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Tropisetron sensitizes α7 containing nicotinic receptors to low levels of acetylcholine *in vitro* and improves memory-related task performance in young and aged animals

Patrick M. Callahan [a, *], Daniel Bertrand [b], Sonia Bertrand [b], Marc R. Plagenhoef [a], Alvin V. Terry Jr. [a]

[a] Department of Pharmacology and Toxicology, Augusta University, Augusta, GA 30912, United States
[b] HiQScreen Sàrl, 6, rte de Compois, 1222 Vésenaz, Geneva, Switzerland

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ABSTRACT

Tropisetron, a 5-HT_3 receptor antagonist commonly prescribed for chemotherapy-induced nausea and vomiting also exhibits high affinity, partial agonist activity at α7 nicotinic acetylcholine receptors (α7 nAChRs). α7 nAChRs are considered viable therapeutic targets for neuropsychiatric disorders such as Alzheimer's disease (AD). Here we further explored the nAChR pharmacology of tropisetron to include the homomeric α7 nAChR and recently characterized heteromeric α7β2 nAChR (1:10 ratio) and we evaluated its cognitive effects in young and aged animals. Electrophysiological studies on human nAChRs expressed in *Xenopus* oocytes confirmed the partial agonist activity of tropisetron at α7 nAChRs (EC_{50} ~2.4 μM) with a similar effect at α7β2 nAChRs (EC_{50} ~1.5 μM). Moreover, currents evoked by irregular pulses of acetylcholine (40 μM) at α7 and α7β2 nAChRs were enhanced during sustained exposure to low concentrations of tropisetron (10 and 30 nM) indicative of a "priming" or co-agonist effect. Tropisetron (0.1–10 mg/kg) improved novel object recognition performance in young Sprague-Dawley rats and in aged Fischer rats. In aged male and female rhesus monkeys, tropisetron (0.03–1 mg/kg) produced a 17% increase from baseline levels in delayed match to sample long delay accuracy while combination of non-effective doses of donepezil (0.1 mg/kg) and tropisetron (0.03 and 0.1 mg/kg) produced a 24% change in accuracy. Collectively, these animal experiments indicate that tropisetron enhances cognition and has the ability to improve the effective dose range of currently prescribed AD therapy (donepezil). Moreover, these effects may be explained by tropisetron's ability to sensitize α7 containing nAChRs to low levels of acetylcholine.

1. Introduction

Tropisetron (Nabovan) is a potent 5-hydroxytryptamine type 3 (5-HT_3) receptor antagonist widely used outside the United States to treat patients with chemotherapy-induced nausea and vomiting (Simpson et al., 2000). In addition to its prominent effects at 5-HT_3 receptors (Barnes and Sharp, 1999), tropisetron is also a high affinity (Ki = 6.9 nM) partial agonist at α7 nicotinic acetylcholine receptors (α7 nAChRs) with an EC_{50} of ~800 nM (Macor et al., 2001; Papke et al., 2004). The homomeric α7 nAChR has been considered as an important therapeutic target in Alzheimer's disease (AD) and schizophrenia for several years due to deficits in α7 nAChR protein that have been observed in brains of patients who suffered from these disorders (for review see Bertrand et al., 2015). Neuronal α7 nAChRs are abundant in brain regions (e.g., prefrontal cortex and hippocampus) important for cognition where they modulate a number of calcium-dependent events including neurotransmitter release (Huang et al., 2014; Radcliffe et al., 1999), synaptic signaling (Berg and Conroy, 2002; Hefft et al., 1999) and neuroprotection (Bitner et al., 2009, 2010; Roncarati et al., 2009). Moreover, α7 nAChR agonists (e.g., A-582941, ABT 107 and S24795) have been shown to increase the phosphorylation of ERK and CREB (signaling pathways linked to

Abbreviations: ACh, acetylcholine; AChEI, acetylcholinesterase inhibitor; DMTS, delayed match to sample; MCI, mild cognitive impairment; MLA, methyllycaconitine; nAChR, nicotinic acetylcholine receptor; ORT, object recognition task.

* Corresponding author. Department of Pharmacology and Toxicology, Augusta University, CB 3540, 1459 Laney Walker Blvd, Augusta, GA 30912, United States.
E-mail address: pcallahan@augusta.edu (P.M. Callahan).

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long term potentiation and memory formation) in rodent brain (Bitner et al., 2007, 2010), to produce beneficial disease-modifying effects (e.g., reductions in tau hyperphosphorylation) and to improve cognitive function in animal models of AD (Bitner et al., 2010; Medeiros et al., 2014; Wang et al., 2009, 2010).

There is considerable preclinical and some clinical evidence that tropisetron (most likely via its effects at α7 nAChRs) could be repurposed as a therapeutic agent for AD and other neuropsychiatric disorders (e.g., mild cognitive impairment (MCI), Lewy-body dementia and schizophrenia). For example, in the preclinical literature, tropisetron exhibited neuroprotective effects in retinal ganglion cells against glutamate-induced excitotoxicity (Swartz et al., 2013) and it attenuated β-amyloid-induced inflammatory and apoptotic responses in the hippocampus of rats (Rahimian et al., 2013). Moreover, Spilman et al. (2014) demonstrated that tropisetron increased the ratio of the protective soluble amyloid precursor protein alpha to the neurotoxic Aβ1-42 peptide (i.e., the sAPPα/Aβ ratio) and improved spatial and working memory in J20 (PDAPP, huAPP Swe/Ind) AD mice. In these studies, tropisetron was efficacious during both the symptomatic, pre-plaque phase (5–6 months) and in the late plaque phase (14 months) in the J20 mice suggesting therapeutic utility of tropisetron throughout the various stages of AD. Tropisetron has also been shown to improve learning and memory (Hashimoto et al., 2006) and sensory gating deficits (Hashimoto et al., 2005; Kohnomi et al., 2010) in animal models of schizophrenia. These tropisetron-related improvements appeared to be mediated by α7 nAChRs since they were attenuated by the α7 nAChR antagonist methyllycaconitine (MLA; Nirogi et al., 2012). In clinical studies, tropisetron has been shown to improve auditory sensory gating (P50) deficits, cognitive impairments and negative symptoms in patients with schizophrenia (Noroozian et al., 2013; Shiina et al., 2010; Zhang et al., 2012).

The purpose of the present study was to explore the nAChR pharmacology of tropisetron *in vitro* and to evaluate its effects on cognition in both young and aged rats and aged nonhuman primates. To this end, we were particularly interested in characterizing the novel effects of tropisetron at the recently characterized heteromeric α7β2 nAChR (1:10 ratio) *in vitro*. This receptor is now known to be expressed in both rodent and human brain regions (e.g., basal forebrain, cortex and hippocampus) involved in learning and memory and may be involved in the pathogenesis of AD (Liu et al., 2009, 2012; Moretti et al., 2014; Thomsen et al., 2015). In the basal forebrain, it has been shown that α7 and β2 mRNAs are co-expressed on cholinergic neurons (i.e., neurons commonly damaged or lost in AD) (Azam et al., 2003). Moreover, nanomolar concentrations of the neurotoxic Aβ1-42 peptide blocked activation of α7β2 nAChR heteromers, suggesting that this receptor could also play a role in the pathology of AD (Liu et al., 2009, 2012). Additionally, we were interested in determining whether tropisetron produces "priming" (or co-agonist) effects at nAChRs (see Discussion for further details). Priming (as opposed to classical agonist or partial agonist) effects have been observed *in vitro* with the α7 nAChR ligands encenicline, FRM-17874 and RG 3487 (Prickaerts et al., 2012; Stoiljkovic et al., 2015; Wallace et al., 2010, 2011a) at concentrations that were much more relevant to the doses (and consequent brain levels) that were associated with improved cognitive function in animal models. Lastly, we were interested in characterizing the effects of tropisetron in young and aged rats performing the object recognition task and the delayed-match-to-sample (DMTS) task in aged monkeys. We also sought to characterize the "adjunctive" therapeutic strategy of combining sub-effective doses of the AChEI donepezil and tropisetron in aged monkeys.

2. Materials and methods

2.1. Electrophysiological recordings

Electrophysiological experiments were carried out with the human homomeric α7 and heteromeric α7β2 nAChR expressed in *Xenopus* oocytes as previously described by Prickaerts et al. 2012 and Wallace et al. 2011a,b. Oocytes were prepared, injected with cDNA encoding either the human α7 receptor subunit or a mixture of the human α7 and β2 receptor subunits in a 1:10 ratio, and recorded using standard procedures (Hogg et al., 2008). Briefly, ovaries were harvested from *Xenopus laevis* females that were deeply anesthetized by cooling at 4 °C and with tricaine mesylate (3-aminobenzoic acid ethyl ester, methane sulfonate salt, 150 mg/l). Small pieces of ovary were isolated in sterile Barth solution (88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 10 mM HEPES, 0.82 mM $MgSO_4 \cdot 7H_2O$, 0.33 mM $Ca(NO_3)2 \cdot 4H_2O$, and 0.41 mM $CaCl_2 \cdot 6H_2O$, pH 7.4) and supplemented with 20 μg/ml kanamycin, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Injections of cDNAs encoding for the receptors were performed in at least one hundred oocytes using an automated injection device (Roboinject, Multi Channel Systems, Reutlingen, Germany); and receptor expression was examined at least two days later. Oocytes were impaled with two electrodes filled with 3 M KCl, and their membrane potentials were maintained at −80 mV throughout the experiment. All recordings were performed at 18 °C and cells were superfused with OR2 medium (88 mM NaCl, 2.5 mM KCl, 5 mM HEPES, 1.8 mM $CaCl_2 \cdot 2H_2O$, and 1.8 mM $MgCl_2 \cdot 6H_2O$, pH 7.4). Currents were recorded using an automated process equipped with a standard two-electrode voltage-clamp configuration (HiClamp, Multi Channel Systems). The principle of this system differs from standard electrophysiology because instead of applying the compound in the perfusion, the oocyte is moved into a well from a 96 well microtiter plate containing the desired solution. Data were captured using Matlab (Mathworks Inc., Natick, MA) software.

Concentration activity studies were conducted to determine the dose-response activation and inhibition profiles for α7 and α7β2 nAChRs. Tropisetron responses were determined using a protocol of 9 concentrations with a reference response before and after compound testing, whereas the inhibition responses were determined using a protocol of sustained exposure to 6 increasing concentrations of tropisetron and a brief co-application of ACh. All experiments were carried out using three or more cells. Test compounds were prepared as 100 mM stock solutions in water and then diluted in the recording medium to obtain the desired test concentration.

2.2. Rodent behavioral studies

2.2.1. Test subjects

Experimentally naive, male Sprague-Dawley, (young 3–4 month old) and male Fischer 344 (young 3–4 month old and aged 24–25 month old) rats were obtained from Hilltop Lab Animals (Scottdale, PA) and housed 2–3 per cage (45 × 30 × 18 cm polycarbonate cage with corncob bedding) in a vivarium of constant temperature (21–23 °C) and humidity (40–50%) for at least 1 week prior to behavioral testing. Lighting was maintained on a 12-hr light-dark cycle (7:00 a.m.–7:00 p.m.) and food and water were available *ad libitum*. All behavioral testing was performed Monday thru Friday during the light portion (9 a.m.-5 p.m.) of the light/dark cycle. The strains of rats, Sprague-Dawley and Fischer 344 were selected to allow direct comparisons to our previously published results (Callahan et al., 2013, 2014). Animals were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* (U.S. National Institutes of Health Publications No. 80-23) and all