REVIEWS

Experimental Modeling of Damaging and Protective Hypoxia of the Mammalian Brain

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Abstract—Currently, there is a new surge of interest in the problem of hypoxia, almost lost in recent decades. Due to the fact that the circle of competent specialists in this field has significantly narrowed, it is necessary to carry out an intensive exchange of knowledge. In order to inform a wide range of interested researchers and doctors, this review summarizes the current understanding of hypoxia, its pathogenic and adaptogenic consequences, as well as key physiological and molecular mechanisms that implement the response to hypoxia at various levels—from cellular to organismic. The review presents a modern classification of forms of hypoxia, the understanding of which is necessary for the formation of a scientifically based approach to experimental modeling of hypoxic states. An analysis of the literature covering the history and current level of hypoxia modeling in mammals and human experiments, including methods for creating moderate hypoxia used to increase the resistance of the nervous system to severe forms of hypoxia and other extreme factors, is carried out. Special attention is paid to the discussion of the features and limitations of various approaches to the creation of hypoxia, as well as the disclosure of the potential for the practical application of moderate hypoxic effects in medicine.

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With all the typological diversity of hypoxia and the extensive classification of its forms, the brain is the most sensitive target of its harmful effects.

Hypoxic factor is one of the leading in the pathogenesis of cardiovascular and neurological diseases, which occupies the first place in prevalence and mortality among other pathological conditions of the body. With all the etiological diversity of hypoxia and the extensive classification of its forms, the brain is the most sensitive target of its harmful effects. Accordingly, the study of mechanisms and development of new ways to increase brain tolerance to the damaging effects of hypoxia is one of the central problems of theoretical and applied physiology, starting from the pioneering works under the leadership of L.A. Orbeli up to the present day. Two main approaches to solving this problem can be distinguished. The first one consists in using pharmacological medications (nootropics, neuroprotectors, antihypoxants, neurotrophins, etc.). Although it should be recognized that the molecular mecha-
nisms of many existing pharmacological drugs are poorly understood, their effectiveness is not always high, and the side effects can initiate additional pathological processes. The second approach is based on the use of non-drug means, mobilizing endogenous, evolutionarily acquired and genetically fixed defense mechanisms. In particular, it includes moderate but sublethal hypoxia. The present review demonstrates the evolution of ideas about hypoxia, ways of experimental modeling of its various forms and mechanisms of both pathogenic and adaptogenic effects on the body as a whole and, especially, on the central nervous system. The authors expect that coverage of this invariably actual topic will serve to improve ways to increase the resistance of the human brain to damaging influences.

THE CONCEPT OF HYPOXIA

By the 1960s, the studies that began as early as the 19th century had formed the concept of hypoxia as the most important manifestation of tissue and cell pathology caused by a complex of physical, chemical and biological factors. By the end of the century ideas were formed not about pathogenic, but about the adaptogenic properties of moderate hypoxia, which can be used as a preconditioning (preventive, protective) procedure to reduce the damaging effect of the subsequent, more severe form of hypoxia on the brain [1]. The main pathogenetic element in hypoxia of any etiology is not the reduced oxygen level as such, but the limitation of the cell ability to produce a sufficient amount of macroergic compounds to ensure the complex of its endergonic processes (energy deficiency). In mammalian nerve cells, the main energy production is provided by mitochondria during oxidative phosphorylation and carbohydrate degradation in the Krebs cycle. O₂ is the final electron acceptor in the chain of redox catabolism of glucose. Thus, the body is required to have a constant supply of substrate (glucose) and final oxidant (oxygen) to the cells. Obviously, this is why hypoxia is often referred to as a condition associated with O₂ deficiency alone, and ischemia is referred to as hypoxia aggravated by glucose deficiency. The phenomenon of ischemia attracts more interest of researchers than “pure” hypoxic hypoxia, because it is more often observed in clinical situations (myocardial infarction, stroke, thrombosis, edema, tumors, traumatic brain injuries, etc.). The most vulnerable in hypoxia are nerve cells of the central nervous system, because their energy consumption is extremely high, and the reserves of respiratory substrates in the form of glycogen (such as in muscles) are extremely limited or absent. Moreover, under hypoxia, brain neurons, unlike the cells of peripheral tissues, cannot get energy by triggering glycolysis processes for a long time [2]. Therefore, when discussing the mechanisms of hypoxic cellular pathogenesis and the ways to protect against it, brain neurons are primarily meant.

Since the function of supplying cells with oxygen-substrate is performed by the circulatory system, any causes limiting oxygen transfer from atmosphere to lungs, from alveoli to blood, from blood to cells and from cytosol to mitochondria, as well as reasons limiting blood glucose level and efficiency of its transport to mitochondria, cause development of hypoxic (ischemic) state. The “dose” of hypoxia or ischemia, i.e. the degree and duration of oxygen-substrate deficiency, determines the development in the body, tissues and cells of either compensatory-adaptive reactions or pathogenesis leading to death.

When choosing an experimental model to study these processes, the above-mentioned difference between hypoxic and ischemic conditions should be taken into account. Namely, the fact that in the latter case the oxygen supply limitation is aggravated not only by hypoglycemia, but also by impaired blood flow functions such as integrative neurohumoral signaling, metabolite transport, water-salt metabolism, etc.

PATHOGENIC AND ADAPTOGENIC EFFECTS OF HYPOXIA

The first changes in the body during hypoxia development are characterized by the inclusion of a complex of compensatory-adaptive reactions of different levels, aimed at the preservation of homeostasis (compensation phase). In this phase, we distinguish between reactions aimed at adapting to short-term acute hypoxia (urgent) and reactions providing stable adaptation to less
severe, but prolonged or repeated hypoxia (the reactions of long-term adaptation). If these reactions are insufficient, and hypoxic factors continue to act, structural and functional disorders develop in the body (decompensation phase). Both phases are manifest themselves at all levels from the organismal to the cellular. The basis of urgent compensatory reactions of the organismal level to hypoxia is the cardio-respiratory reflex, which begins with depolarization of O₂-sensitive glomerular cells of carotid bodies, triggered by mitochondrial signaling. [3, 4]. The complex reflex response is expressed in: increase of alveolar ventilation through deepening and more frequent breathing and mobilization of reserve alveoli; in heart rate increase; in blood release from reserve blood depots; in increase of heart stroke and minute volume; in increase of blood flow rate (primarily in brain); in decrease of blood supply to muscles, skin and other “noncritical” consumers; in limiting activity of organs and tissues, not directly involved in oxygen transport [5].

In response to hypoxia blood oxygen capacity increases due to increased erythrocyte washout from the bone marrow and activation of erythropoiesis; the oxygen capacity of hemoglobin increases and its dissociation in the peripheral blood stream is facilitated; the conjugation of the processes of biological oxidation and phosphorylation increases; anaerobic synthesis of ATP enhances due to the activation of glycolysis; in various tissues the production of nitric oxide is upregulated leading to the dilation of precapillary vessels and reduction of platelet adhesion and aggregation [6].

An important adaptive response in hypoxia is the activation of the hypothalamic-pituitary-adrenal system (stress-syndrome), whose hormones (in particular, glucocorticoids) stimulate various intracellular signaling systems, leading to the expression of genes and their protein products, whose activity is aimed at compensating energy deficiency, stimulating neuroplasticity and resisting neurodegenerative processes [7, 8].

If the organismal and tissue compensatory reactions are unable to withstand the continuing and/or deepening hypoxia/ischemia, deenergization develops in the nerve cells, disturbing homeostatic balance of the most important electrolytic and regulatory cations (K⁺, Na⁺, Ca²⁺). This leads to depolarization of plasma and internal membranes; glutamate turnover in the neuron-glia system and mechanisms controlling its reception by neuromembranes are destroyed. This results in hyperexcitation of glutamate receptors and development of neuronal excitotoxicosis, accompanied by their overload with extracellular Ca²⁺. The increase in intracellular Ca²⁺ concentration is reinforced by its release from the endoplasmic reticulum and mitochondria, as well as by additional entry through nonspecific cationic plasmolemma channels. Pathogenic Ca²⁺-dependent phosphatases and kinases are activated, triggering mechanisms of neurodegeneration. The pathogenic situation is deepened by the accumulation of toxic “unstructured” proteins, hyperactivation of free-radical processes, mitochondrial disorganization and other molecular processes leading to neuronal death [9].

In humans, acute hypoxia, caused, for example, by reduction of oxygen content in the inhaled air to 15–10% or altitude hypobaric hypoxia (mainly in mountaineers or pilots) is accompanied by distinct neuropsychiatric disorders. There have been many studies of such disorders occurring at moderate, high and extreme altitudes. At moderate altitude, a decrease in accuracy and speed of motor reactions and psychomotor efficiency has been found, there are changes in handwriting and skipping of letters, the perception thresholds for taste, smell, pain are reduced. Aesthetic illusions, euphoria and visual hallucinations often occur. At altitudes of 6000 m and higher, distinct cognitive violations occur. Learning ability, short-term memory, speech, and cognitive flexibility decrease, and there are signs of depression [10]. Meanwhile, it is noted that the severity of the mentioned disorders depends on the personal psycho-type, work motivation, experience and other individual psychophysiological characteristics. Under hypoxia, this specificity is supported by the pattern of local cerebral blood flow regulation, in which signaling within the neuron-astrocyte-microvascular system plays an important role [11]. Psychophysiological disorders begin to be accompanied by somatic ones. In particular, the compensatory mechanism of hypoxic pulmonary vasoconstriction during deepening and pro-
longation of hypoxic state causes pulmonary edema [12]. Hypoxic tachycardia continues to increase, but the pulse amplitude decreases up to thread, atrial and ventricular fibrillation occurs. Systolic pressure after compensatory rise starts to fall [13].

CLASSIFICATION OF HYPOXIC CONDITIONS

To choose an adequate experimental hypoxic model on animals in vivo or on isolated tissues and cells (ex vivo, in vitro), it is necessary to take into account the actually occurring hypoxic conditions, which may have different causes and course of development. In this regard, it is important to characterize the forms of hypoxia occurring in humans in extreme situations or in pathological states. Domestic medical literature usually uses the classification proposed by Sirotinin and Kolchinskaya [14].

One of the most typical forms of hypoxia, according to the generally accepted classification, is hypoxia associated with decreased \( O_2 \) content in inhaled air, referred to as “exogenous” and called hypoxic hypoxia. Its two varieties are hypobaric and normobaric hypoxia.

**Hypobaric hypoxia.** This condition occurs when in a rarefied atmosphere the oxygen partial pressure decreases. It can occur during mountain climbing (mountain sickness) or during decompression (leakage) of aircraft (altitude sickness). During rapid decompression, which is encountered by flight personnel, a person develops an altitude-dependent dysbarism symptom complex. At an altitude of 3000–4000 m there is an expansion of gases and a relative increase in their pressure in enclosed body cavities (nasal appendages, frontal sinuses, middle ear cavities, pleural cavities, gastrointestinal tract) which leads to irritation of corresponding receptors, causing sharp “altitude pains”. At the altitude of 9000 m blood desaturation and gas embolism develops, resulting in ischemia, vision disorders, mechanical barriers to air entry. Respiratory hypoxia is usually combined with hypercapnia. Hypoxic conditions of the above forms are usually divided according to their severity. This gradation is based on oxygen tension in arterial blood, which normally is 90–100 mm Hg. Mild hypoxia is characterized by a decrease in \( pO_2 \) to 60–50 mm Hg, medium—to 50–40 mm Hg, severe—to 40–20 mm Hg, and extremely severe—below 20 mm Hg.

**Circulatory (ischemic) hypoxia.** Hypoxic condition associated with disorders of the circulatory system, resulting in inadequate supply of organs and tissues with oxygen and glucose. The most important indicator of its development is a decrease in the minute blood volume. Circulatory hypoxia can be caused by cardiac disorders (heart attack, cardiosclerosis, cardiac overload, disorders of neurohumoral regulation of cardiac function) or hypovolemia due to massive blood loss. Brain ischemia occurs as a result of sclerosis, thrombosis, or rupture of cerebral vessels. A sign of ischemic hypoxia is a reduced oxygen tension
in the venous blood while its content in the arterial blood is normal (high arteriovenous oxygen ratio).

**Hemic hypoxia.** A form of hypoxia associated with blood pathology, leading to a decrease in its oxygen capacity. Causes are anemia (decreased hemoglobin content in blood) or impaired ability of hemoglobin to bind, transport and deliver oxygen to tissues (formation of carboxyhemoglobin, methemoglobin, genetic anomalies of hemoglobin). A sign of hemic hypoxia is a decrease of oxygen level in both arterial and venous blood.

**Tissue (cytotoxic) hypoxia.** A form of hypoxia associated with inhibition of mitochondrial energy production. This type includes the following conditions: (a) toxin-induced downregulation of respiratory enzymes activity or synthesis; (b) death or structural abnormalities of mitochondria; (c) separation of biological oxidation and phosphorylation processes, when tissue oxygen consumption is not reduced or may increase, but macroenergetic production is not enough to cover their energy requirements. A sign of tissue hypoxia is increased oxygen content in the venous blood (low arterio-venous oxygen ratio).

**Substrate (hypoglycemic) hypoxia.** A form caused by a lack of glucose as a substrate of cellular respiration. This condition results in disruption of all links of biological oxidation. Substrate hypoxia usually occurs in disorders of carbohydrate metabolism (diabetes mellitus, etc.), as well as in severe starvation.

**Overload hypoxia.** A special form of hypoxia caused by excessive energy demand. This condition occurs during intensive activity of an organ or tissue, when functional reserves of oxygen transport and utilization mechanisms without pathological changes are insufficient to meet the sharply increased demand for oxygen (excessive muscular work, cardiac overload). A sign of overload hypoxia is the phenomenon of “oxygen debt”, i.e. the development of venous hypoxemia and hypercapnia with increased rate of oxygen delivery and consumption.

Human hypoxic conditions are sometimes classified by the rate of onset and progression. **Instant hypoxia** can occur during decompression of high-altitude aircraft and develops within seconds. **Acute hypoxia,** resulting from altitude sickness, asphyxia, or profuse blood loss, develops within minutes. **Subacute hypoxia** develops within hours, days, or weeks. This type is seen with a short stay in high altitude conditions, with acute pneumonia or acute cardiac or respiratory failure. **Chronic hypoxia** can last for months or years. It is characteristic for a long stay in the highlands or in some chronic diseases.

**EXPERIMENTAL MODELS OF HYPOXIA**

In experimental practice, many models of hypoxia are used on animals (mostly rodents) to simulate and study pathological or adaptive mechanisms initiated in humans by various forms and doses of hypoxia. Relatively safe forms and doses of hypoxia are used on primates and human subjects. These are usually dosed exposures to hypoxic or overload hypoxia. Two main groups of experimental hypoxic models are applied to animal studies. In the first one, hypoxic exposure is given to an intact or anaesthetized animal (in vivo hypoxia). This group allows to emulate human pathophysiology, to observe the experimental animal for a relatively long time, estimate systemic and long-term effects of hypoxia, and finally, it is a necessary research step before starting clinical trials. In the second group, hypoxia is created in vitro in isolated and incubated tissue section or cell culture. Such models can be applied to human cells as well. The cell culture approach allows estimate functional specificity of different cell types, identify hypoxic reactivity of individual intracellular signaling systems, and apply genomic editing. All these generates a large body of data. Finally, such models are easier to standardize, automate, and overcome ethical restrictions. Meanwhile, for each group of models, the list of limitations is as extensive as the advantages [15].

It should be noted that each model corresponds to its own technical or surgical procedure and arbitrarily chosen experimental protocol. It provides, first of all, for the dose of hypoxia. Often, moderate-dose hypoxia is used and studied as adaptogenic stimuli that induce tissue and cell tolerance to damaging influences, including hypoxic ones. If such hypoxia is used as a “preventive” treatment followed by severe hypoxia or other exposure known to be pathogenic, we speak...
of hypoxic preconditioning. If, on the other hand, it is used as a “therapeutic” treatment after a pathogenic exposure, it is called postconditioning [16]. Some researchers applying a combination of these forms introduce the term periconditioning [17]. Models of severe hypoxia can reproduce either temporary or irreversible hypoxic state. The first paradigm is called hypoxia/reoxygenation or ischemia/reperfusion. The second one refers to terminal (lethal) exposures and aims to investigate the mechanisms of cell death.

A modification of hypoxic models can be considered the use of a series of short-term hypoxia alternating with periods of reoxygenation or hyperoxygenation. Depending on the frequency of alternation and the number of procedures in the series, such scheme is called either repetitive hypoxia or intermittent hypoxia. However, these terms are not clearly distinguished by everyone [18].

Models of hypoxia in vivo

Global cerebral ischemia model. Models of this type are designed to simulate whole-brain ischemia, which can occur in situations that restrict (stop) blood flow in the main arteries. Global cerebral ischemia of the experimental animal is achieved by cardiac arrest or blockage of cerebral blood flow. A classic model of complete cerebral ischemia used in rats consist in “4-vessel occlusion”, in which both vertebral arteries are first coagulated, and a day later the blood flow in the carotid arteries is temporarily blocked [19]. The “2-vessel carotid artery occlusion” can be used in the Mongolian gerbil, since in this species the Willis’ circle is not closed and this procedure is sufficient to deprive the entire brain of blood supply [20]. In mice or rats, 2-vessel occlusion should be accompanied by a decrease in total arterial pressure [21]. In models of ischemia/reperfusion, in addition to investigating the pathological processes that develop after restoration of blood supply to the brain, the mechanisms of cellular tolerance to hypoxia can be investigated.

Models of global hypoxic hypoxia. Depending on the research context, these models can be used in acute or chronic experiments. In acute experiment with anesthesia, myorelaxation and artificial lung ventilation it is reasonable to use respiratory gas hypoxic mixtures or stop artificial ventilation (anoxia). In particular, the model of “living preparation” of the brain cortex of anesthetized cat or rabbit, developed in the 70’s by Samoilov and Semenov and not repeated by anyone, gave interesting results [22, 23]. A special neurosurgical operation partially isolated a portion of the sensorimotor cortex with preserved blood supply through the pial arteries. It was rotated at an angle of 90° so that the transverse incision became accessible for contact microscopy and application of microelectrodes for recording bioelectrical activity or oxygen tension. Introduction of vital dyes into the washing solution made it possible to observe individual neurons and measure in them the dynamics of redox processes as well as the manifestations of calcium metabolism during anoxic and postanoxic exposure of the animal for different durations. The dose of hypoxic impact is determined exclusively by the duration of exposure. An obvious limitation of such models is the artifact effect of anesthesia and myorelaxation, impossibility to study delayed reoxygenation processes and other factors of the acute experiment.

Popular models on intact animals placed in special chambers in a chronic experiment seem to be less complicated and more reproducible. Both normobaric and hypobaric hypoxia are applicable. For the former, the dose is determined by the percentage of oxygen, and for the latter by the degree of air rarefaction. A certain exposure, number of exposures and time interval between them are also chosen. This significantly increases the variability of parameters determining the effect of hypoxia. However, this effect can be assessed either in vivo by behavioral methods during reoxygenation or by histochemical studies of samples post mortem.

Normobaric exposure is usually applied to adult animals (mice or rats) placed in a chamber which is purged with an oxygen-depleted gas mixture (sometimes up to 5–8%) for 1–5 h. Material for analysis is taken at different periods of hypoxia or reoxygenation. Studies of posthypoxic dynamics of several nonlinearly changing characteristics (e.g., gene expression), require sample collection at short time steps (5 to 6 points in the interval from 1 to 24 h of reoxygenation) [24]. In addition to studying the pathogenic effect of hypoxia, this
approach is often used, including in humans, to study the neuroprotective effect of repeated moderate hypoxia or hypoxic training. In this case, a gas mixture with 8–15% O₂ is administered daily with a specific session duration. A mixture of one composition or in alternation with another hypoxic, normoxic or hyperoxic mixture can be used [25].

The creation of hypobaric hypoxia takes into account the fact that most people on Earth live up to 3000 m above sea level, but 15 million people live in the band from 3000 m to 4800 m. At altitudes above 5500 m a person cannot live for long periods of time. According to this, two degrees of air rarefaction are traditionally used in experimental pressure chambers for people: either at the line between “mid-altitude” and “high-altitude”, i.e. 3000–4000 m (as a training and adaptive procedure), or above 7000–8000 m (for a short time, as a test for the effectiveness of antihypoxic means). It should be noted that these limits are taken for an “average” person untrained by special methods. They differ in various populations of people, have individual distinctions, and also differ in people previously acclimatized to altitude or subjected to hypoxic training. In addition, they are also specific for the different mammalian species used in the experiment.

For rats, in a number of studies, exposure at the level of severe hypobaric hypoxia (SHH)—7600 m was performed from 3 h [26, 27]. After “descent”, the characteristics revealing the damaging effect of SHH, from genomic level to cognitive functions, are usually investigated. Stronger SHH exposure in rats—10000–11000 m without pretreatment and with sufficient duration is obviously lethal and serves as a control for the application of preconditioning neuroprotective procedures. Specifically, prior to SHH, rats are given a series of moderate, short-term daily hypobaric exposures (4000–5000 m) that form hypoxic tolerance and attenuate or prevent the development of SHH-induced pathology. An intermediate level of hypobaric hypoxia (6000–6500 m) is sometimes used in the form of repeated more or less prolonged sessions (intermittent hypobaric hypoxia). Acute, “impulse” exposures also can be used. For example, 1 session of 1 h every day, in which hypobaric hypoxia (6500 m) is created with 1 min exposure every 5 min. In another variant, in a study of disturbances in monoamine levels in different parts of the rat brain, a daily hypobaric (5500 m) of 8 h duration was created for a week [28].

The translational potential of SHH models is low, and such publications in the open press are few, since the problems associated with extreme mountaineering are not widespread, and situations of catastrophic decompression usually belong to the sphere of military aviation medicine. At the same time, models of moderate hypobaric exposure are widely used on animals to study the mechanisms of hypoxic/ischemic brain tolerance [29].

Models of focal ischemia. In the clinic, the most frequently encountered condition is incomplete (focal) cerebral ischemia or stroke. One of the frequently used models since 80–90s is the model of middle cerebral artery occlusion (MCAo) [30]. The model was proposed in 1986 [31] and then somewhat upgraded [32]. In this model, the area of the right or left carotid artery is exposed under anesthesia, the external carotid artery is cut and used as an entrance to the common and internal carotid arteries to introduce an elastic siliconized thread. The thread guided through the carotid artery system achieves the middle cerebral artery and blocks the blood flow in it. In rats, this occlusion is usually created on 60–120 minutes. The thread can be removed and perfusion restored [33]. If the occlusion is long enough, apoptotic neurodegeneration develops in the reperfused cerebral cortex after some time. The disadvantages of the model include frequent cases of subarachnoid hemorrhage and negative consequences of ischemization of facial and jaw regions of the head caused by the occlusion of the external carotid artery. A variant of MCAo is its embolization. In this technique, a solution containing silicone macrospheres is injected into the middle cerebral artery instead of a thread. This allows ischemizing less extensive parts of the brain and not damaging the hypothalamus, which prevents ischemic hyperthermia [30]. However, reperfusion is not possible with this method. A variant of the embolic model is the acute photothrombotic model of focal ischemia proposed and developed in the 70–80s, which uses the phenomenon of
local photooxidation of special photosensitive dyes (e.g., Bengal pink) [34]. The dye is injected intravenously and spreads along the system of brain pial vessels. Focused illumination of the cerebral cortex by stereotaxic coordinates through the cranial bone can induce local singlet oxygen damage of the vascular endothelium, platelet activation and complete ischemia in the irradiated area. This minimally invasive technique, does not require anesthesia, and creates a very small, "targeted" area of ischemization. However, it simulates only irreversible ischemia and generates too small area of "shadow ischemia" (zona penumbra). Meanwhile, it is this zone, which appears in natural situations of stroke due to the activation of collateral blood supply around the focus of complete ischemia, is characterized by the development of a certain pattern of functional disorders and is of particular interest for clinicians. Combined models are used to investigate the mechanisms of brain hypoxic tolerance. One of them combines short-term preconditioning focal ischemia and subsequent (24 h later) severe global ischemia [35].

Despite the variety of in vivo hypoxia/ischemia models used in animals, so far none of them can be recognized as perfectly simulating real clinical situations in the human brain [36]. Therefore, new models are constantly appearing and old ones are being improved [37, 38].

**Models of hypoxia used in humans.** For obvious reasons, the study of hypoxic effects on humans has several limitations. Firstly, the possibility to analyze isolated tissue samples is excluded, and the researcher is left with more or less extended clinical blood analysis (determination of erythropoietin, erythrocytes, reticulocytes, hemoglobin, hematocrit, soluble transferrin-receptor, etc.). [39], as well as assessment of general physiological or psychological characteristics. Second, relatively harmless models of reversible and clearly dosed exposure that can be interrupted at any time are used. Usually such models are reduced to three groups: normobaric, hypobaric or overload hypoxia or their combinations. Third, the choice of the model is determined by practical (professional) tasks, implying the retention of human performance in a hypoxic environment or at excessive energy expenditure. Such studies are often carried out in sports or military medicine. It should be noted that due to the known ethical and economic reasons, the application of experimental hypoxia to primates as the final object of preclinical studies has almost the same limitations as experiments on humans. In addition to studying somatic effects of hypoxia, special attention is paid to its effect on cognitive functions of these animals [40, 41].

Some "human" models aim to simulate the gradual development of hypoxia and long-term stay in hypoxic conditions (mountaineering, high altitude training). For these tasks studies and training are usually carried out in "middle mountain" conditions (2500–4000 m) for 3–5 weeks [42]. Other models are used to study acute forms of hypoxia developing under sharp and relatively short-term reduction of oxygen level and/or blood supply (decompression of aircrafts, malfunctions in security systems in confined spaces, gravitational overload). As in animals, these models, firstly, widely apply the principle of repetitive hypoxic effects with different duration, number and interval of exposures in the series and the repetitiveness of the series themselves; secondly, they are used to study both pathogenic and adaptogenic effects.

A special area of application of adaptogenic hypoxic procedures on humans is given to practical medicine (both preventive and therapeutic). One of the common schemes in the treatment of a number of respiratory, cardiac, allergic, etc. disorders is the model of repeated sessions of moderate normobaric hypoxic therapy (10–30 sessions over several weeks) [25]. Hypobaric therapy sessions are often used to treat bronchial asthma, hypoplastic and iron deficiency anemia, chronic leukemia, cardiovascular disorders, hypertension, postinfarct cardioclserosis, endocrine diseases (thyrotoxicosis, diabetes mellitus), chronic inflammatory gynecological diseases, neurasthenia, etc. [43]. Recently, it has been shown that interval hypobaric hypoxia combined with physical activity significantly facilitates rehabilitation from COVID-19 [44]. In preventive aspect (hypobaric prophylaxis) hypobaric treatment is applied to people who have risk factors in everyday life or in professional activity (overweight, stressful environment, physical overload, expo-
sure to electromagnetic fields, the need to improve physical and mental performance in extreme situations).

Remote ischemia model. This model has become very popular in recent years in translational research as a neuroprotective effect in the form of remote ischemic preconditioning (RIPC). Its essence is to create a series of short-term ischemia/reperfusion of peripheral areas of the body (usually limbs). Such procedure initiates a protective phenomenon in the vital organs whose functions are impaired by persistent ischemia/reperfusion. Preclinical studies using RIPC indicate protection against focal and global brain ischemia/reperfusion. It is also shown that conditioning with RIPC of the hind limb has a pronounced stress-protective, antidepressant-like and anxiolytic effect [45]. The involvement of HIF-1α, oxidative stress, glucocorticoid receptors, platelet response, adenosine A1 receptors, AMP-activated protein kinase, etc. is investigated in search of mechanisms of neuroprotective phenomenon of RIPC. Early and late phases of neuroprotective RIPC and differences in their mechanisms are revealed [45, 46].

In vivo hypoxia models accepted in pharmacology. After evaluation of drug metabolism and pharmacokinetics, usually performed on a cascade of in vitro models, proceed to the evaluation of the efficacy of the selected candidate drug on in vivo models, first on rodents, then on “higher” species (primates). When a group of such models is used sequentially, the safety level of the drug and the degree of adverse effects are determined. [47].

An antihypoxant under study is usually injected subcutaneously or intraperitoneally into the experimental animal for 5 days before the hypoxic test. The control group receives placebo in the same mode. The criterion for successful selection of a drug is a significantly increased quality of function recovery during reoxygenation after hypoxic test. In cases of lethal hypoxic tests (without reoxygenation), the criterion for the effectiveness of the drug is the prolonged survival time of animals under hypoxic conditions compared to the survival of those who received placebo. In pharmacological screening, acute normobaric hypercapnic hypoxia is often used. This simple model involves placing an animal in a sealed small-volume vessel where, as the animal consumes O₂, its concentration decreases and the CO₂ concentration increases. After some time, the animal dies [48]. Acute hemic hypoxia is created using subcutaneous administration of sodium nitrite (NaNO₂) at a dose of 150–250 mg/kg. This salt oxidizes divalent hemoglobin iron to trivalent iron. The formed methemoglobin loses the ability to reversibly bind O₂, and hemic hypoxia develops [49]. Acute SHH—single “ascent” in a vacuum chamber to the altitude of 11000–12000 m at high speed (about 30 m/s) is also used on mice and rats. In this case the total time of reaching the lethal hypobaric plateau is about 6 min. As in the cases of the described models, the criterion of pharmacological efficacy is the increase of animal life expectancy compared to the control [50].

In pharmacological screening, any variants of the above-described model of focal cerebral ischemia are often used with irreversible MCAo [51, 52]. In some cases, on the second day of cerebral ischemia, the psycho-physiological state of rats is assessed in behavioral tests “Open Field” and “Elevated Plus-maze”. Histochemical and histological examination of the brain is then performed after euthanasia. According to the classic technique, 0.5% Evans blue solution is used to assess the level of ischemia. The stained tissue is placed in dimethyl sulfoxide for dye extraction and spectrophotometry. The extent of ischemic damage is judged by the intensity of staining of the extract in the spectral region of 630 nm. There are a number of techniques using dyes that detect necrotic or apoptotic neurons on fixed histological brain preparations. Biochemical parameters characterizing energy metabolism (lactate and pyruvate content, ATP, ADP and AMP), lipid peroxidation state (malonic dialdehyde content) and antioxidant system state (superoxide dismutase activity and reduced glutathione content) are determined in brain homogenates.
models of isolated brain tissue have been developed.

Fragments of brain tissue of young animals (for rats no more than 1 week old) can grow in incubators and be suitable for the experiment for up to a month. Such an organotypic tissue culture can be created from a particular part of the brain (e.g., hippocampus, cerebellum, cerebral cortex). It has been proven that this system relatively steadily reproduces postnatal brain development with establishment of inter-neuronal connections and manifestation of specific physiological and biochemical properties [53]. Donors for such objects can be mice, rats, guinea pigs, rabbits, and human embryo. The hippocampal culture is more often explored, as it has clear structural and functional features of its areas, including CA1 zone with neurons highly sensitive to hypoxia. To simulate ischemia, the oxygenated nutrient incubation medium is replaced with oxygen and glucose deprived (OGD) solution [54, 55]. One of the drawbacks of this model is the need to work with tissue that is in early ontogenesis and, therefore, has specific metabolic and regulatory processes characteristic of a very young organism. Another possible limitation of cultures is the difficulty in controlling the oxygen tension in the pericellular micro-environment under “normoxic” and, even more so, under “hypoxic” conditions. Despite the standard recommendations for the incubate and ambient atmosphere composition (18.6% O2 and 5% CO2), there are significant difficulties in determining its real composition near individual cells [56, 57]. At least two reasons are seen. First, the specificity of culture incubation requires not flow media, but a periodic changeable one. In this case, as the cells consume O2, its content by the time of the next change can decrease to “hypoxic” (according to some data, already within an hour). Secondly, cells of the culture due to mutual adherence and adhesion to the substrate supplied with oxygen in varying degrees. Countering these disadvantages requires the use of complex incubation systems and constant pO2 control in different culture microsites [58].

If it is necessary to study “adult”, highly differentiated tissue, a model of acute slices freshly prepared from the brain of a decapitated animal is used [59]. Both OGD of hippocampal slices [60, 61] and normoglycemic hypoxia of different depths, up to cortical slices anoxia, can be created on this object [62, 63]. This model has several advantages over cell cultures already because it assumes flow-through incubation medium. In addition, it makes it possible to observe the inter-position and interactions between functionally different cells of the selected object using contact or confocal microscopy, bioindicator dyes, and microelectrode techniques. However, such models also have their limitations. “Adult” slices are difficult to maintain in an active functional state for longer than a day, so they are used to study the most rapidly developing effects of hypoxic exposure. In addition, even in thin sections (less than 300 μm), oxygen diffuses from the washing solution with a gradient falling from the surface to the substrate. For confocal microscopy and tissue spectrofluorometry under such conditions inverted microscopes are of little use, because their working “field of view” includes first of all poorly washed lower regions of the slice. Application of microscopic technique with upper lens location creates constructive problems for microelectrode technique.

For both groups of in vitro models used in hypoxic experiments, the difficulty in providing “normoxic” and “hypoxic” oxygen saturation of the incubation medium that would simulate the levels of real oxygen supply in vivo remains a critical limitation. This problem is particularly acute when there is a need for rapid change from one medium to another in models of fast-rate intermittent hypoxia simulating obstructive sleep apnea [64]. To overcome these limitations special bioreactors for replaceable media, are used, allowing them to be exposed for tens of seconds [65]. Another problem of open cell culture technology, namely slow diffusion from the surrounding gas environment, was partially solved by the use of oxygen-permeable substrates at the bottom of the cells. In this case, oxygen shifts in the gas phase become directly available to the cells and the cellular liquid medium throughout the entire preparation volume [66].

CONCLUSIONS

The data presented in this review allow us to
conclude that to date, a wide arsenal of different approaches and methods of modeling hypoxic conditions in experiments on mammalian animals and humans has been developed. Studies using these models will largely reveal the high potential of moderate hypoxic effects for medicine, sports, extreme tourism, as well as the training of workers, whose work is associated with the risk of exposure to damaging factors, including in the aerospace industry.

According to the accumulated data provided by various models of experimental hypoxia, the use of hypoxic conditioning stimulating evolutionarily ancient mechanisms of hypoxic/ischemic tolerance of the mammalian brain seems to be an important non-drug component of preventive and therapeutic strategies to combat neurodegenerative, neurological and mental diseases, cognitive deficits and premature aging of humans.

AUTHORS’ CONTRIBUTION

Text writing and manuscript design—D.G.S. Literature selection—D.G.S., A.V.B., E.A.R. Review idea, text editing, introduction, conclusion—E.A.R., D.G.S

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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