

Oxime Reactivators and Their *in Vivo* and *in Vitro* Effects on Nicotinic Receptors

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Summary

Current treatment of organophosphorus poisoning, resulting in overstimulation and desensitization of muscarinic and nicotinic receptors by acetylcholine (ACh), consists of the administration of atropine and oxime reactivators. However, no versatile oxime reactivator has been developed yet and some mortality still remains after application of standard atropine treatment, probably due to its lack of antinicotinic action. In our study, we focused on the interesting non-acetylcholinesterase property of oximes, i.e. antinicotinic effect of reactivators. Two standard reactivators (HI-6, obidoxime) and two new compounds (K027 and K203) were chosen for *in vitro* (patch clamp) and *in vivo* (nerve-evoked muscle contraction) testings. Both examinations showed antinicotinic effects of the reactivators. *In vitro* inhibition of acetylcholine-evoked currents by obidoxime, HI-6 and K203 was equivalent while K027 was less potent. Similar order of potency was observed by the *in vivo* examinations. We thus confirm previous *in vitro* results, which describe antinicotinic effects of oxime reactivators, and furthermore, we show *in vivo* antagonism of oxime reactivators exerted by the inhibition of ACh effect on the nicotinic receptor in the neuromuscular junction. Taking together, the effects of tested oxime reactivators indicate an antagonism on both embryonic and adult form of the muscle nicotinic receptors.

Key words

Organophosphates • Patch-clamp • Nicotinic receptors • Reactivator • Isometric muscle contraction • TE671 cells

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Introduction

Organophosphates (OP) are compounds, which inhibit the enzyme acetylcholinesterase (AChE, EC 3.1.1.7) *via* phosphorylation or phosphonylation of serine hydroxyl group at its active site (Radic and Taylor 2006). AChE inhibitors, according to their potency, can be grouped into either nerve agents (such as sarin, soman, tabun and VX) or pesticides (such as paraoxon, parathion and chlorpyrifos) (Marrs 1993). AChE inhibition results in accumulation of acetylcholine (ACh) at cholinergic receptor sites, leading to the overstimulation of cholinergic receptors in both the central and the peripheral nervous system (Bajgar 2004).

The treatment is ensured by two functionally different types of drugs: (1) Reactivators of AChE -

oximes - are able to restore the physiological function of inhibited AChE and (2) Anticholinergics, such as atropine, are able to antagonize the effects of excessive ACh by a blockade of muscarinic receptors. Anticholinergics and reactivators may be administered together because of their synergistic effect (Kassa 2002, Bajgar 2004).

Current treatment of organophosphorus poisoning consists of the administration of atropine and oxime reactivator (mostly HI-6 or obidoxime), and, for anticonvulsant effects, diazepam may occasionally be employed. Atropine has been used for 50 years in clinical practice to control the overstimulation caused by AChE inhibitors (Sivagnanam 2002). However, 20 % mortality still remains after application of standard atropine treatment (Eddleston *et al.* 2008). An explanation to this persisting mortality could be the lack of *in vivo* nicotinic antagonism of the compound (Tobin *et al.* 1991, Luo *et al.* 2010).

HI-6 and obidoxime are the most commonly used oxime reactivators in the treatment of organophosphorus poisoning. HI-6, a broad-spectrum antidote, is considered to be the most effective and is effective against soman, sarin and VX (Kassa 2002), but it is less effective against tabun (Puu *et al.* 1986). On the other hand, obidoxime is used in the treatment of both poisoning by tabun as well as by pesticides (Kassa 2002).

Despite of all this knowledge, the treatment of organophosphorus poisoning is still limited due to the following reasons: 1. No versatile antidote capable to effectively restore activity of AChE inhibited by random organophosphates has been developed. 2. Oxime reactivators are efficient only when administered before the “aging” of AChE-OP complex. 3. Anticholinergics, like atropine, are effective only on muscarinic and not on nicotinic receptors (nAChRs). Owing to this fact, new AChE reactivators, capable of reactivating AChE irrespective of the type of nerve agent used are thus required. Alternatively, other treatment approaches need to be introduced.

Such an alternative approach presumes other mechanisms of oximes, not related to the reactivation (Hamilton and Lundy 1989, van Helden *et al.* 1992, 1996, Tattersall 1993, Soukup *et al.* 2010a). Oximes have been reported to act at several levels of the cholinergic transmission including synthesis, release, inactivation and re-uptake of the transmitter, but the interaction with cholinoreceptors has been put forward as the most plausible alternative of mechanism of action (Tattersall

1993, van Helden *et al.* 1996). Both standard reactivators, obidoxime (Soukup *et al.* 2010b) and HI-6, (Hamilton and Lundy 1989, Soukup *et al.* 2008) have been reported to exert some antimuscarinic properties. Antinicotinic action, which atropine lacks and which can prevent respiratory failure, has also been reported. Both neuromuscular blocking (Schlagmann *et al.* 1990, Tattersall 1993, Chiou and Chang 1994) and ganglioblocking (Lundy and Tremblay 1979, Schlagmann *et al.* 1990) types of antinicotinic action have been reported. However, the reported *in vitro* properties do not correlate with the antidotal observations *in vivo*.

In our study, we have chosen two standard reactivators (HI-6, obidoxime) and two newly synthesized promising compounds (K027 and K203) in order to investigate their antinicotinic properties (for structure see Fig. 1). K027 may become the preferred antidote in the treatment of poisoning by pesticides (Petroianu *et al.* 2007) and K203 is an effective compound in cases of inhibited AChE by tabun (Kovarik *et al.* 2009). We examined the differences in antinicotinic efficacy of the individual reactivators *in vitro*, and further, we wondered if the *in vitro* findings had any *in vivo* significance.

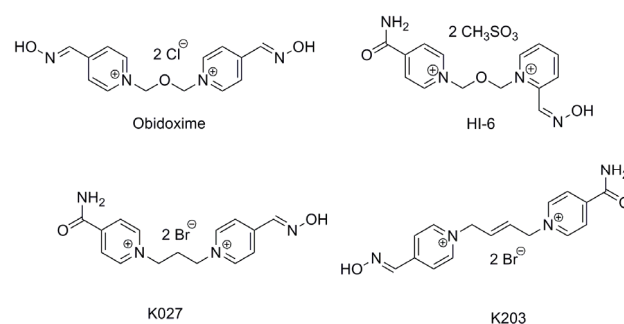


Fig. 1. Structures of tested oximes.

Materials and Methods

In vivo measurement of neuromuscular block

The animal ethics committee at the University of Gothenburg approved the experiments of the present study.

Male rats of the Sprague-Dawley strain (200-300 g) were anaesthetized with pentobarbitone (45 mg/kg, IP) and ketamine (50 mg/kg, IM) followed by supplementary doses injected intravenously as required during the experiments. Nerve-evoked maximal twitches of the tibialis anterior muscle of the right lower limb of

the rat was monitored as described previously (Tran *et al.* 1982). The free tendon of the tibialis anterior muscle was attached to a force transducer (Biopac, Goleta, USA) by a thread, while the right lower limb was kept immobilized. The resting tension of the muscle was adjusted to approximately 20–30 mN. A 3 cm incision was made from the gluteal region to the middle of the thigh. The muscle was carefully dissected to expose 2.0–2.5 cm of the sciatic nerve. The sciatic nerve was stimulated (1 Hz or 5 Hz for 10 s every 60 s, 4 V, 0.8 ms) with an electrode connected to a stimulator (Grass stimulator S88, Grass technologies, USA) to produce maximal twitches of the tibialis anterior muscle. Twitches were recorded in the presence and in the absence of an oxime reactivator. Reactivators were administered in a cumulative manner, I. V. (femoral vein) in four doses (10 µg, 100 µg, 1 mg and 10 mg per kg) and the stimulation (recording) was performed after 60 s from administration by stimulation frequencies at 1 Hz and 5 Hz applied for 10 s. The recording was done in duplicate, 60 s after the previous recording. The isometric twitches or tetanic tensions were continuously recorded. Data were recorded by MP100WSW data acquisition system and ACQKnowledge software (Biopac, Goleta, USA).

In vitro measurement of direct nicotinic receptor inhibition by the patch-clamp technique

The direct inhibitory effects of compounds were measured as the diminution of cationic current responses induced by 10 µM acetylcholine on the nicotinic receptors. The ability of reactivators to inhibit ACh responses was then quantified.

The experiments were performed on TE671 cell line, which is medulloblastoma cell line endogenously expressing human embryonic muscle-like acetylcholine receptor (Schoepfer *et al.* 1988). Even though the cell line is of neuronal origin, rhabdomyosarcoma properties has been described too (Stratton *et al.* 1989). The whole-cell mode of patch-clamp method using an Axopatch 200A amplifier (Axon Instruments, Foster City, CA, USA) was applied. TE671 cells (kindly provided by Dr. Jan Rícný) were cultivated at 37 °C under 5 % CO₂ atmosphere in a minimal essential medium (D-MEM), which was supplemented with 10 % of fetal calf serum (Dr. Kysilka, Brno). Nicotine (100 µM) was added to cultivation medium 2–3 days before measurement to increase nAChRs expression (Ke *et al.* 1998).

Fire-polished glass micropipettes with an outer diameter of approx. 3 µm were filled with a solution of

the following composition (in mM): CsF 110, CsCl 30, MgCl₂ 7, Na₂ATP 5, EGTA 2, HEPES-CsOH 10, pH 7.4. The resistances of the microelectrodes were 3 to 5 MΩ. The cell bath solution contained (in mM): NaCl 160, KCl 2.5, CaCl₂ 1, MgCl₂ 2, HEPES-NaOH 10, glucose 10, pH 7.3. Solutions of drugs were applied using a rapid microperfusion system (Mayer *et al.* 1989) consisting of an array of 12 parallel quartz-glass tubes each approximately 400 µm in diameter. The tubes were positioned and the flow of different solutions was switched on/off under microcomputer control (Mayer *et al.* 1989, Vyklický *et al.* 1990). A complete change of the solution around the cell could be carried out in 20 to 60 ms. For signal recording and evaluation of data, an Axon Instruments Digidata 1440A digitizer and pCLAMP10 software package (Axon Instruments, Foster City, CA) were used. Data were low-pass filtered at 1 kHz and digitized at 2 kHz. Cells were held at –40 mV during recordings.

Ionic current was induced by application of 10 µM ACh for 5 s. The solution of inhibitor was applied as 10 s preapplication followed by the application together with 10 µM ACh.

Data analysis

All data values are expressed as mean ± S.E.M. Statistical significance of *in vivo* experiment was determined by one-way analysis of variance (ANOVA) followed by the Dunnett's multiple-comparison test. All statistical analyses were performed on raw data, but the graphs are presented in percentage. P-values less than 0.05 were regarded as statistically significant. Graphs were generated by using the GraphPad Prism software (GraphPad Software, Inc., San Diego, USA).

As current responses of TE671 cells vary with time, regular periodic applications of 10 micromolar ACh were used as a control of cell sensitivity in the patch clamp technique. Corrected relative value of inhibition was calculated by dividing the amplitude of inhibited response by the average of two surrounding control responses.

Chemicals

HI-6, (1-(2-hydroxyamino-methylpyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane dimethanesulfonate); obidoxime, (1,3-bis(4-hydroxyiminomethylpyridinium)-2-oxapropane dichloride); K027, (1-(4-hydroxyiminomethyl pyridinium)-3-(4-carbamoylpyridinium) propanedibromide); and K203 ((E)-1-(4-carba-

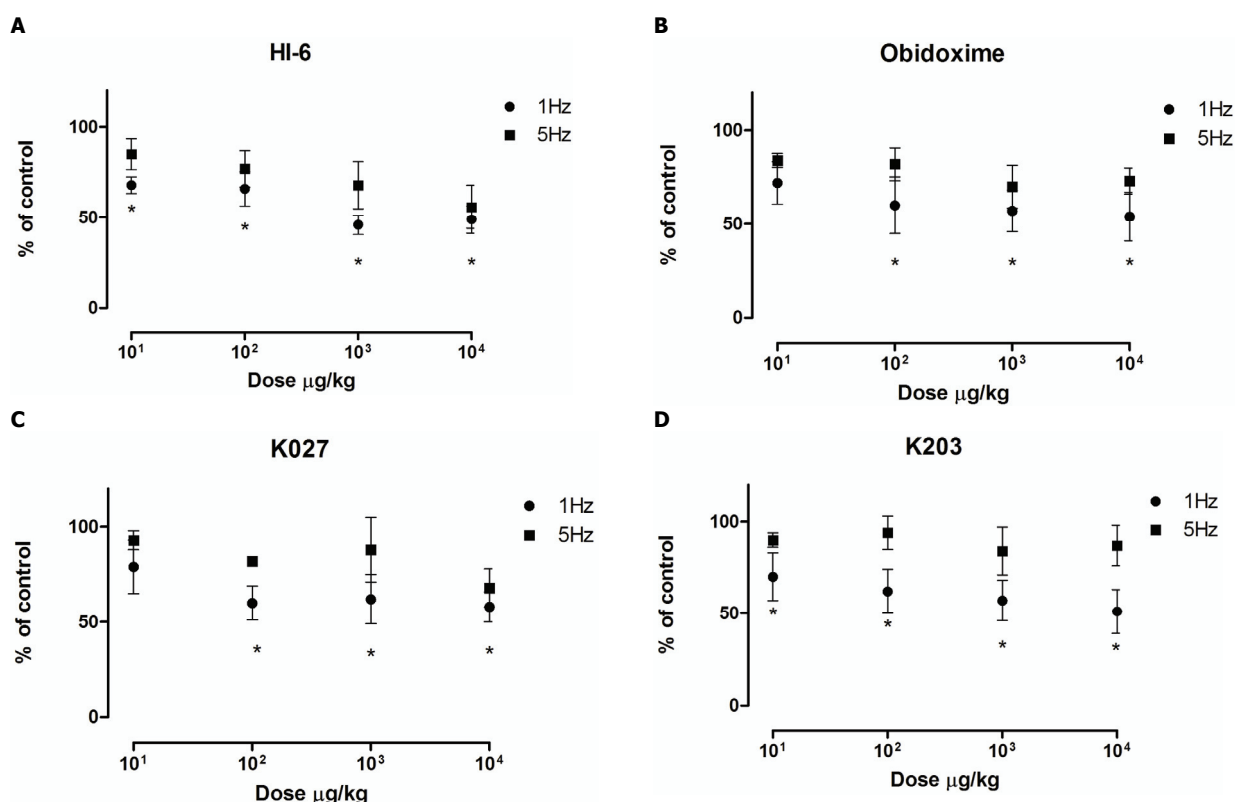


Fig. 2. The effect of various reactivators on the amplitude of the muscular twitches produced by electrical stimulation (1 Hz or 5 Hz). Data are shown in percentage. All values are expressed as mean \pm S.E.M. * indicates the statistically significant difference ($p < 0.05$) from the basal response (absence of tested compound).

moylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide) were synthesized at the Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic. Pentobarbitone, Ketalar (Apoteket AB, Sweden) were obtained from the commercial sources.

Acetylcholine chloride was from Merck (Darmstadt, Germany), tissue culture media D-MEM was from GIBCO (Invitrogen, Carlsbad, California, USA) and all other chemicals were from Sigma Aldrich (St. Louis, MO, USA).

Results

In vivo measurement of neuromuscular block

The basal amplitude of muscle twitches was 512 ± 25 mN ($n=20$) at 1 Hz and 902 ± 88 mN ($n=19$) at 5 Hz stimulation; an illustrative typical muscle twitches recording is shown (Fig. 3). The lowest dose of reactivators (10 µg/kg I.V.) caused reductions by 28 ± 5 %, 32 ± 4 %, 21 ± 6 %, 30 ± 6 % at 1 Hz ($n=5$, $p < 0.05$) and only 16 ± 2 %, 15 ± 8 %, 7 ± 2 %, 10 ± 2 % at 5 Hz in obidoxime groups, HI-6, K027 and K203, respectively ($n=5$). The largest dose administered (10 mg/kg I.V.)

caused at 1 Hz even larger reductions, namely 46 ± 6 %, 51 ± 8 %, 42 ± 4 %, 49 ± 5 % ($n=5$, $p < 0.05$) for the same groups. On the other hand, at 5 Hz, no such clear progress occurred (37 ± 3 %, 46 ± 12 %, 32 ± 5 %, 13 ± 5 %) in the same groups ($n=5$). The inhibition, if any, was less; statistical significance was attained only at 1 Hz (Fig. 2).



Fig. 3. Illustration of twitches recordings. Basal response at 1 Hz (left) and response at 1 Hz after administration of HI-6 10 mg/kg (right).

In vitro measurement of direct nicotinic receptor inhibition by the patch-clamp technique

The application of 10 µM of acetylcholine to TE671 cells, clamped at -40 mV membrane potential, produced inward currents in the range of 200 pA–7 nA. Superfusion of TE671 cells with ECS containing reactivator did not evoke any inward current (not shown).

When reactivators were pre-applied and then co-applied together with ACh 10 μ M, they reversibly inhibited ACh responses in a concentration-dependent manner. The inhibitory effect of tested drugs at the concentration of 200 μ M has been illustrated (Fig. 4). The comparison of reactivators' inhibitory activity at the concentration of 200 μ M is summarized in Table 1.

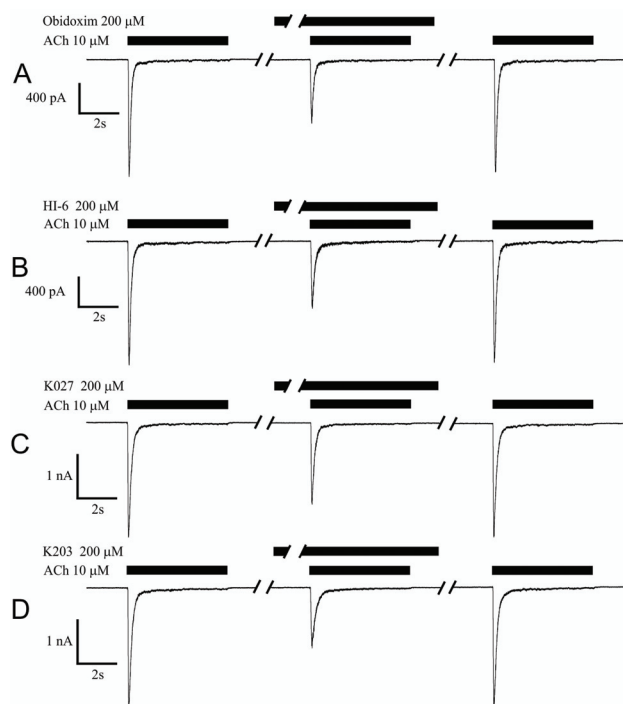


Fig. 4. Inhibition of human embryonic muscle receptors by oxime reactivators. Whole-cell membrane currents evoked by acetylcholine were recorded in the absence and in the presence of different oxime reactivators. Drugs, at 200 μ M, were preapplied for 10 s and then coapplied with 10 μ M ACh for 5 s as is indicated by bars. TE671 cells were clamped at -40 mV. **A)** Obidoxime 200 μ M, **B)** HI-6 200 μ M, **C)** K027 200 μ M, **D)** K203 200 μ M.

Table 1. Inhibition shows the drop (in percentage) in response caused by an individual reactivator at the concentration of 200 μ M on the ACh 10 μ M- evoked response (patch-clamp) (0 % = no effect, 100 % = complete inhibition). N stands for the number of cells used. S.E.M stands for the standard error of the mean.

Reactivator	Inhibition (200 μ M) (%)	S.E.M (%)	N
<i>Obidoxime</i>	51	2	4
<i>HI-6</i>	45	1	4
<i>K027</i>	31	1	3
<i>K203</i>	50	1	3

Discussion

The principal mechanism of action of oxime antidotes is the reactivation of inhibited AChE. But still, the pharmacological effect of oximes is an open question. Doubtless, oximes affect the cholinergic transmission at several levels, and antimuscarinic and antinicotinic effects are two such sites (van Helden *et al.* 1996). Today, atropine is frequently used in order to reduce effects connected with excessive ACh stimulation during the OP poisoning. ACh is the transmitter both at the ganglionic level in the autonomic nervous system and at the skeletal neuromuscular junction, and a blockade caused by atropine in the therapeutical doses is not sufficient to hinder all ACh effects (Tobin *et al.* 1991, Luo *et al.* 2010). On the other hand, atropine-induced shortening of the endplate postsynaptic potentials has been reported in the frog neuromuscular junction (Beranek and Vyskocil 1967).

Previously, oximes have been shown to inhibit the nicotinic ion channel at the mouse muscle endplate and at the guinea-pig diaphragm preparations (Tattersall 1993). Furthermore, ganglioblocking properties has been suggested too (Lundy and Tremblay 1979, Kirsch and Weger 1981). However, contradictory observations exist and HI-6 and pralidoxime have been reported to also increase the opening probability of nicotinic receptors that are activated by ACh (Alkondon *et al.* 1988). It has also been shown that all cholinesterase inhibitors, in addition to their well-known anti-AChE activity, have multiple effects on the nicotinic ACh receptor-ion channel macromolecule. The effects on the nicotinic ACh receptor have been reported to be exerted by competitive antagonism and by different types of noncompetitive blockade (van Helden *et al.* 1996). In this context, it may be worth noting that binding and functional studies of the antagonism on muscarinic receptors have indicated a complexity in the mechanisms of action of oxime reactivators, which may be valid for the nicotinic antagonism as well (Amitai *et al.* 1980). Two elements for ligands are necessary to interact with nAChR in the orthosteric site: H-bond acceptor group and positively charged nitrogen (Romanelli and Gualtieri 2003). Both requirements are fulfilled by our oxime reactivators. However, only small changes in the chemical structure may shift activity from agonist to antagonist. On the other hand, allosteric modulators belong to structurally very heterogeneous group. Furthermore, the dose employed may differentiate between opposite effects (Romanelli

and Gualtieri 2003). From our observations, it is hard to deduce oximes' mechanism of inhibition; more mechanistically focused experiments should be performed.

In the present study, nicotinolytic effects were shown to occur *in vivo* when studied on a tibial muscle preparation. This confirms previous *in vitro* observations (Tattersall 1993). Taking experimental data together, oxime reactivators show an antagonism on both embryonic and adult form of the nicotinic receptors.

Furthermore, when the oxime effects were studied on the nicotinic channel in the TE671 cell line, inhibitory effects were also observed. *In vivo*, a difference between 1 Hz and 5 Hz stimulation was obvious. At 5 Hz stimulation, smaller inhibitory effects appeared, probably due to a larger availability of ACh at this frequency. Even though no statistical significance was shown, still dose-dependent effects seem to occur. It should be stressed that spontaneous fading of twitches may occur during a repetitive stimulation, probably due to changed sensitivity of the perceptual receptors (Van der Kloot *et al.* 1994). However, a decrease by 23 % was reported after delivery of 3000 stimuli. In our study, only about 1000 stimuli were delivered and the reduction observed was up to 50 %. Concerning individual compounds only small differences in potency appeared. If any difference in potency occurred between the various oximes, it was somewhat less for K027. However, the same pattern occurred when examined in the TE671 cell line. The compounds examined are hydrophilic. Therefore, it may be assumed that they mainly distribute into the water compartment. Estimation of the IC₅₀ values in the *in vivo* experiments based on such distribution volumes renders concentration ranges between 30 and

150 µM, which roughly correspond to the estimative *in vitro* IC₅₀ values (140-250 µM in case of obidoxime, HI-6 and K203; over 600 µM in case of K027; data not shown). To the best of our knowledge, this is the first time that an oxime inhibition on nicotinic receptors is demonstrated *in vivo*.

However, since the nicotinic receptor activation was evoked by electrical nerve stimulation, effects on neuronal receptors may have influenced the results. As muscarinic receptors (M2) are present in the presynaptic part of neuromuscular synapse it is possible that this antimuscarinic action of the oxime reactivators could have played a role by the enhancing the release of neurotransmitters (Santafe *et al.* 2004)

In conclusion, we confirm previous results showing *in vitro* inhibitory effect of oximes on nicotinic receptors, and further, we show that the nicotinic neuromuscular antagonism of oxime reactivators occurs *in vivo*, as well. Obidoxime, HI-6 and K203 show similar potency, while K027 is less potent to inhibit ACh-evoked current mediated by stimulation of the nicotinic ACh receptor. Unselective nicotinic antagonism is indicated by the fact, that similar antinicotinic action was observed in two different sources of nicotinic receptors.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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References

- ALKONDON M, RAO KS, ALBUQUERQUE EX: Acetylcholinesterase reactivators modify the functional properties of the nicotinic acetylcholine receptor ion channel. *J Pharmacol Exp Ther* **245**: 543-556, 1988.
- AMITAI G, KLOOG Y, BALDERMAN D, SOKOLOVSKY M: Interaction of bis-pyridinium oximes with mouse-brain muscarinic receptor. *Biochem Pharmacol* **29**: 483-488, 1980.
- BAJGAR J: Organophosphates/nerve agent poisoning: mechanism of action, diagnosis, prophylaxis, and treatment. *Adv Clin Chem* **38**: 151-216, 2004.
- BERANEK R, VYSKOCIL F: The effect of atropine on the frog sartorius neuromuscular junction. *J Physiol* **195**: 493-503, 1968.
- EDDLESTON M, BUCKLEY NA, EYER P, DAWSON AH: Management of acute organophosphorus pesticide poisoning. *Lancet* **371**: 597-607, 2008.
- HAMILTON MG, LUNDY PM: HI-6 therapy of soman and tabun poisoning in primates and rodents. *Arch Toxicol* **63**: 144-149, 1989.

- CHIOU LC, CHANG CC: Obidoxime antagonizes the neuromuscular failure induced by neostigmine and diisopropyl fluorophosphate *via* different mechanisms. *J Biomed Sci* **1**: 19-27, 1994.
- KASSA J: Review of oximes in the antidotal treatment of poisoning by organophosphorus nerve agents. *J Toxicol Clin Toxicol* **40**: 803-816, 2002.
- KE L, EISENHOUR CM, BENCHERIF M, LUKAS RJ: Effects of chronic nicotine treatment on expression of diverse nicotinic acetylcholine receptor subtypes. I. Dose- and time-dependent effects of nicotine treatment. *J Pharmacol Exp Ther* **286**: 825-840, 1998.
- KIRSCH DM, WEGER N: Effects of the bispyridinium compounds HGG12, HGG42, and obidoxime on synaptic transmission and NAD(P)H-fluorescence in the superior cervical-ganglion of the rat *in vitro*. *Arch Toxicol* **47**: 217-232, 1981.
- KOVARIK Z, VRDOLJAK AL, BEREND S, KATALINIC M, KUCA K, MUSILEK K, RADIC B: Evaluation of oxime k203 as antidote in tabun poisoning. *Arh Hig Rada Toksikol* **60**: 19-26, 2009.
- LUNDY PM, TREMBLAY KP: Ganglion blocking properties of some bispyridinium soman antagonists. *Eur J Pharmacol* **60**: 47-53, 1979.
- LUO WQ, GE XL, CUI WY, WANG H: Targeting cholinesterase inhibitor poisoning with a novel blocker against both nicotinic and muscarinic receptors. *Neurotox Res* **18**: 173-186, 2010.
- MARRS TC: Organophosphate poisoning. *Pharmacol Ther* **58**: 51-66, 1993.
- MAYER ML, VYKLYCKY L, CLEMENTS J: Regulation of NMDA receptor desensitization in mouse hippocampal-neurons by glycine. *Nature* **338**: 425-427, 1989.
- PETROIANU GA, HASAN MY, NURULAIN SM, NAGELKERKE N, KASSA J, KUCA K: New K-oximes (K-27 and K-48) in comparison with obidoxime (LuH-6), HI-6, trimedoxime (TMB-4), and pralidoxime (2-PAM): survival in rats exposed IP to the organophosphate paraoxon. *Toxicol Mech Methods* **17**: 401-408, 2007.
- PUU G, ARTURSSON E, BUCHT G: Reactivation of nerve agent inhibited human acetylcholinesterases by HI-6 and obidoxime. *Biochem Pharmacol* **35**: 1505-1510, 1986.
- RADIC Z, TAYLOR P: Structure and function of cholinesterases. In: *Toxicology of Organophosphate & Carbamate Compounds*. R GUPTA (ed), Elsevier Academic Press, London, 2006, pp 161-186.
- ROMANELLI MN, GUALTIERI F: Cholinergic nicotinic receptors: competitive ligands, allosteric modulators, and their potential applications. *Med Res Rev* **23**: 393-426, 2003.
- SANTAFE MM, SALON I, GARCIA N, LANUZA MA, UCHITEL OD, TOMAS J: Muscarinic autoreceptors related with calcium channels in the strong and weak inputs at polyinnervated developing rat neuromuscular junctions. *Neuroscience* **123**: 61-73, 2004.
- SCHLAGMANN C, ULBRICH H, REMIEN J: Bispyridinium (oxime) compounds antagonize the ganglion blocking effect of pyridostigmine in isolated superior cervical-ganglia of the rat. *Arch Toxicol* **64**: 482-489, 1990.
- SCHOEPFER R, LUTHER M, LINDSTROM J: The human medulloblastoma cell line TE671 expresses a muscle-like acetylcholine receptor. Cloning of the alpha-subunit cDNA. *FEBS Lett* **226**: 235-240, 1988.
- SIVAGNANAM S: Potential therapeutic agents in the management of organophosphorus poisoning. *Crit Care* **6**: 260-261, 2002.
- SOUKUP O, POHANKA M, TOBIN G, JUN D, FUSEK J, MUSILEK K, MAREK J, KASSA J, KUCA K: The effect of HI-6 on cholinesterases and on the cholinergic system of the rat bladder. *Neuro Endocrinol Lett* **29**: 759-762, 2008.
- SOUKUP O, TOBIN G, KUMAR UK, BINDER J, PROSKA J, JUN D, FUSEK J, KUCA K: Interaction of nerve agent antidotes with cholinergic systems. *Curr Med Chem* **17**: 1708-1718, 2010a.
- SOUKUP O, TOBIN G, KUMAR UK, JUN D, FUSEK J, KUCA K: Characterization of the anticholinergic properties of obidoxime; functional examinations of the rat atria and the urinary bladder. *Toxicol Mech Methods* **20**: 428-433, 2010b.
- STRATTON MR, DARLING J, PILKINGTON GJ, LANTOS PL, REEVES BR, COOPER CS: Characterization of the human cell line TE671. *Carcinogenesis* **10**: 899-905, 1989.
- TATTERSALL JE: Ion channel blockade by oximes and recovery of diaphragm muscle from soman poisoning *in vitro*. *Br J Pharmacol* **108**: 1006-1015, 1993.

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- TOBIN G, EKSTROM J, BLOOM SR, EDWARDS AV: Atropine-resistant submandibular responses to stimulation of the parasympathetic innervation in the anaesthetized ferret. *J Physiol* **437**: 327-339, 1991.
- TRAN DQ, AMAKI Y, OHTA Y, NAGASHIMA H, DUNCALF D, FOLDES FF: Simultaneous *in vivo* measurement of nm block on three muscles. *Anesthesiology* **57**: A276, 1982.
- VAN DER KLOOT W, BALEZINA OP, MOLGO J, NAVES LA: The timing of channel opening during miniature end-plate currents at the frog and mouse neuromuscular junctions: effects of fasciculin-2, other anti-cholinesterases and vesamicol. *Pflugers Arch* **428**: 114-126, 1994.
- VAN HELDEN HP, VAN DER WIEL HJ, DE LANGE J, BUSKER RW, MELCHERS BP, WOLTHUIS OL: Therapeutic efficacy of HI-6 in soman-poisoned marmoset monkeys. *Toxicol Appl Pharmacol* **115**: 50-56, 1992.
- VAN HELDEN HP, BUSKER RW, MELCHERS BP, BRUIJNZEEL PL: Pharmacological effects of oximes: how relevant are they? *Arch Toxicol* **70**: 779-786, 1996.
- VYKLICKY L JR, BENVENISTE M, MAYER ML: Modulation of N-methyl-D-aspartic acid receptor desensitization by glycine in mouse cultured hippocampal-neurons. *J Physiol* **428**: 313-331, 1990.
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