THE EFFECT OF HOMOCYSTEINE THIOLACTONE ON ACETYLCHOLINESTERASE ACTIVITY IN RAT BRAIN, BLOOD AND HEART

Milan Petrovic[1], Ivan Fufanovic[2], Iris Elezovic[2], Mirjana Colovic[3], Danijela Krstic[4], Vladimir Jakovljevic[5], Dragan Djuric[6]
[1]Clinic of Abdominal and Endocrine Surgery, Military Medical Academy, Belgrade, [2]School of Medicine University of Belgrade,
[3]Institute for Nuclear Sciences "Vinca", [4]Institute for Chemistry in Medicine, School of Medicine University of Belgrade,
[5]Department of Physiology, Faculty of Medicine University of Kragujevac,
[6]Institute of Medical Physiology „Richard Burian", School of Medicine University of Belgrade

EFEKTI HOMOCISTEIN TIOLAKTONA NA AKTIVNOST ACETILHOLINESTERAZE U MOZGU, KRVI I SRCU PACOVA

Milan Petrović[1], Ivan Fufanović[2], Iris Elezović2, Mirjana Čolović[3], Danijela Krstić[4], Vladimir Jakovljević[5], Dragan Đurić[6]
[1]Klinika za abdominalnu i endokrinu hirurgiju, Vojnomedicinska akademija, Beograd, [2]Medicinski fakultet Univerziteta u Beogradu,
[3]Institut za nuklearne nauke "Vinca", [4]Institut za hemijsku medicinu, Medicinski fakultet Univerziteta u Beogradu,
[5]Katedra za fiziologiju, Medicinski fakultet Univerziteta u Kragujevcu ,
[6]Institut za medicinsku fiziologiju „Richard Burian", Medicinski fakultet Univerziteta u Beogradu

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ABSTRACT

Limited data exist in the literature regarding the effects of homocysteine thiolactone on the activity of the acetylcholinesterase (AChE) in the blood, and practically no data exist regarding the influence of homocysteine thiolactone on the enzyme in the brain and heart. Taking into consideration the importance of hyperhomocysteinemia in clinical practice, it has been thought to be of particular interest to examine the effect of homocysteine thiolactone on the activity of AChE in the rat's blood, brain and heart. In this study, male Wistar rats (weighing 250-300g) were used, and they were divided into two groups; one served as a control group and receieved a placebo (1 ml 0.9 % NaCl, i.p.), while the other group received a homocysteine thiolactone solution (5.5 mmol/kg b.m., i.p.). An hour after the administration, the rats were euthanized by decapitation, heir tissues were harvested, buffered, and homogenized in a phosphate buffer (pH 8). The concentration in the tissue homogenates was 20 mg of tissue per 1 ml of buffer. The buffered and homogenized parts of the tissues were used as substrates for spectrophotometric measurements. The AChE activity was then measured by the Ellman method. Statistical analysis was conducted using a one-way ANOVA test, and the intergroup comparisons were performed using a Bonfferoni test. The results showed a significant reduction in AChE activity in all tissues obtained from the animals treated with homocysteine thiolactone compared to the enzyme activity of the control group. In addition, the results also showed that the blood enzyme activity inhibition was the lowest (12%), while the enzyme activity was slightly higher in the brain (27.8%) and heart specimens (86.3%). It was concluded that homocysteine thiolactone significantly inhibited AChE activity in the heart and brain tissue, but not in the blood of the rat.

Keywords: *acetylcholinesterase, homocysteine thiolactone, specific enzyme activity*

SAŽETAK

U literaturi je nađeno malo podataka o uticaju homocisteina na aktivnost acetilholinesteraze u krvi, a direktnih nalaza o uticaju homocistein tiolaktona u mozgu i srcu nema. S obzirom na medicinski značaj pojave hiperhomocisteinemije, smatrali smo da je od interesa da se ispita uticaj D,L-homocistein tiolaktona na aktivnost acetilholinesteraze u krvi, mozgu i srcu pacova. U eksperimentu su korišćeni pacovi mužjaci soja Wistar (telesne mase 250-300g) podeljeni u dve grupe: jedna grupa je bila kontrolna i dobijala placebo (1ml 0,9 % NaCl, i.p.), dok je druga grupa dobijala rastvoreni homocistein tiolakton (5,5mmol/kg t.m, i.p.). Sat vremena po aplikaciji pacovi su dekapitovani, dobijena tkiva su zatim puferovana i homogenizovana u fosfatnom puferu (pH 8). Koncentracija tkiva u homogenatu iznosila je 20mg tkiva po ml pufera. Puferovani i homogenizovani delovi tkiva su korišćeni kao supstrat za spektrofotometrijska merenja. Zatim se pristupilo merenju aktivnosti acetilholinesteraze, koja je merena metodom po Ellmanu. Statistička obrada podataka urađena je jednofaktorskom analizom varijanse, a međugrupna poređenja Bonferonijevim testom. Rezultati pokazuju da postoji značajno smanjenje aktivnosti enzima acetilholinesteraze u svim tkivima uzetih od pacova tretiranih homocistein tiolaktonom, za razliku od aktivnosti enzima kontrolne grupe koja je dobijala placebo, i to: u krvi je utvrđena najmanja inhibicija specifične aktivnosti (12%), u mozgu nešto veća (27,8%), dok je u srcu najveća (86,3%). Na osnovu dobijenih rezultata zaključeno je da homocistein tiolakton u značajnom procentu inhibira aktivnost acetilholinesteraze u mozgu i srcu, ali ne i u krvi pacova.

Ključne reči: *acetilholinesteraza, homocistein tiolakton, specifična enzimska aktivnost*

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Correspondence to:
Prof. Dr. Dragan Đurić / Institute of Medical Physiology "Richard Burian" / School of Medicine University of Belgrade / Višegradska 26/II, 11000 Belgrade
Tel. +381 11 36 07 074 / Fax. +381 11 36 11 261 / drdjuric@eunet.rs

INTRODUCTION

Acetylcholinesterase (AChE, EC 3.1.1.7) is an enzyme that rapidly hydrolyzes the neurotransmitter acetylcholine in cholinergic synapses, including the neuromuscular junction. Recent surveys have highlighted the enormous importance of AChE in processes such as the growth of cholinergic and non-cholinergic neurons, as well as in processes related to extraneural tissues, including the inhibition of hematopoietic stem cell differentiation, connection of amyloid fibres, the influence on apoptosis, and neoplasma growth (1,2). AChE is transcribed from only one gene, but due to a variety of post-translational processes, it exists in a variety of isoforms and is also present in numerous tissues. By combining a number of different isoforms, it is possible to acquire more complex structures, mainly in the form of dimers and tetramers. These forms are connected to the plasma membrane with an "anchor" of a glycophosphatidylinositol structure, while the form on a neuromuscular connection is represented by appropriate anchor proteins (sequence WAT, consisting of aromatic amino acids on the enzyme) to a collagen-like domain (PRAD, CoQ protein) (3,4). Homocysteine thiolactone is the cyclic metabolite of homocysteine, generated in an organism under oxidative stress conditions and the lack of vitamin B12 and/or folic acid. The most common route of its creation comes from the metabolism of folic acid accompanied by vitamin B12 where homocysteine is created from methionine. Another route for generating homocysteine is by methylation with betaine homocysteine-methyltransferase. Homocysteine thiolactone is a very reactive metabolite that has been viewed to be enormously important in the pathogenesis of cardiovascular diseases, diabetes, and osteoporosis, as well as in various diseases of the central nervous system including Alzheimer, neural tubus defects and schizophrenia (5). The mechanisms of the effect of homocysteine thiolactone on the above mentioned disorders are not known, but it is possible that homocysteine acts, as a reducing agent, reacts with the sulfhydryl groups of certain molecules, thereby changing their structure, adhesion and signalling within cells. Another mechanism could be an increase in the quantities of S-adenosyl methionine resulting in a reduction of gene methylation, and consequently a reduction in gene expression (6).

Little is known about the influence of homocysteine thiolactone on AChE activity in the blood, while descriptions of the effect of homocysteine thiolactone on the brain and heart are practically non-existent. With respect to the clinical importance of hyperhomocysteineamia, it is crucial to investigate the influence of D,L-homocysteine thiolactone on the activities of the AChE enzyme in the blood, brain and heart of a rat.

MATERIALS AND METHODS

Animals

Male Wistar albino rats weighing 250-300g were used in the experiment and were studied at 10 weeks of age. The animals were kept in a standard laboratory environment at a temperature of 22oC. Water and food were provided ad libitum.

Tissue preparation

The animals were divided into two experimental groups, six rats per group. The first group was the control group, and the second was treated with homocysteine thiolactone (Sigma Chemical Co. USA). The animals were cared for in accordance with the codes for laboratory animals established by the School of Medicine, University of Belgrade, and in compliance with the Committee of Ethics related to the work with experimental animals. Homocysteine thiolactone was dissolved in buffered 0.9% NaCl at pH 7.4. A solution (5.5 mmol/kg of body weight) of homocysteine thiolactone (1 ml) was administered intraperitoneally. The control group was given a placebo intraperitoneally (1 ml 0.9% NaCl).

Sixty minutes later, the rats were euthanized by decapitation. Whole brains and hearts isolated from the rats were rinsed in a phosphate buffer pH 8.0, and the blood was stored in test tubes coated in heparin. The brains and the hearts were homogenized in cold phosphate buffer (pH 8.0). The final tissue concentration was 20 mg tissue per ml buffer.

Biochemical determination

AChE activity was determined by Ellman's method (7). The incubation mixture contained: 20µl brain homogenate in 600µl of the phosphate buffer pH 8.0; 40µl heart homogenate in 580µl of the phosphate buffer pH 8.0; 50µl heparinized blood (diluted in sodium chloride 1:100) in 570µl of the phosphate buffer pH 8.0. The mixture was incubated at 37 oC for 10 minutes. A volume of 20µl 5,5'-dithionitrobenzoic acid (DTNB) (Sigma Chemical Co, USA) and 10µl of acetylcholine iodide (Sigma Chemical Co, USA), used as substrates, was added, and the reaction was started. The reaction was monitored spectrophotometrically (Gilford Instrument, Model 250) by an increase in the absorbance (ΔA) at 412nm. An assay, without the tissue homogenate, was used as a blank probe. The measurements were assessed with double probes, and the specific AChE activity was calculated as ΔA (min x mg tissue) for the brain and heart, and ΔA (min x µLblood) for blood.

Statistical analyses

Values are presented as means ± SD. Statistical analyses were performed using a monofactorial analysis of variance, as well as Bonferroni test. P values less then 0.05 were considered to be significant.

Chemicals used

All chemicals were of p.a. grade quality. D,L-homocysteine thiolactone, acetylcholine-iodide (ASChI) and 5,5-dithiobis(2 nitrobenzoic acid) (DTNB) were purchased from Sigma Chemical Co. (USA).

RESULTS

The AchE activity determined in homogenized whole brain, heart and blood from non-treated rats (control group) are presented in Table 1. The AchE activities are significantly different between the different types of rat tissues. Higher enzyme activities were recorded in the brain

Acetylcholinesterase activity (means ± SD)

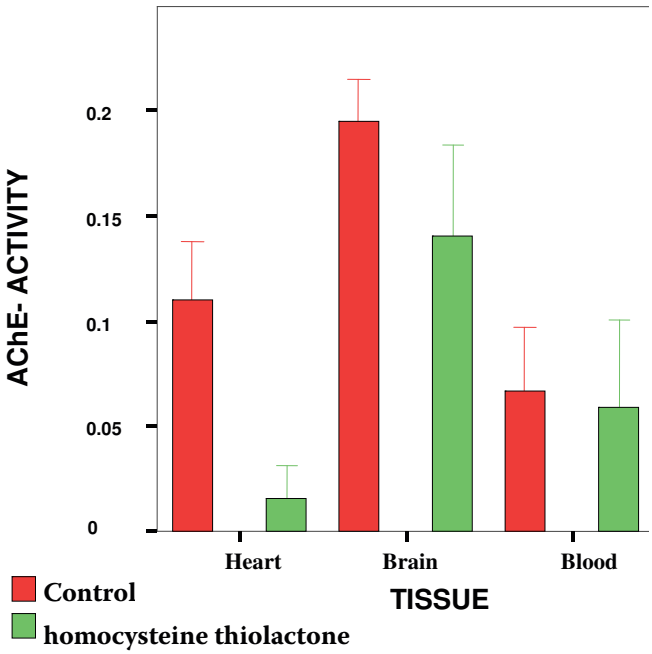
n=6		p-value[a]	p-value[b]		p-value[a]	p-value[b]
Tissue	Control			Treated		
Heart	0.110±0.028	< 0.001	< 0.05 vs. brain and blood	0.015±0.016**	<0.001 vs. control	<0.001 vs. control
Brain	0.194±0.020	< 0.001	< 0.05 vs. brain and blood	0.140±0.044*	<0.01 vs. control	NS
Blood	0.067±0.030	< 0.001	< 0.05 brain and heart	0.059±0.041	NS	NS

a- statistical test applied : ANOVA
b- statistical test applied : Bonferroni test

(0.194±0.020) and in the heart (0.110±0.028). The lowest activity was recorded in the blood (0.067±0.030). All enzyme activities from homogenized tissues of rats treated with homocysteine thiolactone were decreased compared to the control group. Moreover, these activities were significantly lower compared to control values for the heart (by 86.3%) and the brain (by 27.8%) (Table 1, Fig 1). However, there was no evidence of a significant difference in the AchE activity for blood in the treated group (0.059±0.041) compared to control values (0.067±0.030) (Table 1, Fig 1).

DISCUSSION

Homocysteine thiolactone inhibits the activity of AChE, and specific AChE activities are different in different types of tissues (brain, heart and blood) in the rat (8). A similar ration of the specific AChE activities in the experimental animals has been observed (9). In this paper, we described the influence of homocysteine thiolactone on AChE activities in the brain, heart and blood of the rat. Our results clearly show that an acute treatment with homocysteine thiolactone causes a decrease in the AChE activity, while the sensitivity of AChE in the brain, heart and blood in the presence of the tested substance mutually differ.



The highest sensitivity of AChE, i.e., the highest inhibitory effect of homocysteine thiolactone on the enzyme activity, was obtained in the heart, where the activity of this enzyme was decreased by 86.3% compared to the control value. This treatment resulted in the inhibition of AChE in the brain by 27.8% compared to the control group, while the lowest sensitivity was obtained for AChE in the blood. The observed decrease in the specific activity of AChE in the blood was 12% compared to the control group, and cannot be considered a statistically significant change compared to the control group. Homocysteine thiolactone in vitro inhibits the activity of butyrylcholinesterase (containing a similar structure to AChE) in the blood of the rat; this inhibition is proportional to the concentration of the inhibitor (10). Furthermore, according to the kinetic analysis of inhibition, homocysteine thiolactone and the substrate bind to the same site in the enzyme (10).

Another group of researchers believe that homocysteine thiolactone produces tight thioester connections (8). A significant decrease of AChE activity corroborates our results. In addition to the stated (un) competitive inhibition of AChE with homocysteine thiolactone, the obtained inhibition of the tested enzyme could be explained by the fact that the presence of the increased concentration of homocysteine thiolactone produces an increase in the production of oxidative free radicals, i.e., the obtained decrease of the enzyme activity tends to be a consequence of the oxidative stress in the functional groups on the enzyme (8, 11). Providing that hyperhomocysteinemia (as well as the increased concentration of homocysteine in tissues) is present in a variety of pathological disorders (5,11). In addition to the fact that the mechanistic role of this metabolite in the initiation and development of disease processes is not known, the results obtained here suggest that the inhibition of AChE with homocysteine could be one of the most possible mechanisms responsible for the pathogenesis of the diseases stated herein.

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