1 Summary in English

Currently, organophosphorus (OP) poisoning is a threat particularly in war conflicts in connection with terrorism or a poisoning caused by OP pesticides in agriculture. The poisoning is caused by inhibition of the enzyme acetylcholinesterase (AChE), which, under physiological conditions, cleaves acetylcholine (ACh) and regulates the transmission of nerve signals. The inhibition of AChE leads to excessive stimulation of cholinergic receptors and clinically it manifests as so-called cholinergic syndrome. According to the structures affected, symptoms are divided into nicotinic, muscarinic and central. Organophosphates are lethal compounds, when death is caused by suffocation. Current treatment is mainly based on the application of anticholinergics (atropine) and /or on the application of AChE oxime reactivators (HI-6, obidoxime, pralidoxime etc.) Atropine inhibits excessive neural transmission by blocking muscarinic receptors. However, it is only symptomatic cure not causal. Whereas oximes, which represent a causal therapy, are able to unbind an OP compound from the AChE and restore its splitting function.

Using reactivators has two major drawbacks; there is no universal reactivator against all types of OP compounds (soman, sarin, paraoxon etc.) and reactivation is possible only within a certain time after the exposure ("aging"). Based on these limiting factors, it is essential to keep searching for a universal oxime or apply different therapeutic approaches.

One possible approach is a deeper examination of the reactivators´ mechanism of action since they have a very complex effect on the whole cholinergic system. In particular, their ability to influence the nervous signal transmission at the receptor level.

The aim of this dissertation thesis is to study the cholinergic effects of oxime reactivators (especially their effect on the muscarinic and nicotinic receptors) and the significance of non-reactivating properties in the treatment of organophosphate poisoning. We selected two commonly used reactivators (HI-6 and obidoxime) and 2 newly synthesized reactivators (K027 and K203) for this purpose. These 4 compounds were studied by using various techniques involving *in-vitro* and *in-vivo* experiments. Specifically, reactivators' affinity for muscarinic receptors, effect on signal transduction, functional experiments on the tissue samples (isolated heart atrium, urinary bladder in the organ bath) and on the whole body (impact on rat heart rate). Nicotinic effects were also studied using *in-vitro* (cell patch-clamp) and *in-vivo* (rat neuromuscular effects) methods. Other experiments were performed in order to describe a direct effect on AChE or on the choline re-uptake as the other cholinergic structures that may be affected by reactivators.

The experimental data showed that AChE reactivators have a very complex mechanism of action. They exert the inhibitory effect on AChE with the efficacy at least 3-fold lower than the commonly used central inhibitors of AChE. Furthermore, they display inhibitory effect on the high-affinity choline uptake transporter (HACU), which is a key-regulatory step in the ACh synthesis *de novo.* I*n-vitro* and *in-vivo* experiments comprehensively proved inhibitory effects of studied oximes on nicotinic receptors. Although, the IC_{50} values were in the hundreds of micromoles, even a weak inhibition may speak in

favor of the treatment with reactivators. Moreover, atropine does not affect nicotinic receptors at all and unfortunately nicotinic symptoms are usually responsible for the death of a victim. Muscarinic antagonism has also been verified by both *in-vitro* and *in-vivo* experiments. Although, the mechanism of binding has not been satisfactorily explained a weak inhibition was observed (atropine in all experiments possesses at least 3-fold higher efficacy). However, even a weak inhibition may play an important role in the treatment of OP poisoning, in particular, in the case of "aging".

Focusing on individual reactivators- HI-6, obidoxime, and K203 usually demonstrated a higher inhibitory efficiency than K027. Surprisingly, HI-6, that exerted the lowest affinity for muscarinic receptors, showed notably higher efficiency in more complex systems (the tissue level, the whole organism level). This fact suggests other mechanisms, than performed in this study, may be involved (e.g. the effect on presynaptic receptor, synthesis or the release of ACh). Moreover, it also explains why HI-6 seems to be currently the most effective reactivator with the broadest spectrum of action.

Review article and results from this dissertation thesis were published in Czech and international scientific journals (8x first-author papers, 7x IF). Furthermore, results were also presented at Czech and international conferences in the form of lectures or poster presentations.

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