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Counteracting desensitization of human α7-nicotinic acetylcholine receptors with bispyridinium compounds as an approach against organophosphorus poisoning

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(±)-epibatidine (PubChem CID: 3073763)
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ABSTRACT

Irreversible inhibition of acetylcholinesterase (AChE) resulting in accumulation of acetylcholine and overstimulation of muscarinic and nicotinic receptors accounts for the acute toxicity of organophosphorus compounds (OP). Accordingly, the mainstay pharmacotherapy against poisoning by OP comprises the competitive muscarinic acetylcholine receptor antagonist atropine to treat muscarinic effects and, in addition, oximes to reactivate inhibited AChE. A therapeutic gap still remains in the treatment of desensitized nicotinic acetylcholine receptors following OP exposure. Hereby, nicotinic effects result in paralysis of the central and peripheral respiratory system if untreated. Thus, these receptors pose an essential target for therapeutic indication to address these life-threatening nicotinic symptoms of the cholinergic crisis. Identification of ligands regulating dynamic transitions between functional states by binding to modulatory sites appears to be a promising strategy for therapeutic intervention. In this patch clamp study, the ability of differently substituted bispyridinium non-oximes to "resensitize" i.e. to recover the activity of desensitized human homomeric α7-type nAChRs stably transfected in CHO cells was investigated and compared to the already described α7-specific positive allosteric modulator PNU-120596. The structures of these bispyridinium analogues were based on the lead structure of the *tert*-butyl-substituted bispyridinium propane MB327, which has been shown to have a positive therapeutic effect due to a non-competitive antagonistic action at muscle-type nAChRs *in vivo* and has been found to have a positive allosteric activity at neuronal receptors *in vitro*. Prior to test compounds, desensitization of hα7-nAChRs was verified by applying an excess of nicotine revealing activation at low, and desensitization at high concentrations. Thereby, desensitization could be reduced by modulation with PNU-120596. Desensitization was further verified by dose-response profiles of agonists, carbamoylcholine and epibatidine in the absence and presence of PNU-120596. Although less pronounced than PNU-120596 and the lead structure MB327, bispyridinium compounds, particularly those substituted at position 3 and 4, resensitized the nicotine desensitized hα7-nAChRs in a concentration-dependent manner and prolonged the mean channel open time. In summary, identification of more potent compounds able to restore nAChR function in OP intoxication is needed for development of a putative efficient antidote.

1. Introduction

Acetylcholine receptors (AChRs) are a heterogeneous family of ligand-gated cationic ion channels consisting of five heteropentameric or homopentameric subunits organized around an ionic pore and have been found in the peripheral (PNS) and central nervous system (CNS), the immune system and non-neuronal tissues (Fagerlund and Eriksson, 2009). These receptors are implicated in several pathophysiological situations comprising CNS (e.g. Alzheimeŕs and Parkinsońs disease, schizophrenia, depression) and PNS (e.g. myasthenia gravis) disorders (Kalamida et al., 2007; Albuquerque et al., 2009; Gotti et al., 2009; Millar and Gotti, 2009). Since this discovery, a new area of drug

Abbreviations: AChE, acetylcholinesterase; ACh, acetylcholine; BP, bispyridinium; CNS, central nervous system; 7α-nAChRs, human α7-nicotinic acetylcholine receptor(s); mAChR, muscarinic acetylcholine receptor; nAChRs, nicotinic acetylcholine receptor(s); OP, organophosphorus compound; PAM(s), positive allosteric modulator(s); PNS, peripheral nervous system; PNU-120596, N-(5-chloro-2,4-dimethoxyphenyl)-N'-(5-methyl-3-isoxazolyl)-urea

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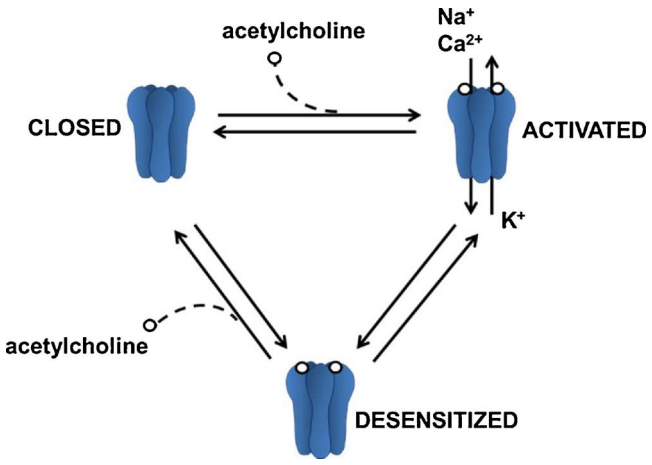

Fig. 1. Simplified schematic model of conformational transitions of nAChRs including closed, open and desensitized states consequent to binding of acetylcholine (Faghih et al., 2008). For further details see text.

development has been initiated in the past decades emerging the development of several drugs including agonists, antagonists and, more recently, allosteric modulators of nAChRs (Williams et al., 2011b). Multiple conformational states of nAChRs and the equilibria among these states are regulated by ligand binding (Campling et al., 2013). In the model presented in Fig. 1, nAChR transitions required to account for a closed, open and desensitized state are shown (Faghih et al., 2008). Hereby, the probability of converting the receptor state from the closed, unbound to the open receptor state increases with the agonist binding sites occupied (Williams et al., 2011b). When the fractional occupancy of the agonist binding sites increases due to higher agonist concentrations, the non-conducting desensitized state is favored (Arias, 1998; Uteshev et al., 2002). The following considerations derive from investigations of the muscle-type nAChR wherein desensitization poses a severe life-threatening event after exposure to organophosphorus (OP) compounds, including pesticides and nerve agents, as it can cause skeletal muscle fasciculation with subsequent weakness (Grob and Harvey, 1953; Wright, 1954). If untreated, continued accumulation of the endogenous neurotransmitter acetylcholine may lead to continued overstimulation of nAChR and subsequently produce muscle paralysis due to prolonged depolarization of the endplate, a phenomenon referred to as depolarization block (Aldridge and Reiner, 1972, Holmstedt, 1959).

The major acute toxicity of OP compounds derives from irreversible inhibition of acetylcholinesterase (AChE) (Aldridge and Reiner, 1972) resulting in accumulation of the neurotransmitter acetylcholine in the synaptic cleft and subsequent overstimulation of muscarinic and desensitization of nicotinic receptors leading to disturbance of numerous body functions (Holmstedt, 1959).

Current therapy focuses on competitive antagonism at muscarinic acetylcholine receptors (mAChR) by atropine and reactivation of inhibited AChE by mono- and bisquarternary pyridinium oximes (Thiermann et al., 2013).

Functional recovery of nAChRs is only indirectly achieved by oxime-induced AChE reactivation (Eyer and Worek, 2007). Moreover, the ability of commonly used oximes to reactivate AChE inhibited by certain OP compounds is limited due to a therapeutically inaccessible enzyme-organophosphate complex (tabun) or due to a rapid dealkylation reaction of the OP-inhibited AChE that is resistant to oxime reactivation (soman) (Fleisher and Harris, 1965; Worek et al., 2004; Worek et al., 2007b). To overcome this therapeutic gap, novel therapeutic strategies against nicotinic dysfunction following intoxication by OP compounds focus on the intervention of ligands at nAChRs (Sheridan et al., 2005; Turner et al., 2011; Niessen et al., 2016). In this context, previous studies with the bispyridinium (BP) non-oxime compound MB327 (4-*tert*-butyl bispyridinium) demonstrated a recovery of the neuromuscular transmission postulating an open channel block, which is a mechanism based on direct non-competitive antagonistic interaction of MB327 with nAChRs, wherein antagonism becomes greater as activation of nAChRs increases (Tattersall, 1993; Seeger et al., 2012; Sheridan et al., 2005).Hence, MB327 was identified as a promising compound for the treatment of OP poisoning. However, the efficacy of MB327 in protection of OP poisoning appears to be rather modest according to *in vivo* studies by Kassa et al. (2016). Furthermore, muscle-type nicotinic receptors have been mainly addressed in previous studies investigating the effect of different ligands on nicotinic receptor dysfunction, in particular desensitization of these receptors. Neuronal nicotinic receptors have only merely been addressed so far although acute clinical symptoms are indicative for the implication of both, muscle and neuronal nicotinic receptors, in respiratory failure in OP poisoning (Rickett et al., 1986; Marrs, 2007). Furthermore, the pronounced desensitization of neuronal nicotinic receptors may be useful to investigate desensitization and the effect of test compounds on desensitized nicotinic receptors. Therefore, the present study uses an automated patch clamp system to investigate MB327 used as a template and structurally related BP non-oximes bearing different substituents at varying positions for the ability to recover the functional activity of desensitized human α7-nAChRs (hα7-nAChRs). The α7-nAChR is a subtype abundantly expressed in neuronal and non-neuronal tissues (Fagerlund and Eriksson, 2009) and was used as a representative example of neuronal nicotinic receptors to elucidate the *in vitro* effect of test compounds on desensitization of neuronal nicotinic receptors. For the investigation of the mode of action of compounds on α7-nAChR function, PNU-120596, the well-known allosteric type II modulator (Hurst et al., 2005), was used as a model compound able to reverse desensitization. Thereby, PNU-120596 used as a positive control in this study is known to be an exclusive α7-selective PAM which has no further potential use as a therapeutic drug in OP poisoning because different, centrally and peripherally expressed nAChR subtypes are implicated in paralysis of the respiratory system in OP poisoning. Response profile of PNU-120596 was compared to that of the BP compounds in order to estimate their mode of action. In accordance with previous studies (Arias et al., 2010; Arias et al., 2012; Arias et al., 2013), the desensitized state of AChRs could be experimentally induced *in vitro* by an excess of the orthosteric agonist nicotine and desensitization could be reversed by PNU-120596 (Hurst et al., 2005). To verify desensitization and positive allosteric modulation by PNU-120596, response profiles of further orthosteric agonists including carbamoylcholine and epibatidine in the absence and presence of PNU-120596 were generated. In summary, this study serves to identify structural requirements of substituted BP non-oximes responsible to resensitize nAChRs i.e. to reverse desensitization for an improved, predictive drug design against OP poisoning.

2. Materials and methods

2.1. Materials

Cell culture media and supplements were purchased from Gibco, distributed by Invitrogen (Darmstadt, Germany). Carbamoylcholine dichloride, (−)-nicotine and PNU-120596 were purchased from Sigma Aldrich (Taufkirchen, Germany). (±)-epibatidine was obtained from Tocris (Bristol, UK). All other chemicals were purchased from Merck Eurolab GmbH (Darmstadt, Germany) and from Carl Roth GmbH (Karlsruhe, Germany) at the purest grade available. MB327 and the tested BP analogues having different substitution patterns of *tert*-butyl, methoxy and isopropyl groups on both pyridinium moieties were referred to as PTM compounds and were synthesized at the Department of Pharmacy − Center for Drug Research of the Ludwig-Maximilians-Universität München, (Munich, Germany) (Rappenglück et al., 2017a; Rappenglück et al., In preparation). Stock solutions of PTM compounds,