REVIEWS

Bioenergetic Hypoxia: Definition, Mechanisms, and Methods of Correction

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Bioenergetic hypoxia is defined as a phasic process starting at the substrate site of the respiratory chain with injury to the mitochondrial enzymatic complex I and involving, as oxygen insufficiency progresses, the terminal cytochrome site of the respiratory chain. Different stages of the process, determined experimentally, are described from a bioenergetic viewpoint. The development of bioenergetic hypoxia in tissues of animals with different resistance to hypoxia is analyzed. Modern concepts of the trigger mechanisms of bioenergetic hypoxia and approaches to correcting the function of the energy system at different stages of hypoxia are discussed.

Key Words: *bioenergetic hypoxia; energy metabolism; respiratory enzymatic complexes; individual sensitivity to hypoxia; adaptation; antihypoxants*

Hypoxia is a highly prevalent phenomenon, which develops both under conditions of oxygen deficiency in the environment and as a result of a variety of diseases involving the respiratory and cardiovascular systems and impairing blood transporting function. All such disorders lead to a decrease in oxygen delivery to tissues to a level which is insufficient for maintaining function, metabolism, and structure of the cell. Thus, hypoxia is an important problem of practical and theoretical medicine.

This problem has been researched for more than a hundred years. In Russia it attracted the attention of I. M. Sechenov, V. V. Pashutin, P. M. Al'bitskii, and E. A. Kartashevskii. Later it became the main object of research for N. N. Sirotinin, A. M. Charnyi, I. R. Petrov, Z. I. Barbashova, M. N. Gaevskaya, and many others. Due to these investigations, study of hypoxic states became an important branch of fundamental and applied medicine, which still remains a priority trend for the Russian science. At present, a vast data bank on the mechanisms of hypoxia has been accumulated, permitting the creation of classifications of hypoxic states, development of prognostic criteria for assessing them, and analysis of the succession of disorders occurring under conditions of oxygen deficiency.

The intricate time course of this process and involvement of a wide spectrum of functional and metabolic systems regulating it at different levels of organization determine the multiplicity of the limiting sites and mechanisms underlying hypoxia. This fact explains why, despite an almost 100-year history of investigation, many pathogenetic aspects of hypoxia and many questions in antihypoxic defense are still not solved.

The crucial factor leading to the development of hypoxic states is oxygen delivery from the environment to the cell, where it participates in reactions of aerobic energy production as the substrate of cytochrome oxidase (CCO) — the terminal enzyme of the mitochondrial respiratory system. That is why oxygen deficiency under some conditions can limit or completely suppress aerobic energy production. The levels

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of macroergic substances — adenosine triphosphate (ATP) and creatine phosphate — are decreased in different forms of hypoxia, this decrease being one of the main signs of the condition. Oxygen and respiratory system directly interact only at the terminal site of the respiratory system with CCO, and therefore this enzyme is believed to limit the energy-producing function of mitochondria under conditions of oxygen deficiency. This phenomenon is known as **tissue** or **bioenergetic hypoxia**; it is associated with virtually any form of hypoxia and represents one of its stages.

The notion of **tissue hypoxia** was introduced by P. M. Al'bitskii in 1905. It denoted disorders of the oxidative processes in tissues caused by toxins. Later, Peters and Van Slyke in their classifications denoted it as **histotoxic anemia,** although they meant the same phenomena as P. M. Al'bitskii. The notion was extended at a conference devoted to problems of hypoxia, which was held in Kiev in 1949: since then, tissue hypoxia implied not only toxin suppression of oxidative capacity of the respiratory system, but its inactivation under the action of endogenous factors as well. The changes occurring during this process were still believed to take place only at the terminal CCO-site of the respiratory chain.

The term **bioenergetic hypoxia** was introduced by D. M. Jones [42]. It is the synonym of tissue hypoxia, disclosing its origin. Like his predecessors, D. M. Jones regarded this type of hypoxia as a result of impaired kinetic properties of CCO under conditions of oxygen deficiency. However, a phenomenon called **hypoxic paradox** was revealed in 1959. It was found that disorders of energy metabolism begin earlier and result in a critical concentration of oxygen and decrease in oxygen consumption, i.e., a decrease in CCO activity. This fact suggested the existence of other than CCO limiting sites of aerobic energy production in hypoxia, but could not be explained at that time because of the traditional ideas about the leading role of CCO in this process. Reports which help understand this phenomenon were published during two recent decades.

Time course of respiratory dysfunction in hypoxia. At the end of the eighties, we formulated the principles underlying the development of energy deficiency in hypoxic hypoxia [12-17,23,27,35,44,45]:

1) Hypoxia is a phasic process depending on the severity and/or duration of hypoxic exposure and leading to a complex of functional metabolic disorders in the cells, the principal of which is changed energy metabolism.

2) Impaired energy-producing function of the respiratory system observed in hypoxia and characteristic of it results from a series of successive changes in the activities of its different enzymatic complexes.

3) Changes in the respiratory function in hypoxia begin not at the terminal (cytochrome), but at the substrate site, involving the mitochondrial enzymatic complex I (MEC I). In response to a decrease in oxygen concentration the activity of the NADH-oxidase oxidation pathway is at first enhanced and then suppressed, disordering the electron transfer at the NADH—CoQ site and the conjugated oxidative phosphorylation process.

4) As the severity or duration of hypoxic exposure increases, disorders of electron-transporting function of the respiratory chain involve not only its substrate, but also the cytochrome site (the ***b-c*** cytochromes) and, finally, the CCO, which is the last to be inactivated.

5) CCO is not the limiting link of the process in a wide spectrum of Po_2 values and even anoxia; this is explained by the kinetic properties of CCO (low $K_m(O_2)$ determining its high affinity for oxygen). The decrease in its activity in the presence of rather high oxygen concentration in the environment may be due to limited electron supply from the substrate site of the respiratory chain through cytochromes ***b-c*** (Fig. 1).

This succession of changes in the activities of different enzymatic complexes of the respiratory system in hypoxia contradicts the traditional concept about primary role of CCO in suppression of aerobic energy production under conditions of oxygen deficiency and is based on considerable experimental data. It has been proven that the earliest response to hypoxic exposure is intensification of the NADH-dependent oxidation pathway and its increased contribution to total respiration, as estimated by the increase in respiration sensitivity to MEC I [4,10,11, 19,23,35]. An increase in the maximum activity (V_{max}) of rotenone-sensitive NADH—cytochrome ***c*** oxidoreductase at the early stages of hypoxia is one more proof of it [4]. The associated increase in electron transport from NAD-dependent substrates can be the cause of the increase in CCO reduction during this period, which was demonstrated for different cell models [18] but not yet explained. Moreover, this reduction correlates with an increase in the ATP content [21,35] and intensification of cellular functional activities: pulsed activity of neurons [19,20], myocardial contractility [10,11,22,32], hepatocyte capacity to urea production [2], etc.

The signs of MEC I inactivation after increase of its activity in hypoxia are as follows: decreased intensity of oxidation of NAD-dependent substrates and oxidative phosphorylation related to it; decreased sensitivity of respiration to specific inhibitors of NAD-dependent site of the respiratory chain [13-17,35,44,45]; recovery of respiratory carriers of MEC

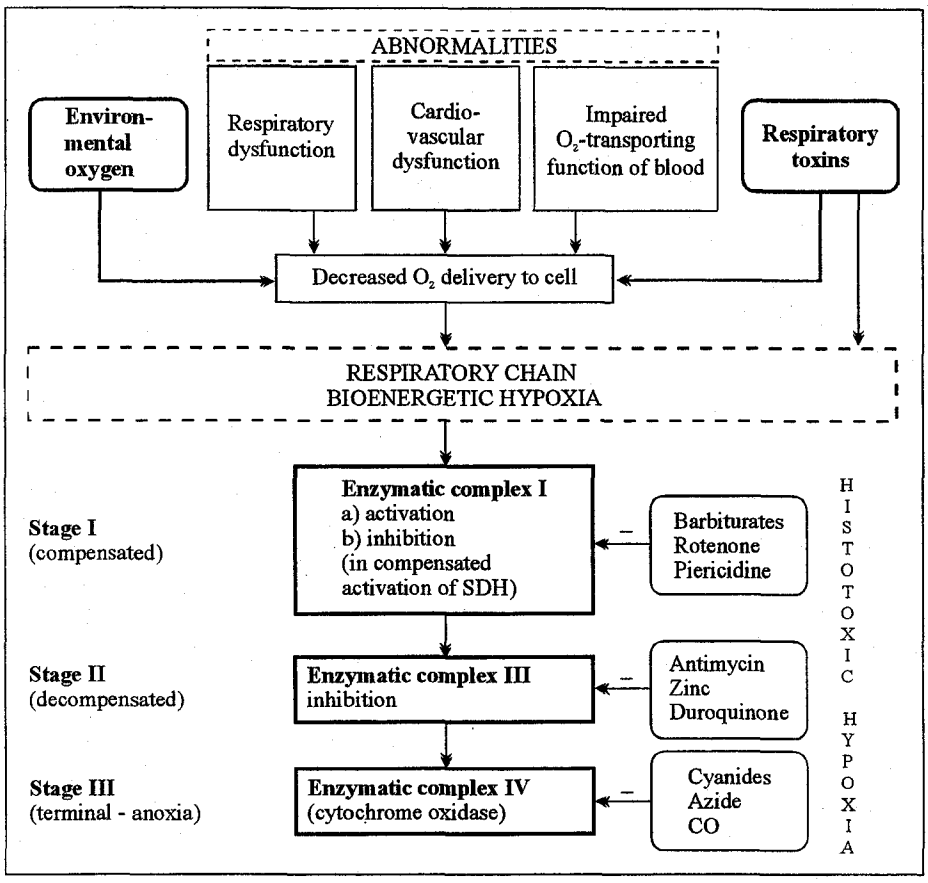

Fig. 1. Disorders of respiratory chain function in bioenergetic hypoxia.

I (pyridine nucleotides and flavins) reflecting the suppression of transfer of restored equivalents via this site [10,18,23]. The substances shunting electron transfer at the NAD—CoQ site are capable of restoring the respiration and redox status of respiratory carriers [10,11,14-16,32], which, in turn, indicates that the cytochrome site is intact at this period. There is direct evidence that the activity of rotenone-sensitive NADH—cytochrome ***c*** reductase is decreased during this stage of hypoxia, while the activities of other respiratory enzymes is retained [4]. Probably, it is this mechanism that is responsible for accumulation of the Krebs' cycle NAD-dependent substrates during the early stages of hypoxia [34].

Despite disturbed MEC I function during this period, intracellular ATP concentration and cell function are either unchanged or slightly decreased [2,15-17,19,21,35]. This is possible only if suppression of the main oxidation pathway involves activation of compensatory metabolic flows in the respiratory chain, preserving energy-producing function of the cytochrome site.

Previously, we showed that succinate oxidase oxidation plays a special role in this process; due to activation of this pathway, electron transport through the cytochrome site to CCO and capacity of oxidative phosphorylation are retained [10,11,13-17,27,35]. The probability of a compensatory increase in succinate oxidase oxidation during the early stages of hypoxia has been proven [9,31,33]. Therefore, alteration of the metabolic flows delivering the recovering equivalents into the respiratory system is an early sign of oxygen insufficiency.

If oxygen deficiency increases or duration of hypoxic exposure is prolonged, the above-mentioned disorders are followed by suppression of electron transfer at the cytochrome ***b-c*** site [10,11,14-17,44,45]. Although ATP still can be produced at the expense of MEC IV activity, the activity of CCO apparently decreases because electron delivery from the substrate part of their respiratory chain (both NAD-dependent and succinate oxidase) ceases or drops. As a result, the ATP content drops, and a linear relationship appears between respiration and ATP concentration, on the one hand, and Po_2, on the other. The membranes are labilized and cytosolic enzymes released, free-radical products are actively produced, energy-dependent processes and specific function of the cell are almost completely suppressed, and the adenine nucleotide degradation products (adenosine, inosine, and hypoxanthine) appear at this period, which is followed by cell death.

However, complete inactivation of CCO is observed only under conditions approximating the anoxic. Respiration and oxidative phosphorylation are completely suppressed in this case.

It is noteworthy that the status of respiratory enzymes in hypoxia was studied by few scientists; hypoxic activation of the NADH-dependent oxidation was described in several reports [4,34,49]. There are only two papers devoted to subsequent phasic changes in the respiratory system [4,34]. By contrast, many researchers studied the time course of energy metabolism disorders in ischemia of different organs, which is similar to the above-mentioned changes [41,46-48,50-52]. Experiments demonstrated not only decreased activity of the NADH-oxidase oxidation, but also confirmed that inhibition of MEC I precedes the inactivation of other mitochondrial enzymes [52]. The first data on disorders in the NADH-oxidase oxidation in ischemia were reported in 1957, but were neglected by specialists and not explained by the authors. Only two decades later these scientists came to a conclusion about the probable suppression of the MEC I function under the same conditions [41]. However, all these data did not help the researchers understand that phasic changes of respiratory enzymes observed in hypoxia are the components of one process eventuating in CCO inhibition.

Thus, **bioenergetic hypoxia** is a **complex phasic process,** developing in different forms of oxygen insufficiency. Successive changes in MEC properties disordering the energy-producing function of the respiratory system underlie this process; these changes start at the substrate and involve the terminal site of the chain. Three stages of bioenergetic hypoxia can be distinguished, differing by the mechanism of action. **Stage I** is related to inactivation of the NAD-dependent oxidation and the concomitant enhancement of the succinate oxidase pathway (the **compensatory stage of bioenergetic hypoxia**). **Stage II (noncompensated) of bioenergetic hypoxia** is characterized by suppression of the electron-transporting function of the respiratory chain at the site of cytochromes ***b-c***. **Stage III (terminal) of bioenergetic hypoxia** is characterized by CCO inhibition under conditions of anoxia. We showed that all these stages of bioenergetic hypoxia correlate with phasic changes in the content of ATP and the leading energy-dependent processes in the cells, anticipating disorders of other functional metabolic parameters regulating cell life [2]. For example, increased membrane permeability and intensity of lipid peroxidation and adenine nucleotide degradation are observed only during the terminal stage. Our estimations demonstrate that glycolysis does not notably contribute to replenishment of the ATP pool during the compensatory phase of bioenergetic hypoxia, nor does its sharp activation during the decompensation phase prevent the drop of ATP level.

We should like to draw attention to the fact that MEC I inhibition is preceded by activation of NAD-dependent oxidation, which is generally paralleled by increased production of ATP. Can we regard this reaction as a sign of hypoxia, although it does not lead to cell de-energization? We believe that this stage should be regarded as a peculiar urgent nonspecific compensatory reaction of the energy system to decreased oxygen delivery to the cell and that changes similar to those leading to exercise hypoxia or stress hypoxia underlie it. In the latter case, a decrease in intracellular Po_2 in response to intensification of exercise leads to compensatory activation of energy production.

We should like to emphasize that limitation of oxidation of NAD-dependent substrates at the early stages of oxygen insufficiency followed by disorders of electron transfer through the cytochrome site, leads to the loss of cell capacity to oxidize a number of even available energy substrates. Thus, the "substrate hunger" is associated with bioenergetic hypoxia at the early stages of oxygen insufficiency. In other words, ischemia is a component of bioenergetic hypoxia.

Bioenergetic disorders similar to the above-mentioned are not only various forms of oxygen insufficiency, but different substances of exo- and endogenous origin, termed as the "respiratory system toxins." As already mentioned, tissue or bioenergetic hypoxia was regarded previously as specific inhibition of CCO by toxic agents such as cyanides, azide, or CO, which inhibit electron transfer at the terminal site of the respiratory chain and suppress the enzyme capacity to react with oxygen. Such a form of hypoxia was sometimes called **chemical, histotoxic,** or **cytotoxic.** Now we know that electron transfer inhibition by different toxic agents of exo- or endogenous nature can occur at any site of the respiratory chain.

For example, many chemical compounds are capable of suppressing the activity of MEC I, thus impairing electron transport at the NAD—CoQ site. Among these agents are barbiturates, rotenone, and piericidine, as well as numerous substances exerting rotenone-like effects, capable of reacting with the hydrophobic site of this complex [8]. Obviously, changes caused by these agents are similar to stage I of bioenergetic hypoxia. There are inhibitors of other sites of the respiratory chain. For example, malonate, a competitive inhibitor of succinate dehydrogenase, suppresses succinate-dependent oxidation which can be activated in hypoxia. Antimycin, Zn^{2+}, and other compounds block the respiratory chain near the ***b-c*** cytochromes (MEC III). KCN, azide, and CO in-

hibit CCO. Therefore, it is obvious that the notion of **hypoxic hypoxia** is wider than its previous definition. It is not only a form of bioenergetic hypoxia, but can develop during toxin inactivation of CCO or any other site of the respiratory chain. Inhibitory analysis is widely used for *in vitro* simulation of disorders at various sites of the respiratory chain, that is, for studies of different stages of bioenergetic hypoxia.

Development of bioenergetic hypoxia in animals with different resistance to oxygen deficiency. There are individuals characterized by different resistance to oxygen deficiency in any population of inbred animals. This feature is determined by genetic and phenotypic characteristics: metabolism, maturity of the regulatory mechanisms, and their capacity to restructuring under conditions of hypoxia in order to retain the host viability. Individual reactions of the organism to hypoxia play an essential role in the development, course, and outcome of the resultant pathological state. Individual differences in reaction to hypoxia, assessed by changes of the functional activity, are retained at the tissue and cell levels [2,4-7,10,13-17,21-23,35,36,45].

For example, 2/3 of neurons are resistant to oxygen deficiency in the cerebellum of animals high-resistant (HR) to hypoxia, whereas in low-resistant (LR) rats, 2/3 of the cerebellum neurons are sensitive to it [20]. The time course of pulsed activity of the neurons during Po_2 decrease is different in HR and LR animals. According to electroencephalography, the reaction of brain cortex during "lifting" in a pressure chamber differs in HR and LR animals in exactly the same way [30]. Myocardial contractility of HR rats is impaired under conditions of induced hypoxia later and to a lesser extent than in LR animals [10,11,15,35,45]. These findings imply different organization of metabolism, including the energy metabolism, in HR and LR rats [14-17,45].

Our experiments demonstrated that the initially similar efficiency of oxidative phosphorylation in the brain mitochondria of HR and LR rats is due to a higher velocity of phosphorylating respiration and strain of energy production in the LR animals [25]. This indicates that the processes of oxidative phosphorylation are less economic in them. Moreover, during oxidation of the NAD-dependent substrates by the brain mitochondria of LR rats, the rate of electron transfer in the respiratory chain is the maximum and leads to exhaustion of the reserve potential of their respiratory activity, which is not observed in the brain of HR animals. The animals with different sensitivity to acute hypoxia under normoxic conditions and different organization of energy metabolism differ by the parameters of higher nervous activity [43].

These differences do not disappear in hypoxia. Activation of energy metabolism under conditions of hypoxia associated with intensification of NAD-dependent oxidation, being higher in the brain of HR animals [14,15,35,45]. By contrast, inhibition of NAD-dependent oxidation, following its intensification with aggravation of hypoxia, develops earlier and is more pronounced in the brain of LR rats and is associated with a more pronounced recovery of the respiratory transmitters of this site [14]. These changes in MEC I activity correlate with the time course of the relationship between ATP content and Po_2. The gradient of ATP drop in the brain of LR animals at low Po_2 values is much more expressed than in HR animals [14,15,35]. Thus, changed activity of NAD-dependent oxidation directly correlates with brain resistance to hypoxia.

The differences in oxidative capacity of the NAD-dependent site of respiratory chain in the brain of HR and LR animals reflect kinetic characteristics of MEC I. The V_{max} and K_m values for mitochondrial NADH—cytochrome *c* reductase in the brain of LR rats are significantly lower than in HR animals [6]. This means that MEC I in the brain of LR animals is more rapidly saturated with the substrate (NADH) and oxidizes it slower, which can be the cause of lower activity of NADH-oxidase oxidation of substrates in the brain of LR animals.

The same kinetic features of the enzyme underlie the lower oxidative efficacy of NADH—cytochrome *c* reductase of LR rat brain at high values of the NADH/NAD ratio, which are observed in hypoxia. Due to them, the NADH-oxidase pathway of oxidation in the brain of LR rats is limited earlier and is more expressed than in HR animals [13-17,25,35,45]. Thus, although this route of oxidation is limiting at the early stages of hypoxia and determines the disorders of energy production in the brain of both HR and LR animals, the degree of its suppression in hypoxia is different.

The kinetic characteristics of succinate-cytochrome *c* reductase and cytochrome *c* reductase in the brain of HR and LR animals differ just negligibly, as is the content of mitochondrial cytochromes [5,6]. Still, the contribution of the succinate-oxidase oxidation to general respiration in the brain of intact LR rats is greater than in HR rats. The contribution of this oxidation pathway increases in hypoxia, when the signs of suppressed NAD-dependent oxidation become manifest, the increase being much greater in LR than in HR rats; this explains the different reaction of the animals to acute hypoxia.

The differences in the activities of NAD-dependent and succinate-oxidase oxidation can be observed in the myocardium of HR and LR rats [10,11,22,32],

but the contribution of NAD-dependent oxidation of substrates to cardiac energy metabolism is appreciably lower in HR rats under normoxic conditions than in LR rats. By contrast, the activity of succinate-oxidase oxidation is higher in the myocardium of HR than in LR rats. During the early stage of hypoxia, inactivation of NAD-dependent oxidation in the myocardium of LR animals (where this metabolic pathway predominates) determines a more rapid drop of ATP content and myocardial contractility.

Electron transfer to CCO is retained at the early stages of hypoxia in the myocardium of HR rats with the initially predominant succinate-oxidase oxidation, possessing kinetic advantages under conditions of high recovery of MEC I transmitters, thus preventing a drop of ATP content and suppression of myocardial contractility.

Therefore, tissue-specific features of aerobic energy formation are of crucial importance for the formation of individual resistance of the organism to oxygen deficiency.

As for CCO, we showed that at the same V_{max}, K_m values for CCO of the brain of LR rats are lower than in HR rats [6]. Therefore, CCO affinity for cytochrome *c* is higher in the brain of LR than HR animals. This is probably important in hypoxia, when the mitochondria lose cytochrome *c* due to membrane labilization. In this case, a decrease in the brain CCO activity in LR rats occurs later than in HR rats, because lower concentrations of cytochrome *c* are needed to maintain it. The same is true for limited electron flow to the substrate site of the respiratory chain and the relevant decrease in recovery of the cytochrome site. Since in the LR rat brain CCO is saturated with lesser amounts of cytochrome *c* in comparison with the brain of HR rats, the enzyme functions more effectively in LR rats.

Similar changes in the kinetic characteristics of mitochondrial enzymes were observed in the liver of HR and LR rats [6].

Mechanisms of dynamic disorders in the activities of the respiratory enzymatic complexes in hypoxia. The triggering mechanism of metabolic disorders in the cell under conditions of oxygen deficiency leading to inactivation of the respiratory enzymes is still unknown.

Changed intracellular pH is one of the main candidates to this role [40]. Different effects of mild and stringent acidosis associated with hypoxia were reported [3]. The former activates many metabolic processes in the cell, including respiration, which may indicate the probability of increased activity of NAD-dependent oxidation. In stringent acidosis, the accumulation of intracellular NADH, one of whose sources can be increased production of fatty acids at the early stages of hypoxia, and deceleration of NAD regeneration needed for the Embden—Mayerhoff cycle, can be among the causes responsible for decreased triose oxidation and increased recovery of MEC I respiratory transmitters. Recent reports indicate that changes in pH virtually do not affect the energy metabolism in hypoxia [38].

Free radicals may be another factor contributing to the pathogenesis of mitochondrial damage and respiratory dysfunction in hypoxia and ischemia.

MEC I and MEC III are capable of generating superoxide radicals ($O_2^{\cdot}$) and H_2O_2 in the presence of NADH even under normoxic conditions. NADH-dependent generation of free radicals is increased in ischemized tissues [47,48,52]. Higher degree of recovery of the respiratory chain transmitters and the presence of relatively high oxygen concentrations in the environment create particularly favorable conditions for their formation. There is considerable evidence that active oxygen forms produced in the MEC I and MEC III are responsible for the loss of their activity under such conditions [39,47,48,52]. MEC I is the most sensitive to toxic action of free radicals, in contrast to MEC II and MEC III which are far less sensitive to it. Thus, free-radical reaction products intensely inactivate electron transport between NADH-dehydrogenase and ubiquinone and far less intensely between ubiquinone and cytochrome *c*.

H_2O_2 is the most important factor in this process, that is why catalase stimulating H_2O_2 degradation possesses an antihypoxic effect. Mitochondrial enzymes or NADH-oxidase of the external mitochondrial membrane (not bound to the respiratory chain) can be the sources of H_2O_2. In the brain or myocardium, it is the mechanism participating in the oxidation of cytoplasmic NADH and formation of H_2O_2 and activated in hypoxia or ischemia [28]. Free-radical processes are particularly important in chemical hypoxia. Other sources of free radicals cannot be ruled out, for example, xanthine oxidase, whose activity increases under conditions of high recovery in the cell at the expense of proteolytic conversion from xanthine dehydrogenase. However, since hypoxanthine (an adenine nucleotide degradation product) is a substrate of this reaction, it may be realized only during energy decompensation.

Numerous oxygen-dependent monoamine oxidase oxidation reactions with K_m (O_2) 2-4 orders of magnitude higher than that of CCO are another potent source of free radicals. Due to this fact, they are suppressed even at a slight decrease in the oxygen content in the environment. Intermediate products of oxygen reduction can be formed and accumulated in this case, which can alter physicochemical characteristics of the membrane lipids, specifically, their

microviscosity and density, and even modify the conformation motility and functional activity of membrane-bound proteins, transport proteins — transmitters, receptors, enzymes, ionic channels, surface charge values, etc. Free radicals can aggravate the damage to water and ionic balance in the cell, swelling of the mitochondria, tissue edema, disorders of membrane phospholipid metabolism, and increase their fluidity and permeability, leading to leakage of CoQ and cytochrome *c* at late stages of hypoxia (ischemia), which can be the cause of impairment of the MEC III electron-transporting function [1,51]. Ubiquinone deficiency boosts the production of free radicals, causing additional damage to biological membranes. Previously, we demonstrated an increase in peroxidase activity, paralleled by production of OH^- and H_2O_2 [24].

Disorders of calcium metabolism occupy a special place in the mechanism of hypoxic disorders, as Ca is an important regulator of cellular metabolism. Calcium is released in hypoxia from intracellular depots, leading to activation of the arachidonic acid cycle, followed by release of prostaglandins, leukotrienes, thromboxane, and prostacyclins, that can also stimulate the free-radical processes. An immediate result of calcium metabolism disorders is the changed status of many Ca-dependent mitochondrial enzymes (pyruvate dehydrogenase, isocitric dehydrogenase, and α-ketoglutarate dehydrogenase). This leads to Ca-dependent depression of respiration and energy production and an increase in the NADH/NAD ratio. Calcium participates in MEC I inactivation [38], implying that it regulates inhibition of electron-transporting function of the respiratory chain in hypoxia.

Correction of bioenergetic disorders in hypoxia. Aerobic energy metabolism is the key factor in the cascade of metabolic transformations occurring in the cell under conditions of hypoxia and regulating the process in general. Therefore, correction of mitochondrial function and elimination or prevention of bioenergetic hypoxia is the major problem in protecting the organism from oxygen insufficiency. Since virtually any disease involves bioenergetic hypoxia, the significance of such protection cannot be overrated. There are two approaches to the solution of this problem: drug therapy and natural increase of cell resistance to oxygen deficiency.

Drug correction of energy disorders caused by hypoxia is based on the knowledge of the mechanisms of bioenergetic hypoxia. The respiratory function recover at the early stages of hypoxia includes either restoration of electron-transporting and conjugated function of its NAD-dependent site or activation of compensatory metabolic pathways other

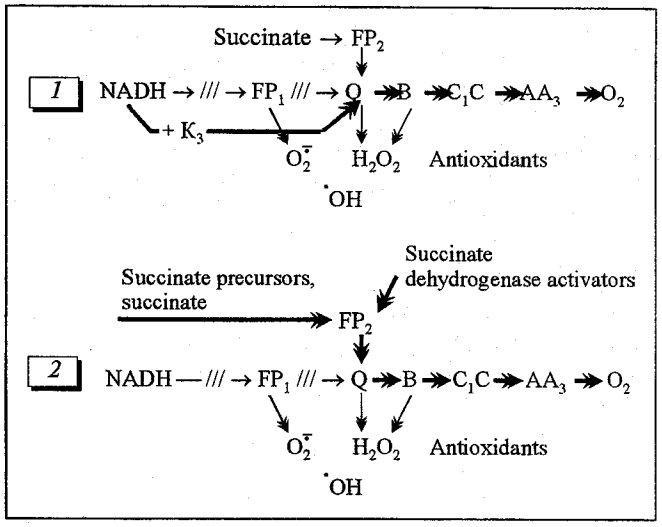

Fig. 2. Correction of the substrate site of respiratory chain at the early stages of hypoxia. *1*) repair of the function of NAD-dependent site of respiratory chain by vitamin K_3; 2) activation of succinate-oxidase oxidation. FP — flavoprotein; Q — coenzyme Q; B, C_1C, AA_3 — cytochromes.

than NADH-oxidase pathway and ensuring electron flow to the cytochrome site, thus maintaining its capacity to energy production [14,16].

In the former case drugs with the donor-acceptor action are used, for example, quinones. Vitamin K_3 (menadione or 2-Methyl-1,4-naphthoquinone), which exhibits high antihypoxic activity, has been well described. After incorporation in the respiratory chain by means of menadione reductase (DT-diaphorase), which is present in the majority of tissues in sufficiently high amounts, it shunts the electron flow at the NADH—CoQ site and repairs the electron flow from NADH to CCO disrupted by hypoxia (Fig. 2) [10,11,13-15]. Vitamin K_3 is used abroad for the treatment of some myopathies associated with congenital MEC I insufficiency. Protective action of other synthetic quinones has been demonstrated, for example, aminobenzoquinones [3], but high toxicity impedes their practical use.

Another approach to repair the function of the respiratory chain at the early stages of hypoxia is the use of agents activating compensatory metabolic production of ATP, alternative to the NADH-oxidase oxidation. One such a method is the succinate-oxidase oxidation. However, administration of exogenous succinic acid is ineffective because it poorly penetrates through biological membranes. Succinate-oxidase oxidation in hypoxia can be activated by increasing the activity of succinate dehydrogenase; by activating the enzymes of reactions with endogenous production of succinate, or by administration of its precursors which are metabolized in these reactions; by various organic succinate-containing compounds

which facilitate its penetration in the cell [14-16]. As for the latter agents, we showed that many succinate-containing derivatives of hydroxypyridine elicit strong antihypoxic effects [26,37]. Mexidol (3-hydroxy-6-methyl-2-ethylpyridine succinate), like all 3-hydroxypyridines, is capable of membrane labilization, thus promoting penetration of the molecule into the cell and utilization of succinate as energy substrate of respiratory chain. We proved that the succinate-oxidase oxidation is activated in the presence of mexidol, and under conditions of limited NAD-dependent oxidation at the early stages of hypoxia, this activation helps preserve the capacity of the cytochrome site to energy production. Antihypoxic effects of mexidol and its advantages over other antihypoxants were demonstrated experimentally [26,37].

At later stages of hypoxia, when electron transfer at the ***b-c*** cytochrome site is limited because of membrane labilization and CoQ and cytochrome ***c*** loss, exogenous cytochrome ***c*** and CoQ promote the recovery of mitochondrial respiratory function [1]. It was reported that both substances elicit sufficiently high protective effects under conditions of hypobaric hypoxia and a wide spectrum of concomitant pharmacological effects (antioxidant and psychotropic activities, antiepileptiform action, etc.). Their antihypoxic activity increases in combinations with analogs and other antihypoxants with direct energizing action (mixtures of NAD^+, cytochrome ***c*** and inosine; succinate and ubiquinone; succinate and cytochrome ***c***). These data indicate that the targets of these agents in hypoxia are different.

Another nonpharmacological approach: increase of the natural resistance of the organism to hypoxia by adaptation cannot be neglected. Prolonged periodical and particularly intermittent adaptation to hypoxia leads to rearrangement of energy metabolism so that not only oxidative capacity of main NAD-dependent oxidation is restored, but kinetic properties of respiratory enzymes are changed, specifically, MEC I and MEC IV. It is a mechanism of increasing resistance of respiratory chain under conditions of low oxygen content. Thus, energy metabolism plays the key role in the mechanisms of adaptation and its transformation under such conditions increases the efficacy of energy production and utilization in the cells [4-6,29,45]. The contribution of NADH-oxidase oxidation to aerobic energy production during long adaptation increases similarly as the enzyme capacity to oxidize NADH. Kinetic characteristics of CCO change in parallel with this [6]. This prevents the inactivation of these enzymes in hypoxia. Thus, adaptation is a highly effective method of increasing the resistance of the cell and organism to oxygen insufficiency.

This review demonstrates that studies of energy metabolism under conditions of oxygen deficiency are of paramount importance for practical medicine and open new prospects for preventing or alleviating bioenergetic hypoxia and its consequences.

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