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Rhesus monkey α7 nicotinic acetylcholine receptors: Comparisons to human α7 receptors expressed in *Xenopus* oocytes

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Abstract

An α7 nicotinic acetylcholine receptor sequence was cloned from Rhesus monkey (*Macaca mulatta*). This clone differs from the mature human α7 nicotinic acetylcholine receptor in only four amino acids, two of which are in the extracellular domain. The monkey α7 nicotinic receptor was characterized in regard to its functional responses to acetylcholine, choline, cytisine, and the experimental α7-selective agonists 4OH-GTS-21, TC-1698, and AR-R17779. For all of these agonists, the EC_{50} for activation of monkey receptors was uniformly higher than for human receptors. In contrast, the potencies of mecamylamine and MLA for inhibiting monkey and human α7 were comparable. Acetylcholine and 4OH-GTS-21 were used to probe the significance of the single point differences in the extracellular domain. Mutants with the two different amino acids in the extracellular domain of the monkey receptor changed to the corresponding sequence of the human receptor had responses to these agonists that were not significantly different in EC_{50} from wild-type human α7 nicotinic receptors. Monkey α7 nicotinic receptors have a serine at residue 171, while the human receptors have an asparagine at this site. Monkey S171N mutants were more like human α7 nicotinic receptors, while mutations at the other site (K186R) had relatively little effect. These experiments point toward the basic utility of the monkey receptor as a model for the human α7 nicotinic receptor, albeit with the caveat that these receptors will vary in their agonist concentration dependency. They also point to the potential importance of a newly identified sequence element for modeling the specific amino acids involved with receptor activation.

Keywords: Voltage-clamp; Alzheimer's disease; Acetylcholine binding protein; α7 nicotinic receptor

1. Introduction

The α-bungarotoxin sensitive α7-type nicotinic acetylcholine receptor is expressed throughout the brain and also in the peripheral nervous system and some peripheral tissues (Sharma and Vijayaraghavan, 2002). In the brain, α7nicotinic acetylcholine receptor are located in high concentrations in the hippocampus, neocortex, and hypothalamus as seen by binding sites (Clarke et al., 1985). The use of nicotinic agonists has improved delay matching in primates (Terry et al., 2002), eye blink memory in rabbits, and spatial-memory related behavior in rats, as well as social memory relationships in rats (Arendash et al., 1995a,b; Meyer et al., 1994; Van Kampen et al., 2004). Some mutations and/or splice variants of the α7 gene have been linked to a decrease in hippocampal auditory gating, which is a symptom of some schizophrenics and approximately 50% of their family members (Freedman et al., 1994, 2000). This may be due to the roles played by α7nicotinic acetylcholine receptor in the activation of GABAergic inhibitory interneurons in hippocampus (Adler et al., 1998; Frazier et al., 2003). The α7 nicotinic receptor may also be involved with the etiology and/or possible treatment of other conditions such as Alzheimer's Disease and Down's Syndrome. Nicotinic receptor agonists have been shown to improve memory and are neuroprotective. Moreover, α7 has been found to coprecipitate with the $A\beta_{1\text{-}42}$ within the histopathological amyloid beta plaques (Wang et al., 2000) and the functional interactions between $A\beta_{1\text{-}42}$ and α7

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nicotinic acetylcholine receptor (Liu et al., 2001) further support α7 nicotinic acetylcholine receptor as a therapeutic target for Alzheimer's Disease.

The adverse side effects of nicotine or other non-selective cholinergic agonists have promoted the development of more selective α7 agonists for therapeutics. GTS-21 (2,4-dimethoxybenzylidene anabaseine or DMBX), one such selective agonist was tested in phase 1 clinical trials and was found to have no adverse side effects and to increase cognitive functioning in healthy subjects (Kitagawa et al., 2003). With the identification of α7 nicotinic receptors as potential therapeutic targets has also come the need to develop animal models for the testing of novel therapeutic agents. While rodent models are most commonly used, there are numerous pharmacological differences between rat and human α7 nicotinic receptors (Papke and Papke, 2002). Monkey models have the intrinsic advantage of being amenable to more complex behavioral testing than rodents and therefore may have special usefulness for evaluating potential drugs for human therapeutics. We report the cloning and functional characterization of a Rhesus monkey (*Macaca mulatta*) α7 nicotinic acetylcholine receptor (mkα7) in regard to its responses to a series of nicotinic agonists including acetylcholine (acetylcholine), choline, and cytisine, as well as the α7-selective agonists 4OH-GTS-21 (4-hydroxy 2-methoxybenzylidene anabaseine), TC-1698 (2-(3-pyridyl)-1-azabicyclo[3.2.2]nonane) and AR-R17779 ((−)-spiro[1-azabicyclo[2.2.2]octane-3′,5′-oxazolidin-2′-one) (Marrero et al., 2003; Meyer et al., 1998; Papke et al., 2004). These agonists had lower EC_{50}s for activating human α7 (hα7) nicotinic receptors than for monkey α7 (mkα7) nicotinic receptors. Only two amino acids differ between the mkα7 and hα7 sequence in the extracellular domain. Mutations were made of mkα7 sequence to the amino acids present in hα7, and the resulting mutants were tested to determine if changing either or both of the amino acids shifted the concentration–response relationships towards that of hα7.

2. Methods

2.1. Rapid amplification of cDNA ends (RACE) for the 5′- and, 3′-ends of rhesus monkey α7

To identify the 5′- and 3′-ends of rhesus monkey α7, four primers, mkα7-5′R (CTCATCTCCACGCTGGCCAGGTGCAG), mkα7-5′N (CGCACCTTATCCTCTCCCGGCCTCTTCATG), mkα7-3′R (CATGAAGAGGCCGGGAGAGGATAAGGTGCG) and mkα7-3′N (CTGCACCTGGCCAGCGTGGAGATGAG), were designed based on Genbank sequence AJ245976 and a polymerase chain reaction (PCR) was used with a Gene Racer cDNA library generated using rhesus monkey brain mRNA (Biochain). The cDNA fragments from the nested PCR were cloned and sequenced. The 3′ fragment contained a stop codon (TAA) and a polyA+ signal (AATAAA) indicating the end of the transcript. The 5′ fragment extended further upstream of AJ245976, however, failed to reach the starting Met. Additional primers were designed based on new sequence information and used for the second round of 5′ RACE. After the third 5′ RACE, the resulting cDNA fragment contained a Met and an in-frame stop codon, suggesting identification of the starting Met. Additional 5′ RACE primers: mkα7-5′R1 (GACCAGCCTCCATAAGACCAGGATCCAAACTTCAG), mkα7-5′N1 (CGCACGTCGATGTAGCAGGAACTCTTGAATATGC), mkα7-5′R2 (GGTTCTTCTCATCCGCGTCCATGATCTGCAG), and mkα7-5′N2 (GTAGACGGTGAGCGGTTGCGAGTCATTGG).

2.2. Full-length cloning of rhesus monkey α7

A cDNA contig of rhesus monkey α7was built by combining the sequences of the 5′ and 3′ RACE fragments. Two primers spanning the coding sequence of the monkey α7, a 5′ primer CTCAACATGCGCTGCTCGCAGGGAGG and a 3′ primer CCAAGCCAGAGGCCTTGCCCATCTGTGAG, were designed based on the contig and were used to PCR a monkey brain cDNA library. The resulting PCR product was cloned into pcDNA3.1 TOPO vector (Invitrogen) and confirmed by sequencing.

The full length clone we isolated has a predicted amino acid sequence that is 100% identical to that of the unpublished Genbank *M. mulatta* sequence AF486623, although differing by a total of 5 nucleotides in the open reading frame.

2.3. Sequence comparisons and selection of mutations

Two differences were noted within the extracellular region of the rhesus monkey α7 and human α7. Numbering the amino acids as for human α7 (vicinal cysteines at positions 190 and 191), these differing residues were located at positions 171 and 186. Two single point mutants were made in mkα7 (mkα7S171N and mkα7K186R) as well as the double mutant (mkα7S171N,K186R). Mutants were constructed using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions, and were confirmed by automated fluorescent sequencing (University of Florida ICBR core facility, Gainesville, FL, USA).

2.4. Preparation of RNA

The hα7 clone was obtained from Dr. Jon Lindstrom (University of Pennsylvania), and the *M. mulatta* (rhesus) α7 cloned as described above. After linearization and purification of cDNA templates, RNA was prepared using the appropriate mMessage mMachine kit from Ambion, Inc. (Austin, TX, USA), according to the manufacturer's instructions.

2.5. Expression in Xenopus oocytes

The preparation of *Xenopus laevis* oocytes for RNA expression was conducted as previously described (Papke and Papke, 2002). In brief, mature (>9 cm) female *X. laevis* African frogs (Nasco, Ft. Atkinson, WI) were used as a source of oocytes. Prior to surgery, the frogs were anesthetized by placing the animal in a 1.5g/l solution of MS222 (3-aminobenzoic acid