Effect of Prenatal Hypoxia on Cholinesterase Activity in Blood Serum of Rats

Daria I. Kozlova[1,3], Ekaterina G. Kochkina[1], Nadezhda M. Dubrovskaya[1,4], Igor A. Zhuravin[1], Natalia N. Nalivaeva[1,2]

[1]Sechenov Institute of Evolutionary Physiology and Biochemistry of RAS, 44 Thorez av., 194223, St. Petersburg, Russia; [2]School of Biomedical Sciences, Faculty of Biological Sciences, School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, United Kingdom; [3]LLC Scientific and Production Firm "ABRIS +", St. Petersburg, Russia; [4]Saint-Petersburg State Pediatric Medical University, St. Petersburg, Russia. e-mail: di.kozlova.official@gmail.com

Introduction

Reactions of organisms to various external stimuli are accompanied by activation of a wide spectrum of receptors allowing them to react adequately to the constantly changing environment. During this process the external signals are transmitted to the receptors via different mediator systems which determine the specificity of the host response. Among those the cholinergic system and its major mediator acetylcholine (ACh) determine the organismal response to stress both at the levels of the central and peripheral nervous systems. Both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) degrade ACh although they have other non-cholinergic functions. The soluble form of AChE also possess neurotrophic and neuritogenic properties while BChE's main function is inactivation of various toxic compounds. There are convincing data that in the process of ageing or under permanent stress, as well as in the case of neurodegenerative disorders, the ratio of different molecular forms of AChE and BChE in the tissues can be significantly changed, however, there are no consistent literature data on the dynamics of AChE and BChE activity in blood serum of animals during their ontogenesis or under the effects of various stressors. As such, the present study was aimed at the analysis of AChE and BChE activity in blood serum of rats during normal postnatal ontogenesis as well as after prenatal hypoxia or administration to animals of pharmacological substances (L-carnitine and imatinib (Gleevec)) used clinically to treat various pathological conditions.

Methods

Prenatal hypoxia. Pregnant Wistar rats (200g) on the 14th day of gestation were exposed to normobaric hypoxia in a special chamber with a capacity of 100 liters containing systems of thermoregulation, ventilation, gas analysis and adsorption of exhaled CO_2. During the experiment, the oxygen content in the chamber was reduced from 20.7 to 7.0% and kept at this level for 3 hours. Concentration of carbon dioxide in the chamber did not exceed 0.2%, and the temperature was maintained at 22° C. No more than 10 rats were simultaneously placed in the chamber. Intact control pups were taken from the offspring of females not exposed to hypoxia.

To obtain serum plasma, after blood after decapitation was collected in vials without anticoagulant. After formation of a clot the serum was separated by centrifugation at 20,000 g. Serum not containing blood cell elements was aliquoted into Eppendorf tubes, frozen and kept at -80°C for not longer than 2 months to avoid loss of activity.

Drug administration. L-carnitine syrup (Ampule 3000, BioTech, USA) was dissolved in water and each animal weighing approx. 300 g received 50 ml of the solution (40 mg/kg of weight). The solution was placed in drinking bowls and for ensuring that each rat consumed the whole volume they were given dry chow. In experiments the mature animals (8 months old) subjected to prenatal hypoxia have been used. Naïve rats have been used as controls. Rats (4-6 animals per cage) have been kept under standard conditions and received either water or L-carnitine solution for 2 or 6 weeks. Imatinib (Glevec) administration was performed to adult (9 months old) control rats and rats subjected to prenatal hypoxia daily with an interval over the weekend (9 times in total) via intranasal drops (5 μl per nostril; STI57I, 2×10^{-2} M). Control rats received sterile water.

Enzyme assay. AChE and BChE activity was assayed by our modification of the Ellman's procedure in 96-well plates in the medium containing: 0.025 ml of 0.002 M DTNB (5,5'-dithio-bis-(2-nitrobenzoic acid), dissolved in 200 mM Na-phosphate buffer (pH 7.5), 0.0125 ml of 0.01 M reaction substrates (acetylthiocholine iodide – ATCh, or butyrylthiocholine iodide - BTCh) in water, 20-50 μl of enzyme fractions, and bi-distilled H_2O up to a volume of 0.125 ml. The reaction was initiated by addition of the substrate. Samples were incubated for 20 min at room temperature and the reaction was terminated by addition of 0.0125 ml of 3% SDS in water. In the blank samples SDS was added before the substrate. Each sample was analysed in triplicate. The AChE assay was performed in the presence of 20 μM of a BChE inhibitor ethopropazine hydrochloride (Sigma). The absorption of the coloured product developed in the samples was measured at a wavelength of 410 nm using a Microplate Reader (NorthStar Scientific Limited, UK). The calibration curve was plotted using cysteine as a standard. The results were presented as the specific activity of the enzymes in nmoles of converted substrate (nmoles of substrate/mg protein × min).

Selection of active and passive rats was performed by analysing horizontal activity of each animal in the "open field" test for 5 min calculating the sum of the squares crossed. The animals were considered active (n=13) if their average number of crossed squares was higher than the upper quartile of values calculated for the whole group. The passive rats (n=12) had their average number of closed squares lower than the fourth quartile of values calculated for the whole group (n=52). Other animals with the average values of crossed squares in the range between the first and third quartiles were considered as having intermediate activity and have not been used in further experiments.

Radial 2-level 8-arm maze test. Following 24 h of food deprivation, animals (P90) were taken for 14 daily trials of memory test in a two-level 8-arm maze as described in detail previously [Dubrovskaya et al., 2006]. Briefly, the rats were placed in a platform of the two-level eight-arm maze from which they could enter any of the eight arms of the maze. Each arm had a feeder with food pellets (35 mg, containing sugar and starch). Entrances to the arms on the second level and exits from the arms on the first level were equipped with light-weight self-shutting doors opening only in one direction (inside or outside). Rats were allowed to perform eight visits of the arms, and any repeat visit to the same arm was counted as an error. The ability of rats to perform and remember the task was evaluated and expressed as percent of erroneous visits out of 8.

Novel object recognition test. At the beginning of the test, an experimental animal was placed in a 100x100 cm box with non-transparent 20 cm high walls for 15 min adaptation in the absence of any specific behavioural stimuli. In the first training session, after 2 hours acclimatisation to the experimental arena, the animal was presented with two novel objects (1 and 2) and left to explore them for 10 min. The test was repeated 10 min later to analyse STM and 60 and 24 hours later to analyse LTM. In these tests, which lasted 10 min each, one of the objects (object 2) was replaced by a new object (numbered 3 for testing STM, and 4 and 5 for LTM). The object 1 stayed unchanged in all tests. The time spent to explore each object was recorded by an observer blind to the treatment and expressed as a percentage of the total exploration time computed in seconds. The same scheme of experiments was employed for testing animals from all experimental groups (n=12 for each group). Usually, in this test, animals prefer new objects and spend more time exploring them during subsequent repeat challenges (at 10, 60 min and 24 hours). Absence of statistically significant differences in exploration time between the familiar object 1 and any of the new objects (3, 4 or 5) testifies to the deterioration of memory.
* - p<0.05 - compared to 50% recognition time.

Developmental profile of AChE and BChE activity in rat blood serum
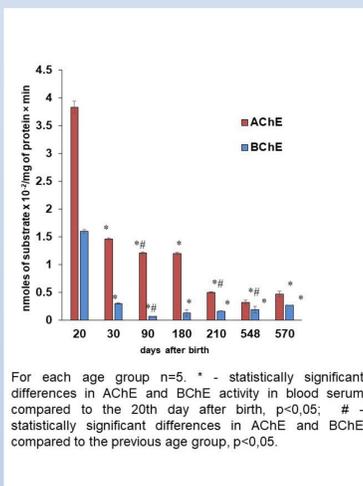
For each age group n=5. * - statistically significant differences in AChE and BChE activity in blood serum compared to the 20th day after birth, p<0,05; # - statistically significant differences in AChE and BChE compared to the previous age group, p<0,05.

Analysis of age-dependent dynamics of acetylcholine- and butyrylcholinesterase (AChE and BChE) in blood serum of rats demonstrated that their enzyme activities significantly decrease during ageing.
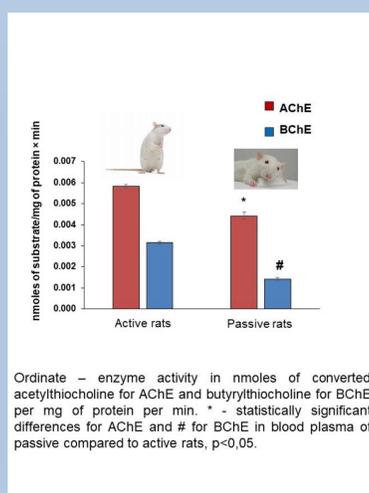
Effect of prenatal hypoxia on the prenatal days 14 (E14) and 18 (E18) on AChE and BChE activity in rat blood serum
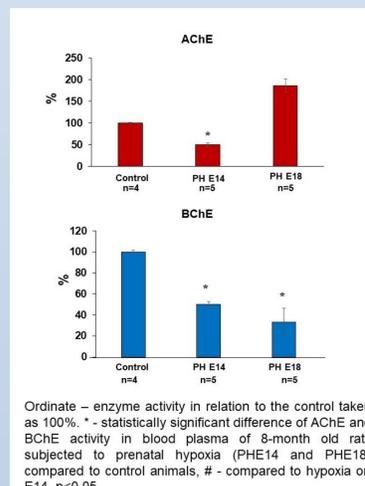
Ordinate – enzyme activity in relation to the control taken as 100%. * - statistically significant difference of AChE and BChE activity in blood plasma of 8-month old rats subjected to prenatal hypoxia (PHE14 and PHE18) compared to control animals, # - compared to hypoxia on E14, p<0,05.

In mature rats (5 and 8 months) subjected to prenatal hypoxia during the period of active formation of the brain (E14, 7% O2, 3 h) there was a two-fold decrease in the activity both of AChE and BChE compared to controls. Prenatal hypoxia at a later stage of pregnancy (E18) also resulted in decreased activity of BChE (down to 30% of the controls) in the blood serum of mature rats. However, the activity of AChE in the serum of such animals was significantly higher (by 80%) than in controls.

AChE and BChE activities in blood serum of mature rats with different levels of motor activity

Ordinate – enzyme activity in nmoles of converted acetylthiocholine for AChE and butyrylthiocholine for BChE per mg of protein per min. * - statistically significant differences for AChE and # for BChE in blood plasma of passive compared to active rats, p<0,05.

Levels of cholinesterase activity in blood serum also correlated with the motor activity of rats. In active mature rats, AChE activity was, on average, 20% and BChE, 70% higher than in passive rats.

Effect of L-carnitine and imatinib administration on AChE and BChE activities in blood serum of mature rats
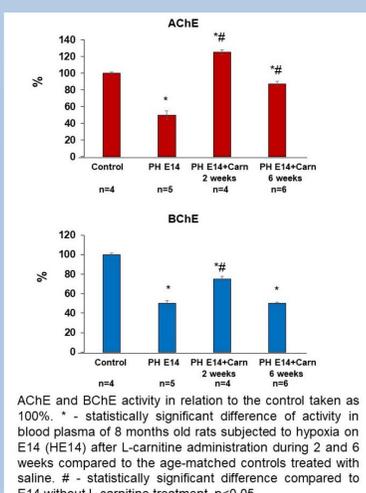
AChE and BChE activity in relation to the control taken as 100%. * - statistically significant difference of activity in blood plasma of 8 months old rats subjected to hypoxia on E14 (HE14) after L-carnitine administration during 2 and 6 weeks compared to the age-matched controls treated with saline. # - statistically significant difference compared to E14 without L-carnitine treatment, p<0,05.

Administration to animals of a natural antioxidant L-carnitine resulted in an increase in the activity of both cholinesterases in the serum of adult rats subjected to prenatal hypoxia.
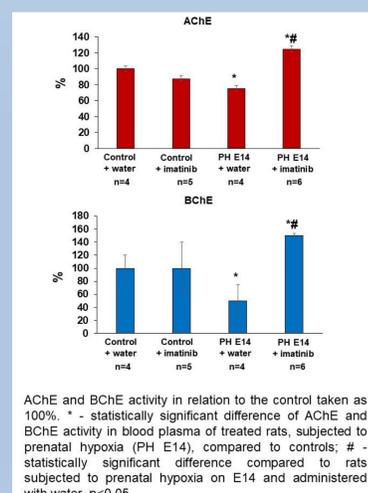
AChE and BChE activity in relation to the control taken as 100%. * - statistically significant difference of AChE and BChE activity in blood plasma of treated rats, subjected to prenatal hypoxia (PH E14), compared to controls; # - statistically significant difference compared to rats subjected to prenatal hypoxia on E14 and administered with water, p<0,05.

Administration to animals of a synthetic tyrosine kinase inhibitor imatinib (Gleevec), which affects expression of some neuronal genes including AChE, resulted in an increase in the activity of both cholinesterases in the serum of adult rats subjected to prenatal hypoxia.

Effect of imatinib on cognitive functions of rats subjected to prenatal hypoxia
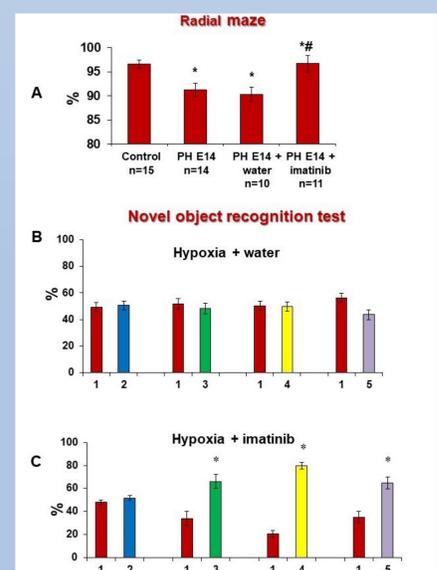
A - % of correct (single) visits of maze arms by rats of the control group and hypoxic rats (PH E14) either treated with water or imatinib solution. * - statistically significant differences compared to controls, # - compared to animals subjected to hypoxia and treated with water, p<0,05.
B,C - % of time spent investigating the familiar (1) and new (2-5) objects for rats, subjected to prenatal hypoxia on E14 and treated with water (B, n=4) or imatinib (C, n=6). * - statistically significant differences between the time spent investigating the familiar and new objects, p<0,05.

Administration to animals of a synthetic tyrosine kinase inhibitor imatinib (Gleevec) improves cognitive functions of rats impaired by prenatal hypoxia.

Conclusions:

- Activity of AChE and BChE in serum plasma of rats declines with age and is reduced after prenatal hypoxia.
- Active rats have higher levels of AChE and BChE in their blood plasma.
- Administration of L-carnitine or imatinib for 2 weeks increases levels of both enzymes and improves cognitive functions of rats impaired after prenatal hypoxia.