European Journal of Pharmacology 726 (2014) 77–86

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Molecular and cellular pharmacology

Unique pharmacology of heteromeric α7β2 nicotinic acetylcholine receptors expressed in *Xenopus laevis* oocytes

Ruud Zwart*, Merrick Strotton, Jennifer Ching, Peter C. Astles, Emanuele Sher

Lilly Research Centre, Eli Lilly and Company Limited, Erl Wood Manor, Sunninghill Road, Windlesham, Surrey GU20 6PH, United Kingdom

ARTICLE INFO

Article history:
Received 6 September 2013
Received in revised form
13 January 2014
Accepted 21 January 2014
Available online 28 January 2014

Keywords:
Nicotinic acetylcholine receptor
Pharmacology
Voltage-clamp
Heteromeric
Xenopus oocyte

ABSTRACT

α7β2 is a novel type of nicotinic acetylcholine receptor shown to be uniquely expressed in cholinergic neurons of the basal forebrain and in hippocampal interneurons. We have compared the pharmacological properties of recombinant homomeric α7 and heteromeric α7β2 nicotinic acetylcholine receptors in order to reveal the pharmacological consequences of β2 subunit incorporation into the pentamer. The non-selective agonist epibatidine did not distinguish α7β2 from α7 nicotinic acetylcholine receptors, but three other non-selective agonists (nicotine, cytisine and varenicline) were less efficacious on α7β2 than on α7. A more dramatic change in efficacy was seen with eight different selective α7 agonists. Because of their very low intrinsic efficacy, some compounds became very efficacious functional antagonists at α7β2 receptors. Three α4β2 nicotinic receptor selective agonists that were not active on α7, were also inactive on α7β2, and dihydro-β-erythroidine, an α4β2 receptor-preferring antagonist, inhibited α7 and α7β2 in a similar manner. These results reveal significant effects of β2 incorporation in determining the relative efficacy of several non-selective and α7 selective agonists, and also show that incorporation of β2 subunits does not cause a shift to a more "β2-like" pharmacology of α7 nicotinic acetylcholine receptors.

1. Introduction

Homomeric α7 and heteromeric α4β2 nicotinic acetylcholine receptors are the predominant subtypes of nicotinic acetylcholine receptor in the central nervous system (Flores et al., 1992; Piciotto et al., 2001; Gotti and Clementi, 2004) and are possible targets for drugs to treat various disorders, including cognitive impairment associated with Alzheimer's disease and schizophrenia (Jensen et al., 2005; Arneric et al., 2007; Haydar and Dunlop, 2010, Hurst et al., 2013).

In the basal forebrain transcripts for α7 and β2 are the predominant nicotinic acetylcholine receptor subunit mRNAs present, raising the possibility that these two subunits co-assemble in a so far unknown subtype of nicotinic acetylcholine receptor (Azam et al., 2003). Recently, it has been found that neurons from the basal forebrain and hippocampal interneurons express a novel type of functional heteromeric nicotinic acetylcholine receptor which contains α7 and β2 subunits (Liu et al., 2009, 2012). In these neurons α7 subunits are co-expressed, co-localized, and co-assembled with β2 subunits. α7β2 nicotinic acetylcholine receptors are functionally and pharmacologically different from α7 nicotinic acetylcholine receptors, because they desensitize slower and are more sensitive to blockade by the β2 subunit-containing nicotinic acetylcholine receptor-selective antagonist dihydro-β-erythroidine (dHβE). In heterologous expression systems, it has also been shown that α7 can co-assemble with other subunits and form heteromeric nicotinic acetylcholine receptors which also differ functionally and pharmacologically from homomeric α7 nicotinic acetylcholine receptors (Palma et al., 1999; Khiroug et al., 2002; Criado et al., 2012; Murray et al., 2012).

The presence of a unique nicotinic acetylcholine receptor subtype in the cholinergic basal forebrain is intriguing since this system plays an essential role in cognitive function. Cholinergic projections from the basal forebrain innervate the entire cortex and hippocampus (Woolf, 1991). In schizophrenic patients and in patients suffering from Alzheimer's disease, the cholinergic system is impaired and cognitive functions of these patients are severely reduced. Attempts to treat cognitive symptoms have therefore focused on cholinomimetic strategies (Money et al., 2010) such as the non-selective increase in acetylcholine by inhibiting acetylcholine esterase. In addition, a number of muscarinic and nicotinic receptor agonists have been characterized over the years. In particular, α4β2 (Radek et al., 2010) and α7 (Freedman, 2014) subtype-selective nicotinic agonists have been developed and shown to be moderately effective at enhancing cognitive function in preclinical animal models and in early clinical studies. However, the efficacy of these compounds in patients has been generally disappointing (Wallace and Bertrand, 2013). This could be due to

* Corresponding author. Tel.: +44 1276 483554.
E-mail address: zwart_ruud@lilly.com (R. Zwart).

0014-2999/$ - see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ejphar.2014.01.031

targeting the wrong receptor subtypes and/or the inability to achieve an efficacious dose due to on-target and off-target side effects. The new α7β2 nicotinic acetylcholine receptors, which are expressed by neurons of the basal forebrain, provide a potential novel target to treat symptoms of AD and schizophrenia. A compound that activates or potentiates α7β2 nicotinic acetylcholine receptors in the basal forebrain is hypothesized to stimulate acetylcholine release in the hippocampus and cortex, where acetylcholine will activate not only locally expressed nicotinic acetylcholine receptors, but also local muscarinic acetylcholine receptors, two mechanisms which are thought to be beneficial for the symptomatic treatment of cognitive deficits associated with AD and schizophrenia.

In the present study we have investigated the pharmacological effects of a number of non-selective, α7-selective and α4β2-selective agonists in *Xenopus* oocytes transfected with α7 subunits and oocytes cotransfected with α7 and β2 subunits. We found that α7β2 nicotinic acetylcholine receptors have a very unique pharmacology compared to both homomeric α7 and heteromeric α4β2 nicotinic acetylcholine receptors.

2. Materials and methods

2.1. Nicotinic acetylcholine receptor expression in Xenopus oocytes

Xenopus oocyte expression and electrophysiological recordings were performed as described previously by us (Zwart et al., 2002) and stage V and VI *Xenopus* oocytes were prepared using standard procedures. Rat α7 and β2 subunit cDNAs were ligated into the pcDNA3 (Invitrogen) expression vector, and dissolved in distilled water at a concentration of 0.1 mg/ml (spectrophotometric determinations). α7 cDNA or mixtures of α7 and β2 cDNA at 1:3 and 1:10 ratios were injected into the nuclei of oocytes in a volume of 18.4 nl/oocyte, using a Nanoject Automatic Oocyte Injector (Drummond, Broomall, PA). The total amount of cDNA injected per oocyte was kept constant at 2 ng. After injection, oocytes were incubated at 18 °C for 3–5 days in a modified Barth's solution containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.3 mM Ca $(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 15 mM Hepes and 5 mg/l neomycin (pH 7.6). Recordings were performed 3–5 days post-injection. Oocytes were placed in a 0.1 ml recording chamber and

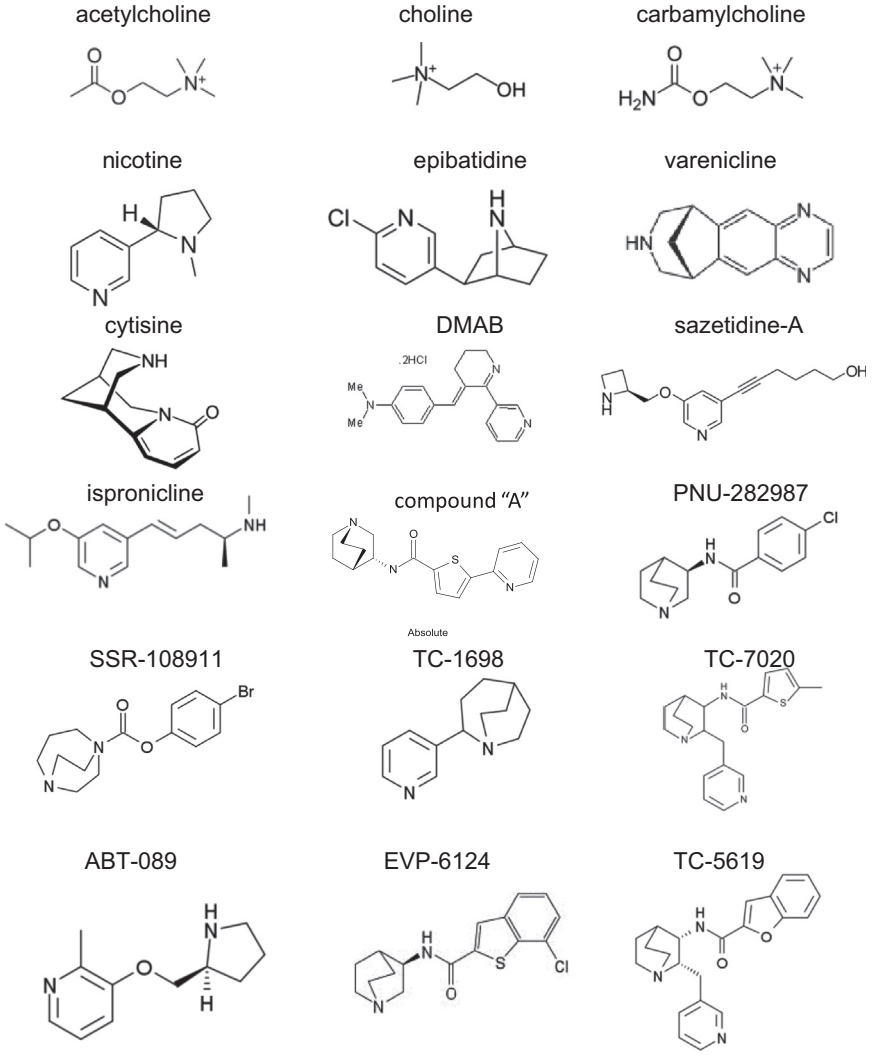

Fig. 1. Chemical structures of the nicotinic agonists used in this study. The chemical names of the agonists used are for **varenicline**: 7,8,9,10-Tetrahydro-6,10-methano-6*H*-pyrazino [2,3-h][3]benzazepine; **DMAB**: 4-[5,6-Dihydro[2,3′-bipyridin]-3(4*H*-ylidene)methyl]-*N*,*N*-imethylbenzenamine-dihydrochloride; **sazetidine A**: 6-[5-[(2*S*)-2-Azetidinylmethoxy]-3-pyridinyl]-5-hexyn-1-ol; **ispronicline**: (2*S*,4*E*)-5-(5-isopropoxypyridin-3-yl)-*N*-methylpent-4-en-2-amine; **compound "A"**: [R-N-(1-azabicyclo[2.2.2]oct-3-yl)(2-pyridyl)]thiophene-2-carboxamide; **PNU-282987**: N-[(3R)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide; **SSR-108911**: 1,4-Diazabicyclo[3.2.2]nonane-4-carboxylic acid, 4-bromophenyl ester; **TC-1698**: 2-pyridin-3-yl-1-azabicyclo[3.2.2]nonane; **TC-7020**: 5-methyl-*N*-[2-(pyridin-3-ylmethyl)-1-azabicyclo[2.2.2]oct-3-yl]thiophene-2-carboxamide; **ABT-089**: 2-methyl-3-([(2*S*)-pyrrolidin-2-yl]methoxy)pyridine; **EVP-6124**: (*R*)-7-chloro-*N*-(quinuclidin-3-yl)benzo[b]thiophene-2-carboxamide; and **TC-5619**: *N*-[(2*S*,3*S*)-2-(pyridin-3-ylmethyl)-1-azabicyclo [2.2.2]oct-3-yl]-1-benzofuran-2-carboxamide.