Neuropharmacology 56 (2009) 821–830

Contents lists available at ScienceDirect

Neuropharmacology

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

Positive modulation of α7 nAChR responses in rat hippocampal interneurons to full agonists and the α7-selective partial agonists, 4OH-GTS-21 and S 24795

Gretchen Y. López-Hernández [a,*], Jeffrey S. Thinschmidt [a], Philippe Morain [b], Caryn Trocme-Thibierge [b], William R. Kem [a], Ferenc Soti [a], Roger L. Papke [a]

[a] Department of Pharmacology and Therapeutics, University of Florida, College of Medicine, Gainesville, FL, USA
[b] Institut De Recherches Internationales Servier, 6 Place des Pléaides, 92415 Courbevoie Cedex, France

ARTICLE INFO

Article history:
Received 20 May 2008
Received in revised form
24 December 2008
Accepted 12 January 2009

This paper is dedicated to the late Dr. Philippe Morain, whose knowledge of the field and enthusiasm for research was, and will continue to be, an inspiration to all of his co-authors and colleagues.

Keywords:
Brain slices
Patch-clamp
nAChRs
Partial agonists
Full agonists
Hippocampus
Positive allosteric modulators

ABSTRACT

One approach for the identification of therapeutic agents for Alzheimer's disease has focused on the research of α7 nAChR-selective agonists such as the partial agonists 3-(4-hydroxy,2-methoxybenzylidene)anabaseine (4OH-GTS-21) and, more recently, 2-[2-(4-bromophenyl)-2-oxoethyl]-1-methyl pyridinium (S 24795). An alternative approach for targeting α7 nAChR has been the development of positive modulators for this receptor. In this study we examined the interactions between full or partial agonists and positive modulators of α7 nAChRs *in situ* in brain tissue. Three positive modulators were used, 5-hydroxyindole (5-HI), 1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxanol-3-yl)-urea (PNU-120596), and genistein. Whole-cell recordings were performed in stratum radiatum interneurons from rat brain slices. Hippocampal interneurons were stimulated by ACh, choline, S 24795, or 4OH-GTS-21, before and after bath perfusion with the positive modulators. 5-HI was not effective at potentiating 200 μM 4OH-GTS-21-evoked responses, however 5-HI induced a sustained potentiation of responses evoked by 30 μM 4OH-GTS-21. When 1 mM ACh and 200 μM 4OH-GTS-21 were applied alternately α7-mediated responses to both agonists were reduced, suggesting that high concentration of 4OH-GTS-21 produces residual inhibition or desensitization and that 5-HI is not effective at overcoming receptor desensitization. Similar results were obtained with α7 receptors expressed in *Xenopus* oocytes. Interestingly, responses evoked by S 24795 were potentiated by 5-HI but not by genistein. Additionally, PNU-120596 was able to potentiate α7-mediated responses, regardless of the nature of the agonist. We demonstrated that the potentiation of α7 nAChR response would depend on the nature and the effective concentration of the agonist involved and its particular interaction with the positive modulator.

1. Introduction

The α-bungarotoxin-sensitive, α7 nicotinic acetylcholine receptors (nAChRs) are highly expressed in hypothalamus, cortex, and hippocampus (DelToro et al., 1994; Seguela et al., 1993). Brain α7 nAChRs are homomeric receptors displaying very rapid desensitization to high concentrations of agonist, high levels of calcium permeability, and activation by choline (Castro and Albuquerque, 1995; Papke et al., 1996; Peng et al., 1994; Seguela et al., 1993).

The function and number of α7 receptors can be affected in several pathological conditions, such as schizophrenia, Alzheimer's disease (AD), and Parkinson's disease (Burghaus et al., 2003; Freedman et al., 1995; Guan et al., 1999, 2000). AD is characterized by loss of cholinergic projections; therefore drugs that act on or modulate nAChRs are likely to have great therapeutic potential. It has been shown that both nAChR agonists and partial agonists can enhance cognition in aged rats (Arendash et al., 1995; Socci et al., 1995). An alternative approach for targeting α7 receptors is the use of positive allosteric modulators (PAMs) that potentiate receptor function without causing direct activation.

Several positive modulators of α7 nAChRs have been documented, including ivermectin (Krause et al., 1998), 5-hydroxyindole (5-HI) (Grilli et al., 2006; Gurley et al., 2000; Mannaioni et al., 2003; Mok and Kew, 2006; Zwart et al., 2002), 1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea (PNU-120596) (Hurst et al., 2005), genistein (Charpantier et al., 2005; Cho et al., 2005), and compound 6 (Ng et al., 2007). Emergence of distinct PAMs has prompted a proposed classification into two types, based on their pharmacological profiles (Grønlien et al., 2007). Both types of PAMs increase the potency and efficacy of agonists. The effects of

* Corresponding author. Department of Pharmacology and Therapeutics, University of Florida, P.O. Box 100267, Gainesville, FL 32610-0267, USA. Tel.: +1 352 392 3257; fax: +1 352 392 9696.
E-mail address: gylopez@ufl.edu (G.Y. López-Hernández).

0028-3908/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuropharm.2009.01.011

Type I, exemplified by 5-HI and genistein, are mostly observed in the agonist-evoked peak amplitude. Type II prolongs the agonist-evoked response in addition to increase peak amplitude. PNU-120596, the prototypical Type II PAM, has the apparent ability to reactivate receptors that are in a desensitized state (Hurst et al., 2005), while 5-HI potentiation was occluded by pretreatment with desensitizing concentrations of nicotine (Mok and Kew, 2006).

We performed whole-cell patch-clamp recordings in stratum radiatum interneurons from hippocampal rat brain slices, which robustly express functional α7 nAChRs (Alkondon et al., 1998; Frazier et al., 1998; Jones and Yakel, 1997), to examine the effect of 5-HI, PNU-120596, and genistein on the evoked responses by ACh and choline. Also to determine potential interactions that might occur in combinational therapies we investigated the effects of these modulators on the activity of two partial agonists proposed for clinical development, 3-(4-hydroxy,2-methoxybenzylidene)anabaseine (4OH-GTS-21) and 2-[2-(4-bromophenyl)-2-oxoethyl]-1-methyl pyridinium (S 24795). 4OH-GTS-21 is the active metabolite of GTS-21, one of the first α7-selective agonists to be identified (de Fiebre et al., 1995; Woodruff-Pak et al., 1994), while S 24795 is a second generation α7-selective partial agonist (López-Hernández et al., 2007). Through our studies we hope to provide new insights for the development of strategies to enhance nicotinic transmission in the brain and to better evaluate alternative approaches of using direct-acting agonists and/or positive modulators of receptor function.

2. Methods

2.1. Chemicals

4OH-GTS-21 was provided by Taiho Pharmaceuticals (Tokyo, Japan) and S 24795 by Servier (Paris, France). PNU-120596 was synthesized at the University of Florida and all other chemicals were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. cDNA clones

The human α7 nAChR clone was obtained from Dr. Jon Lindstrom (University of Pennsylvania, Philadelphia, PA). The RIC-3 clone was obtained from Dr. Millet Treinin (Hebrew University, Jerusalem, Israel).

2.3. Preparation of RNA

Subsequent to linearization and purification of cloned cDNAs, RNA transcripts were prepared *in vitro* using the appropriate mMessage mMachine kit from Ambion, Inc. (Austin, TX).

2.4. Expression *in* Xenopus laevis *oocytes*

Mature (>9 cm) female *X. laevis* African frogs (Nasco, Ft. Atkinson, WI) were used as a source of oocytes. Before surgery the frogs were anesthetized by placing them in a 1.5 g/l solution of MS222 for 30 min. Oocytes were removed from an incision made in the abdomen.

Harvested oocytes were treated with 1.25 mg/ml collagenase (Worthington Biochemical Corporation, Freehold, NJ) for 2 h at room temperature in calcium-free Barth's solution (88 mM NaCl, 1 mM KCl, 2.38 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 15 mM HEPES (pH 7.6), 12 mg/l tetracycline) in order to remove the follicular layer. Stage-5 oocytes were isolated and injected with 50 nl (5–20 ng) of α7 cRNA, usually in combination with human RIC-3 to accelerate and increase the level of nAChR expression (Halevi et al., 2003). Recordings were conducted 2–5 days post-injection.

2.5. Electrophysiology

Experiments were conducted using OpusXpress6000A (Molecular Devices, Union City, CA). OpusXpress is an integrated system that provides automated impalement and voltage clamp of up to eight oocytes in parallel. Both the voltage and the current electrodes were filled with 3 M KCl. The oocytes were clamped at a holding potential of −60 mV.

Data were collected at 50 Hz and filtered at 20 Hz. The oocytes were bath-perfused with Ringer's solution. Agonist solutions were delivered from a 96-well plate using disposable tips. Flow rates were set at 2 ml/min. Drug applications usually alternated between ACh controls and test solutions of ACh or other experimental agonists at varying concentrations. PAMs were applied in the bath solution and then co-applied with agonist during the evoked responses.

2.6. Experimental protocols and data analysis

Responses of α7 receptors were calculated as net charge (Papke and Papke, 2002). Each oocyte received two initial control applications of ACh, then experimental drug applications, and follow-up control applications of ACh. The control ACh concentration was 60 μM, a concentration which is sufficient to evoke approximately 50% maximal net charge response (Papke and Papke, 2002). The initial ACh control responses from each cell were used to normalize the data for all subsequent responses and compensate for differences in the levels of channel expression among the oocytes. Mean values and standard errors (SEM) were calculated from the normalized responses of at least four oocytes for each experimental concentration, except where otherwise noted. For concentration–response relations, data were plotted using Kaleidagraph 3.0.2 (Abelbeck Software; Reading, PA), and curves were generated from the Hill equation:

$$\text{Response} = \frac{I_{\max}[\text{agonist}]^n}{[\text{agonist}]^n + (\text{EC}_{50})^n}$$

$I_{\max}$ denotes the maximal response for a particular agonist/subunit combination, and n represents the Hill coefficient. $I_{\max}$, n, and the EC_{50} were all unconstrained for the fitting procedures.

2.7. Brain slice preparation and patch-clamp recording

All procedures involving animals were approved by the University of Florida Institutional Animal Care and Use Committee and were in accord with the NIH *Guide for the Care and Use of Laboratory Animals*. Male Sprague Dawley rats (p16–p30) were anesthetized with Halothane (Halocarbon Laboratories, River Edge, NJ) and swiftly decapitated. Transverse (300 μm) whole brain slices were prepared using a vibratome (Pelco, Redding, CA) and a high Mg^{2+}/low Ca^{2+} ice-cold artificial cerebral spinal fluid (ACSF) containing (in mM) 124 NaCl, 2.5 KCl, 1.2 NaH_2PO_4, 2.5 $MgSO_4$, 10 D-glucose, 1 $CaCl_2$, and 25.9 $NaHCO_3$, saturated with 95% O_2–5% CO_2. Slices were incubated at 30 °C for 30 min and then left at room temperature until they were transferred to a submersion chamber (Warner Instruments, Hamden, CT) for recording. During experiments, slices were perfused at a rate of 2 ml/min with normal ACSF containing (in mM) 126 NaCl, 3 KCl, 1.2 NaH_2PO_4, 1.5 $MgSO_4$, 11 D-glucose, 2.4 $CaCl_2$, 25.9 $NaHCO_3$, and 0.004 atropine sulfate, saturated with 95% O_2–5% CO_2 at 30 °C. Interneurons of the stratum radiatum were visualized with infrared differential interference contrast microscopy using a Nikon E600FN microscope. Patch-clamp recording pipettes were pulled from borosilicate glass (Sutter Instruments, Novato, CA) using a Flaming/Brown micropipette puller (P-97; Sutter Instruments, Novato, CA). Recording pipettes were filled with an internal solution of (in mM) 125 K-gluconate, 1 KCl, 0.1 $CaCl_2$, 2 $MgCl_2$, 1 EGTA, 2 MgATP, 0.3 Na_3GTP, and 10 HEPES (pH 7.3 using KOH). The resistance of the recording pipette when filled with the internal solution was 3–5 MΩ. Cells were held at −70 mV, and a −10 mV/10 ms test pulse was used to determine access resistance, input resistance, and whole-cell capacitance. Cells with access resistances > 60 MΩ or those requiring holding currents > 200 pA were not included in the final analyses. Signals were digitized using an Axon Digidata1322A and sampled at 20 kHz on a Dell computer using Clampex version 8 or 9. Data analysis was done with Clampfit version 8 or 9 (Axon Instruments, Union City, CA), Excel 2000 (Microsoft, Seattle, WA), GraphPad/Prism version 4.02 (GraphPad Software, San Diego, CA), and SigmaStat version 3.5 (Systat Software, Inc., San Jose, CA). Data are reported as mean ± SEM. Statistical analyses were done using two-tailed Student's *t*-test and one-way analysis of variance (ANOVA).

2.8. Drug application

Local somatic applications of ACh (1 mM pipette concentration), choline (2 mM pipette concentration), S 24595 (1 mM pipette concentration), and 4OH-GTS-21 (200 μM or 30 μM pipette concentration) were made using single- or double-barrel glass pipettes attached to a picospritzer (General Valve, Fairfield, NJ) with Teflon tubing (10–20 psi for 5–15 ms). ACh and either choline or S 24795 were alternately applied every 30 s, while ACh and 4OH-GTS-21 were alternately applied with an interstimulus interval of 1 min. Single-barrel pipettes were pulled from borosilicate glass with an outer diameter (o.d.) and inner diameter (i.d.) of 1.5 mm and 0.86 mm, respectively (Sutter Instruments, Novato, CA). Pipette opening size of the single barrel was typically 2–3 μm. Double-barrel pipettes were pulled from borosilicate theta glass with an o.d. of 1.5 mm; pipette opening size was around 3–4 μm. The application pipette was usually placed within 10–15 μm of the cell soma. For each cell, four baseline agonist-evoked responses were recorded followed by bath application of the positive modulator. 5-HI was bath-applied at a final concentration of 1 mM. PNU-120596 and genistein were bath-applied at a final concentration of 10 μM. Evoked responses were then recorded for 15–22 min. In some experiments 4OH-GTS-21 was bath-applied at a concentration of 1 μM.

When pipettes were loaded with 1 mM ACh, the average net charge of evoked responses did not differ significantly between single- and double-barrel