The International Journal of Biochemistry & Cell Biology 76 (2016) 19–30

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

Positive allosteric modulators of α7 nicotinic acetylcholine receptors affect neither the function of other ligand- and voltage-gated ion channels and acetylcholinesterase, nor β-amyloid content

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ARTICLE INFO

Article history:
Received 23 March 2016
Received in revised form 22 April 2016
Accepted 25 April 2016
Available online 26 April 2016

Keywords:
α7 Nicotinic acetylcholine receptor positive allosteric modulators
Serotonin type 3 and glutamate receptors
Acetylcholinesterase
β-Amyloid
Molecular docking and molecular dynamics

ABSTRACT

The activity of positive allosteric modulators (PAMs) of α7 nicotinic acetylcholine receptors (AChRs), including 3-furan-2-yl-*N*-*p*-tolyl-acrylamide (PAM-2), 3-furan-2-yl-*N*-*o*-tolylacrylamide (PAM-3), and 3-furan-2-yl-*N*-phenylacrylamide (PAM-4), was tested on a variety of ligand- [i.e., human (h) α7, rat (r) α9α10, hα3-containing AChRs, mouse (m) 5-HT_{3A}R, and several glutamate receptors (GluRs)] and voltage-gated (i.e., sodium and potassium) ion channels, as well as on acetylcholinesterase (AChE) and β-amyloid (Aβ) content. The functional results indicate that PAM-2 inhibits hα3-containing AChRs (IC_{50} = 26 ± 6 μM) with higher potency than that for NR1aNR2B and NR1aNR2A, two NMDA-sensitive GluRs. PAM-2 affects neither the activity of m5-HT_{3A}Rs, GluR5/KA2 (a kainate-sensitive GluR), nor AChE, and PAM-4 does not affect agonist-activated rα9α10 AChRs. Relevant clinical concentrations of PAM-2–4 do not inhibit Na_v1.2 and K_v3.1 ion channels. These PAMs slightly enhance the activity of GluR1 and GluR2, two AMPA-sensitive GluRs. PAM-2 does not change the levels of $A_{β42}$ in an Alzheimer's disease mouse model (i.e., 5XFAD). The molecular docking and dynamics results using the hα7 model suggest that the active sites for PAM-2 include the intrasubunit (i.e., PNU-120596 locus) and intersubunit sites. These results support our previous study showing that these PAMs are selective for the α7 AChR, and clarify that the procognitive/promnesic/antidepressant activity of PAM-2 is not mediated by other targets.

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); m5-HT3AR, murine serotonin type 3A receptor; AChR, nicotinic acetylcholine receptor; ACh, acetylcholine; NMDAR, *N*-methyl-*D*-aspartate receptor; AMPAR, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; NIC, nicotine; MLA, methyllycaconitine; ACTh, acetyltiocholine; AChE, acetylcholinesterase; ECD, extracellular domain; TMD, transmembrane domain; PAM, positive allosteric modulator; PAM-2, 3-furan-2-yl-*N*-*p*-tolyl-acrylamide; PAM-3, 3-furan-2-yl-*N*-*o*-tolylacrylamide; PAM-4, 3-furan-2-yl-*N*-phenylacrylamide; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-*D*-aspartic acid; KA, kainate; Nav1.2, voltage-gated sodium channel type 1.2; Kv3.1, voltage-gated potassium channel type 3.1; RT, room temperature; Vmax, maximum rate of enzymatic reaction; Km, Michaelis constant; IC50, ligand concentration that produces 50% inhibition; Ki, inhibition constant; nH, Hill coefficient; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum.

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http://dx.doi.org/10.1016/j.biocel.2016.04.015
1357-2725/

1. Introduction

In recent years, many agonists, antagonists, and positive allosteric modulators (PAMs) with selectivity for the α7 nicotinic acetylcholine receptor (AChR) have been recently studied as potential treatments for different neurological disorders (reviewed in Arias, 2010; Malysz et al., 2009; Chatzidaki and Millar, 2015). In particular, PAMs have generated a lot of expectation since these compounds can potentiate the α7 AChR activity elicited by endogenous neurotransmitters, ACh and choline, potentially producing fewer side effects than exogenous agonists. In previous studies, the activity of three α7-PAMs (PAM-2–4) was characterized (Arias et al., 2011; Targowska-Duda et al., 2014; Andersen et al., 2016). These PAMs enhance agonist-induced α7 AChR activity (Arias et al., 2011) and reactivate desensitized α7 AChRs (Targowska-Duda et al., 2014) supporting a type II PAM classification. Additional microscopic current results showed that these PAMs behave as type I PAM at 22 °C, whereas this activity decreased at higher temperatures (i.e., 34 °C), coincident with type II PAMs (Andersen et al., 2016). In conclusion, PAM-2–4, present pharmacological properties of both type I and type II PAMs, that make them different to other α7 PAMs.

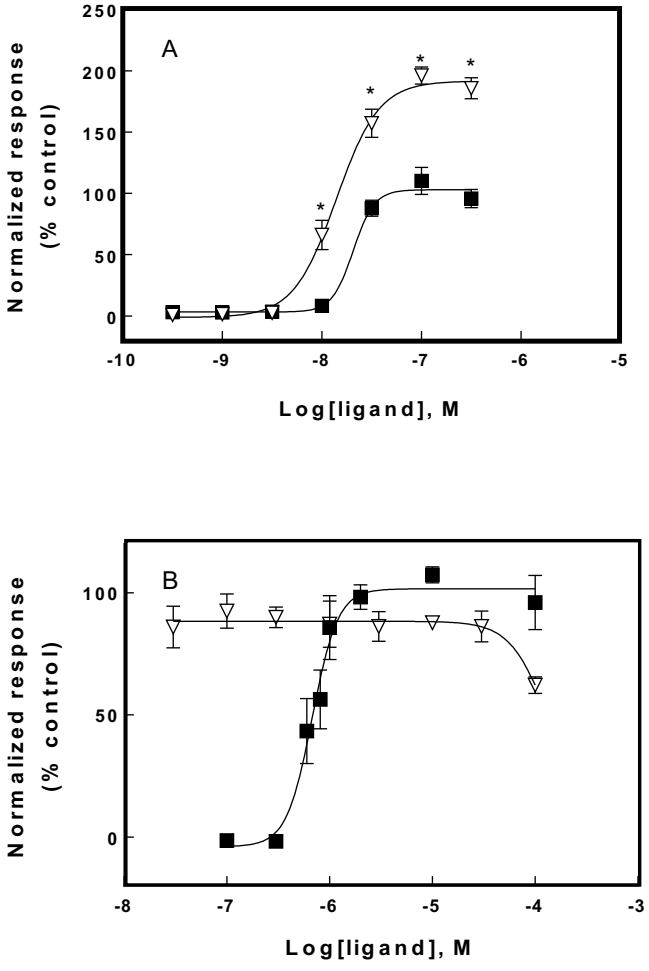

Fig. 1. Effect of PAM-2 on agonist-induced Ca^{2+} influx in CH3-hα7 and N1E115-m5-HT_3 cells. (A) Increased concentrations of (±)-epibatidine (■) activate hα7 AChRs with potency EC_{50} = 52 ± 4 nM (n = 28). PAM-2 (10 μM) (▽) increases (±)-epibatidine-induced hα7 AChR activity with apparent EC_{50} = 17 ± 5 nM and E_{max} = 190 ± 5% (n = 3). Statistical analysis was performed by a paired *t*-test with Sidak-Bonferroni correction for repeated measurements. The results indicated that both curves are different (P < 0.02) in the concentration range labeled with *. (B) Increased concentrations of 5-HT (■) activate m5-HT_3Rs with EC_{50} = 0.55 ± 0.03 μM (n = 47). Subsequently, cells were pre-treated with several concentrations of PAM-2 (▽) followed by addition of 1 μM 5-HT (n = 3). Agonist responses were normalized to the maximal response which was set as 100%. Each data point is the mean ± SEM. The apparent EC_{50}, E_{max}, and n_H values are summarized in Table 1.

To complete the initial studies on receptor selectivity performed on several human (h) AChR subtypes (Arias et al., 2011, 2015a), the activity of PAM-2 was tested on other members of the Cys-loop family, including rat (r)α9α10 and hα3-containing AChRs as well as mouse (m)5-HT_{3A}Rs. We also determined whether PAM-2 can modulate choline-evoked α7 nAChR currents when low concentrations of choline are used.

Since PAM-2 has procognitive (Potasiewicz et al., 2015) and promnesic (Targowska-Duda et al., 2016) activities, and glutamate receptors (GluRs) are important for learning and memory processes (Gécz, 2010; Collingridge et al., 2013), the activity of PAM-2 was determined on several GluR subtypes, including those sensitive to *N*-methyl-D-aspartate (NMDA) (i.e., hNR1aNR2A and hNR1aNR2B), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (i.e., GluR1 and GluR2), and kainate (KA) (i.e., GluR5/KA2).

Since $Na_v1.2$ (Ahn et al., 2007) and $K_v3.1$ (Perney et al., 1992) channels are found in the hippocampus, and this brain area is considered very important for cognitive and memory functions, the activity of these PAMs was tested on these channels.

Taking into account that some acetylcholinesterase (AChE) inhibitors used in the treatment of Alzheimer's disease (i.e., tacrine and galantamine) are also PAMs at different AChRs (Storch et al., 1995; reviewed in Arias, 2010, 2011), the activity of PAM-2 was also tested on the AChE. Since the chronic application of galantamine reduces the plaque density in 5XFAD transgenic mice (Bhattacharya et al., 2014), an Alzheimer's disease animal model (Oakley et al., 2006), the effect of PAM-2 on brain levels of $A_{\beta 42}$ (i.e., active species) was also determined in the same transgenic mice.

Our previous molecular docking results suggest that PAM-2–4 may bind to either the extracellular domain (ECD), transmembrane domain (TMD), and/or the ECD-TMD junction (Arias et al., 2011). To determine what domain is the most important, PAM-2 activity was correlated with the structural components (i.e., determined by molecular docking and molecular dynamics) of the binding sites at the hα7 AChR and mouse (m)5-HT_{3A}Rs. Based on our study, PAM-2–4 are selective for the hα7 AChR, where they bind to the TMD at the intrasubunit (i.e., the PNU-120596 locus) and intersubunit sites.

2. Material and methods

2.1. Materials

(±)-Epibatidine hydrochloride and serotonin hydrochloride (5-HT) were obtained from Tocris Bioscience (Ellisville, Missouri, USA). Fetal bovine serum (FBS) and trypsin/EDTA were purchased form Gibco BRL (Paisley, UK). Acetylthiocholine (ACTh), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), acetylcholinesterase from *Electrophorus electricus*, poly-ornithine coated 24-well dishes, methyllycaconitine (MLA), (−)-nicotine hemisulfate (NIC), *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate (KA), glycine hydrochloride, choline chloride, PEG200, and acetylcholine chloride (ACh) were obtained from Sigma-Aldrich (St. Louis, MO, USA), whereas probenecid was obtained from Sigma-Aldrich (Buchs, Switzerland). Trypsin–EDTA was obtained from Euroclone (Milan, Italy). Dulbecco's Modified Eagle Medium (DMEM) was obtained from Seromed (Biochrom, Berlin, Germany), and Fluo-4 was purchased from Molecular Probes (Eugene, Oregon, USA). PAM-2–4 were synthesized as described previously (Arias et al., 2011). Salts, solvents, and reagents were purchased from commercial suppliers and used as received.