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MECHANISMS OF THE ACTION OF NA-NITROPRUSSIDE AND SOME CYANIDES ON CHOLINESTERASES IN INVERTEBRATES AND VERTEBRATES ANIMALS

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Abstract: This study aims to reveal some mechanisms of the inhibitory effect of Na-Nitroprusside (Na-NP) on cholinesterase (ChE) activity in vertebrate and invertebrate animals. Na-NP is a synthetic exogenous nitric oxide (NO) donor and is a very effective medicine for the treatment of acute hypertension. Overdose, however, may cause intoxication and death. This is one of the reasons that has prompted our attention to its mechanism of intoxication. As we have found in other studies, the effects of Na-NP on cholinesterases are biphasic – moderate activation of enzyme activity at very low concentrations and progressive concentration-dependent inhibition of the enzyme. This inhibitory effect is due to specific metabolic products released during the decomposition of Na-Nitroprusside. This influence largely resembles the effects of anticholinesterase agents. Under these circumstances, the hypotonic efficacy of Na-NP can be explained by vagotonic activity (increased acetylcholine content and blood vessel relaxation). In this work, the influence of various cyanide products has also been traced. It was established for the first time that nitro-ferri-cyanides concentration-dependently inhibit cholinesterase activity in invertebrates and vertebrates. Certain scientific findings may be used for treatment of severe mental illness.

INTRODUCTION

The cholinergic system is one of the most important neurotransmitter systems. It controls almost all physiological processes, behavior, serious neurological and neuropsychiatric disorders and diseases. The cholinesterases – acetylcholinesterase

(AChE) and butyrylcholinesterase (BChE) are very important components of this system. The main targets of these esterases are many anticholinesterase agents, pesticides and insecticides, plant and animal poisons, narcotic substances, medicines, etc. The esterases, through the cholinergic system, control the state, all physiological processes, behavior, some serious neuropsychiatric disorders and diseases, etc. (Ivanov, 2006; Colovic et al., 2013).

In our previous studies (Ivanov and Dencheva, 2016; Dencheva and Ivanov, 2017) it has been shown that Na-NP at very low concentrations moderately stimulates cholinesterase activity in different brain fractions of two mammalian species – rat (Rattus rattus) and rabbit (Oryctolagus cuniculus) Its increasing concentrations, however induced progressive inhibition of the enzyme activity.

The Na-NP molecule contains one radical of nitrogen oxide (NO), 5 cyanide ions and one iron atom in third valence. There is no evidence in the literature about the mechanisms of influence of cyanide preparations on cholinesterase activity. Therefore, our focus has been put on the role of ferricyanide radicals as probable inhibitors of cholinesterase activity.

All anticholinesterase agents are potent neurotoxins, pesticides, insecticides, medicines, chemical warfare agents, etc. (Henk et al., 2012; Cowel, 2013; Čolović et al., 2013). Cyanides are potent poisons for all organisms. They are also used as insecticides. The aim of the present study is to compare the effectiveness of Na-NP and several cyanide radicals on cholinesterase activity (in particular on AChE) in invertebrates. Another aspect of the research is to reveal some of the mechanisms of the inhibitory action of this agent.

MATERIAL AND METHODS

Preparation of enzyme fractions. Isolation of membrane and mitochondrial fraction of brain of two mamalian species (Rattus rattus and Oryctolagus cuniculus), and of *Vespula germanica*, Apis mellifera and other invertebrates, has been performed by the method of differential centrifugation. The medium for homogenization and isolation of the respective fractions consisted of 0.1 M KCl, 1,0 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 from the vivarium of the Faculty of Biology, Sofia University and from natural sources. The research has conformed to the international and national rules and regulations for the ethical treatment of animals.

Determination of acetylcholinesterase and butyrylcholinesterase activity. We have estimated the AChE and BChE activity by the classical method of Elman et al. (1961). The activity of AChE and BChE has been determined based on the thiocholine released during the hydrolysis of acetylthiocholine iodide (AChE) and butyrylthiocholine iodide (BChE). The thiocholline content has been determined spectrophotometrically at λ= 412 nm. The cholinesterase reaction has been stopped by adding the specific blocker of AChE or BChE – serine salicylate (500 μM).

Calculation of enzyme activity. The principle of measurement of cholinesterase activity is by determining the contents of the SH-group. Therefore, we use L-cysteine (C3H7NO2S) in different molar concentration to prepare the standard curve. The molar content of SH-groups in cysteine and thiocholine (TCh) is the same. The SH-groups of TCh react with dithionitrobensonic acid (DTNB) which is reduced in a yellow-colored product – thionitrobenzoic acid (mercaptonitrobenzoate; MNB), which is spectrophotometrically measured.

According to the methodology for calculating the enzyme activity and according to the data from the standard curve. The enzymatic 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 % relative to the respective controls.

The formula for calculation of enzyme activity is: A (μM or μg hydrolised substrate (acetylcholine or butyrylcholine)/mg protein/min=E (optical density). k1 or k2 /mg protein/min (time of incubation).

Determination of the contents of the protein. The protein content in the samples has been determined by the method of Lowry (1961). Standard curve was prepared with lyophilized albumin (10-100μg/ml). The spectrofotometrical density (E) of the samples has been determined at λ=750 nm. According to data of this calibration curve has been calculated the factor for recalculation (k).

Statistic calculations and analysis. The significance of differences between the control and experimental samples has been estimated by the Student`s t-test.

RESULTS AND DISCUSSION

The influence of Na-Nitroprusside on the activity of AChE and BChE in the mitochondrial-membrane fraction in different areas of the brain of white rat and rabbit.

Na-nitroprusside (Na_2 [Fe $(CN)_5NO$] • $2H_2O$) is a pharmacological substance and potent peripheral vasodilator. The active components of the drug are nitrosil metal complex ([Fe $(CN)_5NO]_2$-) containing 5 cyanide radicals and a NO ligand. A major metabolic pathway to eliminate cyanide poisoning is the formation of thiocyanate in the liver under the influence of the rhodanidase enzyme (Butler, Megson, 2002). In our work we show data for the role of NO and other metabolic products of Na-nitroprusside (cyanide radicals, thiocyanates, thiosulphates) on the activity of AChE and BChE in various brain fractions in mammalian species.

The half-life of Na-NP in the body is in the order of several minutes, while cyanide ions, thiocyanate and thiosulphate remain in the body for hours and days. This means that some of the obtained experimental data may be due to NO and to other metabolic products, such as cyanide or thiocyanate, especially at higher concentrations of the Na-NP. These series have first traced the influence of Na-NP at concentrations 0.01-1.0 mM on the activity of AChE and BChE of forebrain of white rat. The results of the study are presented as activity of the enzyme (A) and as percentage of control (fig. 1 and Table 1).

The results in the graph are presented in % versus control accepted for 100%. The other data is shown in a table format (see below). The concentration-

dependent inhibition factor (CDI50, i.e. 50% inhibition) and the initial effective concentration (IEC; Table 1) are also shown.

Table 1. Influence of Na-NP on the activity of AChE and BChE in the different fractions of the brain of Rattus rattus

Cholinesterases; Object: Cerebrum; Na-NP, mM	AChE		BChE	
	Forebrain	Cerebellum	Forebrain	Cerebellum
0,01	-5%	9%	18% **	3%
0,1	-18% **	-3%	-41% **	-13% *
1	-95% **	-31% **	-67% **	-35% **
CDI_{50}	Around 0.3 mM	Around 5-6 mM	Around 0.2 mM	Around 4-5 mM
IEC	Around 0.01 mM	0,1 mM	Around 0.1 mM	Around 0.13 mM

Other notes: (-) – inhibition; (+) – stimulation; ** - p <0,001; n= 8-10;

The general conclusion of this study is that this pharmacological agent (Na-NP) has induced biphasic effect – stimulation at very low concentrations (0.005 mM 0.01 mM) and concentration-dependent inhibition with the increase of molar concentration (0.1 mM-1.0 mM) for both enzyme forms. It is believed that the effect of stimulation is due to NO, which is inhibited by increasing the concentration of Na-NP or cyanide radicals respectively.

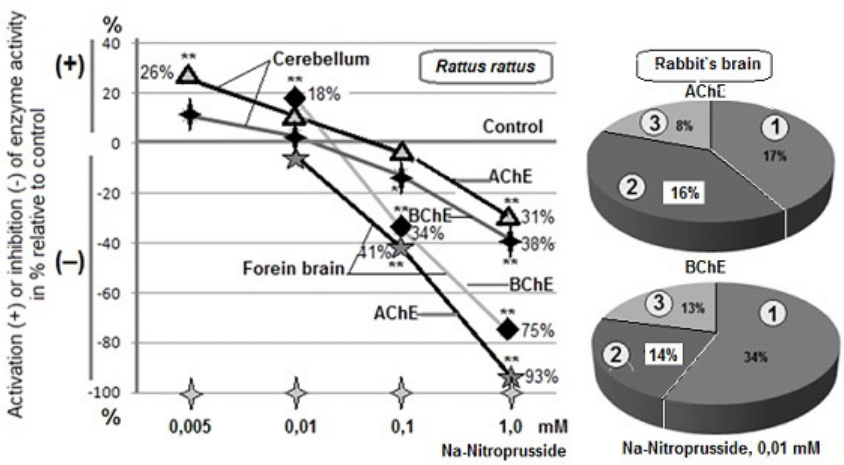

Fig. 1. Influence of Na-NP as a ferrinitrocyanide complex on the activity of AChE and BChE in fractions of forebrain and cerebellum of Rattus rattus and brain cortex (1), brain steam (2) and cerebellum (3) of rabbit`s brain

The activation levels of Na-NP activity (0.01 mM) are different in the different parts of the brain of rats and rabbits. They are high (17-18%), for example, for AChE in the cerebral cortex and the brain steam of rabbits, as well as for BChE of brain cortex (about 14%) (Figure 1). Another reason for this assertion is the biomonitoring index (CDI50) - very low for the rat's forebrain – 0.2-0.3 mM and very high (10-15 times) – 4.0-6.0 mM for the cerebellum of the rat (Table 1). These data suggest different sensitivity of different animal structures to toxic products.

In the latter case (inhibition of enzyme activity) may be conditionally assumed that NO is a "typical" anticholinesterase agent. Such an opinion, that Na-NP or NO inhibits AChE activity has been suggested by some authors (Butler and Megson, 2002).

Initial effective concentrations (IEK) of Na-Nitroprusside (0,01-1,0 mM) (both in activation and inhibition) are much lower than those in the series with L-Arginine (1,0-50 mM). This is due to a very complex structure of Na-Nitroprusside, which is metabolised to several products such as NO, metal cyanide radicals, thiocyanates and others.

The stimulating action of Na-NP on AChE and BChE activity in fractions of different brain areas of rats and rabbits occurs only at very low concentrations of the agent. We believe that this is due to the release of NO. Similar opinion has been expressed by others, who have studied the influence of Na-NP on the activity of the mitochondrial ATPase in rat liver (Shkodrova et al., 2013).

Concentration-dependent inhibition of the enzyme activity under the influence of Na-NP is much higher in the forebrain than in the cerebellum of rat brain. This is valid for both enzyme types. For example, Na-NP (1.0 mM) suppresses AChE activity to 90% and BChE to 75%, whereas this inhibition in cerebellum fractions ranges from 30-40% for both enzymes. When using this medicine we must be very careful and responsible since this may lead to severe consequences, even death, when overdosed.

Effectiveness of Eserine salicilate and Na-NP as inhibitors of AChE activity in fractions of *A. mellifera*

The use of Na-NP in clinical practice has begun in 1928 (Friederich, Butterworth, 1995) In this section, the effectiveness of Na-NP on the activity of AChE from *A. mellifera* and *V. germanica* fractions has been monitored. The aim was to compare the effect of this medicine in invertebrate and vertebrate tissues on the enzyme activity and estimate the role of L-arginine as a reactivator of AChE in cyanide and other poisoning.

First, the inhibitory efficacy of a classic anticholinesterase agent eserine (phisostygmin) and a synthetic drug Na-Nitroprusside has been monitored. The two substances have common features in terms of chemical composition, e.g. NO group, but also significant differences. In a specific concentration range, the two substances cause concentration-dependent inhibition of AChE activity in vertebrates and invertebrates.

Ultimately, the goal of the study has been to reveal some of the mechanisms of the inhibitory efficiency of Na-NP. The object of this study were fractions of

A. mellifera and *V. germanica.* Some of the results are shown in Figure 2 and Table 2. According to the data, eserine salicilate and Na-NP cause concentration-dependent inhibition on AChE. The suppression of enzyme activity is more strongly manifested by eserine than by Na-NP. This suggests a direct action of eserine on the enzyme active center (competitive inhibition). At a concentration of 100 μM eserine inhibits the enzyme activity in the order of 90% (relative to the controls) and Na-NP – at about 70%.

There is no literature evidence about cyanide influence on cholinesterase activity.

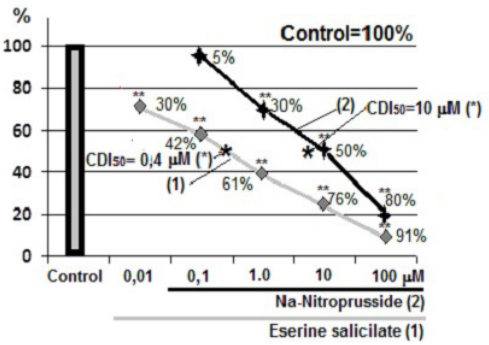

Fig. 2. Influence of Ezerin salicylate (1) as a specific inhibitor of cholinesterases and Na-NP (2) as a ferrinitrocyanide complex on the activity of AChE in *Apis mellifera* fractions

The results in the figure are presented in % inhibition versus control, accepted for 100%. The specific data are shown in a table format (see below). Other indications: n = 8; ** p≤ 0.001 (compared to the control). The concentration-dependent inhibition factor (CDI50) is shown in the figure and table. See also Table 2.

Table 2. Influence of Na-NP and Ezerin salicylate on the activity of AChE in *Apis mellifera* fractions

(C); Control (Eserine) (A)	43,50±1,60 (100%)				
+ Eserine salicilate (A) (% relative to the control; C)	31.05±1,20 (71%) **	25.29±0,59 (58%) **	16.93±0,150 (39%) **	10.03±0,200 (24%) **	5.10±0,70 (12%) **
CDI_{50}	0.4 μM				
Control (Na-Nitroprusside) (A)	44,12±1,55 (100%)				
+ Na-Nitroprusside) (A) (% relative to the control; C)	-	42.06±0,90 (95%)	29.85±1,20 (68%) **	22.18±1,20 (50%) **	10.15±0,08 (23%) **
CDI_{50}	10 μM				
Eserine salicilate or Na-Nitroprusside (μM)	0,01	0.1	1	10	100

Other data: A–enzyme activity in μg hydrolyzed acetylcholine/mg protein/min; % - relative to the control (100%); n= 8-10; ±m about AchE activity = 5-15; *-p<0.05, **-p<0.001

Another indicator on the action of reagents is the KDI50 index, which for eserine is 0.4 μM, for Na-NP is about 10 μM (i.e. 25 times higher). This means that ezerine AChE inhibitor is much effective compare to Na-NP.

In the work of Badiou et al. (2008) estimated the effectiveness of neonicotinoids, pyrethroids and carbamates in ecotoxicological conditions by analyzing the activity of AChE in dead and live bees. Toxicity criteria (DL50 and CDI50) are markers for the pesticide effectiveness of insecticides. For example, compounds containing the nitro group (NO) are very toxic for bees with LD50 in order of 22 ng (clothianidin – neonicotinoid). On the other hand insecticides containing cyano group are with very low toxicity – DL50 = 7.0 mg/bee (for acetamiprid). The conclusion of another study (Iwasa et al., 2004) is that the resistance of bees to certain insecticides depends on the high activity of P450. This cytochrome converts cyanide into metabolites with very low toxicity (Danielson, 2002; Guengerich, 2008; Siegel et al., 2007). Our data also indicate the lower toxicity of Na-NP than that of eserin.

Influence of Na-NP on AChE activity in fractions of *A. mellifera* and *V. germanica*

Na-NP is a typical pharmaca that contains cyanide ingredients. The purpose of the study is to illustrate the toxic potential of Na-NP in different biological objects. Other new data from this material is the comparative analysis between activities of AChE in two evolutionarily close species (*V. germanica* and *A. mellifera*) (Figure 3).

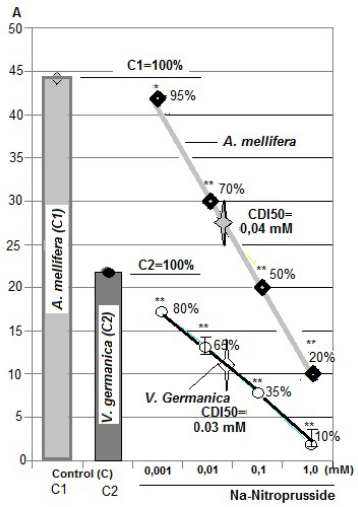

Fig. 3. Influence of Na-NP on the activity of AChE in *Apis mellifera* and *V. germanica* fractions

The results in the graph are presented in A (real enzyme activity) and % relative to the control (C1 and C2). Other indications: n = 8; ** p≤ 0.001. The concentration dependent inhibition factor (CDI50) is shown in the figure.

The effect of Na-NP (0.001-1.0 mM) on AChE in *A. mellifera* and *V. germanica* fractions is a concentration dependent inhibition, which at concentration of

1.0 mM is around 80% and 90%, respectively for both species. However, this inhibition is much less than the influence of organophosphates and carbamate pesticides. For example, KDI50 for the inhibitory effect of eserine salicylate and Na-NP on AChE activity in A. mellifera has been 0.4 μM and 10 μM, respectively. This means that the affinity of this enzyme for eserine is about 25 times higher.

Another fact (Fig. 3) is the almost twofold higher activity of AChE in *A. mellifera* compared to that of *V. germanica* under equal conditions.

Role of L-Arginine as an antidote against poisoning with carbamate pesticides and Na-NP (*A. mellifera*)

An important point in the study is 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, Denceva, 2016). The results of this study are shown in Fig. 4 and in Table 3.

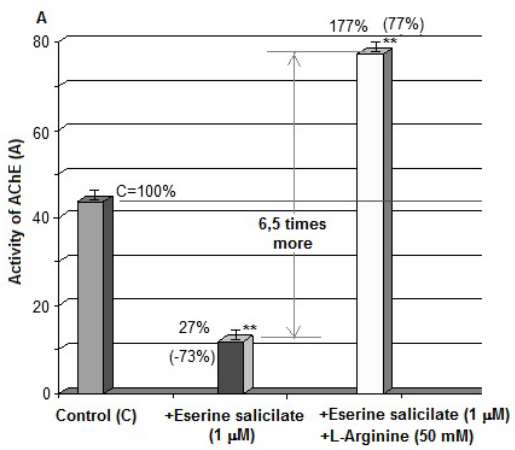

Fig. 4. The role of L-Arginine (50 mM) as an antidote against carbamate pesticides and reactivator to normal AChE activity of *A. mellifera*

A – mg hydrolysed acetylcholine/mg protein/ min; other data: n = 8;
** p≤ 0.001; see also Table 3.

The specific cholinesterase inhibitor, in the case of eserine salicylate at a concentration of 1.0 μM suppresses the activity of AChE in the order of 70%. This inhibition of enzyme activity is very easily removed by adding L-Arginine (50 mM) (combination between eserine and L-arginine). The suppressed AChE in this case is reactivated approximately 2 times over the control and about 6.5 times more than the suppressed activity by Eserine. Almost the same pattern is seen with pre-introduction of L-arginine. Reactivation of enzyme activity suppressed by eserine (1.0 μM) is also valid at concentrations of L-Arginine of 30 mM.

Table 3. The influence of L-Arginine and Eserine on AChE activity in fraction of *A. mellifera*

Control (C)=100%	43.67 ±1.21				
+L-Arginine, mM	1,0	10	30	50	mM
	38.18±1.06	**40.12±0.90**	46.50±0.76	79.94±3.64	A
%	87%	92%	107%	183%	%
+Eserine, μM	0,1	1	10	100	μM
	42.06±0.90	**29.85±1.20**	22.18±1.20	10.15±0.08	A
%	95	68	50	20	%

The results are shown as real values (A) and as % relative to the control; see also Fig. 4.

The data presented above is the main objective of the study - evidence of the role of L-arginine as a reactivator of AChE and antidote against cholinesterase intoxication by anticholinesterases, other esters, poisons, insecticides, etc. This pattern, demonstrated in *A. mellifera*, is similar also in *V. germanica* or in mammalian brain fractions (Ivanov, Dancheva, 2018). This means the possibility of using L-arginine and other similar endogenous and exogenous products as a means of preventing organisms, including humans, from specific pesticides, poisons, narcotic agents, etc.

Influense of K-ferricyanide, K-ferrocyanide and KCN on the AChE activity in fraction of A. mellifera

Na-NP (Na_2 [Fe $(CN)_5NO].2H_2O$), as a ferri-nitro-cyano compound, which in certain concentration ranges suppresses the activity of AChE in various animal species. The following section attempts to identify the components of the chemical structure of Na-NP that directly affect enzyme activity.

The compounds we have selected are potassium ferricyanide (K-Fe^3CN, three valent iron and 6 cyanide radicals), potassium ferrocyanide (K-Fe^2CN, two valent iron and 6 cyanide radicals) and potassium cyanide (KCN) – without a nitro group and a metal ion. Potassium ferrocyanide and potassium ferricyanide are synthetic ferrocyanide complexes that are used for technical needs, in various scientific studies, in the clinical practice, etc. The active groups in all these compounds are: NO, cyanide ions, fericyanide complexes, iron ions at different valences (ferrites ($^{3+}$) and ferrous ($^{2+}$)). This means that they are deficient in electrons, which makes them radicals with increased reactivity. Accordingly their chemical structures of Na-NP and K-Fe^3CN have different toxicity – much higher for Na-NP than for sodium ferricyanide.

Influence of K-ferricyanide and Na-NP on AChE activity in *A. mellifera* fractions

K-ferricyanide (K_3[Fe $(CN)_6$]) is chemically similar to Na-NP, but is without a nitro group. The purpose of these part of the study is to find an explanation for the concentration-dependent inhibition of AChE from Na-NP as a result of the influense of ferricyanide radicals or iron ions as components of a powerful redox system (Fe^{3+}/Fe^{2+}). The different valences of the iron ions are responsible for the

various coefficients for dissociation of the ferrocomplexes. A brief comparison between the levels of toxicity of K-Fe^{3}CN and Na-NP is presented bellow.

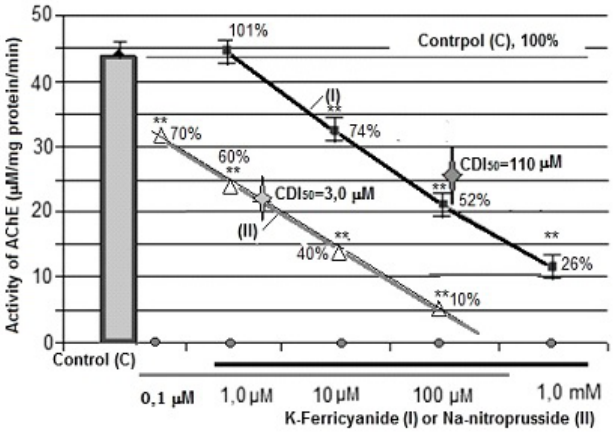

Fig. 5. Influences of K-ferricyanide (I) and Na-Nitroprosside (II) on the activity of AChE in *A. mellifera* fractions

Other data: n = 6; ** p≤ 0.001; IDI50 – level of concentration-dependent inhibition (in this case about 100 μM for K-ferricyanide and 3,0 μM for Na-NP). More information about K-ferricyanide – see below data presented in table format.

Control (C)	44.06±0.120 (M±m)			
+K3[Fe(CN)6}	1.0 μM	10 μM	100 μM	1.0 mM
Activity	44.52±0.060	32.49±1.80	21.06±1.50	11.52±1.20
%vs. C (C=100%)	101%	74%	52%	25%

The data in the figure 5 and the table indicate that K-ferricyanide in concentration 1,0 μM-1.0 mM concentration-dependent inhibits the activity of AChE in A. mellifera fractions. One of the integral toxicity index (CDI50) in this model is about 100 μM. If we compare this index to that of the Na-NP inhibitory effect (CDI50=3,0 μM), it appears that the toxicity of the Na-NP is about 30 times more potent than that of the K-ferricyanide.

This could be explained with the presence in the Na-NP molecule of ferri-nitro-cyanide components, a different degree of dissociation of the ferricyanide complex, the presence or absence of a NO group, the ferrous ion valence, etc.

Influence of K-ferrocyanide on AChE activity

Potassium ferrocyanide ($K_4[Fe(CN)_6].3H_2O$) is a substance used in the food industry, in various technologies for oxidation of metal surfaces, in photography, in the textile industry, etc. Potassium ferricyanide and potassium ferrocyaide are important because many people have day-to-day contact with them. A comparative analysis has been made between the effects of K-Fe^{3}CN and K-Fe^{2}CN on the activity of AChE (Fig. 6).

Data in literature on the influence of cyanide, hydrogen cyanide, ferri- and fero-cyanides and nitro ferricyanides on the activity of AChE is not available. For example, the role of metal ions (Fe^{2+} and Fe^{3+}), the ferrocomplex dissociation coefficients and their biological role, the efficiency of other compounds with similar or close structure, etc., are similar.

Although the relatively low toxicity of Na-NP, K-Fe^3CN and K-Fe^2CN , these products form highly toxic products in acidic environment:

$$(6\ HCl + K_3[Fe(CN)_6] \rightarrow 6\ HCN + FeCl_3 + 3\ KCl).$$

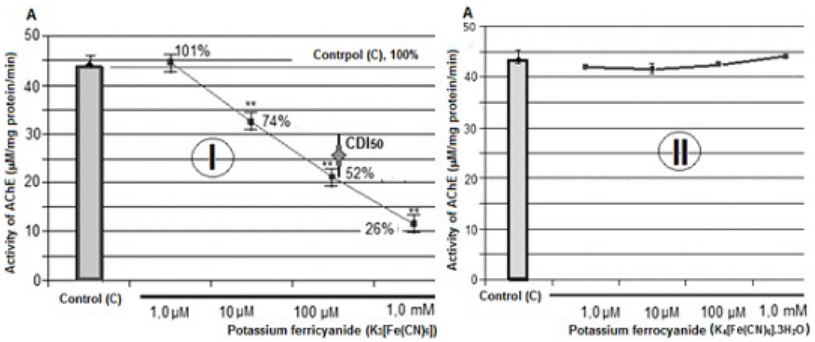

Fig. 6. Influence of K-ferricyanide (I) and K- ferrocyanide (II) on the activity of AChE in *A. mellifera f*ractions

The final result of the study has been that K-Fe^2CN in a broad concentration range has not affected the activity of AChE in *A. mellifera.*

Influence of KCN on AChE activity in fraction of *A. mellifera*

In this aspect, it is reasonable to analyze the effect of KCN on the activity of cholinesterases in organisms. The experimental results, surprisingly show that KCN concentration-dependently stimulates the activity of AChE in fractions of *A. mellifera.* (Fig. 8).

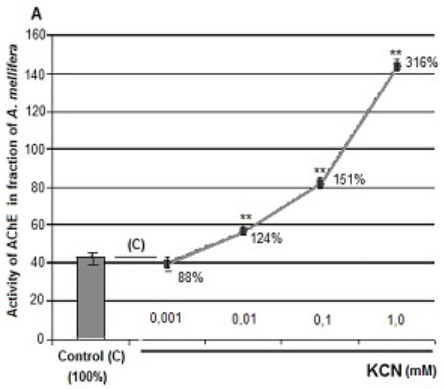

Fig 7. The influence of KCN on the activity of AChE in *A. mellifera* fraction

A – mg hydrolysed acetylcholine/mg protein/min; % – % relative to control; n=10; ** p <0.001

The effect of 1 mM cyanide concentration is about 300% and of 0,1 mM –about 150%.

Potassium cyanide has been discovered in 1762. It is a highly toxic substance. The human lethal dose is 3-4 mg/kg. An antidote for cyanide poisoning is sodium thiosulphate, which produces low toxic products (rhodanides). Such antidotes are also hydrogen peroxide and sodium hypochlorite. Blood cyanide levels of 0.5-1 mg/l are estimated to be mild, 1-2 mg/L – moderate, 2-3 mg/L – heavy and above 3 mg/l – lethal (Anseeuw et al, 2013; Hamel, 2011).

Toxicity of cyanides is due to the blockage of electron transport in the mitochondrial respiratory chain by inactivation of cytochrome c oxidase (E.C. 1.9.3.1). The effectiveness of KCN as a powerful toxic product is manifested in acidic medium to form toxic hydrogen cyanide. More details on the protector and reactivating function and as antidote of L-arginine on cholinesterases in case of damage by different inhibitors can be found in Ivanov and Dencheva, (2018).

CONCLUSION

In this work, some effects and mechanisms of the action of some endogenous and exogenous cyanide-containing compounds (Na-Nitroprusside, K-ferricyanide, K-ferrocyanide, KCN, thiocyanides, cyanomethyl hemoglobin, etc.) on cholinesterase activity in various fractions in vertebrates and invertebrates were analyzed for the first time,.

It has been found that cyanides inhibit cholinesterase activity (AChE and/or BChE). This inhibition by kinetic parameters resembles the effects of typical anticholinesterase preparations (pesticides, insecticides, poisons) but is realized at higher concentrations.

Our data showed that L-arginine has reactivated partially or completely the cholinesterase activity in various animal species. This means that this amino acid, in combination with other components and technologies, can be successfully used as an antidote against cyanide poisoning, for improvement of cyanide resistance, for monitoring and prophylaxis in ecotoxic environment.

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