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EXPERIMENTAL PAPERS

Effect of Prenatal Hypoxia on Activity of the Soluble Forms of Cholinesterases in Rat Brain Structures during Early Postnatal Ontogenesis

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Abstract—The dynamics of soluble AChE and BChE (EC 3.1.1.7; EC 3.1.1.8) in the hippocampus, cortex, cerebellum and blood serum of control rats and rats exposed to prenatal hypoxia was studied on days 5, 10 and 30 of postnatal development. The activity of soluble AChE in all brain structures was found to reach its maximum on postnatal day 10, and then either persisted at this level (in the cerebellum and cortex) or decreased by day 30 (in the hippocampus). Similar changes were found in the activity of BChE, which was roughly one level of magnitude lower than of AChE in all the brain structures studied in this work. Prenatal hypoxic exposure on day 14 of embryonic development led to statistically significant changes in the activity of soluble AChE and BChE in all the brain structures studied, as well as in blood serum. In rats exposed to prenatal hypoxia, serum AChE and BChE activities on postnatal days 5 and 10 were significantly lower while, on day 30, they were indistinguishable from the control values. Thus, oxygen deficit in the maternal organism during pregnancy significantly affects the activity of soluble forms of the key enzymes of the central and peripheral cholinergic systems, indicating possible changes in the formation of these systems in early ontogenesis. This may lead both to impaired neurogenesis and malformation of the motor and cognitive functions and general homeostatic imbalance during animal and human development.

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INTRODUCTION

Recent years have witnessed a dramatic rise in children's nervous system disorders, most of which are associated with developmental CNS pathologies in the prenatal period. These pathologies include intrauterine growth retardation/restriction (IUGR) which develops due to placental insufficiency [1, 2]. The effect of pathological factors on the maternal organism impairs the transport, trophic and antioxidant functions of the placenta, which may lead to fetal hypoxia and IUGR [1]. Infants with such a diagnosis are characterized by suppressed locomotor activity, reduced learning ability, inattention, increased anxiety, and some other cognitive impairments. It should also be particularly noted that IUGR infants suffer from neurological disorders not only in the perinatal period but in later years as well [3]. In this regard, it is of current importance to study the pathogenesis of prenatal hypoxia and to develop diagnostic tests for the timely assessment of CNS dysfunctions in IUGR newborns. At present, the long-term effects of prenatal hypoxia are being actively investigated on animals, specifically on rats. These studies add to the already available information on the biochemical mechanisms of prenatal hypoxia and promote revealing new indicators that allow assessment of the impact of prenatal hypoxia on the brain. One of these indicators is the activity of cholinesterases, which characterizes the state of the cholinergic system, one of the major neurotransmitter brain systems that plays a pivotal role in the regulation of locomotor activity, as well as learning and memory, which are severely affected in IUGR infants.

Acetylcholinesterase (AChE) is one of the principal enzymes of the cholinergic system. It can be employed as a marker, the activity dynamics of which allows the enzyme's functional state to be adequately evaluated. In an animal organism, AChE occurs in two forms: membrane-bound and soluble. The latter form is not only responsible for hydrolysis of the main neurotransmitter, acetylcholine (ACh), but also displays neurotrophic properties, which are of particular importance during the ontogenesis of the nervous system [4–6]. For instance, soluble forms of AChE are involved in cell adhesion, neurogenesis and axonal growth [7], and exert an influence on proliferation and differentiation of nerve cells [8, 9]. These processes ensure normal functioning of the CNS, while changes in the level and activity of soluble AChE can accompany and even cause varied pathologies. Dysfunction of the cholinergic system and changes in the properties of the relevant enzymes of ACh metabolism are concomitant to stress, brain stroke and ischemia [10, 11], as well as such nervous system pathologies as Parkinson's and Alzheimer's diseases [12, 13]. Besides AChE, there is another type of cholinesterase, butyrylcholinesterase (BChE), which is localized mainly to the cerebral white matter, glia and neuronal bodies, while AChE occurs in neurons and nerve endings [14, 15]. During ontogenesis, expression of these enzymes manifests itself in different ways. For instance, BChE appears far earlier than AChE and may even induce AChE expression [16]. At the same time, in an adult organism BChE performs a protective function, eliminating various toxins and xenobiotics [17], although in the absence of AChE it can also play a "classical" role, the cleavage of ACh [18].

Neurotrophic properties of AChE and BChE are crucial for the formation and development of interneuronal links in the CNS, especially during early ontogenesis, which is necessary for the formation in offspring of such vital functions as learning and memory. Despite ample data on the general properties of the cholinergic system's enzymes, there are only a few studies devoted to soluble AChE and BChE forms, especially in the cerebellum responsible for motor functions, which are the first to suffer in IUGR newborns. An integrated assessment of the activity dynamics of these enzymes in the brain structures responsible for the formation of motor behavior, learning and memory, as well as in blood serum, during early ontogenesis are virtually absent. In this regard, it is of great interest to address the activity dynamics of the soluble AChE and BChE forms in the rat cortex, hippocampus, cerebellum and blood serum of animals during their early ontogenesis, as well as to assess the influence of prenatal hypoxia on their activity.

The present work is aimed at exploring the activity of soluble cholinesterases in the hippocampus, cortex, cerebellum, and blood serum at

the early stages of ontogenesis under normal conditions and following prenatal hypoxia.

MATERIALS AND METHODS

The study was carried out on Wistar rats of different ages from the Sechenov Institute's vivarium, both exposed (experiment) and unexposed (control) to prenatal hypoxia. All experimental procedures complied with the approved international regulations on conducting research with experimental animals [19], as well as the recommendations by the Ethics Committee at Sechenov Institute of Evolutionary Physiology and Biochemistry (Russian Academy of Sciences). In the experimental rat group, pregnant females were exposed to normobaric hypoxia on prenatal day 14 (E14) in a 100-L chamber equipped with the systems for ventilation, thermoregulation and exhaled CO_2 adsorption, as well as with a gas analyzer. During experiments, the O_2 content was being reduced from 21% to 7% over 10 min and retained at this level for 3 h. The control group of pregnant females were kept in a chamber with an intact gas medium during the same period of time.

Further experiments involved the offspring from the hypoxic and control pregnant rat groups on days 5, 10 and 30 after birth. The choice of the prenatal (embryonic) day 14 for hypoxic exposure owed to the fact that the proliferative activity of brain cells peaks by the end of the second prenatal week and then declines [20–22]. Because within this lapse there occurs a generation of neocortical, hippocampal and cerebellar neurons [21], hypoxic exposure was applied exactly during the critical period, when these cells are most vulnerable. The choice of postnatal time points for examining offspring was determined for the following reasons: day 5—completion of neuroblast migration, neuritogenesis; day 10—onset of myelination, maximal blood flow, synaptogenesis; day 30—completion of intense CNS formation [23]. The number of rats in the control and experimental groups was no less than 6 animals.

At the above time points, animals were decapitated, and the brain was extirpated in the cold (+4°C). The area matching the cortex, hippocampus and cerebellum was identified according to the atlas of the rat brain in stereotaxic coordinates [24]. If a repeated examination was needed, samples were frozen and stored at −80°C. Tissues of the brain structures were homogenized in a buffer containing 0.02 M Tris-HCl, 0.01 M $MgCl_2$ and 0.05 M NaCl and then centrifuged at 100000 *g* for 10 min. The resulting supernatant was separated from the sediment and used to assay the activity of soluble forms of AChE and BChE. Serum samples were obtained by allowing the blood collected during rat decapitation to settle for 30 min at 37°C with subsequent centrifugation at 2500 rpm for 20 min. For a repeated examination, samples were frozen and stored at −80°C.

Cholinesterase activities were assayed by the modified Ellman's method in 96-well plates [25]. As an incubation medium, we used a solution containing 0.025 mL of 0.002 M dithionitrobenzoic acid (DTNB) dissolved in 0.2 M sodium phosphate buffer (pH 7.5), 0.0125 mL of an aqueous solution of 0.01 M reaction substrates: acetylthiocholine iodide (ATC) or butyrylthiocholine (BTC), and 20–50 μL of fractions containing the enzymes to be analyzed. The reaction was triggered by adding a substrate. Samples were kept for 20–30 min at ambient temperature until the appearance of yellow staining; after that, the reaction was stopped by adding a 3% sodium dodecyl sulfate (SDS) solution. Each sample was analyzed in triplicate. To consider the level of endogenous thiols in the samples, for each of them we prepared parallel control samples with SDS added in advance (before adding a substrate), and the resulting values were subtracted from those obtained after a completion of the enzymatic reaction. AChE activity was assayed in the presence of the BChE inhibitor etopropazine hydrochloride (20 μM; Sigma, USA), while BChE activity was assayed in the presence of the AChE inhibitor phenylmethylsulfonyl fluoride (25 μM; Sigma, USA). Staining intensity was measured at $\lambda = 405$ nm. A calibration curve was plotted using cysteine as a standard. The results are presented as enzyme specific activity values and expressed in nmol of substrate/mg of protein/min. The protein level was quantified by the Bradford protein assay [26].

Statistical treatment was carried out using the Statistica for Windows 6.0 software (StatSoft). Data were tested for the distribution normality using the Shapiro–Wilk W-test; the uniformity of

Table 1. Age-related dynamics of the activity of soluble AChE in brain structures after prenatal hypoxia (nmol substrate/mg protein/min) compared to control values

Brain structure	Postnatal day	AChE	
		Control	Hypoxia
Hippocampus	5	12.90 ± 1.25	4.70 ± 0.84^^^
	10	24.70 ± 3.80**	9.30 ± 1.40***^^^
	30	4.10 ± 0.17*###	5.20 ± 0.40###^^^
	F-test	26.1335; $p < 0.001$	17.4174; $p < 0.001$
Cortex	5	6.10 ± 1.05	3.10 ± 0.60^^
	10	10.80 ± 0.60***	7.20 ± 0.33***^^^
	30	2.60 ± 0.30**###	4.10 ± 0.30###^^
	F-test	41.9911; $p < 0.001$	40.5684; $p < 0.001$
Cerebellum	5	7.70 ± 0.84	10.20 ± 0.38^^
	10	10.10 ± 0.90*	7.40 ± 0.39***^
	30	10.20 ± 0.60*	6.70 ± 0.35***^^^
	F-test	5.7679; $p < 0.05$	27.501; $p < 0.001$

*—$p < 0.05$; **—$p < 0.01$; ***—$p < 0.001$—significance of differences in AChE activity *vs.* P5.

#—$p < 0.05$; ##—$p < 0.01$; ###—$p < 0.001$—significance of differences in AChE activity *vs.* P10.

^—$p < 0.05$; ^^—$p < 0.01$; ^^^—$p < 0.001$—significance of differences in AChE activity in hypoxic group *vs.* control.

The number of rats in each age group was ≥ 6.

the group dispersions was checked by the Levene's test. The significance of intergroup differences was assessed by the Student's *t*-test for two independent samples. When comparing several groups, a one-way ANOVA was used, in which a statistical significance of differences among mean values of the parameters studied in groups was evaluated using the F-test; the Bonferroni test was applied for multiple comparisons. Data were considered statistically significant at $p < 0.05$. Results are presented as $M \pm SEM$.

RESULTS

In control rats, activities of soluble AChE and BChE forms in the hippocampus, cortex and cerebellum during early ontogenesis differ in their magnitude and to a certain extent in the dynamics, as evidenced by the data presented in Tables 1 and 2. For instance, on postnatal days 5 and 10, the highest AChE activity was observed in the hippocampus, being twice as high on day 10 as on day 5, while by day 30 it decreased threefold. In the cerebral cortex, AChE activity on day 10 was 1.8 times higher and on day 30 2 times lower than on day 5, while in the cerebellum the activity of soluble AChE gradually increased from days 5 to 30 (Table 1). During the first month of rat pups' life, BChE activity in all the structures examined was almost an order of magnitude lower than that of AChE; changes in activities of both enzymes shared a similar dynamics, although by day 30 BChE activity in the cortex and cerebellum remained higher than on day 5 (Table 2). Thus, activities of soluble AChE and BChE in the cortex and hippocampus of control animals peak on postnatal day 10. In 1-month-old pups, AChE activity either declined in the cortex and hippocampus or remained at the same level in the cerebellum, while BChE activity in the hippocampus declined, in the cortex remained at the level achieved by day 10, and in the cerebellum gradually rose. A comparison of activities of these enzymes in the above structures shows that on days 5 and 10 maximum activities of AChE and BChE characterize the hippocampus, while on day 30 activities of both enzymes are higher in the cerebellum.

In animals that experienced prenatal hypoxia, cholinesterase activities in all the brain structures

Table 2. Age-related dynamics of the activity of soluble BChE in brain structures after prenatal hypoxia (nmol substrate/mg protein/min) compared to control values

Brain structure	Postnatal day	BChE	
		Control	Hypoxia
Hippocampus	5	0.80 ± 0.07	0.60 ± 0.06^^^
	10	1.80 ± 0.30**	1.00± 0.20**^^
	30	0.80 ± 0.02###	1.10 ± 0.15***^^
	F-test	14.3921; $p < 0.001$	18.3566; $p < 0.001$
Cortex	5	0.40 ± 0.05	0.60 ± 0.06^^
	10	0.81 ± 0.11**	0.87 ± 0.20
	30	0.71 ± 0.06*	1.10 ± 0.06**^^
	F-test	8.2719; $p < 0.01$	7.2469; $p < 0.01$
Cerebellum	5	0.60 ± 0.07	1.01 ± 0.14^^
	10	1.40 ± 0.05***	1.60 ± 0.15
	30	1.90 ± 0.07***###	1.90 ± 0.22**
	F-test	143.326; $p < 0.001$	9.2784; $p < 0.01$

*—$p < 0.05$; **—$p < 0.01$; ***—$p < 0.001$—significance of differences in BChE activity *vs.* P5.

#—$p < 0.05$; ##—$p < 0.01$; ###—$p < 0.001$—significance of differences in BChE activity *vs.* P10.

^—$p < 0.05$; ^^—$p < 0.01$; ^^^—$p < 0.001$—significance of differences in BChE activity in hypoxic group *vs.* control.

The number of rats in each age group was ≥ 6.

change, indicative of the cholinergic system's sensitivity to prenatal hypoxia. We found that the impact of prenatal hypoxia led to a statistically significant decrease in the activity of soluble AChE: in the hippocampus by 2.7 and 2.6 times and in the cortex by 2 and 1.5 times on days 5 and 10, respectively (Table 1). At the same time, by the end of the first month of life, in rats that endured prenatal hypoxia, AChE activity increased by 1.3 times in the hippocampus and 1.6 times in the cortex as compared to control animals. In the cerebellum of 5-day-old rat pups, AChE activity in hypoxic rats was 1.33 times higher compared to control animals, while on days 10 and 30, it was 1.4 and 1.5 times lower, respectively. An analysis of the impact of prenatal hypoxia on the activity of soluble BChE shows a different pattern of changes compared to AChE. For instance, in the cortex, BChE activity was significantly higher than in control animals on days 5 and 30, while in the cerebellum, the enzyme activity was significantly higher (by 1.7 times) on day 5 only. On day 10, there was only an upward trend (by 1.14 times), and by day 30, no significant changes in BChE activity were observed between control and experimental rat groups (Table 2).

Apart from the activity of soluble cholinesterases in the brain structures, we also examined the dynamics of AChE and BChE activities in rat blood serum on postnatal days 5, 10 and 30 (Table 3). It was revealed that serum AChE activity is almost twice as high as that of BChE at all time points studied in this work. In control animals, AChE activity significantly increased almost 1.6 times during the first month of life compared to postnatal day 5, while BChE activity virtually did not change and remained at the level of 5-day-old rat pups at all time points. The AChE activity dynamics in rat pups that experienced prenatal hypoxia was similar to that in control animals, although by the end of the first month of life it increased almost 3 times. BChE activity in hypoxic rats also increased 3 times by postnatal day 30 and reached the values of this indicator in control animals in this ontogenetic period (Table 3). However, prenatal hypoxia led to decreased activities of both enzymes in the rat blood serum. For instance, AChE activity on day 5 was 1.7 times and on day 10 1.4 times lower

Table 3. Serum AChE and BChE activities during the first postnatal month

Postnatal day	AChE		BChE	
	Control	Hypoxia	Control	Hypoxia
5	0.0044 ± 0.0001	0.0023 ± 0.0004^^^	0.0030 ± 0.0004	0.0011 ± 0.0005^^^
10	0.0056 ± 0.0004*	0.0040 ± 0.0001***^^	0.0030 ± 0.0008	0.0020 ± 0.0005^
30	0.0069 ± 0.0003***##	0.0070 ± 0.0030***###	0.0026 ± 0.0002	0.0030 ± 0.0003
F-test	27.541; $p < 0.001$	91.2195; $p < 0.001$	2.1537; $p > 0.05$	2.05382; $p > 0.05$

*—$p < 0.05$; **—$p < 0.01$; ***—$p < 0.001$—significance of differences in cholinesterase activities *vs.* P5.

#—$p < 0.05$; ##—$p < 0.01$; ###—$p < 0.001$—significance of differences in cholinesterase activities *vs.* P10.

^—$p < 0.05$; ^^—$p < 0.01$; ^^^—$p < 0.001$—significance of differences in cholinesterase activities in hypoxic group *vs.* control.

The number of rats in each age group was ≥ 8.

compared to the control, while on day 30 no significant differences were detected. Analogous changes were observed in BChE. On day 5, its activity was 3 times and on day 10 1.5 times lower, while on day 30 the enzyme activity level was indistinguishable from control values.

DISCUSSION

The cholinergic system is one of the major neurotransmitter systems in the mammalian brain. Its development plays a pivotal role in establishing cognitive functions. The activity of AChE, a principal hydrolytic enzyme that cleaves acetylcholine, determines the speed of synaptic transmission. In addition, AChE is involved in the formation, growth and establishment of synaptic contacts [9, 27]. Any changes in the level or activity of this enzyme, arising during brain morphogenesis, can influence learning and memory formation, as well as the development of the nervous system in general. A possible involvement of BChE in cholinergic neurotransmission is evidenced by data indicating that, in the absence of the AChE gene, BChE can take over its role in neurotransmission and also participate in the formation of cognitive functions [18]. In this regard, the dynamics of changes in cholinesterase activity during early ontogenesis can reflect the degree of the enzymes' involvement in the above-mentioned physiological processes.

It is well known that development of neurons and their axons in the rat brain is most intensive in the first two weeks of postnatal development, during which the establishment of cholinergic synapses intensifies, and that the formation of neural connections is mostly completed by the end of the first month. Therefore, the increased activity of soluble AChE and BChE detected here in the rat cortex, hippocampus and cerebellum on postnatal day 10, as well as its decline toward day 30, both in control animals and those that experienced prenatal hypoxia reflect a specific role of these enzymes in the formation of the brain structures examined in this study. Our data indicate that in the rat cerebral cortex and hippocampus the activity of soluble AChE is the highest on postnatal day 10, which is the period of most intense development of neural connections and synaptic contacts, while by postnatal day 30, when the intense formation of neural connections comes to an end, AChE activity in both brain structures declines sharply. Nevertheless, in the cerebellum, the activity of soluble AChE by the end of the first month of life remains at the day 10 level.

The BChE activity dynamics indicates that BChE and AChE have different functions as we found no significant changes in BChE activity during the first month of rat life. We assume that BChE may play a reserve role, being involved when required in ACh hydrolysis and breakdown/removal of toxic compounds from the brain, which is currently considered to be one of the major functions of this enzyme [28]. If considering AChE and BChE as proteins having neurotrophic properties, namely involved in proliferation and development of neurons [9], then our data are consistent with the ideas on the role of neurotrophic factors in brain development,

according to which nascent neurons are laid in excess during early ontogenesis competing for trophic factors, and those neurons that do not receive appropriate trophic support are eliminated via apoptosis [29, 30]. The data obtained in this study are also in agreement with those indicating a higher level of apoptosis in the hippocampus during the first 7 days of postnatal ontogenesis [31], since the lowest level of activity of soluble AChE and BChE, having neurotrophic functions, in the hippocampus and other brain structures falls on day 5 of the rat pups' life. Importantly, activities of the enzymes in the hippocampus, cortex and cerebellum are quite different in their magnitude. The brain structures are well known to develop heterochronously [32], hence the activity of AChE and BChE during ontogenesis may vary, as observed in our experiments, with the activity of soluble AChE in control animals being the highest in the hippocampus, as compared to the cortex and cerebellum, on postnatal days 5 and 10.

As accepted, prenatal hypoxia is one of the causes of brain malformation and dysfunction during ontogenesis [33]. Pathological alterations observed under oxygen deficiency most often occur exactly in those structures that were examined in this work (hippocampus, cortex, cerebellum), and this affects the formation of their cholinergic innervation during postnatal development [34–36]. These structures are associated with organizing animal behavior which includes motor acts developed at early stages of postnatal ontogenesis. Moreover, the cerebral cortex and hippocampus are involved in the formation of cognitive functions, such as learning and memory, which actively form in the first month of life [34, 37]. At the same time, the pattern of impairments in the functional development of the nervous system depends on the period of embryogenesis, during which the pathological factors came into action [33, 36, 38]. This is what determined the choice of prenatal day 14 (E14) for studying the effect of prenatal hypoxia in this work because, exactly in this period, large cerebellar, cortical and hippocampal neurons begin to form.

Analysis of the influence of prenatal hypoxia on the activity of soluble cholinesterases in the brain structures shows that the pathological effect of hypoxia at E14 leads to significant changes in AChE and BChE activities during postnatal ontogenesis. For instance, the hippocampal activity of AChE and BChE was lower compared to control animals on postnatal days 5 and 10. Nevertheless, by the end of the first month of life, the activity of soluble forms of both enzymes was higher in animals exposed to prenatal hypoxia, which may either represent a compensatory effect or lead to an increase in ACh breakdown and influence the formation of synaptic plasticity. Our data on the altered activity of soluble AChE in the rat cerebral cortex are in line with the literature data indicating a reduced activity both of the soluble and membrane-bound forms of AChE on days 5 and 10 in animals that experienced prenatal hypoxia [39]. In addition to changes in the cortex, we revealed a decrease in the activity of soluble AChE in the cerebellum at all time points of postnatal life, which also reflects the pattern of influence of hypoxia on the formation and functions of this brain structure. Besides coordinating motor activity, the latter is currently assigned an important role in many cognitive and emotional processes [40]. The changes that we revealed in the activity of the soluble neurotrophic form of AChE in the brain structures may not only reflect the developmental dynamics of the cholinergic system, which affect the formation of cognitive and motor functions in animals, but also lead to morphological abnormalities of nervous tissue [34, 41, 42]. For instance, it is reported in the literature that prenatal hypoxia at E14 leads to developmental retardation of the sensorimotor cortex in 5-day-old rat pups, which reflects itself in the large volume of intercellular space, insufficient number of differentiated neurons, as well as in the lack of mature synapses. In 2-week-old animals the signs of nerve cell destruction were also observed [43]. On the other hand, increased activity of soluble AChE in the cortex and hippocampus of hypoxic rat pups aged 30 days can induce increased hydrolysis of ACh, thereby leading to its deficiency and impaired cognitive functions [44, 45].

Apart from changes revealed in the activity of soluble AChE during the postnatal ontogenesis of rats exposed to prenatal hypoxia, we also demonstrate that in the cortex and cerebellum of these

animals BChE activity rises compared to control values. An increase in BChE activity may be due to the fact that this enzyme is able both to compensate AChE deficiency [18] and to take part in the degradation of a variety of other substrates by acting as a serine hydrolase. At present, BChE is an important therapeutic target in various types of pathology, and therefore studying its dynamics in different brain structures and blood serum during postnatal ontogenesis under normal conditions and after prenatal stress is of great interest [46].

Analysis of AChE and BChE in rat serum shows that during early ontogenesis AChE activity is twice as high as that of BChE and significantly increases almost one and a half times by the end of the first month of life, whereas BChE activity remains practically intact. In rats exposed to prenatal hypoxia, the activity of both enzymes on days 5 and 10 was lower than in the control, which is comparable with the results obtained for the brain structures. Nevertheless, on day 30, no significant differences between control and hypoxic animals were already observed. Since peripheral ACh is involved in the regulation of crucial cellular functions, such as division, cell–cell interactions, hormone secretion and immunity [47, 48], serum AChE and BChE activities are essential for the maintenance of homeostasis and the response readiness to different types of stress. In this connection, it is noteworthy that the level and activity of AChE and BChE in blood plasma are widely used as diagnostic criteria to assess the presence of brain pathologies, specifically, cognitive disorders and Alzheimer's disease [47–49].

CONCLUSION

Thus, prenatal hypoxia on day 14 of embryonic development in rats evokes statistically significant changes in the activity of soluble AChE and BChE forms in the cerebellum, hippocampus and cerebral cortex, which indicates a malformation and impaired functioning of their cholinergic systems. Similar impairments may lead to retardation in the formation of motor activity and cognitive functions during early ontogenesis of animals and humans. A decreased activity of AChE and BChE in blood serum of rats exposed to prenatal hypoxia indicates the impairment not only of the central but also peripheral cholinergic systems, which may lead to altered homeostasis of the whole organism. This suggests that levels of the AChE and BChE activity in blood serum can be used as markers of the cholinergic system's dysfunction in offspring after prenatal hypoxia, specifically in infants with intrauterine growth retardation.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare no conflict of interest.

All procedures involving laboratory animals were carried out in compliance with the requirements of the Ethics Committee at the D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology and Sechenov Institute of Evolutionary Physiology and Biochemistry, as well as the European Communities Council Directive 1986 (86/609/EEC) and "Guide for the Care and Use of Laboratory Animals".

This study did not involve humans as research subjects.

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