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L-ARGININE AS A REACTIVATOR OF CHOLINESTERASES AND ANTIDOTE FOR INTOXICATION BY ANTICHOLINESTERASE AGENTS AND CYANIDES

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Abstract: This study demonstrates the potential, through preventive and therapeutic techniques, to manage and regulate the activity of cholinesterases.

L-Arginine has been found to affect significantly the activity of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in various brain structures in vertebrates and the activity of AChE in total fractions of invertebrates. An interesting relation was observed – the stimulating effect of L-Arginine on cholinesterases is proportional to the level of inhibition of their activity. Such a stimulation is a special characteristic of BChE, which eliminates all choline esters, many poisons of plant and animal origin, narcotic agents, drugs and others. This enzyme is the major factor protecting the nervous system and synapses from damage.

Major damaging factors in this aspect are anticholinesterase pesticides, insecticides, nerve poisons and others. This work also analyzes the toxic action and mechanisms of action of various cyanide-containing products. The list includes Na-Nitroprusside, which contains 5 cyanide radicals in its molecule, K-ferricyanide and others. L-Arginine has been shown to reactivate cholinesterase activity, which is inhibited by cyanides.

INTRODUCTION

In some of our works (Ivanov and Dencheva, 2016; Dencheva and Ivanov 2017), it was shown that L-Arginine as a source of NO specifically activates or reactivates the cholinesterases in different tissue and animal species, inhibited by anticholinesterase agents (pesticides, insecticides, etc.) .

In other studies, it was shown that a number of cyanide-containing products also in a concentration-dependent manner inhibit the activity of cholinesterases (Dencheva and Ivanov, 2018). L-Arginine reactivates the cholinesterases inhibited by cyanides in vertebrates and invertebrates.

It also focuses on insect resistance to organophosphate, carbamate insecticides and cyanides and some technologies for their protection and survival in an ecotoxic environment.

Previous studies of one of the authors of this paper (Ivanov et al., 2001, Ivanov et al., 2003, Vukova et al., 2005) showed that in the case of cholinesterase damage from microwave electromagnetic fields (2,4 GHz) induced serious conformational changes of the enzyme protein (mainly despiralization).

The main findings of the study are: Arginine in varying degrees activated cholinesterases in invertebrates and vertebrates; this effect was tissue and species specific; arginine is reactivator of cholinesterases damaged by various toxic agents, including various cyanides; the data obtained is the basis for the development of biomonitoring technics for assessing the state of organisms and the environment.

MATERIALS AND METHODS

Preparation of enzyme fractions. Isolation of membrane-mitochondrial fraction from brain of two mamalian species – Rattus rattus and Oryctolagus cuniculus, as well as a total body fraction from invertebrate species – *Vespula germanica* and Apis melliferawas were made by the method of differential centrifugation. The medium for homogenization and isolation of the respective fractions contained 0.1 M KCl, 1 mM $MgCl_2$, 0.1 mM EDTA-Na salt, 50 mM Tris-HCl (pH = 7.6). For more details see Ivanov, Dencheva (2016); Dencheva, Ivanov (2017).

The animals used in the laboratory tests were obtained from the vivarium of the Faculty of Biology of Sofia University, while the insects were obtained from natural sources. This investigation has conformed to the international and national rules and regulations regarding the ethical treatment of animals.

Determination of acetylcholinesterase and butyrylcholinesterase activity.

The activity of AChE and BChE was determined by the classical method of Elman et al. (1961). The activity of cholinesterases was determined depending on the thiocholine released by the hydrolysis of acetyl thiocholin iodide or butyrylthiocholniodide. The thiocolline content was measured spectrophotometrically at λ= 412 nm. The reaction was stopped by adding the specific cholinesterases inhibitor – eserine salicylate (0.1-0.5 mM).

Calculation of enzyme activity. The principle of measurement of cholinesterase activity is by measuring the contents of the SH-group. As a source of SH-groups to prepare the standard curve, L-cysteine ($C_3H_7NO_2S$) was used in different molar concentrations. The molar content of SH-groups in cysteine and thiocholine is the same.

The enzyme activity of the respective fractions in the article is presented in real values (μg or μM hydrolyzed substrate/mg protein/min), in relative units (r.u.) or in % versus controls.

Determination of the contents of the protein. The protein content in the samples was determined by the classical method of Lowry et al. (1951) using lyophilized bovin albumin.

Statistical analysis. The significance of differences between the control and experimental samples has been estimated by Student's t-test.

RESULTS AND DISCUSSION

Some basic biomonitoring criteria that characterize the state of the cholinergic system in different animal species

Cholinesterases as biomonitoring objects. Cholinesterases (ChE) are important biomonitoring objects through which the state of the nervous system and the body can be evaluated. Numerous toxic agents (endogenous and exogenous) attack the nervous system by influencing the activity of ChE (choline esters, drugs, narcotics, cyanide-containing compounds, chemical poisonous substances, various pesticides and insecticides, etc.). In 2016, we presented a scientific report on the role of butyrylcholinesterase (BChE) as a protection factor for the nervous system from toxic products. For example, in literature there are evidences that BChE hydrolyzes 95% of heroin and other narcotic agents (Dencheva, Ivanov, 2016; Brownee et al., 1998; Zhan et al. 2003; Zheng et al., 2008; Pohannka, 2013).

BChE was always on the second place to discuss its functions in the body. In recent years, however, this enzyme was revealed as an important factor defending the nervous system (NS) from toxic agents. As mentioned below, BChE today is considered to be an "immune system" not only for the NS but also for all tissues and systems in the body (Lockindge et al., 2016; Reaqd et al., 2013; Fernandez-Gomez et al., 2010).

Biomonitoring control requires not only suitable object, but relevant criteria for assessing the condition of the body and the environment. Such criteria are, for example, the "Initial Effective Concentration" (IEC) – the threshold concentration of reactivity of the reagent; "concentration-dependent inhibition" (CDI50 – the concentration of reagents resulting in 50% inhibition of the enzyme; Fig. 1). The indicator of level of reagent's toxicity; criterion "Maximal Efficiency Concentration" (MEC) means the maximal level of influence in determined reagent concentration. For example, the 100% inhibition of AChE by Eserine salicylate or Nivalin ocurrs at concentrations of 0.1 mM.

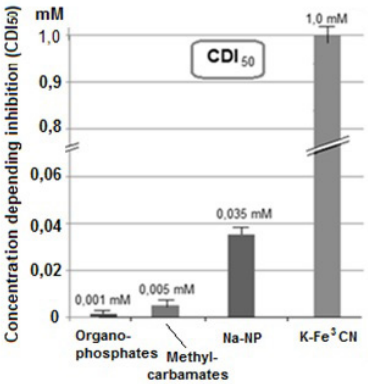

Fig. 1. Concentration-dependent inhibition of AChE activity (CDI_{50}) as a criterion for the sensitivity of acetylcholinesterase in *Vespula germanica* fractions under the influence of various inhibitors (parathion, ezerine salicylate, Na-Nitroprusside and K-Ferricyanide)

The CDI_{50} values are related to demonstrate the sensitivity of the enzyme to the corresponding inhibitors. In this case, AChE has the highest sensitivity to organophosphate preparations and the lowest to K-ferricyanid (K-Fe^3CN).

Another important biomonitoring criterion is the DL_{50} – a universal indicator of intoxication. This factor is usually listed in the Toxicity Manual. For example, the toxic dose (DL_{50}) for mice or rats (intravenous injection) is 8.4 or 11.2 mg/kg (Tinker, Michenfelder, 1976). The toxicity, for example, of Na-Nitroprusside (containing 5 cyanide radicals in its molecule) or of typical anticholinesterase pesticides is tens or hundreds of times lower, i.e. higher than ferric cyanide toxicity (Anseeuw et al. 2013; Hamel, 2011).

We present data on three of the biomonitoring criteria – IEC, CDI_{50} and CDI_{100} or MEC (maximum effective concentration) in a rabbit brain cortex subjected to two powerful anticholinesterase agents – Methyl parathion and Eserine salicylate (Table 1).

Table 1. Biomonitoring criteria, characterizing the changes on AChE and BChE activities A – mg hydrolyzed acetylcholine/mg protein/min) under the influence of Eserine salicylate and Methyl parathion

Anticholinesterase compound/ biomonitoring indicators	Enzyme activity	
	AChE	BChE
Eserine salicylate		
IEC	0.1 μM	0.01 μM
CDI_{50}	1.0 μM	10 μM
CDI_{100} (MEC)	10 μM	0.1 mM
Methyl parathion		
IEC	10 nM	0.1 μM
CDI_{50}	0.1 μM	1.0 μM

The general conclusion is that organophosphates are several times more toxic on BChE and AChE compared to the effects of carbamates. Both enzymes are more resistant to carbamate pesticides than to organophosphates.

These coefficients outline the basic parameters of each enzyme system under the influence of various factors.

This model analysis a specific biomonitoring assessments of the ecotoxic environment, for human and animal pests. It can be used for diagnostics of genetic deficit, etc. A useful book presenting theory and practice in this field is Lyubenova, Kalchev (2007).

Basic controls are the basis of biomonitoring studies and analyzes. The study presents three guidelines for changes in cholinesterase activity - activation, inhibition and reactivation from inhibition (intoxication). In this section, some "normal" values of the enzymatic activity of AChE and BChE are shown as reference controls (Table 2). These indicators were used in the subsequent analyzes.

According to the data in the Table 2, the enzyme activity of ChE (AChE and BChE) in the brain of vertebrates (rats and rabbits) differs depending on the structural and functional organization of the brain areas. The underlying hypothesis here is that the activity of cholinesterases reflects the topographical distribution of the cholinergic system. For example, in the rat brain, the activity of this group of enzymes is higher in the cerebellum than in the forebrain. In the brain of a rabbit, the highest activity of AChE is recorded in the brain stem where many structures and nuclei of cholinergic nature are located.

Table 2. Base enzymic activity of cholinesterases in fractions of different animal species

Subject of the studying	AChE - A	BChE - A
	M±m (±m=5-15)	M±m (±m=0,30-0,90)
Brain of a white rat (*Rattus rattus*)		
Forein brain (Cerebrum)	128.30	мар.67
Cerebellum	156.09	юни.60
Brain of rabbit (*Oryctolagus cuni-culus*).		
Brain cortex	124.05	10.ное
Brain steam	183.75	23.43
Cerebellum	88.13	апр.51
Vespula germanica	21.60±0.90	-
Apis mellifera	44.12±1.55	-

A–enzyme activity in μg hydrolysed acetylcholine/mg protein/min; n = 8-15.

Activity of cholinesterases in the brain tissue of mammals is many times higher than in invertebrates. This is explained by the differences in the nerve tissue content in the respective fractions. The differences in AChE and BChE activity are due to the localization of these enzymes. AChE is mainly found in the postsynaptic membrane, while BChE - in neuroglial cells, interstitial fluid, blood plasma, and partially in the synaptic area.

According to the data, AChE enzyme activity is almost twofold lower in fractions of *V. germanica* compared to *A. mellifera* under the same conditions of isolation and enzyme activity determination. The protein content in fractions of vespa is significantly greater than in honeybee. This means that the nervous system in bees is much more developed than in wasps. Such observations are indirect indicators for the evolutionary stage which each species has reached.

L-Arginine as a regulator, reactivator and antidote on the activity of cholinesterases in fractions of vertebrates and invertebrates

In some of our scientific papers (Ivanov, Dencheva, 2016; Dencheva, Ivanov, 2017) we have presented data on the effect of L-Arginine as a NO donor on cholinesterase activity in fractions of different animal species.

Our primary data showed that L-Arginine stimulates cholinesterase activity at a certain concentration range. Theoretically, this would lead to acceleration of synaptic cycles, depletion of neurotransmiter reserves, behavioral changes, etc.

Influence of L-Arginine on cholinesterases in mammals. According to experimental data (Figure 2), L-Arginine in concentration range from 10 to 50 Mm acivates cholinesteraes (AChE and BChE) enzyme activity in forein brain (Cerebrum) and cerebellum of rat.This effect is much stronger on BChE. L-Arginine, (50 mM) in the foreinbrain increases the activity about eight times and in the cerebellum - about four times.

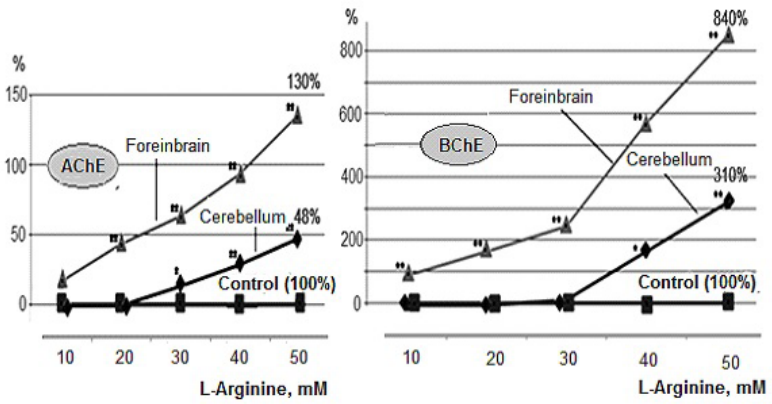

Fig. 2. Influence of L-arginine (50 mM) on the activity of AChE and BChE in different rat`s brain areas

This powerful stimulating effect on BChE and the role of this enzyme as a defending body system offers pharmacological solutions for the prevention and therapy against various kinds of intoxications. The initial (threshold) effective concentration (IEC) of this amino acid varies depending on concentration – less than 10 mM (L-Arginine – 50 mM) and in the range of 30 mM (L-Arginine – 50 mM).

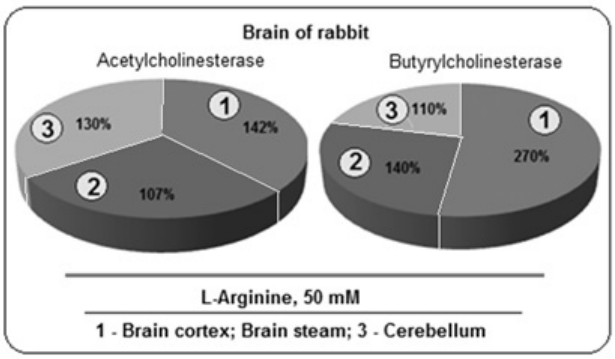

Fig. 3. Influence of L-arginine (50 mM) on the activity of AChE and BChE in different rabbit brain areas

The data are in % relative to the control. The actual values of the enzyme activity of these preparations are given in Table 1. Other data: n = 8-10; $p < 0.001$ (relative to the respective controls).

The activating effect of L-arginine (50 mM) on cholinesterases in rabbit brain fractions (Figure 3) varies depending on the structures studied. It is better for BChE (average total about 170%) compared to the rat foreinbrain (about 25%, relative to the respective controls). This means tissue specificific reactivity to various endogenous substances and xenobiotics. In this setting, the stimulation of the two types' cholinesterases is most pronounced in fractions of the cerebral cortex where neurons are predominantly cholinoreceptive.

Influence of L-Arginine on the activity of AChE in invertebrates. The next step in the study was to analyze the effect of L-Arginine on the activity of AChE in several invertebrate animals. Subject of the study were some of the "most intelligent" species among the insects and invertebrates.

The aim was to study the similarities and differences in the effects of L-Arginine alone or in a combination with different substances, most of them toxins on activities of cholinesterases in vertebrates and invertebrates.

Below data on the effectiveness of L-Arginine as a reactivating factor in various vertebrate and invertebrate species under the conditions of intoxication of cholinesterases from various specific and non-specific toxicants are presented.

The efficiency of L-Arginine on cholinesterase activities from *A. mellifera* and *V. germanica* is shown in Figure 4 and Table 3.

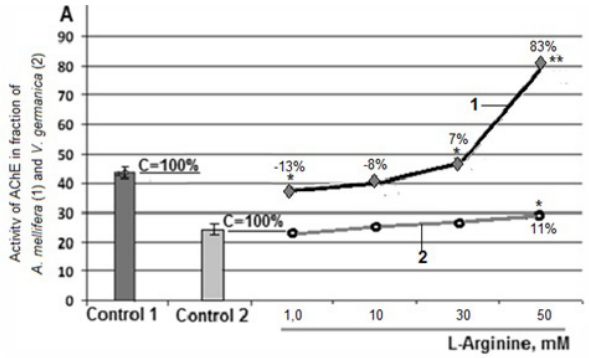

Fig. 4. Influence of L-arginine (1,0-50 mM) on the activity of AChE in fractions of A. mellifera (1) and V. germanica (2)
The results are shown as real values (A) and as % relative to the control; A – enzyme activity in μg hydrolysed acetylcholine/mg protein/min; n = 8-10; p <0.001 (relative to the respective controls). See also Table 3.

The data show that L-Arginine (1,0-50 mM) has not affected the AChE activity in fractions of *V. germanica,* whereas L-Arginine (50 mM) stimulates the enzyme activity in *A. mellifera* about 80%. Another feature is the relatively low value of IEC≥10 mM of stimulation in honey bee fractions. One possibility is the suggestion of low activity of NOS in fractions of *V. germanica.*

Table 3. Influence of L-arginine on the activity of AChE in fractions of *V. germanica* and *A. mellifera*

Control 1 (*A. mellifera*)	+ L-Arginine (30 mM)	+ L-Arginine (50 mM)
43.67±1.21	46.50±0.76 (7%)	79.94±3.64 (83%)
Control 2 (*V. germanica*)		
(A) 21.60±0.15	(A) 22.83±0.32 (6%)	(A) 23.95±0.28 (11%)

Role of L-Arginine and/or probably via NO as an antidote against carbamate pesticide poisoning

An important point of this study was to illustrate the role of L-Arginine as an antidote and reactivator on the activity of cholinesterases, inactivated by carbamate pesticides and Na-Nitroprusside. This phenomenon has already been shown in other objects and test patterns, for example in different brain areas in mammals (Ivanov, Dencheva, 2016).

The data in the Figure 5 and Table 4 refer to two different species – mammals and insects. This is an attempt to make a comparative analysis of the effectiveness of L-Arginine in different groups of animals. The main objective of the study was to provide specific data to support the idea that through the management of the cholinergic system it is possible to create a new approach to protect organisms from certain types of intoxications.

The specific cholinesterase inhibitor, in this case Eserine salicylate at a concentration of 1.0 μM or 10 μM suppresses the activity of AChE in the order of 80%-70% relative to controls. This inhibition of enzyme activity is very easily removed by adding L-Arginine (50 mM) i.e. in a combination of eserine and L-Arginine. Table 4 show data for the effectiveness of L-Arginine as a protector and reactivator of AChE inhibited by eserine salicylate in fraction of *A. mellifera*.

Our data strongly suggests a protective role of L-Arginine on AChE against carbamate pesticides toxicity. In this case, L-Arginine fully reactivated enzyme activity. With adequate concentration, the combinations of a toxicant and L-Arginine is possible to normalize the activity of AChE at a different level of inhibition of the enzyme from both specific anticholinesterases and other toxic substances – cyanides, choline esters, narcotic agents, etc. We also assumed that this approach will be effective in chemical warfare agents.

The data in the table indicates one feature – slight inhibition of AChE activity at low concentrations (0.1-1.0 mM) of L-Arginine in the order of 10%. In concentration of 50 mM this reagent stimulates the enzyme activity to about 80%. The answer to these controversial effect is forthcoming with further experimental studies.

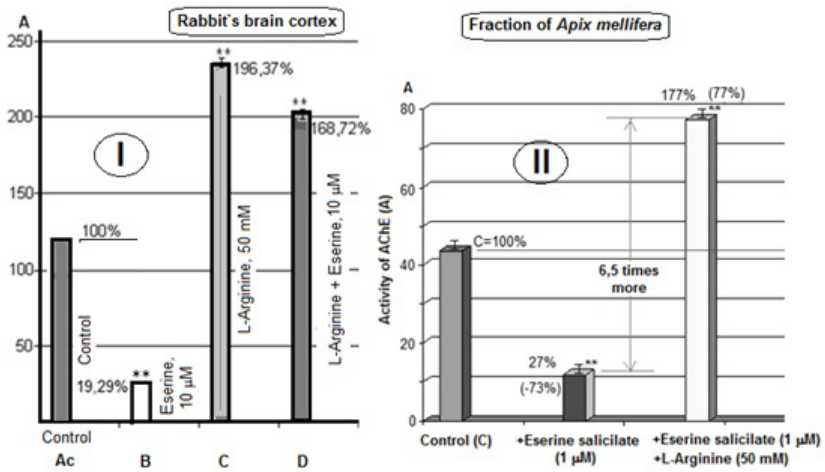

Fig. 5. Effect of Ezerin salicylate (10 μM) (B), L-Arginine (50 mM) (C) and combination of L-Arginine (50 mM) and Eserine (10 μM) (D) on AChE activity in brain's cortex of rabbit (I) and in fraction of A. mellifera (II) (Eserine,1,0 μM and combination of Arginine, 50 mM and Eserine, 1,0 μM)

A – enzyme activity (μg hydrolysed ACh/mg protein/min; n = 8-10; ± m = 4.0-5.50; ** p <0.001; % - percentages relative to controls=100%; see also Table 4.

Table 4. The influence of Eserine and combination between L-Arginine and Eserine on AChE activity in fractions of *A. mellifera*
The results are shown as real values (A) and as % relative to the control; see also Fig. 6.

Control (C)=100%	43.67 ±1.21 (AChE activity)				
+Eserine, μM	42.06±0,90	29.85±1.20	22.18±1.20	10,15±0,20	A
%	95%	68%	50%	23%	%
Concentration	0.1	1	10	100	μM
+L-Arginine, mM	38.18±1.06	40.12±0.90	46.50±0.76	79.94±3.64	A
%	87%	92%	107%	183%	%
Concentration	1.0	10	30	50	mM

The pattern in *A. mellifera* was similar to *V. germanica* and to mammalian brain fractions. This means the possibility to use L-Arginine and other endogenous and exogenous NO-generating substances as a protector in animals and humans.

We think this is a new type of antitoxic technology against anticholinesterase pesticides by controlled management of the activity of AChE and BChE and consequently control over the content of acetylcholine at the synapse in the nervous system and internal organs (protecting the body from the harmful effects of many endogenous and exogenous products).

Role of L-Arginine or NO as a modulator, reactivator and antidote against Na-Nitroprusside, cyanides and other poisoning agents

In some of our papers (Ivanov, Dencheva, 2016; Dencheva, Ivanov, 2018) the effect of Na-NP and other cyanide-containing products on the activity of cholinesterases in various fractions of vertebrates and invertebrates was analyzed. Sodium nitroprusside is an effective medical preparation for controlling high blood pressure (hypertension). Its molecule contains one NO-radical, five cyanide residues and an iron ion, which forms ferricyanide complexes. According to our knowledge, for the first time is reported here that Na-NP, like other cyanides, concentration dependently inhibits the activity of cholinesterases in various mammalian tissues and fractions of invertebrate animals.

Influence of Na-NP and L-Arginine on the activity of AChE in fractions of *V. germanica.* The purpose of this study was to analyze the possibility of using L-Arginine as a modulator, protector and reactivator of cholinesterases in various animal species against cyanide intoxications.

The results of the study with analysis are shown in Figure 6 and Table 5.

In the range 0.1 μM-1.0 mM Na-NP concentration-dependently inhibits the activity of AChE in fractions of V. germanica reaching nearly 100% at 1,0 mM. The stimulation of the enzymatic activity by L-Arginine is statistically unreliable and only manifests a trend (5-10%). Perhaps because of this, the effects of L-Arginine on brain fractions of mammals were different from those in invertebrates. At a mammalian subject, L-Arginine concentration-dependently stimulates the activity of AChE.

The L-Arginine-dependent activation of AChE in the presence of toxic concentrations of Na-NP was induced only by L-Arginine in higher concentration.

The activating effect of L-Arginine was proportional to the administered concentrations (*V. germanica*). For example, 30 mM restored the inhibited enzyme activity about 70%, and 50 mM – about 135%. This also means that with almost 100% inhibition of the enzyme activity of the nitroprusside, arginine restored about 50% of this intoxication.

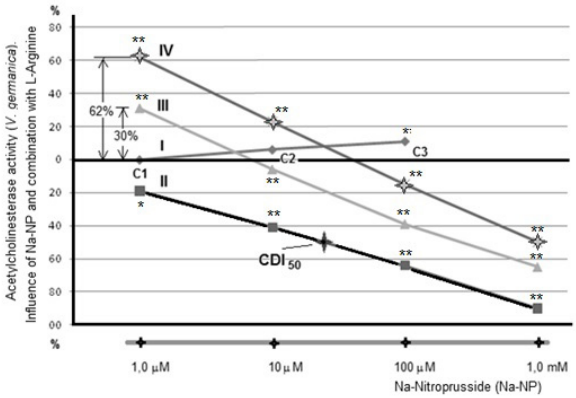

Fig. 6. Influence of Na-NP at a concentration of 0.001 to 1.0 mM on the activity of AChE in *Vespula germanica* (II) and a protective function of L-arginine of 30 mM (III) and 50 mM (IV) against the inhibition of the enzyme activity from Na-HP

Other data: Percentage (inhibition or stimulation relative to the respective control); n = 8; **-p <0.001; CDI_{50} - concentration-dependent inhibition; C – controls (I), respectively: C1 - base control; C2 - + L-Arginine 30 mM and C3 - + L-Arginine 50 mM.

Another indicator of the effects of Na-NP and L-Arginine is CDI50. This Na-NP-ratio (indicator) is about 30 µM and for L-Arginine + Na-NP is about 80-90 µM. This means that the presence of L-Arginine reduces the reactivity of AChE to Na-NP. Protective efficacy in this case occurs only in combination between L-Arginine and Na-NP or their metabolic products. Assuming that the inhibitory effect of Na-NP is due to cyanide components and metabolites, this is an example and proof of the protective efficacy of L-Arginine against cyanide intoxication. Similar patterns have been observed in other insect species, such as honey bee and Colorado potato beetle (*Leptinotarsa decemlineata*, data not shown).

The role of L-Arginine as a reactivator of AChE activity inhibited by K-ferricyanide. A basic result of our study is that K-Fe3CN inhibits enzyme activity of AChE in various fractions from mammals and invertebrates (*A. mellifera* and *V. germanica*). It was also shown that the effectiveness of Na-NP as an inhibitor of AChE (A. mellifera) is more than 30 times stronger if compared to potassium ferricyanide (Figure 7). The inhibitory efficiency of K-Fe3CN is strongly manifested in fractions of *A. mellifera* than in the *V. Germanica*. This statement was supported by CDI_{50} which is 0,4 mM in fractions of *A. mellifera*, and in fractions of *V. Germanica* – 1.0 mM (i.e. about 2.5 times more).

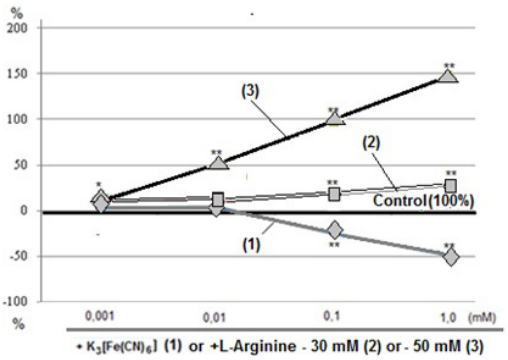

Fig. 7. Influence of K-Fe3CN (1) on activity of AChE in fractions of *V. germanica.* The role of L-Arginine – 30 mM (2) and 50 mM (3) as reactivator on enzyme activity suppressed by potassium ferricyanide at the indicated concentrations. Data are in % relative to baseline control; n=8-10; * p<0.005; ** p <0.001; see also Table 5.

Table 5. Influence of K-Fe3CN on the activity of AChE in fractions of *V. germanica.* The role of L-Arginine – 30 mM and 50 mM as a reactivator on enzyme activity suppressed by potassium ferricyanide at the indicated concentrations.

Basal control (BC)		K-Fe^{3}CN	(mM)	
22.510±0.900 (A)	0.001	0.01	0.1	1.0
+ K-Fe^{3}CN *(A)*	23.14 ±0.640	23.46 ±1.20 **	16.98 ±0.80 **	11.48 ±0.84 **
% спрямо ВС	103% (**+3%**)	104% (**+4%**)	75% (**-25%**)	51% (**-49%**)
+ K-Fe^{3}CN + *L-Arginine, 30 mM* (A)	25.69 ±0.95*	25.58 ±1.29**	19.58 ±0.84**	14.60 ±0.95 **
% спрямо ВС	114% (**+14%**)	114% (**+14%**)	87% (**+13%**)	65% (**+35%**)
+ K-Fe^{3}CN + L-Arginine, 50 mM (A)	26.38 ±0.95*	35.43 ±1.93**	31.92 ±1.48**	?-
% спрямо ВС	117% (**+17%**)	157% (**+57%**)	188% (**+88%**)	? 220% (**+120%**)

The data in the table is presented in real values (A) of enzyme activity (AChE) - M ± m (μg hydrolysed acetylcholine/mg protein/min and as a percentage of the basal control relative to 100%.The true stimulation (+%) and inhibition (-%) values of enzyme activity are shown in brackets; n = 10; * p<0.05; ** p <0.001; ?- inability to measure. See also Fig. 7.

The purpose of these experiments was to determine the effectiveness of L-Arginine as a reactivator on the activity of AChE from K-ferricyanide in fractions of *V. germanica.* 50% inhibition of AChE in this species was achieved at 1.0 mM. This means higher resistance of *V. germanica* to K-ferricyanide compared to the influence of Na-Nitroprusside.

L-Arginine, in all other cases, is a potent reactivator of the activity of AChE, inhibited by various anticholinesterases. L-Arginine (30 mM) reactivated the enzyme activity by 35% relative to basal control or a total stimulation on enzyme

activity at this case is about 85% (35 + 50). This effect when administering L-Arginine of 50 mM is approximately 170%.

More details on the protector and reactivating function and the antidote role of L-Arginine on cholinesterases in case of damage by different inhibitors can be found in another article with authors Dencheva, Ivanov and Ivanov, Dencheva in 2018.

CONCLUSION

In this study, the effectiveness of L-Arginine (Tapiero, 2002) was analyzed as an important factor for the prevention and protection against various toxic products.

It was found that L-Arginine, depending on the applied concentrations or doses (*in vivo*), induces a negligible inhibition of cholinesterase activity at very low concentrations and progressive activation of the enzyme activity in parallel to the increase of the reagent concentration. The mechanisms of this effect have not yet been explained. There are various mechanisms of influence of L-arginine, NO, NOS, Na-NP, etc. on cholinesterase activity. Some of these mechanisms are: activation of different systems of secondary messengers (Ca^{2+}, cAMP, cGMP), conformational changes of enzyme proteins, response to cascades of cellular processes, formation of metabolite products, changes in the oxidation state of membrane structures etc.

This study is a contribution to biomonitoring assessments of the state of organisms and humans in a toxic and ecotoxic environment.

Work has been translated data and analysis that suggest that L-Arginine can be used to protect nervous system from diferent toxic products and as an antidote against some damaging endogenous and exogenous substances (anticholinesterase pesticides, diferent choline esters, narcotic drugs as heroin, cocain, ets.

Another important fact is the possibility of using L-Arginine as a regenerating factor of cholinesterases not only to anticholinesterase agents but also to the toxic action of cyanide products, narcotic agents, certain drugs and other toxicants.

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