the cerebral blood flow.

Thus, 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 apnoea appeared in the pre-tested rats. Apparently, the single pre-testing under SHBH altered synaptic and neuronal preconditioning mechanisms. The variety of neuronal pathways to achieve the same physiological effect demonstrates a great adaptive potential of brain. It seems, such adaptive possibilities are mortgaged by the composite, netted organisation of the respiratory centre. But it is not known whether all these mechanisms will go in the intact rats if theirs to activate, for example, pharmacologically.

In the total, HBH preconditioning eliminates the differences in resistance to SHBH between the intact, high- and low-resistant groups of rats with different innate resistance to severe hypoxia and prior hypoxic experiences. The same preconditioning effects of HBH in the intact rats and pre-tested under SHBH can be explained only by the fact that HBH preconditioning is realised by its own mechanisms, which do not depend on innate resistance to SHBH and prior hypoxic experiences.

At the same time, the resistance to SHBH initiated by HBH showed high rat-to-rat variability. So, the problem appeared to be the absence of methods for prediction of efficiency of hypoxic preconditioning.

Recently, such test was detected. It was a pre-pulse inhibition (PPI) estimated in the model of the acoustic startle reaction.

6. Experiments on the protocol number 2

It was found a correspondence between the values of PPI and T initiated by HBH, and the HBH efficiency was reliably and negatively correlated with PPI (**Figure 7**). The PPI in acoustic sensorimotor startle reaction is a well-known model that was developed in the second half of

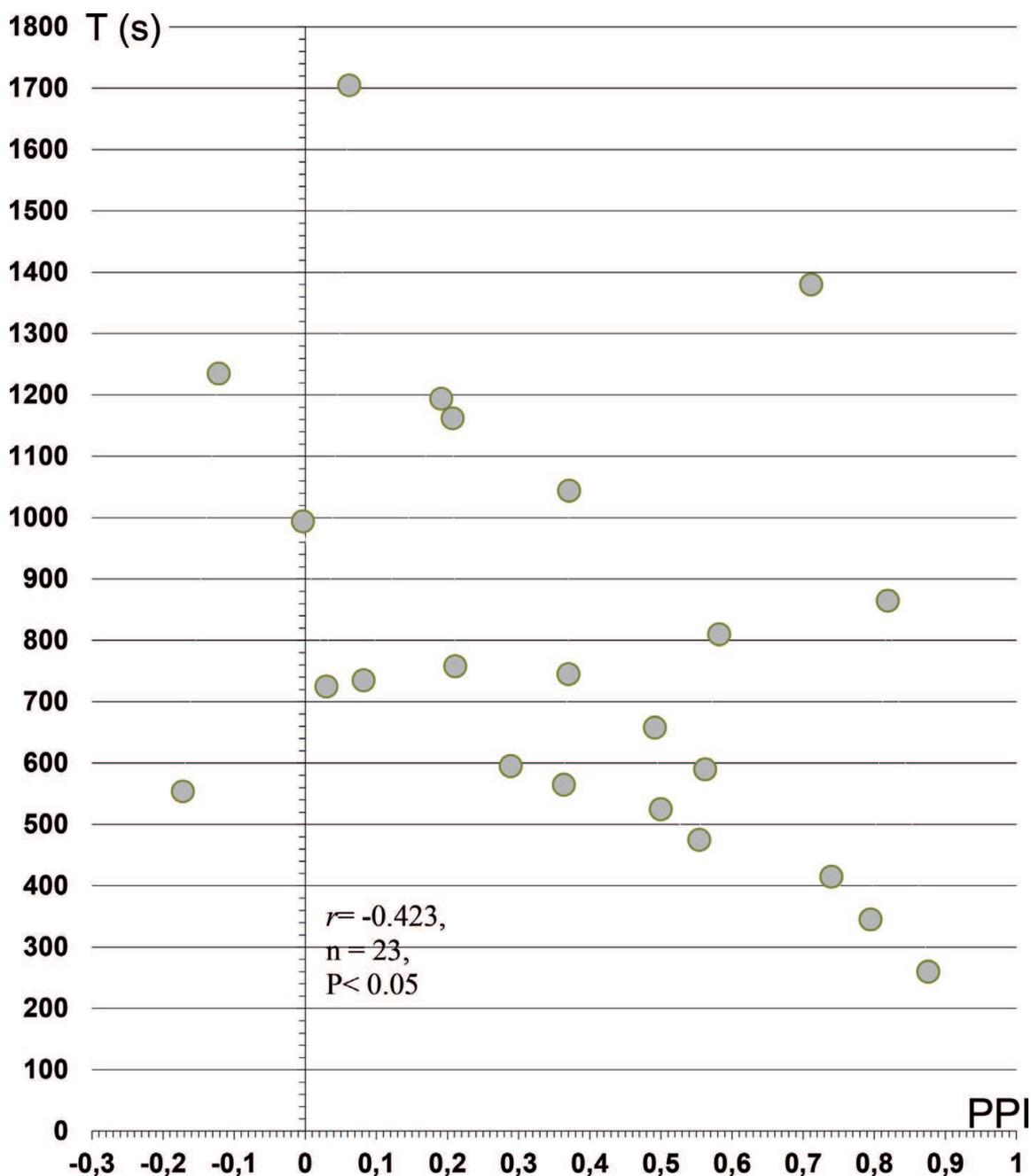


Figure 7. The graph of dependence of the HBH preconditioning efficiency (T) on the rate of PPI. T, a time before apnoea. Grey marks, individual values of the rat resistance to SHBH after HBH. The significant negative correlation takes place between T and PPI values, Pearson's *r*-criterion test.

twentieth century for neurobiology, especially psychiatry [142], and these were the first direct experimental data to report the relationship between PPI and hypoxic preconditioning pathways (RF patent 2571603).

In our recent publication [114] using literary data, we substantiated that acetylcholine, via nAChRs and especially via $\alpha 7$ nAChRs, is involved in hypoxic and ischaemic preconditioning and that an interconnection exists between $\alpha 7$ nAChRs, hypoxic preconditioning and PPI. Thereby in the pharmacological experiments, we investigated the effects of selective agonists

of $\alpha 4\beta 4$ and $\alpha 7$ nAChRs RJR and PNU, respectively, and PNU solvent DMSO on the HBH preconditioning. PPI measures were compared with the HBH-initiated preconditioning (resistance to SHBH) and with the effects of drugs on the HBH preconditioning efficiency (**Figure 2**).

RJR had no effect on the adaptive action of HBH. All the values of resistance in this group of rats ideally fitted into the variation series of T values of the HBH group (**Figure 8**).

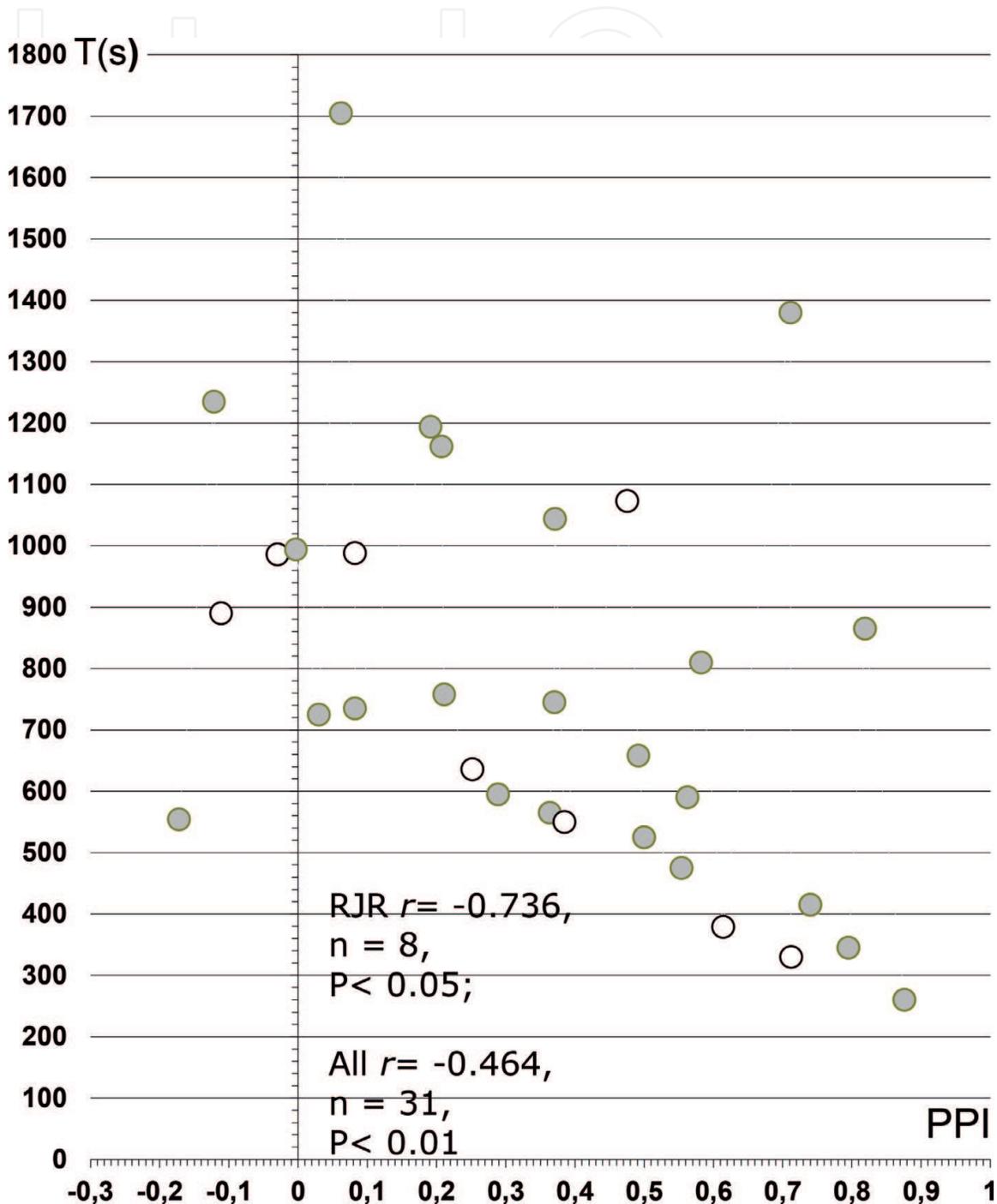


Figure 8. The influence on HBH preconditioning of the selective agonist $\alpha 4\beta 2$ nAChRs metanicotine RJR 2304 (RJR). Grey marks, a time before apnoea after HBH as shown in **Figure 7**; light marks, a time before apnoea after RJR + HBH, Pearson's r -criterion test.

Unlike RJR, PNU inverted the effects of HBH (**Figure 9B**), and it was especially clearly observed in the DMSO group (**Figure 9C**). Moreover, when the graphs of HBH and DMSO groups were combined, the interval of PPI = 0.36–0.40 (36–40%) was found (**Figure 10**). Above these values of PPI, DMSO potentiated the effects of HBH, and lower these values of PPI, DMSO, on the contrary, inhibited them. On the same PPI interval, the directionality of the action of PNU on DMSO effects was divided (**Figure 11**).

Analysis of the literature data revealed the following: (1) PNU in the low doses used (about 2 and 20 nM in the brain) had a desensitising effect on $\alpha 7$ nAChRs, that is, acted as an antagonist [143, 144]; (2) DMSO has the various biological activities, but in the low concentrations used (hundredths or thousandths of a per cent in the brain), it had only anticholinesterase action, that is, activating effect on the cholinergic system [145], and it was found that the anticholinesterase

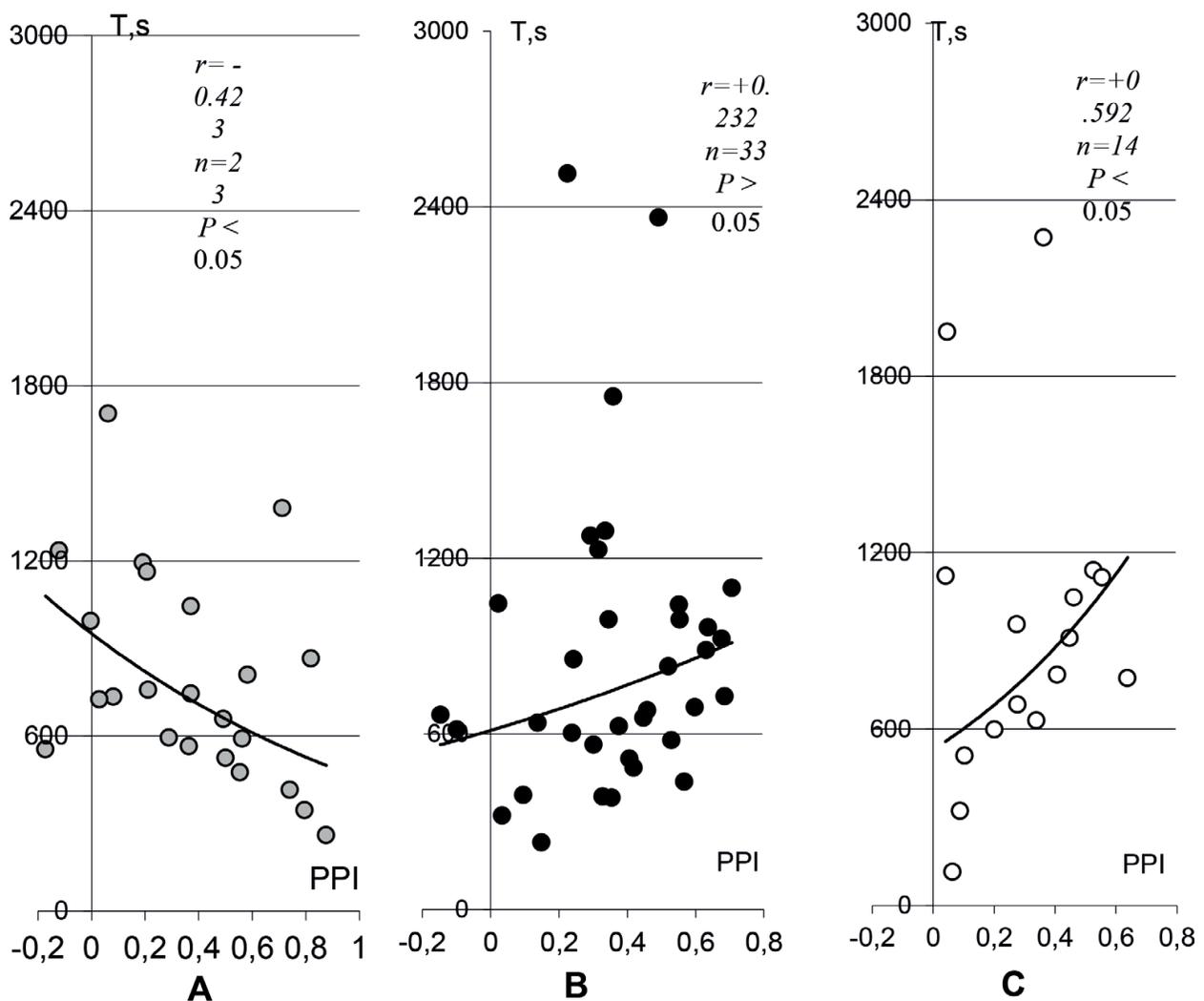


Figure 9. The influence on HBH preconditioning of the selective agonist $\alpha 7$ nAChRs PNU-282,987 (PNU) and its solvent dimethyl sulfoxide (DMSO). Grey marks (A), T values in the HBH rat group as shown in **Figure 7**; black marks (B), T values in the PNU rat group; light marks (C), T values in the DMSO rat group. The significant negative correlation between PPI and T values after HBH inverted into the positive correlation under influence of PNU and significantly under DMSO influence, Pearson's *r*-criterion test.

(neuroprotective) action is realised through the modulation of expression of nAChR genes that were shown for $\alpha 7$ and $\alpha 4$ subunits of nAChRs [146–148]. For details, see [114].

These data indicate the involvement of $\alpha 7$ nAChRs in the mechanisms of HBH preconditioning and explain the antagonism of PNU and DMSO actions. But the literature data do not explain the oppositely directed effects of DMSO and PNU on HBH preconditioning at the PPI boundary = 36–40%. Nevertheless, the existence of the interface does not seem random. Recently, it was found that the predisposed and resistant rats to convulsions in the hippocampal partial kindling model were differed in PPI: the resistant to convulsion rats had PPI of 36–58%, and the unstable to convulsion rats had in all experiments PPI larger, which was selectively susceptible to pronounced variability [149].

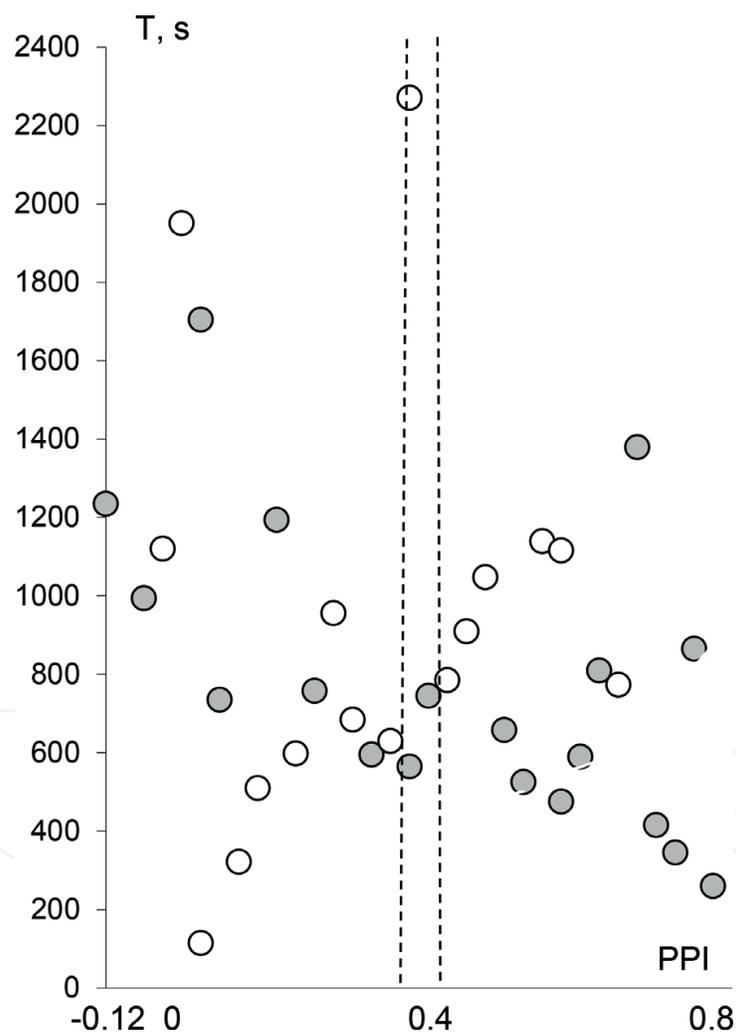


Figure 10. Combined graphs of the dependence of the HBH preconditioning efficiency (T) on the rate of PPI in the HBH and DMSO groups. Grey marks, T values in the HBH rat group as shown in **Figure 7**; light marks, T values in the DMSO rat group. Vertical dotted lines indicate the values of PPI 0.36 and 0.40. The figures under the x -axis are given for orientation and denote the location of the corresponding values of PPI on the axis. The relationship between T values in the compared groups differs on the opposite sides of PPI = 0.36–0.40.

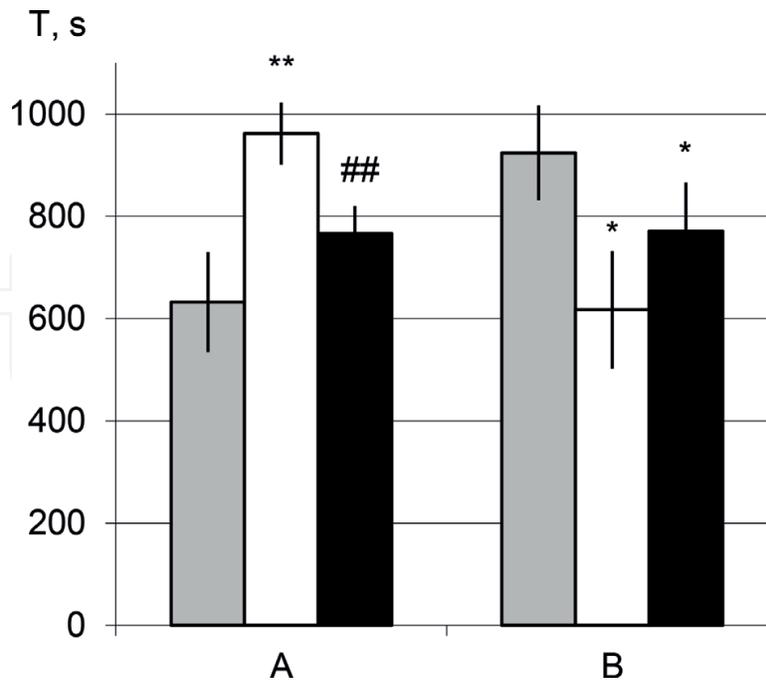


Figure 11. Influence of DMSO and PNU on the HBH preconditioning efficiency in subgroups with PPI > 0.4 (A) and PPI < 0.4 (B). Values are expressed as means \pm SE. Grey bars, HBH rat group; light bars, DMSO group; black bars, PNU group. * p < 0.05 and ** p < 0.025 compared with T values in the relevant HBH groups, ## p < 0.025 compared with T values in the relevant DMSO group, Fisher's exact test.

Also, key brain structures involved in the innate mechanisms of hypoxic preconditioning are unknown. We suppose the obligatory participation of caudal brainstem. In studies related to PPI, the key structure is the hippocampus [149–152]. In our study, in the intact rat group with not arranged related to PPI, no reaction was shown in the hippocampus. We hope to clarify this problem somewhat in the planned neurochemical studies of synaptic pool in the intact rats pre-tested with PPI.

7. Conclusion

1. A search study of the effects and neuronal mechanisms of hypoxic preconditioning were carried out, and the certain results in this direction were obtained using the HBH model.
2. It has been revealed that the model of acoustic start-reaction can be used to predict the efficiency of hypoxic preconditioning and the study of their innate mechanisms because the magnitude of criterion for this model of PPI has the reverse dependence related to the HBH-initiated resistance to SHBH in the intact rats.
3. The pre-testing of intact rats at the PPI revealed the presence of oppositely directed cholinergic mechanisms of hypoxic preconditioning, separated at the border of PPI = 36–40%, and the $\alpha 7$ nAChRs participation in both the mechanisms.

Acknowledgements

We are grateful to the direction of Institute of General Pathology and Pathophysiology that supported and funded our research and to the direction of P.K. Anokhin' Institute of Normal Physiology for the successful cooperation. And we would like to thank the team of the Proof-Reading-Service for editing and proofreading the English of our chapter.

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