Neuropharmacology 62 (2012) 1099–1110

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

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

EVP-6124, a novel and selective α7 nicotinic acetylcholine receptor partial agonist, improves memory performance by potentiating the acetylcholine response of α7 nicotinic acetylcholine receptors

Jos Prickaerts [b,1], Nick P. van Goethem [b,1], Richard Chesworth [a,2], Gideon Shapiro [c], Frank G. Boess [d,3], Christoph Methfessel [e,4], Olga A.H. Reneerkens [b], Dorothy G. Flood [a], Dana Hilt [a], Maria Gawryl [a], Sonia Bertrand [f], Daniel Bertrand [f], Gerhard König [a,*]

[a] EnVivo Pharmaceuticals, Inc., 500 Arsenal Street, Watertown, MA 02472, USA
[b] Maastricht University, School for Mental Health and Neuroscience, 6200 MD Maastricht, The Netherlands
[c] Consultant to EnVivo Pharmaceuticals, Inc., Gainesville, FL, USA
[d] Bayer Healthcare AG, Pharma Research CNS, D-42096 Wuppertal, Germany
[e] Bayer Technology Services GmbH, D-51368 Leverkusen, Germany
[f] HiQScreen Sàrl, 15, rue de l'Athénée, 1211 Geneva 12, Switzerland

ARTICLE INFO

Article history:
Received 31 August 2011
Received in revised form
25 October 2011
Accepted 28 October 2011

Keywords:
α7 nAChR
Object recognition task
Memory
Partial agonist
Novel mechanism of action
Acetylcholine esterase inhibitor

ABSTRACT

EVP-6124, (*R*)-7-chloro-*N*-quinuclidin-3-yl)benzo[b]thiophene-2-carboxamide, is a novel partial agonist of α7 neuronal nicotinic acetylcholine receptors (nAChRs) that was evaluated here *in vitro* and *in vivo*. In binding and functional experiments, EVP-6124 showed selectivity for α7 nAChRs and did not activate or inhibit heteromeric α4β2 nAChRs. EVP-6124 had good brain penetration and an adequate exposure time. EVP-6124 (0.3 mg/kg, p.o.) significantly restored memory function in scopolamine-treated rats (0.1 mg/kg, i.p.) in an object recognition task (ORT). Although donepezil at 0.1 mg/kg, p.o. or EVP-6124 at 0.03 mg/kg, p.o. did not improve memory in this task, co-administration of these sub-efficacious doses fully restored memory. In a natural forgetting test, an ORT with a 24 h retention time, EVP-6124 improved memory at 0.3 mg/kg, p.o. This improvement was blocked by the selective α7 nAChR antagonist methyllycaconitine (0.3 mg/kg, i.p. or 10 μg, i.c.v.). In co-application experiments of EVP-6124 with acetylcholine, sustained exposure to EVP-6124 in functional investigations in oocytes caused desensitization at concentrations greater than 3 nM, while lower concentrations (0.3–1 nM) caused an increase in the acetylcholine-evoked response. These actions were interpreted as representing a co-agonist activity

Abbreviations: A-582941, 2-methyl-5-(6-phenyl-pyridazin-3-yl)-octahydro-pyrrolo[3,4-c]pyrrole; $A\beta_{1-40}$, amyloid peptide; ABBF, *N*-[(3*R*)-1-azabicyclo[2.2.2]oct-3-yl]-7-[2-(methoxy)phenyl]-1-benzofuran-2-carboxamide; ABT-107, 5-(6-[(3*R*)-1-azabicyclo[2,2,2]oct-3-yloxy]pyridazin-3-yl)-1*H*-indole; ACh, acetylcholine; AChEI, acetylcholine esterase inhibitor; AD, Alzheimer's disease; AR-R17779, (−)-spiro[1-azabicyclo[2.2.2]octane-3,5′-oxazolidin-2′-one]; BSA, bovine serum albumin; AZD0328, (2′*R*)-spiro-[1-azabicyclo[2.2.2]octane-3,2′(3′H)-furo[2,3-b]pyridine] D-tartrate; B:P, brain to plasma; CREB, cAMP response element-binding protein; d2, discrimination during trial 2; ERK, extracellular-signal regulated kinase; EVP-6124, (*R*)-7-chloro-*N*-quinuclidin-3-yl)benzo[b]thiophene-2-carboxamide; fu, fraction unbound; GABA, γ-aminobutyric acid; GR65630, 3-(5-methyl-1*H*-imidazol-4-yl)-1-(1-methyl-1*H*-indol-3-yl)-1-propanone; GTS-21, 3-(2,4-dimethoxybenzylidene)anabaseine, (DMXB); 5-HT, 5-hydroxytryptamine; HTS, high-throughput screening; i.c.v., intracerebroventricular; LC-MS/MS, liquid chromatography and tandem mass spectrometry; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor; NMDA, *N*-methyl-D-aspartate; ORT, object recognition task; PBS, phosphate buffered saline; PC, sample concentration in protein-containing side; PF, sample concentration in protein free-side; PHA-543613, *N*-[(3*R*)-1-azabicyclo[2.2.2]oct-3-yl]furo[2,3-c]pyridine-5-carboxamide; PNU-282987, *N*-[(3*R*)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride; RG3487, *N*-[(3*S*)-1-azabicyclo[2.2.2]oct-3-yl}-1*H*-indazole-3-carboxamide hydrochloride; RS-102221, *N*-{5-[5-(2,4-dioxo-1,3,8-triazaspiro[4.5]dec-8-yl)pentanoyl]-2,4-dimethoxyphenyl}-4-(trifluoromethyl)benzenesulfonamide; S 24795, 2-[2-(4-bromophenyl)-2-oxoethyl]-1-methyl pyridinium; SSR180711, 1,4-diazabicyclo(3.2.2)nonane-4-carboxylic acid, 4-bromophenyl ester; T1, trial 1; T2, trial 2; TC-5619, *N*-[2-(pyridin-3-ylmethyl)-1-azabicyclo[2.2.2]oct-3-yl]-1-benzofuran-2-carboxamide; T_{max}, time of maximal concentration.

* Corresponding author. Tel.: +1 617 225 4211; fax: +1 617 225 4267.
E-mail addresses: jos.prickaerts@maastrichtuniversity.nl (J. Prickaerts), n.vangoethem@maastrichtuniversity.nl (N.P. van Goethem), Rchesworth@epizyme.com (R. Chesworth), gshapiro@envivopharma.com (G. Shapiro), Frank.Boess@t-online.de (F.G. Boess), chrismet@t-online.de (C. Methfessel), o.reneerkens@maastrichtuniversity.nl (O.A.H. Reneerkens), dflood@envivopharma.com (D.G. Flood), dhilt@envivopharma.com (D. Hilt), mgawryl@envivopharma.com (M. Gawryl), sonia.bertrand@hiqscreen.com (S. Bertrand), daniel.bertrand@hiqscreen.com (D. Bertrand), gkoenig@envivopharma.com (G. König).
[1] These authors contributed equally to this work.
[2] Present address: Epizyme, Inc., 325 Vassar St., Suite 2B, Cambridge, MA 02139, USA.
[3] Present address: Lilly Germany, Medical Department, Werner-Reimers-Str. 2-4, 61352 Bad Homburg, Germany.
[4] Retired.

0028-3908/$ – see front matter
doi:10.1016/j.neuropharm.2011.10.024

of EVP-6124 with acetylcholine on α7 nAChRs. The concentrations of EVP-6124 that resulted in physiological potentiation were consistent with the free drug concentrations in brain that improved memory performance in the ORT. These data suggest that the selective partial agonist EVP-6124 improves memory performance by potentiating the acetylcholine response of α7 nAChRs and support new therapeutic strategies for the treatment of cognitive impairment.

This article is part of a Special Issue entitled 'Post-Traumatic Stress Disorder'.

1. Introduction

Nicotine has been shown to improve attention, learning, and memory through interaction with neuronal nAChRs (Levin et al., 2006). Several subtypes of nAChRs are expressed in the mammalian brain, each of them displaying distinct physiological and pharmacological properties. Functional nAChRs are assembled from five subunits around an axis of pseudosymmetry and can be composed of identical subunits (homopentamers) or different subunits (heteropentamers) (Dani and Bertrand, 2007). The α4 and β2 subunits are thought to form high affinity brain nAChRs, whereas the homopentameric α7 nAChR, which is expressed throughout the entire central nervous system, is less sensitive to ACh and nicotine (Albuquerque et al., 2009; Dani and Bertrand, 2007).

The α7 nAChRs are highly expressed in the hippocampus, a brain region that is very important for the formation of several types of memory. Hippocampal LTP, the sustained increase in the efficiency of synaptic transmission that is induced by multiple high frequency trains of electrical stimulation, is a potential cellular mechanism for learning and memory. In rat and mouse hippocampal slices, the partial α7 nAChR agonists GTS-21, SSR180711, and S 24795 improved LTP (Biton et al., 2007; Hunter et al., 1994; Lagostena et al., 2008). These effects were blocked by co-application of the α7 nAChR antagonist MLA and were absent in α7 nAChR knock-out mice (Lagostena et al., 2008). Furthermore, activation of the MAPK signaling pathway, with phosphorylation of ERK and CREB, is linked to the establishment of LTP and the formation of long-term memory. The α7 nAChR agonists A-582941 and ABT-107 increased phosphorylation of ERK and CREB in the mouse brain (Bitner et al., 2007, 2010).

Agonists of α7 nAChRs improved performance in learning and memory tasks (for review, see Kem, 2000). GTS-21 improved inhibitory avoidance responding, one-way active avoidance, as well as performance in the Lashley III maze and the 17-arm radial maze in rats (Arendash et al., 1995; Meyer et al., 1994). In addition, GTS-21 facilitated performance in a delayed-matching-to-sample test in monkeys (Briggs et al., 1997). In clinical trials with healthy volunteers, GTS-21 improved attention, working memory, and episodic memory (Kitagawa et al., 2003). In a randomized double-blind crossover trial in nonsmoking subjects with schizophrenia, stably treated with antipsychotics, GTS-21 caused significant cognitive improvement on the Repeatable Battery for the Assessment of Neuropsychological Status total scale (Olincy et al., 2006). GTS-21 is a weak partial agonist of human α7 nAChRs and inhibits α4β2 nAChRs and 5-HT_3 receptors (Briggs et al., 1997). The more selective α7 nAChR agonist, AR-R17779, improved long-term win-shift acquisition in the eight-arm radial maze and social recognition memory in rats (Levin et al., 1999; van Kampen et al., 2004). Improvements in social recognition, object recognition, and water maze performance were observed with several quinuclidine amide α7 nAChR agonists (Boess et al., 2007; Hauser et al., 2009; Wallace et al., 2011; Wishka et al., 2006), as well as with a number of other α7 nAChR agonists, including other types of quinuclidines (Bitner et al., 2010; Feuerbach et al., 2009; Pichat et al., 2007; Sydserff et al., 2009) and novel, structurally unrelated compounds (Bitner et al., 2007; Briggs et al., 2009; Roncarati et al., 2009). These cognitive enhancing effects were blocked by MLA (Boess et al., 2007; Pichat et al., 2007; van Kampen et al., 2004; Wallace et al., 2011), but were maintained by the co-infusion of donepezil, an AChEI (Bitner et al., 2010).

In this work, we examined the physiological and pharmacological properties of a novel and selective quinuclidine amide α7 nAChR agonist, EVP-6124, and its effects on memory in the ORT. Memory loss was either pharmacologically induced (i.e. disruption of the cholinergic system by administration of the muscarinic antagonist scopolamine) or was natural (i.e. a 24 h retention interval). Furthermore, EVP-6124 was co-administered with MLA (i.p. or i.c.v.) to investigate whether the pro-cognitive effects of EVP-6124 could be antagonized. Since α7 nAChR agonists have been suggested for the treatment of AD, and AD patients are often treated with an AChEI, the potential beneficial interaction between AChEIs and EVP-6124 was investigated in the present study at the behavioral level in rodents and at the electrophysiological level in oocytes expressing human α7 nAChRs.

2. Material and methods

2.1. Reagents

EVP-6124 was synthesized by Bayer Healthcare AG (Wuppertal-Elberfeld, Germany) and Ricerca Biosciences (Concord, OH). MLA and scopolamine hydrobromide were obtained from Research Biochemicals International/Sigma-Aldrich (Deisenhofen, Germany). Donepezil was a generous gift from Solvay Pharmaceuticals (Weesp, The Netherlands). Reagents for binding and electrophysiological studies were from Sigma-Aldrich. Reagents used in pharmacokinetic studies were from VWR International, Ltd. (Poole, Dorset, UK).

2.2. Animals

All animal experiments were approved by local ethical committees, followed the principles of laboratory animal care, and were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), or the Guide for the Care and Use of Laboratory Animals from the U.S. Department of the Health and Human Services. All efforts were made to minimize animal suffering, to reduce the number of animals used, and to use alternatives to *in vivo* methods where possible.

2.3. Selectivity profiling

Binding or activity of EVP-6124 was measured at 10 μM in a selectivity panel according to standard validated protocols under conditions defined by the contractor (MDS Pharma Services, Taipei, Taiwan; http://www.mdsps.com). Reference standards were run as an integral part of each assay to ensure the validity of the results.

For the 5-HT_{2A} receptor binding assay, membranes were prepared from HEK293 cells expressing the human recombinant 5-HT_{2A} receptor. For 5-HT_{2B} and 5-HT_{2C} receptor binding assays, membranes were prepared from CHO cells expressing the human recombinant 5-HT_{2B} or 5-HT_{2C} receptor. Affinity was determined by incubating different concentrations of EVP-6124 in binding buffer for 1 h. For 5-HT_{2A} binding, the incubation was at 22 °C in the presence of 0.5 nM [^{3}H]-ketanserin; for 5-HT_{2B}, at 22 °C in the presence of 2 nM [^{3}H]-mesulergine; and for 5-HT_{2C}, at 37 °C in the presence of 1 nM [^{3}H]-mesulergine. Nonspecific binding was determined in the presence of 1 μM ketanserin, 10 μM mesulergine, or 10 μM RS-102221 for 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C}, respectively. All measurements were performed in triplicate. EVP-6124 was also tested in the 5-HT_{2B} rat gastric fundus tissue response assay according to standard protocols under conditions defined by the contractor (MDS Pharma Services). Briefly, inhibition of α-methyl serotonin-induced contraction was isometrically measured. All measurements were performed in duplicate.