DOI 10.1007/s11062-017-9625-y
Neurophysiology, Vol. 49, No. 1, February, 2017

$GABA_A$ Receptors: Involvement in the Formation of Respiratory Reactions to Hypoxic Stimulation under Conditions of Mitochondrial Dysfunction

E. É. Kolesnikova[1]

Received June 20, 2016

In experiments on Wistar rats, the role of the state of $GABA_A$ receptors in the formation of respiratory responses to hypoxic loading was studied under conditions of the norm and experimental mitochondrial dysfunction; the latter was induced by single systemic injections of 3 mg/kg rotenone, a nonselective blocker of complex I in the respiratory chain of the mitochondria. Volume-time parameters of respiration were characterized according to the parameters of respiratory EMG discharges of the diaphragmatic muscle (amplitude, frequency, and integral intensity). Changes in EMG activity of the diaphragm induced by inhalation of a hypoxic gas mixture (12% O_2 + 88% N_2) were estimated prior to and after injections of the blocker of $GABA_A$ and $GABA_B$ receptors bicuculline (bicuculline methiodide, 1.0 mg/kg) in control rats and animals with mitochondrial dysfunction. The development of mitochondrial dysfunction was accompanied by suppression of the respiratory reaction to hypoxic loading, which was manifested in a dramatic decrease in the frequency and integral intensity of EMG discharges of the diaphragmatic muscle. These data can be considered an indication of the considerable involvement of $GABA_A$ receptors localized at the postsynaptic membranes of peripheral chemoreceptors in the formation of respiratory response to hypoxic stimulation (including the stage of depression of ventilation); this was observed in both control rats and animals with mitochondrial dysfunction. The involvement of a GABA-ergic link in the formation of respiratory activity related to hypoxic stimulation acquires special significance under conditions of experimental mitochondrial dysfunction leading to occlusion of afferent impulsation coming from peripheral chemoreceptors.

Keywords: $GABA_A$ receptors, hypoxia, ventilatory response, peripheral chemoreceptors, EMG activity of the diaphragm.

INTRODUCTION

GABA is the main inhibitory neurotransmitter in the CNS of mammals; it plays this role in a considerable part (25–40%, according to different estimates) of the total number of neuron-to-neuron synapses [1]. In the CNS structures localized in the *medulla oblongata*, which are the main link in the system of neural control of respiratory activity, GABA is a key neurochemical component in the system of the respiratory center (RC). The content of GABA in different CNS structures within the CNS varies significantly. Among the structures involved in the regulation of the respiratory function, high contents of GABA are observed in neuronal systems of the brainstem nuclei, in particular in the parabrachial nucleus, *nucl. tractus solitarius* (NTS), *locus coeruleus*, and also within the spinal cord [2–4]. In addition, GABA and GABA receptors (both $GABA_A$ and $GABA_B$) are rather widely presented in the peripheral chemoreceptor structures, carotid bodies (CBs) [5–8]. The activity of precisely these receptor structures provides, obviously, the main contribution to direct determination of the value of the respiratory reaction to hypoxic stimulation. It has been found that GABA is not only present in the CBs but also is released from chemoreceptor type-I cells of these structures [6–8]. In chemoreceptor type-I cells of the CBs, GABA fulfills the function of pre-synaptic modulation (via binding with $GABA_B$ receptors) and also provides post-synaptic effects via the influence on $GABA_A$ receptors of the postsynaptic membranes of afferent fibers of the carotid sinus nerve (CSN) [9]. These fibers are projected to the NTS of the RC.

[1] Bogomolets Institute of Physiology, National Academy of Sciences of Ukraine, Kyiv, Ukraine.
Correspondence should be addressed to E. É. Kolesnikova (e-mail: dr_kolesnikova@ukr.net).

As is known, a decrease in the oxygen content (low partial O_2 pressure, PO_2) in the inhaled air causes a rise in the ventilation intensity. Such a respiratory response to hypoxic stimulation (hypoxic ventilatory response, HVR) demonstrates a clearly pronounced two-phase pattern in both acute (several minutes, acute hypoxia) and longer (10-30 min, sustained hypoxia) hypoxia episodes [10–13]. An increase in the ventilation intensity within the initial stage of the reaction of the organism to hypoxic stimulation is, after a time, changed into a drop in the intensity of ventilation, the so-called roll-off effect, hypoxic ventilatory decline (HVD) [12, 13]. It has been found that the initial enhancement of the intensity of ventilation accompanied by a decrease in the PO_2 in the inhaled air is mediated by the release of some neurotransmitters (catecholamines, GABA, and ATP) from chemoreceptor type-1 cells of the CBs and subsequent intensification of the activity of afferent fibers of the CSN projecting to the NTS [13]. As is believed, a drop in the intensity of ventilation under conditions of the continued hypoxic exposure is, first of all, related to the release of some inhibitory modulators and mediators (adenosine, catecholamines, serotonin, opioids, and, first of all, GABA) within the RC nuclei and accumulation of the mentioned agents in these brainstem structures [12, 14]. The fact that GABA is involved in depression of the HVR (HVD) at the level of the RC casts nearly no doubts. At the same time, there are only limited data on possible participation of GABA-ergic neurons in the formation of the initial (hyperventilatory) phase of the reaction of the respiratory system to a decrease in the PO_2. Simultaneously, there are practically no data on the role of GABA in the functioning of the peripheral PO_2 sensor (CBs) within the late phase of the HVR, i.e., the phase of weakening of ventilation.

For neurons, hypoxia is a factor promoting a rise in the intracellular GABA concentration; this effect depends on the severity and duration of action of the above stimulus [15]. Another but probably no less important factor influencing the rise in the GABA level in neurons is exhaustion of the reserves of macroergs (ATP). Such a shift can result from a significant decrease in the PO_2 in the inhaled air/oxygen tension in tissues [16]. It is also obvious that the content of GABA in nerve cells is closely related to the intensity of energy production provided by the mitochondria (MChs). The level of ATP significantly influences the activity of glutamate decarboxylase (GAD), the enzyme responsible for synthesis of GABA from glutamate [16]. Therefore, any states of the organism, which are accompanied by deviations in the level of mitochondrial energy production, can indirectly but significantly affect the metabolism of GABA as the main inhibitory neurotransmitter. These interactions appear to be the basis of certain rearrangements in different links of the system of respiration control.

Taking into account all the above-mentioned data, we tried to elucidate the role of $GABA_A$ receptors in the formation of respiratory reactions to hypoxic stimulation in the norm and against the background of experimental mitochondrial dysfunction (MChD).

METHODS

Experiments were carried out on Wistar rats (5 to 6 months old, n = 10), which were divided into the control group (n = 5) and the group with experimental MChD (n = 5). During the entire period of examination, rats were on standard food allowance and standard light regime (12/12 h illumination/darkness).

For neurophysiological studies, rats were anesthetized with α-chloralose and urethane (35 and 800 mg/kg, i.p.) and subjected to tracheostomy at the level of the upper third of the trachea. Throughout the experiment, animals maintained spontaneous breathing. The use of a double valve made it possible to divide inspiratory and expiratory volumes; either atmospheric air or a hypoxic gas mixture containing 12% O_2 was fed in the intratracheal cannula.

Volume-time parameters of respiration were estimated indirectly according to the characteristics of EMG discharges of the diaphragmatic muscle [17]. To record EMG activity, two steel insulated (except for the tip) needle-shaped electrodes (interelectrode distance 12-18 mm) were inserted into the diaphragmatic zone adjacent to the chest. EMG signals were amplified (bandwidth 50-5,000 Hz) and subjected to complete rectification and integration (low-frequency filtration) using the modified REO program (Bogomolets Institute of Physiology of the NAS of Ukraine). Analyzing three successive complete respiratory cycles, we estimated the averaged ongoing values of the amplitude of EMG discharges (a.u.), respiratory frequency (min^{-1}), and integral intensity of EMG activity within the recorded segment (areas of rectified EEG discharges, a.u.). The latter index measured within longer time intervals (EMG minute

diaphragmatic output, MDO) strongly correlates with ongoing values of the minute ventilation and can be interpreted as current relative MDO values, while the amplitude of EMG discharges strongly correlates with the respiratory volume (V_T). At the same time, the above-mentioned indices of rectified and integrated EMG samples are correlates of the characteristics of central motor commands (CMCs) arriving at the diaphragmatic muscle along the *n. phrenicus*.

Under conditions of hypoxic stimulation, EMG activity was recorded for 2.5 min (150 sec) after the beginning of inhalation of the mixture containing 12% O_2; the above-mentioned parameters were measured every 30 sec. Alterations of diaphragmatic EMG activity observed in this case corresponded to a classic ventilatory response to hypoxic stimulation. At first, respiratory EMG discharges increased, which provided intensification of ventilation. Then, the above activity weakened, reflecting a gradual drop in the intensity of external respiration. All data characterizing diaphragmatic EMG discharges were normalized with respect to the initial values taken as 100%.

During these acute experiments, we also analyzed the content of gases in the arterial blood and measured the pH of the latter. A polyvinyl chloride catheter was inserted into the rat femoral artery, which made it possible to obtain arterial blood samples with subsequent express evaluation of the gas content using a fluid gas analyzer. Throughout the experiment, the temperature of the animal's body was stabilized at 37.0 to 38.5°C with the accuracy of 1°C.

To block GABA$_A$ receptors, animals were intravenously injected with bicuculline (bicuculline methiodide, BCm; Sigma, USA) in the dose of 1.0 mg/kg.

For partial blocking of the MCh function in neurons of the brainstem and CBs, animals were given a single subcutaneous injection of rotenone (3.0 mg/kg), a selective inhibitor of the activity of complex I of the mitochondrial respiratory chain [18, 19]. Rotenone was dissolved in a minimum amount of the mixture (1:1) of two organic solvents, dimethyl sulfoxide (DMSO) and polyethylene glycol (PEG; Sigma, USA).

The obtained numerical data were treated statistically using standard techniques; the significance of intergroup differences was estimated using the Student's *t*-test.

RESULTS

In our study, we obtained confirmations of significant involvement of the GABA-ergic system in the mechanisms of formation of the HVR against the background of MChD.

EMG Correlates of the HVR in Control Animals. Rats of the control group during inhalation of the hypoxic mixture (12% O_2) demonstrated typical HVRs composed of two phases. The integral intensity of EMG discharges of the diaphragmatic muscle increased very rapidly and peaked on about the 60th sec; then it progressively decreased up to the end of the observation period (150 sec; Fig. 1A). On the 60th sec of hypoxic stimulation, according to the MDO index, the HVR magnitude exceeded the initial level of ventilation by 141%, on average ($P < 0.05$). At the final point of the graph of the respiratory reaction to inhalation of the hypoxic mixture, the integral intensity of EMG activity decreased practically to the initial value (96%).

It should be noted that the amplitude of EMG discharges of the diaphragm under conditions of hypoxic loading underwent more clearly pronounced changes, as compared with those of the frequency of these discharges (Fig. 1B, C). The maximum MDO values coincided in time with analogous values of the frequency, while the amplitude of EMG discharges of the diaphragm were subjected to modulation mostly within the second (late) HVR phase. The value of the amplitude peaked only on about the 120th sec of inhalation of the hypoxic mixture and corresponded to 208%, on average, of the initial value of this index. At the final point of recording of the HVR, the amplitude of EMG activity remained noticeably greater and exceeded the initial index by 40%, on average ($P < 0.05$). The frequency of integral EMG activity of the diaphragmatic muscle underwent significantly smaller changes; the normalized increment of this index was only 25%.

The partial oxygen tension (PaO_2) in the blood and pH of the latter (Fig. 2A, B) were measured in the initial state (which in the figures corresponds to 0 min) and 150 sec after the beginning of inhalation of the hypoxic mixture. As was already mentioned, the latter time interval corresponded to the phase of depression of ventilation. Changes in the above-mentioned parameters (PaO_2 and pH) in both animal groups showed that these indices at the end of the test period did not differ significantly from the initial ones. Thus, the relatively short-term hypoxic loading (2.5 min) was not accompanied by

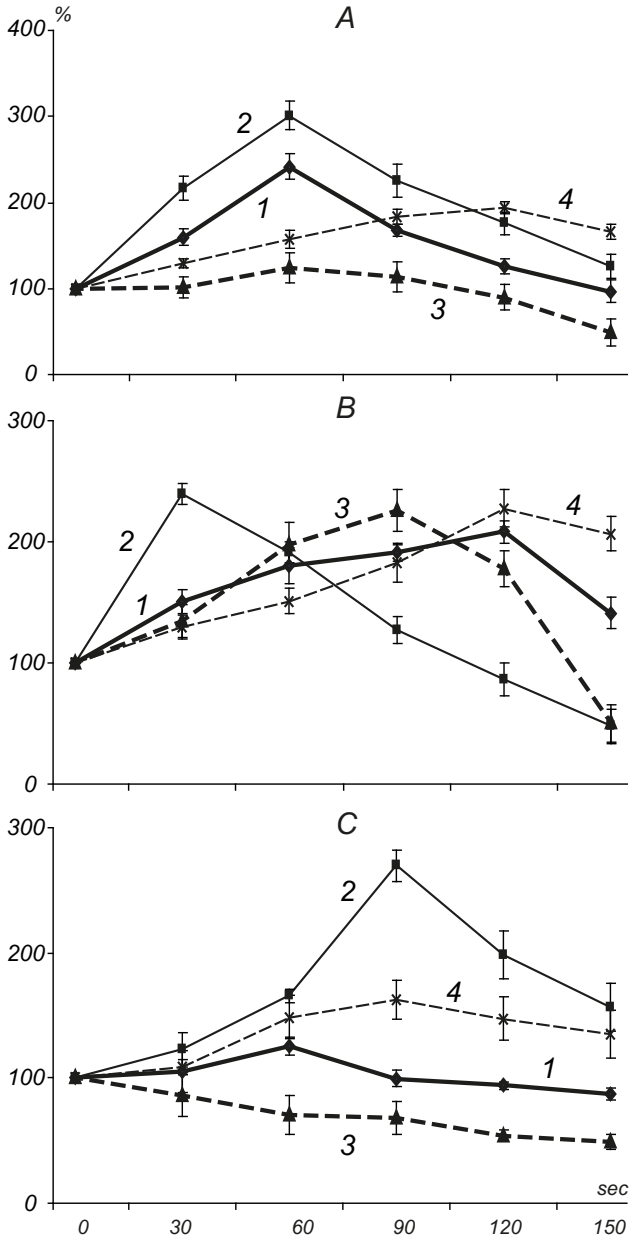

Fig. 1.Changes in the normalized parameters of EMG activity of the diaphragm under the action of acute hypoxia (12% O_2) in the control and after injections of the blocker of $GABA_A$ receptors bicuculline methiodide (BCm). A–C) Dynamics of the integral intensity (A), amplitude (B), and frequency (C) of EMG discharges. Values in the initial state (before the action of hypoxia) were taken as 100%. 1) Control, 2) control after BCm injection, 3) animals with rotenone-induced mitochondrial dysfunction (MChD), and 4) rats with MChD after BCm injection.

considerable alterations of the gas composition and concentration of hydrogen ions in the blood; both prior to and after injection of the blocker of $GABA_A$ receptors (BCm), these indices were also rather similar to the initial ones.

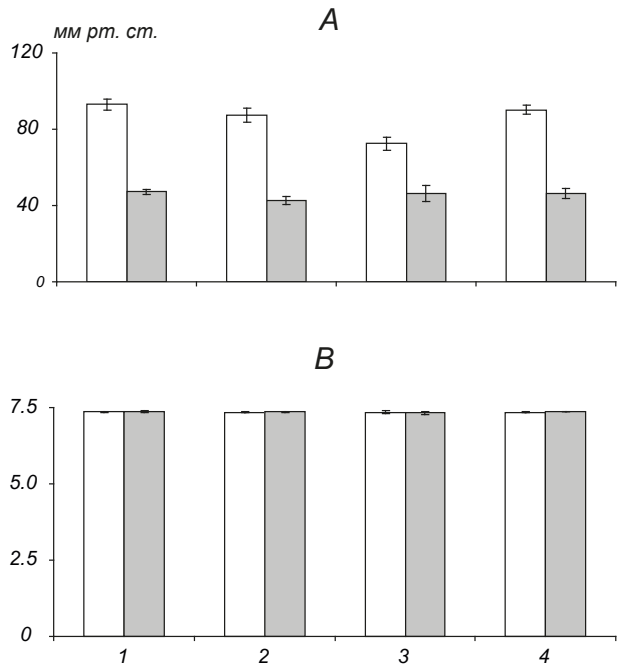

Fig. 2. Values of the partial pressure of oxygen, PaO_2 (A) and pH (B) in the arterial blood of control rats and animals with rotenone-induced mitochondrial dysfunction (MChD) prior to and after injection of the $GABA_A$ receptor blocker bicuculline methiodide (BCm). 1) Control, 2) control after BCm injection, 3) animals with rotenone-induced mitochondrial dysfunction (MChD), and 4) rats with MChD after BCm injection. Open and hatched columns show mean values of the parameters at the moment of the beginning of inhalation of a hypoxic mixture (0 min) and 2.5 min later, respectively.

EMG Indices of the HVR Observed in Control Animals after Injection of Bicuculline. Blocking of $GABA_A$ receptors using BCm induced a number of definite changes in practically all characteristics of EMG activity of the diaphragm. The integral intensity of EMG discharges of the diaphragmatic muscle after injection of BCm significantly exceeded the corresponding values observed without such action during the entire observation period (Fig. 1A). The maximum peak of the MDO, similarly to that in control animals, was observed in about 60 sec from the beginning of inhalation of the hypoxic mixture, but the relative increment of this parameter after injection of BCm became appreciably greater than that prior to such injection (by 24%, $P < 0.05$). The enhanced MDO value was also preserved during the second phase of the HVR development. The latter reaction was characterized by the clearly expressed roll-off effect, but at the end of the observation period (150 sec after the beginning of inhalation of hypoxic mixture) the MDO value

remained increased, on average, by 30% ($P < 0.05$), as compared with the control. The dynamics of the amplitude of EMG discharges generated by the diaphragmatic muscle also demonstrated strong changes after BCm injection. In particular, the amplitude of EMG discharges during hypoxic loading peaked much earlier, 30 sec after the beginning of inhalation of the hypoxic gas mixture, and not on the 120th sec, as was observed in the control (A). The second HVR phase after blocking of GABA$_A$ receptors was characterized by a more significant drop in the amplitude of diaphragmatic EMG discharges of the muscle. At the mentioned final point (150th sec of HVR recording), the averaged value of the discharge amplitude after BCm injection was 53% smaller than that at the initial moment of the observation period ($P < 0.05$). The frequency of EMG activity of the diaphragm under conditions of injection of the GABA antagonist demonstrated opposite shifts. After BCm injection, the value of the frequency peaked not on the 60th sec but only 90 sec after the beginning of inhalation of the hypoxic mixture. In this case, the frequency of respiratory EMG discharges in animals subjected to the action of BCm more than 2.5 times exceeded the initial value. It will be recalled that in the control the second HVR phase (depression of ventilation) began within the above-mentioned time interval (90 sec) (C). On the 150th sec of the action of hypoxic loading, the mean value of the frequency of EMG discharges after BCm injection continued to exceed significantly (by 79%) the initial value of this index ($P < 0.05$).

The values of PaO_2 and pH in the blood of control animals, which were measured against the background of blocking of GABA$_A$ receptors after inhalation of the hypoxic mixture, did not show any significant specific changes (Fig. 2A, B).

EMG Indices of the HVR in Rats with Mitochondrial Dysfunction (MChD). Injection of rotenone, which led to suppression of the activity of complex I of the respiratory chain of the MCh and a subsequent decrease in the production of ATP, was accompanied by the development of the state demonstrating certain signs of MChD. The HVRs during inhalation of the mixture with the low PO_2, which were observed in animals with MChD, were characterized by relatively small changes in the MDO (Fig. 1A). This index rather insignificantly increased during a 60–90 sec segment of the observation period; then it decreased. At the end of HVR recording, the MDO values in animals with MChD became smaller (about two times, $P < 0.05$) than those in control rats. In addition, the responses to hypoxic loading in animals with MChD were characterized by the predominance of changes in the amplitude of diaphragmatic EMG activity but not in its frequency (B). The increment of the amplitude of EMG discharges at the maximum of the reaction was 126%; the greatest increase in the amplitude was observed much earlier (on the 90th sec) than that in control rats (differences of the mean values of this parameter in the above-mentioned groups were obvious but did not reach the confidence level). At the same time, the frequency of EMG activity of the diaphragm under conditions of MChD throughout the entire period of the action of hypoxic loading demonstrated no increase; instead, the frequency progressively decreased (C). The corresponding plot lost its typical two-phase pattern; the frequency of EMG discharges at the end of the period of the above changes was only 48% of the initial index ($P < 0.05$). Moreover, at the end of the HVR, the frequency of EMG discharges of the diaphragm of rats with MChD was more than twice (by 56%) lower than the analogous value in control animals ($P < 0.05$). Therefore, it is obvious that the development of MChD in rats caused clearly pronounced suppression of electrical activity of the main respiratory muscle.

The analysis of PaO_2 and pH in the blood of animals with MChD after the action of hypoxic loading (Fig. 2A, B) showed no considerable deviations from the analogous indices in control animals

EMG Indices of the HVR in Rats with MChD after Injection of Bicuculline. The dynamics of changes in the MDO observed in animals with MChD and blocking of GABA$_A$ receptors looked like a nearly linear increase in this parameter during the most part of the measurement period, with the exception of a short phase of relative depression within the final 30-sec-long segment of the respiratory response. Thus, the MDO value in rats with MChD peaked only on the 120th sec of action of hypoxic loading. The MDO increment against the background of blocking of GABA$_A$ receptors at the HVR maximum was practically twofold ($P < 0.05$), as compared with the initial values in animals with MChD and control rats. The amplitude of EMG discharges of the diaphragm in rats with MChD injected with BCm demonstrated dynamics that

was, to a considerable extent, similar to those of the MDO; the amplitude increased monotonically during 150 sec; then, during 30 sec, it decreased insignificantly (Fig. 1B). After injection of BCm, the amplitude of EMG discharges of the diaphragmatic muscle peaked much later (on the 120th sec but not on the 90th sec, as was observed in rats with MChD prior to injection of the blocker) (B). The difference in this index in control rats and animals with MChD subjected to blocking of $GABA_A$ receptors was insignificant. The effect of injection of BCm in rats with MChD was manifested mostly in changes in the frequency of respiratory EMG discharges (C). The action of BCm caused a significant increase in the frequency of EMG activity of the diaphragm within all time intervals of the HVR. On the 90th sec, blocking of $GANA_A$ receptors promoted a twofold rise of the frequency of EMG discharges ($P < 0.05$), compared with the analogous index under conditions of MChD before blocking, and demonstrated a monotonically increasing drop. Significant differences in the frequency of EMG discharges in rats with MChD under conditions of the presence and absence of $GABA_A$ receptor blockade were rather clearly pronounced at the end of the HVR (on the 150th sec). Therefore, injection of BCm into animals with MChD caused more significant changes in the dynamics of frequency, amplitude, and MDO of EMG activity of the diaphragm, as compared with that in control rats. Under conditions of MChD, the effect of blocking of $GABA_A$ receptors showed a specific intensity during the second HVR phase.

The parameters reflecting the state of the system of endogenous chemical stimuli of respiration present in the blood under conditions of the action of hypoxic loading on rats with MChD, control animals subjected to blocking of $GABA_A$ receptors, and intact rats, demonstrated no significant differences (Fig. 2).

DISCUSSION

As was already mentioned, the HVR is formed due to the activity of two main components of the general system controlling breathing, receptor (CBs) and central (RC) ones [13]. The HVR dynamics are characterized by the presence of two phases, hyperventilation and depression of ventilation (the so-called roll-off effect; under conditions of acute hypoxia, this effect is manifested during several minutes of action of hypoxic loading) [10–13]. There are reasons to believe that precisely the CBs are the crucial link determining the formation of two-phase HVRs [20–22]. Chemodenervation provided by bilateral resection of CBs not only removes the initial phase of the respiratory response to acute hypoxia (hyperventilation phase), but also cancels the roll-off effect; this was observed in many animal species and humans [21, 22]. It was been found that stimulation of CBs after a decrease in the PO_2 causes the release of an excitatory mediator (glutamate) into the caudal NTS part [23, 24]; when chemodenervation (CB resection) is combined with blocking of the release of glutamate into the RC, this cancels completely the development of hyperventilation under conditions of a drop in the PO_2 [23]. Recording of the activity from the *n. phrenicus* during hypoxia indicated that the roll-off effect is associated with a rise in the concentration of inhibitory mediators (GABA and taurine) in the superficial region of the ventrolateral medulla (VLM) [24]. Application of the $GABA_A$ antagonist bicuculline on this surface was accompanied by an increase in the intensity of discharges in fibers of the *n. phrenicus* without its subsequent appreciable lowering, which is typically intrinsic to the final phase of the respiratory reaction to exposure to hypoxia [25, 26]. Therefore, now a general concept has been formed on the close relationship between increase in the intensity of ventilation accompanied by a drop in the PO_2 in the inhaled air and the activity of CBs and, first of all, manifestations of the effect of the main excitatory mediator (glutamate) released in the RC. At the same time, just GABA, the leading inhibitory mediator at the level of the RC, is believed to play the main role in the development of the roll-off phenomenon during exposure to hypoxia under conditions of preservation of afferent activity coming from the CBs.

When we examined the level of involvement of the GABA-ergic link in the mechanisms of HVR formation, we used the ability of rotenone to block complex I in the respiratory chain of the MChs. Rotenone possesses no selectivity in its effects on mitochondrial processes in different central structures; it is capable of influencing the MCh respiratory chain in all cells [18], including cellular RC components localized in the *medulla oblongata* [19] and peripheral chemoreceptor type-I cells localized in the CBs [27].

As was described above, the development of MChD in rats led to a decrease in the integral intensity of diaphragmatic EMG activity, first of all, at the expense of a critical drop in the frequency of EMG discharges of the diaphragmatic muscle. Values of the amplitude of these discharges remained relatively stable, which was combined with rather low values of the HVR, i.e., in general, with a weak intensity of this response. It is obvious that systemic injection of rotenone should exert the effect both at the level of RC structures and at the CB level; the latter is the main source of receptor respiratory activity under conditions of hypoxia [27]. The corresponding changes may, apparently, be interpreted as a sort of occlusion or an equivalent of partial chemical denervation of the CBs, and this was manifested in the peculiarities of formation of the HVR in rats with MChD. Experiments carried out using surgical interruption of the pathways coming from the CBs (transection of the CSN) in rabbits [28] demonstrated that exposure of such animals to hypoxia was accompanied by a significant decrease in the frequency of respiration, drop in the amplitude of respiratory movements, and drop in the minute volume of ventilation. The above-mentioned effects were considered mostly a result of the action of deceased PO_2 in RC neurons. At the same time, the analysis of microdialysates collected from chemosensitive parts of the VLM showed that the GABA concentration in denervated animals increased, as compared with that in samples obtained from intact animals [24]. Later on, the level of GABA on the surface of the VLM of denervated animals under conditions of their exposure to hypoxia demonstrated no increase. It seems possible that the maximum GABA concentration in the studied VLM zones resulted from chemodenervation, and further decrease in the PO_2 did not work as an effective stimulus for the synthesis/release of GABA [24].

Apparently, considering the results of our analysis, it is logical to hypothesize that chemoreceptor dysfunction of the CBs and modifications of the energy/neurotransmitter status of neurons of the *medulla oblongata* determined by MChD lead to shifts in the metabolism/release of GABA in the RC. In rats with MChD, high GABA concentrations are probably present in certain RC nuclei, and this causes a relative decrease in the intensity of electrical activity of the diaphragmatic muscle and rearrangement of the pattern of this activity [29].

Since the CBs are considered polymodal sensory structures [9, 30, 31], where a rather great number of mediator agents work (in particular, acetylcholine, dopamine, serotonin, GABA, and ATP), it should be taken into account that, under conditions of our experiments, a certain contribution to the observed changes is provided by rotenone-induced decrease in ATP production (ATP is the transmitter in chemoreceptor type-I CB cells). This factor, apparently, influences the processes of chemotransduction and modulates the HVR dynamics in rats with MChD. It is believed that ATP is one of the key transmitters in the CBs [31]. The release of ATP from chemoreceptor type-I cells under the action of hypoxia *in vitro* [32] and also a significant weakening of HVR in mice with genetic deficiency of P2X2 subunits of purinergic receptors localized on afferent CNS terminals are facts confirming that the processes of sensory transduction of the PO_2 level in the CBs are significantly involved in modifications of the HVR pattern [33]. It is interesting that P2X3-knockout animals demonstrate practically normal values of the HVR. This, however, is associated with a significant decrease of afferent activity in fibers of the CSN under conditions of normoxia [33]. This fact is an additional indication in favor of the close relation between the state of purinergic P2X receptors and realization of sensory transduction in the CBs. In addition, in experiments carried out on co-culture of rat neurons of the *ganglion pertosum* (GP, the inferior ganglion of the glossopharyngeal nerve) and CBs, interaction between P2X and GABA$_A$ receptors mediated by a shunting mechanism (GABA$_A$-shunt inhibition) was found [9]. It appeared that activation of GABA$_A$ receptors can cause short-circuit fault of excitatory synaptic currents induced by the action of ATP [9]. All the above-mentioned data confirm the existence of close interaction between GABA and ATP in the peripheral mechanisms underlying regulation of respiration.

Apparently, we believe that occlusion of CB activity at reception of the PO_2 in animals with MChD can result in corresponding changes in chemotransduction and transmission of afferent signals in the RC. Due to the development of the above-mentioned alterations in the functioning of peripheral chemoreceptors in rats with MChD, the characteristics of efferent commands (formed in the RC and coming from it) cause considerable weakening of muscle activity of the diaphragm under hypoxic stimulation at all stages of the HVR.

As is known, bicuculline is a selective competitive antagonist of ionotropic $GABA_A$ receptors. The latter receptors possess pentamer ligand-dependent ion chloride channels, the structure of which includes the cysteine loop. Nicotinic acetylcholine receptors, glycine receptors, and serotonin 5-HT3 receptors also belong to the corresponding family. As we already mentioned, we used BCm (bicuculline methiodide) in our study. This agent has a specific property, it does not cross the blood-brain barrier (BBB). At the same time, another frequently used preparation of bicuculline, bicuculline chloride, demonstrates clearly pronounced pharmacological effects with respect to $GABA_A$ receptors on both sides of the BBB, i.e., in both CBs and brain RC structures [34].

In the brainstem, GABA and its agonists suppress the activity of neurons belonging to the dorsal and ventral respiratory groups [35], which is accompanied by depression of ventilation under conditions of both normoxia and hypoxia [36, 37]. Application of bicuculline, i.e., the $GABA_A$ receptor blocker, on the perfused CBs after preliminary injection of GABA agonists restored chemoreceptor activity of these structures generated in response to hypoxic stimulation [37]. This fact rather convincingly indicates that $GABA_A$ receptors participate in the transmission of afferent signals related to a decrease in the PO_2. It has been found that bicuculline applied on the VLM surface promotes a rise in the intensity of ventilation and leads to an increase in the activity recorded from the *n. phrenicus* [25, 26]. Thus, we conclude that the above-mentioned data are indicative of the fact that there is a direct relation between the GABA-ergic apparatus and the mechanisms of formation of spontaneous respiratory activity under conditions of both normoxia and hypoxia.

In our experiments, the BCm-induced blocking of peripheral $GABA_A$ receptors made it possible to reveal a number of peculiarities of formation of diaphragmatic EMG activity in response to inhalation of the hypoxic gas mixture. Such blocking of $GABA_A$ receptors in control animals led to increases in all normalized parameters of EMG activity of the diaphragmatic muscle reflecting the HVR development. In this case, the effect of BCm was especially clearly expressed within the hyperventilation phase, i.e., within the phase mediated, first of all, by the CB activity. This observation allows us to hypothesize that $GABA_A$ receptors are directly involved in the processes of chemotransduction at the level of peripheral chemoreceptors. As was already mentioned, the postsynaptic inhibitory role of GABA fulfilled via $GABA_A$ receptors is revealed well enough on the model of co-culture of CBs and GP [9]. The presence of $GABA_A$ receptors in neurons of the GP terminals of the CSN in slices containing the rat CBs was confirmed immunohistochemically [9]. In co-culture of CBs and GP exposed to hypoxia, BCm exerted a considerable effect on impulse activity, first of all, of GP neurons but not of CB chemoreceptor type-I cells [9]. This fact confirms the assumption on the inhibitory mediator function of GABA in the peripheral mechanisms underlying respiratory control and on the involvement of $GABA_A$ receptors of postsynaptic membranes of afferent fibers of the CSN in the formation of respiratory activity due to the influences coming from the CBs.

Examination of breathing in animals and humans subjected to resection of both CBs [21, 22] showed that, under such conditions, it is difficult to differentiate the hyperventilation phase and the depression phase in the reduced HVR. This circumstance is a convincing evidence of direct involvement of the CBs in the formation of both stages of the respiratory reaction to deviations of the PO_2 in the inhaled air. Examination of patients with unilateral resection of the CB [22] showed that the typical two-phase profile of changes in the intensity of ventilation in response to hypoxic stimulation after such partial denervation was preserved. In such subjects, however, the absolute value of this response became significantly smaller than that in healthy humans. On the other hand, the phase of suppression of the HVR in humans with unilateral CB resection was significantly greater than that in healthy subjects [22]. Thus, we conclude that, after exposure to hypoxia, the inflow of afferent impulsation from the CBs represents a necessary factor not only within the phase of intensification of ventilation but also within the phase of depression of the latter. It appears that the values of normalized indices of integral EMG activity of the diaphragm observed under hypoxic loading are also indicative of the considerable involvement of GABA-ergic apparatus of CBs in the formation of the roll-off effect. Injection of a “peripheral” form of the $GABA_A$ receptor antagonist (BCm) significantly decreases a decrement of the respective graph just at the final stage of the HVR development.

The main effect of injection of BCm into rats with MChD looks as a considerable increase in the integral EMG activity of the diaphragm, which was maximally expressed in the phase of HVR depression. Despite the fact that the dynamics of parameters of EMG discharges within the above-mentioned phase in animals of the studied groups were in general rather similar, the integral EMG activity of the diaphragmatic muscle, observed after the action of BCm in rats with MChD at the stage of HVR depression, significantly exceeded the analogous index in control animals. In this case, both frequency and amplitude of the examined discharges were enhanced.

As was already mentioned, the roll-off phenomenon is obviously due to a certain sum of events realized at the levels of the CB [21, 22] and RC nuclei [24]. The steeper slope of the rising phase of plots of the normalized indices of EMG activity after the action of BCm reflecting the HVR development in animals with MChD was indicative of the fact that occlusion of afferent CB activity, which was not normalized even at blocking of $GABA_A$ receptors, was preserved. If we suppose that rats with MChD are characterized by the enhanced GABA concentration and modified metabolism of this transmitter on the VLM surface due to occlusion of the processes of chemotransduction in the CBs, we should take into account the entire range of factors capable of influencing the parameters of functioning of the GABA-ergic apparatus during PO_2 reception and formation of the corresponding respiratory activity in the above-mentioned situation. Studies of the respiratory reactions in Zucker rats (characterized by obesity and intensification of the synthesis of GABA in the brainstem [34]) showed that blocking of $GABA_A$ receptors after injection of BCm into these animals did not cause any changes in ventilation related to PO_2 lowering in the inhaled air. Bicuculline chloride (which provides both peripheral and central effects) injected into these animals after exposure to hypoxia increased the respiration depth (evoked hyperpnea). This effect, however, practically did not influence the intensity of ventilation because of a drop in the frequency of respiratory movements [34], which was indicative of the ability of the central pool of GABA to significantly modulate the HVR pattern. At the same time, a paradoxical effect of GABA after its injection into the lateral cerebral ventricles was found in other studies [28]. During inhalation of such hypoxic mixture, all parameters of ventilation increased; under conditions of inhalation of the hypoxic mixture after intraventricular injection of GABA, paradoxical hyperventilation was observed in both control and denervated animals [28]. This fact can be explained by the prevention of conversion of glutamate into GABA at the expense of inhibition of GAD, the main enzyme involved in the synthesis of GABA from glutamate, and a subsequent rise in the concentration of the latter.

It is obvious that the effect of excessive/exogenous GABA can affect different classes of GABA receptors in the respiratory medullar nuclei and can influence functionally various neuronal networks of the RC, as well as induce complex modulatory interactions with other transmitters [29] (e.g., with glutamate). Altogether, this can result in complex rearrangements of the volume/time indices of the HVR. At the same time, the phenomenology of the dynamics of hyperventilation and the roll-off effect in animals with MChD subjected to the action of BCm is, apparently, determined by a certain sum of the effects of an increase in the intensity of afferent impulsation coming from the CBs and a paradoxical central effect of GABA.

Therefore, based on the obtained data, we conclude that the GABA-ergic apparatus of the CBs is involved in the formation of both phases of the response to the action of hypoxic stimulation (both hyperventilation and depression of respiration), and this occurs under both control conditions and those of mitochondrial dysfunction. The specificity of the dynamics of the normalized indices of EMG activity of the diaphragmatic muscle during hypoxic loading observed against the background of MChD indicates, apparently, that GABA and ATP closely interact with each other in the peripheral mechanisms of respiratory control in the course of PO_2 reception. The above-mentioned phenomenon is reflected in the functioning of the central link of the system controlling external respiration, which forms efferent commands to the diaphragmatic muscle.

Studies were performed in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1985), and also with those of the Committee of Bioethics at the Bogomolets Institute of Physiology of the NAS of Ukraine.

The author of this study, E. É. Kolesnikova, confirms that, in the course of performance of the experiments, she had

no conflict of interest pertinent to commercial or financial relations and relations with organizations or persons somehow or other related to the study.

REFERENCES

1. F. E. Bloom and L. L. Iversen, "Localizing ^{3}H-GABA in nerve microscopic autoradiography," *Nature*, **229**, No. 5287, 628-630 (1971).
2. R. A. Mueller, D. B. A. Lundberg, G. R. Breese, et al., "The neuropharmacology of respiratory control," *Pharmacol. Rev.*, **34**, No. 3, 255-285 (1982).
3. C. A. Livingston and A. J. Berger, "Immunohistochemical localization of GABA in neurons projecting to the ventrolateral nucleus of the solitary tract," *Brain Res.*, **494**, No. 1, 143-150 (1989).
4. J. Lipski, H. J. Waldvogel, P. Pilowski, and C. Jiang, "GABA-immunoreactive boutons make synapses with inspiratory neurons of the dorsal respiratory group," *Brain Res.*, **529**, Nos. 1/2, 309-314 (1990).
5. I. R. Moss, M. Denavit-Saubie, F. L. Eldrige, et al., "Neuromodulators and transmitters in respiratory control," *Fed. Proc*., **45**, No. 7, 2133-2147 (1986).
6. Y. Oomori, K. Nakaya, H. Tanaka, et al., "Immunohistochemical and histochemical evidence for the presence of noradrenalin, serotonin and gamma-aminobutyric acid in chief cells of the mouse carotid body," *Cell Tissue Res.*, **278**, No. 2, 249-254 (1994).
7. M. Pokorski and S. Ohtani, "GABA immunoreactivity in chemoreceptor cells of the cat carotid body," *Acta Histochem. Cytochem*., **32**, 179-182 (1999).
8. I. M. Fearon, M. Zhang, C. Vollmer, and C. A. Nurse, "GABA mediates autoreceptor feedback inhibition in the rat carotid body via presynaptic $GABA_B$ receptors and TASK-1," *J. Physiol*., **553**, Part 3, 83-94 (2003).
9. M. Zhang, K. Clarke, H. Zhong, et al., "Postsynaptic action of GABA in modulating sensory transmission in co-culture of rat carotid body via $GABA_A$ receptors," *J. Physiol*., **587**, No. 2, 329-344 (2009).
10. P. A. Easton, L. J. Slykerman, and N. R. Antoniesen, "Ventilatory response to sustained hypoxia in normal adults," *J. Appl. Physiol.*, **61**, No. 3, 906-911 (1986).
11. G. E. Bisgard and J. A. Neubauer, "Peripheral and central effects of hypoxia," in: *Lung Biology in Health and Disease*, Marcel Dekker, New York (1995), pp. 617-668.
12. J. Neubauer, J. E. Melton, and N. H. Edelman, "Modulation of respiration during brain hypoxia," *J. Appl. Physiol.*, **68**, No. 2, 1462-1470 (1990).
13. L. J. Teppema and A. Dahan, "The ventilatory response to hypoxia in mammals: mechanisms, measurement, and analysis," *Physiol. Rev*., **90,** No. 2, 675-754 (2010).
14. P. N. McWilliam and S. L. Shepeard, "A GABA-mediated inhibition of neurons of nucleus *tractus solitarius* of the cat that respond to electrical stimulation of the carotid sinus nerve," *Neurosci. Lett.*, **94**, No. 3, 321-326 (1988).
15. J. D. Wood, W. J. Watson, and A. J. Drucker, "The effect of hypoxia on brain gamma-amino butyric acid levels," *J. Neurochem*., **15**, No. 7, 603-608 (1968).
16. J. E. Madl and S. M. Royer, "Glutamate dependence of GABA levels in neurons of hypoxic and hypoglycemic rat hippocampal slices," *Neuroscience*, **96,** No. 4, 657-664 (2000).
17. M. Pokorski, E. Kolesnikova, M. Marczak, and K. Budzinska, "Neurotransmitter mechanisms in the enhancement of the hypoxic ventilatory response by antecedent hyperoxia in the anesthetized rat," *J. Physiol. Pharmacol. (Acta Pol.)*, **56**, 433-446 (2005).
18. R. Betarbet, T. B. Sherer, G. MacKenzie, et al., "Chronic systemic pesticide exposure reproduces feature of Parkinson's disease," *Nat. Neurosci.*, **3**, No. 12, 1301-1306 (2000).
19. E. É. Kolesnikova, V. I. Nosar', I. N. Man'kovskaya, and T. V. Serebrovskaya, "Aging- and experimental mitochondrial dysfunction-related modifications of energy metabolism in brainstem neurons" *Neurophysiology*, **44**, No. 1, 18-24 (2012).
20. J. V. Weil, "Ventilatory response to CO_2 and hypoxia after sustained hypoxia in awake cats," *J. Appl. Physiol*., **76**, No. 6, 2251-2252 (1994).
21. W. Q. Long, G. G. Giesbrecht, and N. R. Anthonisen, "Ventilatory response to moderate hypoxia in awake chemodenervated cats," *J. Appl. Physiol*., **74**, No. 2, 805-810 (1993).
22. H. Kimura, M. Tanaka, K. Nagao, et al., "A new aspect of the carotid body function controlling hypoxic ventilatory decline in humans," *Appl. Human Sci.*, **17**, No. 4, 131- 137 (1998).
23. R. C. Ang, B. Hoop, and H. Kazemi, "Role of glutamate as the central neurotransmitter in the hypoxic ventilatory response," *J. Appl. Physiol.*, **72**, No. 4, 1480-1487 (1992).
24. B. Hoop, J. L. Beagle, T. J. Maher, and H. Kazemi, "Brainstem amino acid neurotransmitters and hypoxic ventilatory response," *Respir. Physiol.*, **118**, 117-129 (1999).
25. J. I. Melton, J. A. Neubauer, and N. H. Edelman, "GABA antagonism reverses hypoxic ventilatory depression in the cat," *J. Appl. Physiol*., **69**, No. 4, 1296-1301 (1990).
26. I. Soto-Arape, M. D. Burton, and H. Kazemi, "Central amino acid neurotransmitters and hypoxic ventilatory response," *Am. J. Respir. Crit. Care Med.*, **151**, 1113-1120 (1995).
27. P. Ortega-Saenz, R. Pardal, M. Garcia-Fernandez, and J. Lopez-Barneo, "Rotenone selectively occludes sensitivity to hypoxia in rat carotid body glomus cells," *J. Physiol.*, **548**, No. 3, 789-800 (2003).
28. N. K. Yelmen, "The role of gamma-aminobutyric acid and glutamate for hypoxic ventilatory response in anesthetized rabbit," *Tohoku J. Exp. Med.*, **203**, 219-232 (2004).
29. N. G. Man'shina and O. A. Vedyasova, "Comparative analysis of respiratory reactions to microinjections of GAB and penicillin in the Bötzinger's complex and pre-Bötzinger's complex in rats," *Vest. SamGU (Nat. Sci. Ser.)*, **94**, Nos. 3/1, 210-218 (2012).

30. R. Itturiaga, R. Varas, and J. Alcayaga, "Electrical and pharmacological properties of petrosal ganglion neurons that innervate the carotid body," *Respirat. Physiol. Neurobiol.*, **157**, 130-139 (2007).
31. C. A. Nurse, "Neurotransmitter and neuromodulatory mechanisms at peripheral arterial chemoreceptors," *Exp. Physiol.*, **95**, No. 6, 657-667 (2010).
32. J. Buttigieg and C. A. Nurse, "Detection of hypoxia-evoked ATP release from chemoreceptor cells of the rat carotid body," *Biochem. Biophys. Res. Commun.*, **322**, No. 1, 82-87 (2004).
33. W. Rong, A. V. Gourine, D. A. Cockaine, et al., "Pivotal role of nucleotide P2X2 receptor subunit of the ATP-gated ion channel mediating ventilatory response to hypoxia," *J. Neurosci*., **23**, No. 36, 11315-11321 (2003).
34. T.-B. Lin, M.-J. Lo, C.-Y. Huang, et al., "GABAergic modulation of ventilatory response to acute and sustained hypoxia in obese Zucker rats," *Int. J. Obesity*, **29**, 188-195 (2005).
35. J. Champagnat, M. Denavit-Saubie, S. Moyanova, and G. Rondoum, "Involvement of amino acids in periodic inhibitions of bulbar respiratory neurons," *Brain Res.*, **237**, No. 2, 351-365 (1982).
36. A. M. Taveira-Da Silva, B. Hartley, P. Hamosh, et al., "Respiratory depressant effect of GABA alpha and beta-receptor agonist in the cat," *J. Appl. Physiol*., **62**, No. 6, 2264-2272 (1987).
37. M. Shirahata, "Neurotransmission in the carotid body and anesthesia," *J. Anesth.*, **16**, No. 4, 298-309 (2002).