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Research paper

Conformationally restrained carbamoylcholine homologues. Synthesis, pharmacology at neuronal nicotinic acetylcholine receptors and biostructural considerations

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ABSTRACT

Exploration of small selective ligands for the nicotinic acetylcholine receptors (nAChRs) based on acetylcholine (ACh) has led to the development of potent agonists with clear preference for the $\alpha_4\beta_2$ nAChR, the most prevalent nAChR subtype in the central nervous system. In this work we present the continuation of these efforts aimed at increasing this subtype selectivity by introduction of conformational restriction in the carbamoylcholine homologue, 3-(dimethylaminobutyl) dimethylcarbamate (DMABC). Our results highlight the importance of the *N*-carbamoyl substitution in $\alpha_4\beta_2$-subtype selectivity. Moreover, we have confirmed the non-linear conformation of DMABC bound to nAChRs suggested by recent crystal structures of the compound in complex with the *Lymnaea stagnalis* ACh binding protein.

1. Introduction

Acetylcholine (ACh) is a major neurotransmitter in the central nervous system (CNS) and peripheral nervous system (PNS), where its actions are mediated through two types of ACh receptors (AChRs): the metabotropic G protein-coupled muscarinic AChRs (mAChRs) and a family of ligand-gated ion channels, the nicotinic AChRs (nAChRs). Neuronal nAChRs are located at presynaptic and postsynaptic neuron terminals through which they modulate functions of several major neurotransmitter systems and influence numerous brain functions [1–3]. For example, there is wide evidence supporting that nAChRs play a role in a broad range of neurodegenerative and psychiatric disorders [4–8].

The nAChR complex consists of five subunits embedded in the cell membrane that are symmetrically arranged to form a cation-selective channel pore. In contrast to the muscle nAChRs expressed at the neuromuscular junction which are $\alpha_1\beta_1\gamma\delta$ or $\alpha_1\beta_1\gamma\varepsilon$ assemblies, neuronal nAChRs are homomeric and heteromeric assemblies of α (α_2–α_7, α_9–α_{10}) and β (β_2–β_4) subunits [9]. Among all the possible receptor combinations, the vast majority of the nAChRs in the CNS are heteromeric receptors comprising α_4 and β_2 subunits and homomeric α_7 receptors, whereas the most prevalent subtype in the PNS are $\alpha_3\beta_4$ receptors [10]. The orthosteric binding site is situated in the interface between α and β subunits in the heteromeric pentamer and between neighbouring α subunits in the homomeric receptor [11–13]. Significant advances have been made in the understanding of the complex molecular basis for nAChR signalling [14,15]. In order to investigate the physiological and pathophysiological roles of nAChRs and potentially explore the therapeutic potential in the receptors, the development of tools able to distinguish between the different nAChR subtypes are needed [10,16]. However, the plethora of physiologically expressed nAChR subtypes, and the highly conserved nature of the orthosteric binding site in the receptors make the development of subtype-selective agonists a challenging task [17].

In recent studies, we have developed a series of carbamoylcholine (CCh) homologues that act as functionally $\alpha_4\beta_2$-selective agonists (Fig. 1): 3-(dimethylamino)butyl methylcarbamate (**2**), 3-(dimethylamino)butyl azetidine-1-carboxylate (**3**), [4-(1-

Abbreviations: CNS, central nervous system; PNS, peripheral nervous system; ACh, acetylcholine; nAChR, nicotinic receptor; CCh, carbamoylcholine; *Ls*-AChBP, *Lymnasia stagnalis* acetylcholine-binding protein.

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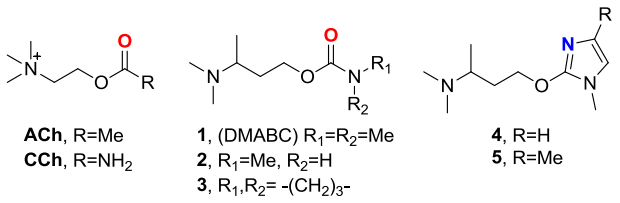

Fig. 1. Structures of acetylcholine (ACh), carbamoylcholine (CCh), and compounds **1**–**5**. Coloured heteroatoms are those involved in hydrogen-bond with a water molecule found in the intersubunit portion of *Ls*-AChBP binding site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

methyl-1H-imidazol-2-yloxy)but-2-yl]-*N*,*N*-dimethylamine (**4**) and [4-(1,4-dimethyl-1H-imidazol-2-yloxy)but-2-yl]-*N*,*N*-dimethylamine (**5**). CCh homologues **1** and **2** exhibit high degree of selectivity for nicotinic over muscarinic receptors, and in addition **2**–**5** display a pronounced degree of selectivity towards β_2-containing nAChRs subtypes [18–20]. Initially, these CCh homologues were thought to bind to the nAChRs in a linear conformation similar to that of ACh or CCh [15]. However, recent crystallographic studies of the *Lymnaea stagnalis* ACh-binding protein (*Ls*-AChBP) in complex with **1** and **4** have found the compounds to bind *Ls*-AChBP in a folded conformation favoured by an intramolecular hydrogen bond established between the protonated dimethylamino group and the ester-like oxygen or the bioisosteric imidazole N3 of these compounds (Fig. 1) [20].

In the observed binding mode in these co-crystal structures, the dimethyl ammonium group of the agonist lies covered by the flexible C-loop of the α subunit within an aromatic box, while the other end of the molecule faces the complementary subunit, the main point of contact being a hydrogen bond from a water molecule to the carbonyl oxygen of **1** (Fig. 2) and the N3 in the imidazole ring of **4** (not shown) [20], respectively. This interaction is also present in the *Ls*-AChBP co-crystal structure with ACh (not shown) and is considered a determinant for agonist binding and function [15].

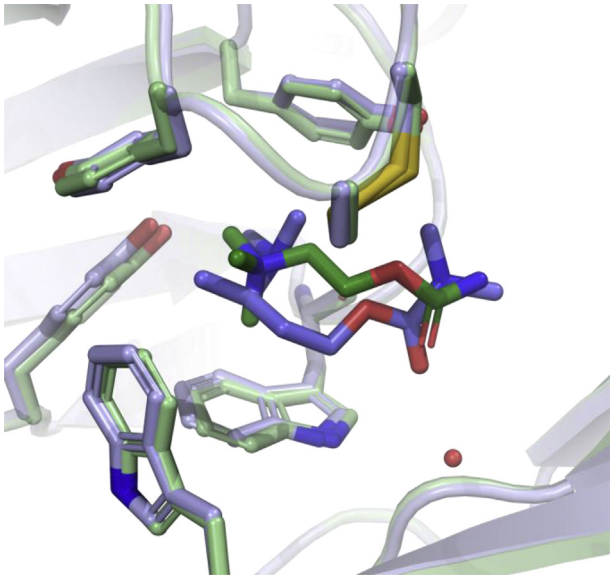

Fig. 2. Superimposition of *Ls*-AChBP co-crystal structures with CCh (green, linear) [21] and (*R*)-**1** (blue, folded) [20]. The carbonyl oxygen of (*R*)-**1** forms a hydrogen bond with a water molecule (red ball) in the binding site and the ester-like oxygen is within hydrogen bond distance to the presumably protonated dimethylamino group (3.0 Å). In the CCh bound structure the water molecule is not resolved and hence not shown in the figure. The conformation of CCh has been changed into a low-energy conformation by a 180 deg. rotation around the O–(CO) bond to comply with the nAChR pharmacophore. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Besides the proposed hydrogen-bond interaction with a water molecule in the interface with the complementary subunit, the carbamate/azole part of the ligands is considered essential for the observed subtype-selectivity profile. Plausible steric clashes against the complementary subunit are thought to be the most relevant interaction for nAChR subtype selectivity [19,20]. Additionally, the interaction with the complementary subunit has been suggested to be responsible for the efficacy displayed by agonists at the $\alpha_4\beta_2$ nAChR [15,22].

The amino part of **1** has already been studied by means of chain elongation, resolution of the enantiomers, alpha methyl deletion or alkyl substitution [19], and incorporation into cyclic structures [23,24]. Herein, we present a systematic introduction of different cycles representing various degrees of constriction of the conformational space of **1**. A cyclopropyl moiety was included within the structure of **1** and **2**, aiming to lock the bio-active conformation of **1**, including the putative intramolecular hydrogen bond described above in the crystal structure of **1** in *Ls*-AChBP. Similarly, larger cycles showing different degrees of bulkiness and flexibility were decorated with the main structural features of the parent compounds giving a series of cyclic urea and thiourea analogues.

2. Chemistry. Synthesis of restricted analogues of 1

The synthetic route that leads to target compounds **13**–**16** is outlined in Scheme 1. Compound *trans*-**8** was synthesized via selective enzymatic mono-hydrolysis of **7** by pig liver esterase [25,26]. The hydrolysis of the inexpensive cyclic anhydride **6** was chosen for the synthesis of *cis*-**8** [27]. The key step in the synthetic route was the Curtius rearrangement [28]; the diphenylphosphorylazide mediated rearrangement worked with excellent yields for both *trans*-**10** and *cis*-**10**. Unlike Csuk et al. reported previously, no isomerization of the *cis* isomer was observed [29–31].

Different reducing agents ($LiBH_4$, $LiAlH_4$ and DIBAL) were tested to selectively reduce the carbonyl group of the ethylcarboxylate to the corresponding alcohols (*trans*-**10** and *cis*-**10**) of which DIBAL displayed the best performance giving the best yields [26]. Subsequently the Boc-protected carbamates *trans*-**11**, *cis*-**11**, *trans*-**12** and *cis*-**12** were synthesized in two steps: first, the imidazolylcarboxylate was obtained from the corresponding alcohol by addition of carbonyldiimidazole and then, the corresponding amine was added, to afford the Boc-protected carbamates in good yields. The cleavage of the Boc group was performed in a mixture of trifluoroacetic acid and dichloromethane [32]. The yields of the primary amines *trans*-**13**, *cis*-**13**, *trans*-**14** and *cis*-**14** ranged from fair to good. The primary amines underwent reductive alkylation with formaline and sodium cyanoboronhydride to afford the dimethylated derivatives *trans*-**15**, *cis*-**15**, *trans*-**16** and *cis*-**16** in excellent yields [33]. Both the primary amines and the dimethylated ones were obtained as oxalate salt.

Compounds **24**–**31**, **36**–**38** were synthesized by addition of a monomethyl or Boc protected diamine to the corresponding isocyanate or isothiocyanate (Scheme 2). The reaction of the diamines with dimethylcarbamyl chloride yielded the dimethylureas **18**–**20**, **35**. Amide **21** was synthesized via carbonyldiimidazole coupling followed by Boc-cleavage in HBr to afford compound **22**. The same Boc-cleaving procedure was employed to obtain compounds **23**, **32** and **33**.