available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/etap

The effect of oxime reactivators on muscarinic receptors: Functional and binding examinations

O. Soukup[a,f,*], U.K. Kumar[c], J. Proska[d], L. Bratova[c], A. Adem[e], D. Jun[a,b], J. Fusek[a], K. Kuca[a,b], G. Tobin[c]

[a] Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Trebesska 1575, Hradec Kralove 50001, Czech Republic

[b] Center of Advanced Studies, Faculty of Military Health Sciences, University of Defence, Trebesska 1575, Hradec Kralove 50001, Czech Republic

[c] Institute of Neuroscience and Physiology, Department of Pharmacology, The Sahlgrenska Academy at University of Gothenburg, Sweden

[d] CTU in Prague, Faculty of Nuclear Sciences and Physical Engineering, V Holesovickach 2, 180 00 Prague 8, Czech Republic

[e] Department of Pharmacology and Therapeutics, Faculty of Medicine, United Arab Emirates University, Al Ain, United Arab Emirates

[f] University Hospital Hradec Kralove, Sokolska 581, Hradec Kralove 500 05, Czech Republic

ARTICLE INFO

Article history:
Received 3 August 2010
Received in revised form
5 January 2011
Accepted 23 January 2011
Available online 28 January 2011

Keywords:
Organophosphates
Muscarinic receptors
Reactivator
beta-Arrestin
Binding study
Isolated atria

ABSTRACT

The antidotal treatment of organophosphorus poisoning is still a problematic issue since no versatile antidote has been developed yet. In our study, we focused on an interesting property, which does not relate to the reactivation of inhibited acetylcholinesterase (AChE) of some oximes, but refers to their anti-muscarinic effects which may contribute considerably to their treatment efficacy. One standard reactivator (HI-6) and two new compounds (K027 and K203) have been investigated for their antimuscarinic properties. Anti-muscarinic effects were studies by means of an *in vitro* stimulated atrium preparation (functional test), the [^{3}H]-QNB binding assay and G-protein coupled receptor assay (GPCR, beta-Arrestin Assay). Based on the functional data HI-6 demonstrates the highest anti-muscarinic effect. However, only when comparing [^{3}H]-QNB binding results and GPCR data, K203 shows a very promising compound with regard to anti-muscarinic potency. The therapeutic impact of these findings has been discussed.

1. Introduction

Organophosphorus compounds (OP), i.e. the nerve agents (e.g. soman, tabun, sarin, and VX) and pesticides (e.g. parathion chlorpyrifos, and paraoxon), inhibit the enzyme acetylcholinesterase (AChE, EC 3.1.1.7) via phosphorylation or phosphonylation of serine hydroxyl group at its active site (Marrs, 1993). AChE inhibition results in the accumulation of acetylcholine (ACh) at cholinergic receptor sites, producing overstimulation of cholinergic receptors throughout the central and peripheral nervous systems (Bajgar, 2004).

Two main groups of drugs are used in the treatment of the poisoning. (1) Anticholinergics, like atropine, that are able

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; BPM, beats per minute; GPCR, G-protein coupled receptor; mAChRs, muscarinic acetylcholine receptors; OP, organophosphorus compounds; QNB, quinuclidinyl benzilate.

* *Corresponding author*. Tel.: +420 973 251 501; fax: +420 495 518 094.
E-mail address: soukup.ondrej@seznam.cz (O. Soukup).

1382-6689/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.etap.2011.01.003

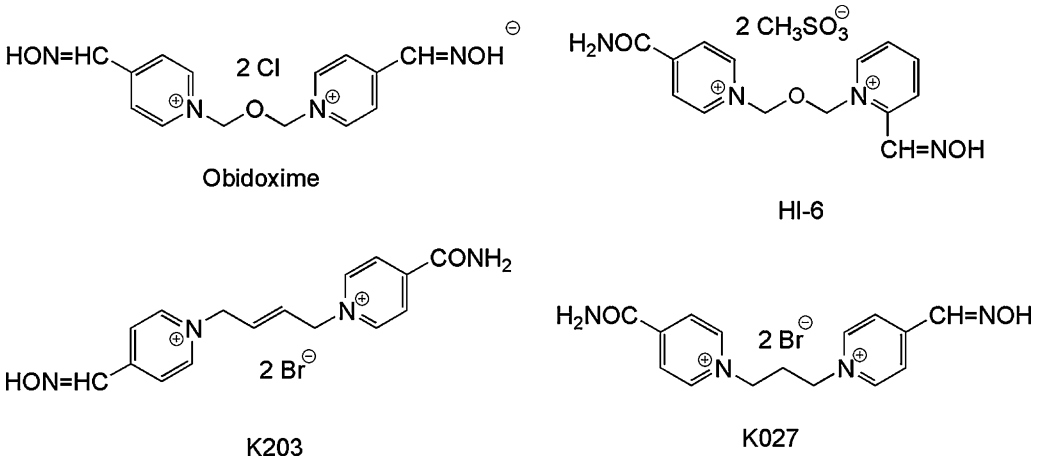

Fig. 1 – Structure of Obidoxime, HI-6, K027, K203.

to antagonize the effects of excessive ACh by a blockade of mainly muscarinic receptors. (2) Reactivators of AChE – oximes – that are able to restore the physiological function of inhibited AChE (Kassa, 2002). Anticholinergics and reactivators can be administered together because of their synergistic effect (Kassa, 2002; Bajgar, 2004). Recently, HI-6 and obidoxime (Fig. 1) have been the most used oxime reactivators in the treatment of organophosphorus poisoning. However, the reactivators differ in their efficacy against individual nerve agents and no universal antidote has been developed yet. Owing to this fact, new AChE reactivators, capable of reactivating AChE irrespective of the type of nerve agent causing the inhibition are thus required, or alternatively, other treatment approaches may be introduced.

An alternative approach is derived from a somewhat obscure property of some oximes, that presumes other mechanisms, not related to the reactivation (Hamilton and Lundy, 1989; Tattersall, 1993; Oydvin et al., 2005; Vesela et al., 2008; Soukup et al., 2010a,b). Oximes have been suggested to act at various levels of cholinergic transmission including synthesis, release, inactivation and re-uptake of the transmitter. However, the interaction with cholinoreceptors, namely, the blocking properties of the oximes on the muscarinic and nicotinic receptors has been put forward as the most plausible mechanism (Tattersall, 1993; van Helden et al., 1996).

Obidoxime is a commonly used reactivator, for which the modulation of muscarinic receptors has been proved (Jakubik and Elfakahany, 2010). In our study, we have chosen three other reactivators in order to investigate their anticholinergic effect. In addition to two newly synthesized compounds, K027 and K203, HI-6 was chosen since it is the most efficacious oxime for the antidotal treatment of acute poisonings (Kassa, 2002; Kassa et al., 2006) (Fig. 1).

HI-6, a bisquaternary oxime, is a very effective antidote against soman, sarin and VX (Kassa, 2002) but it is less effective against tabun (Kuca et al., 2007). Some anticholinergic activity of this compound has already been described (Hamilton and Lundy, 1989). K027 is a very effective reactivator of tabun- and methylparaoxone-inhibited AChE in both *in vitro* and *in vivo* experiments (Kuca et al., 2005; Petroianu et al., 2007a,b). Moreover, since it has a lower toxicity than obidoxime this may lead to a replacement of obidoxime as the preferred antidote in the treatment of poisoning by tabun and pesticides. K203 is also an effective compound in cases of inhibited AChE by tabun. The results from *in vitro* as well as *in vivo* examinations in rats indicate that K203 may have a high therapeutic potency in tabun-poisoning (Kovarik et al., 2009). Furthermore, in combination with atropine it seems to be effective for a decrease in tabun-induced neurotoxicity (Kassa et al., 2009). Since the tabun poisoning is still a problematic issue, these findings place K203 in the spotlight for further investigation and development (Kovarik et al., 2009).

In the present study, one standard oxime (HI-6) and two new oximes (K027 and K203) have been investigated regarding their anti-muscarinic properties. These effects were studied by means of an *in vitro* stimulated atrium preparation (functional test), the [^{3}H]-QNB binding assay and the G-protein coupled receptor assay (GPCR, beta-Arrestin Assay).

2. Materials and methods

HI-6 (1-(2-hydroxyamino-methylpyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane dimethansulfonate), K027 (1-(4-hydroxyiminomethyl pyridinium)-3-(4-carbamoylpyridinium) propanedibromide), K203 ([(E)-1-(4-carbamoylpyridinium)-4-(4-hydroxyiminomethylpyridinium)-but-2-ene dibromide) were synthesized at the Department of Toxicology, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic), methacholine, adrenaline (Sigma–Aldrich, Czech Republic). AChE recombinant (Sigma–Aldrich, Czech Republic), acetylthiocholine, and 5,5′-dithiobis(2-nitrobenzoic) acid (Sigma–Aldrich, Czech Republic) were used for enzymatic study.

For the binding assay [^{3}H]Quinuclidinyl(phenyl-4-3H)benzilate, 1.74 TBq/mmol, 47.0 Ci/mmol ([^{3}H]-QNB) (GE Healthcare, UK), protein standard (BSA), Fluor Universal LSC Coctail for Aeguos Samples and atropine (all Sigma–Aldrich, USA) were used.