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Selective activation of α7 nicotinic acetylcholine receptors augments hippocampal oscillations

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ABSTRACT

Neural α7 nicotinic acetylcholine receptors (α7 nAChRs) emerged as a potential pharmacologic target for treating cognitive deficits in schizophrenia and Alzheimer's disease. Experiments modeling these dysfunctions, as well as clinical evidence, demonstrate the relatively consistent procognitive effects of α7 nAChR agonists. One preclinical observation supporting the procognitive role of α7 nAChRs is their ability to modulate neuronal network oscillations closely associated with learning and memory, especially hippocampal oscillations. Due to the high degree of structural similarity between α7 nACh and 5-HT receptors, the majority of α7 nAChR agonists to date also act as 5-HT_3 antagonists. To address this confounding property and determine the relevance of α7 nAChR agonist binding to 5-HT_3 receptors in modulating hippocampal activity, we tested two well-described α7 nAChR agonists, PNU-282987 and FRM-17874, in mice lacking α7 nAChRs (α7 knock-out, α7KO) using the brainstem simulation-elicited hippocampal theta oscillation assay. Under urethane anesthesia both agonists at equivalent doses demonstrated efficacy in wild-type (WT) mice, significantly enhancing theta power and theta phase-gamma amplitude coupling as compared to saline treated control mice. These effects are comparable to those seen with drugs clinically used to treat Alzheimer's disease. Although α7KO mice showed no alterations in elicited hippocampal oscillations, both α7 nAChR agonists failed to enhance theta power or theta phase – gamma amplitude coupling in these mice. Our findings demonstrate that selective activation of α7 nAChRs can modulate hippocampal oscillation, and these receptors are the primary targets of the tested agonists, PNU-282987 and FRM-17874 and likely underlies their observed procognitive activity.

1. Introduction

Converging preclinical and clinical evidence suggests that α7 nicotinic acetylcholine receptors (α7 nAChRs) could be a potential therapeutic target for cognitive deficits associated with schizophrenia and Alzheimer's disease. Accordingly, several α7 nAChR agonists have been synthesized and successfully tested in different cognitive-related animal models with some advancing to clinical trials (Bertrand et al., 2015; Hajós and Rogers, 2010; Wallace and Porter, 2011).

The α7 nAChRs are ligand-gated ion channels, predominantly formed as a homopentameric complexes and characterized by high permeability to calcium, fast activation, and rapid desensitization kinetics (Albuquerque et al., 2009; Dani and Bertrand, 2007). They have been detected at presynaptic, postsynaptic, and perisynaptic sites and are implicated in mediating various neurotransmitters release, synaptic transmission, and synaptic plasticity upon activation (Wonnacott et al., 2006). α7 nAChRs are widely distributed throughout the brain with particularly dense expression in regions implicated in cognition, attention, and memory formation such as the hippocampus, cortex, and subcortical limbic structures (Gotti et al., 2006). Therefore, agonists of α7 nAChRs have exhibited relatively consistent procognitive effects across species in different behavioral and neurophysiological assays dependent on hippocampal function (Hajós and Rogers, 2010; Levin, 2012; Thomsen et al., 2010). Importantly, however, the close structural homology between the α7 nAChRs and other ligand-gated ion channels such as 5-HT_3 receptors has proven a challenge in developing highly

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selective α7 nAChR compounds. In fact, most of the available α7 nAChR agonists to date, including those under clinical development such as encenicline and RG3487, also exhibit 5-HT_3 antagonist properties (Bertrand et al., 2015; Wallace and Porter, 2011). Interestingly, even though the 5-HT_3 receptors are known to modulate acetylcholine release (Huang et al., 2014) and to be widely expressed in the brain areas implicated in cognitive functions, preclinical and clinical studies have so far produced contradictory results regarding the procognitive-like effects of 5-HT_3 antagonists (reviewed in Thompson and Lummis, 2007; Walstab et al., 2010).

It has been shown that activation of α7 nAChRs can modulate neuronal oscillatory activity (Hajós et al., 2005; Siok et al., 2006), and we previously demonstrated that α7 nAChR agonists can enhance theta power and theta phase – gamma amplitude coupling during brainstem stimulation-elicited hippocampal oscillations in rodents (Stoiljkovic et al., 2015a). Hippocampal theta activity is closely associated with hippocampal-dependent cognitive processes (Buzsáki, 2002), and clinically effective drug treatments for cognitive symptoms of Alzheimer's disease, such as donepezil and memantine, increase the power of elicited hippocampal theta oscillation (Guadagna et al., 2012; Kinney et al., 1999). However, considering the aforementioned cross-reactivity of α7 nAChR agonists with serotonin (5-HT) receptors, which have been shown to influence hippocampal oscillations (Hajós et al., 2003; Ly et al., 2013; Olvera-Cortés et al., 2013; Vertes and Kocsis, 1997), the role of these receptors in the cognitive or behavioral effects of α7 nAChR agonists has not been totally ruled out. Even though there are highly potent and selective α7 nAChR antagonists which could be used *in vitro* (α-bungarotoxin, methyllycaconitine), none are particularly suitable for *in vivo* applications to selectively antagonize α7 nAChRs. As an alternative approach, activity of agonists could be tested in α7 nAChR knock-out (α7KO) mice. In our previous studies using α7KO mice we reported that elicited hippocampal theta oscillation and theta-gamma coupling are comparable to their wild-type (WT) littermates (Stoiljkovic et al., 2015a). Therefore, the effects of two well-described α7 nAChR agonists, PNU-282987 (*N*-[(3*R*)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide) and FRM-17874 ((R)-7-fluoro-N-quinuclidin-3-yl)benzo[b]thiophene-2-carboxamide), were tested in α7KO mice. Both compounds show efficacy in this neurophysiological assay in rats and mice (Siok et al., 2006; Stoiljkovic et al., 2015a,b). Therefore, stimulation-induced hippocampal theta oscillation seemed an optimal assay for determining if modulation of theta oscillation by these agonists are mediated exclusively by α7 nAChRs.

2. Methods

2.1. Animals

All procedures were performed according to the protocol reviewed and approved by the Yale University Institutional Animal Care and Use Committee and in compliance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, revised 1996). Animals were housed in a controlled temperature and humidity environment on a 12-h light/dark cycle with food and water available *ad libitum*. Measures were taken to minimize suffering and to reduce the number of animals used.

Adult, male α7KO mice (B6.129S7-Chrna7^{tm1Bay}/J), and their WT littermates, obtained from the Jackson Laboratory (Bar Harbor, ME) were used in the study. The α7KO mice are homozygous for the α7nAChR null mutation, achieved by deleting the last three exons of the Chrna7 gene (Orr-Urtreger et al., 1997). They were backcrossed for a minimum of 10 generations onto their background strain (C57BL/6J) before being used.

2.2. Drugs and treatment

FRM-17874 is a newly synthesized and characterized compound by Forum Pharmaceuticals (Waltham, MA) (for details see Stoiljkovic et al., 2015b) and PNU-282987 was obtained from Tocris Bioscience (Bristol, UK). Both drugs were dissolved in sterile saline and administered subcutaneously (sc) in an injection volume of 10 ml/kg, as free base equivalents. A dose of 3 mg/kg for each compound was chosen based on previous findings (Stoiljkovic et al., 2015b; Vicens et al., 2011).

2.3. Electrophysiological recordings

Hippocampal oscillations in anesthetized mice were elicited by brainstem stimulation as described previously (Scott et al., 2012). In short, mice were anesthetized with 1.5 g/kg urethane intraperitoneally (ip), and placed in a Kopf stereotaxic frame (Tujunga, CA) on a temperature-regulated heating pad (Physitemp Instruments Inc., Clifton, NJ) set to maintain body temperature at 37–38 °C. After surgical preparation of the animal, two concentric stainless steel bipolar electrodes (NE-100X, Rhodes Medical Instruments, Woodland Hills, CA) were placed in the brain, one in the left hippocampal CA1 region for recording of local field potentials (LFPs) and the other in the nucleus pontis oralis (nPO) for electrical stimulation. Stereotaxic coordinates for each target area were referenced relative to bregma and brain surface and were as follows: CA1, anteroposterior −2.0 mm, lateral 1.5 mm, and dorsoventral 1.5 mm; nPO, anteroposterior −4.0 mm, lateral 1.2 mm, and dorsoventral 3.3 mm (Franklin and Paxinos, 2007). An ear bar of the stereotaxic frame served as ground. Each recording began 30 min following placement of the electrodes. Animals were left in the stereotaxic frame throughout the experiments and their level of anesthesia was checked regularly. The stimulus paradigm consisted of a train of 0.3 msec square pulses delivered at 250 Hz over 6 s, repeated every 100 s and was provided by Isoflex stimulus-isolator (A.M.P.I. Instruments, Jerusalem, Israel). The stimulating current was determined in each individual animal by repeating 3 cycles of increasing currents from 0.0 to 0.1 mA in a stepwise fashion in order to establish a stimulus-response relationship for both peak theta frequency and total theta power. Current inducing theta oscillation between 4 and 6 Hz frequency with an absolute power between 60 and 80% of the maximal response was selected and then held constant for the duration of the experiment. After establishing a stable baseline (about 60 min from the beginning of recording), saline or drug was administered, and recording continued for another 60 min. LFPs were amplified using Grass P55 AC differential amplifier (Grass Technologies, West Warwick, RI, USA) with filters set between 0.3 and 300 Hz. The signal was continuously monitored, digitized at a rate of 1 kHz, and stored on a computer via a CED Micro1401-3 interface and Spike2 software (Cambridge Electronic Design, Cambridge, UK).

At the end of each recording animals were euthanized, and the brains rapidly removed, hemisected and frozen either for histological verification of electrodes placement or for drug exposure analysis. Brain concentrations of the drugs were determined by liquid chromatography with tandem mass spectrometry as described previously (Tang et al., 2014).

2.4. Data analysis

All analyses were performed with custom, built-in, and open source scripts written in Matlab (Mathworks, Natick, MA). All off-line filtering of LFP data was done with *eegfilt.m* from the EEGLAB toolbox (Delorme and Makeig, 2004). The first second of each 6 s long stimulation episode was omitted in all analyses to avoid