PHYSICAL CHEMISTRY 2014

12th International Conference
on Fundamental and Applied Aspects of
Physical Chemistry

The Conference is dedicated to the
25. Anniversary of the Society of Physical Chemists of Serbia

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September 22-26, 2014
Belgrade, Serbia

ISBN 978-86-82475-30-9

Title: PHYSICAL CHEMISTRY 2014 (Proceedings)
Editors: Ž. Čupić and S. Anić
Published by: Society of Physical Chemists of Serbia, Studenski trg 12-16, 11158, Belgrade, Serbia
Publisher: Society of Physical Chemists of Serbia
For Publisher: S. Anić, President of Society of Physical Chemists of Serbia
Printed by: "Jovan" Priting and Publishing Company; 200 Copies;
Number of pages: 6+ 441; **Format**: B5; Printing finished in September 2014.

Text an Layout: "Jovan"

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12th International Conference on
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Organized by
The Society of Physical Chemists of
Serbia

in co-operation
with

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Boreskov Institute of Catalysis of Siberian Branch of the Russian Academy of Sciences

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IN VITRO EVALUATION OF DIAZINON AND ITS DEGRADATION PRODUCTS NEUROTOXICITY POTENTIAL IN RAT BRAIN SYNAPTOSOMES

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ABSTRACT

Toxic effects of diazinon and its degradation products, diazoxon and 2-isopropyl-6-methyl-4-pyrimidinol (IMP), were investigated *in vitro* by determining the inhibition of acetylcholinesterase (AChE), Na^+/K^+-ATPase and ecto-ATPase activity in rat brain synaptosomes after 1 hour exposure toward varying concentrations. Dose-dependent AChE and Na^+/K^+-ATPase inhibition was obtained in the presence of diazinon, while diazinon concentrations below 0.1 mM did not noticeably affect ecto-ATPase activity. Diazinon oxidation product, diazoxon was found as the most toxic investigated compound. Diazoxon induced dose-dependent and almost complete inhibition of AChE, Na^+/K^+-ATPase and ecto-ATPase at the highest investigated concentration (0.1 mM), while hydrolysis product of diazinon, IMP did not remarkably influence their activities.

INTRODUCTION

Organophosphorus pesticides (OPs) are the most widely used pesticides worldwide and their metabolites are widespread across different populations. The primary mechanism of OP toxicity is the inhibition of AChE in the central and peripheral nervous system [1]. Diazinon (O,O-diethyl-O-(2-isopropyl-4-methyl-6-pyrimidinyl phosphorothionate) is a commonly used thionophosphorous OP to control a variety of insects in agriculture and household environment [2]. Diazinon can be transformed to the more toxic diazoxon due to the enzymatic reaction in birds, fish, insects and mammals [3], while IMP and its hydroxylated metabolities were reported to be much less toxic as compared to its parent compound diazinon [4]. Adenosine triphosphatases (ATPases) are a group of enzymes which play an important role in intracellular functions and critical for cellular

viability because they control many essential cellular functions, and are considered to be a sensitive indicator of toxicity [5, 6].
In the present study we investigated *in vitro* toxicity potential of various doses of diazinon and its degradation products (diazoxon and IMP) by determining the activity of AChE and ATPases (Na^+/K^+-ATPase and ecto-ATPase) in rat brain synaptosomes which behave as minicells and represent a suitable model system for *in vitro* toxicity evaluation.

EXPERIMENTAL

Synaptosomes were isolated from the brain of *Wistar albino* rats and incubated at 37^{o}C for 1 hour in the presence of desired concentrations of diazinon, diazoxon and IMP. Activities of AChE and ATPases (Na^+/K^+-ATPase and ecto-ATPase) were measured by standard spectrophotometric methods [7, 8].

RESULTS AND DISCUSSION

The influence of 1 hour exposure to increasing concentrations (within the range 10^{-9}-10^{-4} mol/l) of diazinon and its degradation products (diazoxon and IMP) on the activity of synaptosomal AChE, expressed as a percentage of the control value (obtained without inhibitor), is presented in Figure 1. It is clearly apparent that inhibitor efficiency of diazinon, diazoxon and IMP is quite different. At the concentration of 1×10^{-5} mol/l diazoxon almost completely inhibited AChE activity (85%), while the same concentration of diazinon inhibited only 24% activity. The half-maximum inhibition (IC_{50}, 1h) of the enzyme activity was achieved at $(6.7 \pm 0.2) \times 10^{-8}$ mol/l of diazoxon, while the effects of the same concentration of diazinon as well as IMP on the enzyme activity were negligible. Moreover, the presence of the highest investigated diazinon concentration (1×10^{-4} mol/l) was not able to reach half-maximum inhibition. The dependence of Na^+/K^+-ATPase and ecto-ATPase activity on various concentrations (10^{-7}-10^{-4} mol/l) of diazinon and its degradation products is presented in Figure 2 (a and b). The obtained results (Figure 2a) show that hydrolysis product of diazinon (IMP) did not remarkably alter Na^+/K^+-ATPase activity. Unlike IMP, diazinon and its oxidation products (diazoxon) inhibited the enzyme in concentration-dependent manner, but with various potencies. The presence of the maximal investigated diazoxon concentration (1×10^{-4} mol/l) induced almost complete inhibition, while the same concentration of its parent compound (diazinon) decreased the enzyme activity approximately 50% related to control (Figure 2a). The obtained results for ecto-ATPase (Figure 2b) show similar sensitivity of the enzyme toward diazoxon and IMP as in the case of

Na^+/K^+-ATPase, while diazinon did not cause ecto-ATPase activity inhibition more than 15 %.

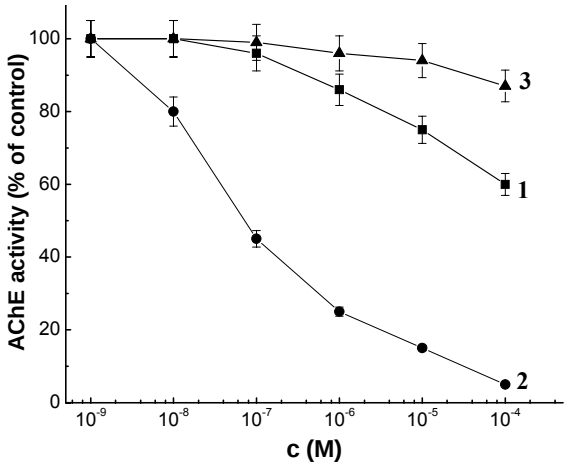

Figure 1. Inhibition of AChE activity in rat brain synaptosomes after 1 hour exposure to different concentrations of diazinon (1), diazoxon (2) and IMP (3).

CONCLUSION

In the present work the activities of AChE, Na^+/K^+-ATPase and ecto-ATPase have been used to investigate neurotoxic effects of diazinon and its decomposition products. The results of our study show that diazinon and its oxidation product, diazoxon, inhibited synaptosomal AChE and ATPases activity in concentration-dependent manner but with varying potencies. Diazoxon demonstrated the strongest neurotoxic effect through the reduction of AChE activity, and the obtained inhibition of Na^+/K^+-ATPase and ecto-ATPase suggests ion exchange as well as purinergic signaling disrupting effect of the oxo analog of diazinon. On the contrary, IMP was found as a non toxic diazinon metabolite.

Considering the obtained changes of the physiologically important parameters, synaptosomes, which functionally behave as minicells, could be recommended as a suitable model system for *in vitro* toxicity evaluation of OPs and their degradation products.

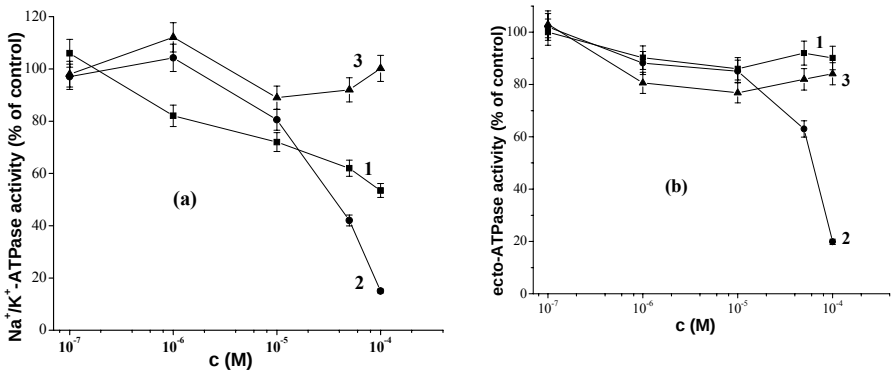

Figure 2. The dependence of rat brain synaptosomal Na^+/K^+-ATPase (a) and ecto ATPase (b) activity on increasing concentrations of diazinon (1), diazoxon (2) and IMP (3).

ACKNOWLEDGEMENT

This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. 172023).

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