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Review

Progress and challenges in the study of α6-containing nicotinic acetylcholine receptors

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ABSTRACT

Recent progress has been made in the understanding of the anatomical distribution, composition, and physiological role of nicotinic acetylcholine receptors containing the α6 subunit. Extensive study by many researchers has indicated that a collection of α6-containing receptors representing a nicotinic subfamily is relevant in preclinical models of nicotine self-administration and locomotor activity. Due to a number of technical difficulties, the state of the art of *in vitro* model systems expressing α6-containing receptors has lagged behind the state of knowledge of native α6 nAChR subunit composition. Several techniques, such as the expression of chimeric and concatameric α6 subunit constructs in oocytes and mammalian cell lines have been employed to overcome these obstacles. There remains a need for other critical tools, such as selective small molecules and radioligands, to advance the field of research and to allow the discovery and development of potential therapeutics targeting α6-containing receptors for smoking cessation, Parkinson's disease and other disorders.

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1. Introduction

The neurotransmitter acetylcholine binds to two main classes of receptors, nicotinic and muscarinic, each named for the prototypical compound that interacts with the class. Nicotinic acetylcholine receptors (nAChRs) play critical physiological roles throughout the body and brain by mediating cholinergic excitatory neurotransmission, modulating the release of neurotransmitters, influencing second messenger systems and gene expression, and contributing to synaptic plasticity [1,2]. Although the action of

Abbreviations: α-Ctx, alpha-conotoxin; CNS, central nervous system; GoF, gain-of-function; KO, knock-out; LC, locus coeruleus; MPTP, 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine; nAChRs, nicotinic acetylcholine receptors; PD, Parkinson's disease; PET, positron emission tomography; SNc, substantia nigra pars compacta; SPECT, single photo emission computed tomography; VTA, ventral tegmental area.

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nicotine at the neuromuscular junction was studied by John Langley in the early 1900s, the role of nAChRs in the central nervous system (CNS) remained in dispute for nearly 90 years. This occurred because expression of nicotinic receptors in the brain was considerably less dense than muscarinic receptors and because the field lacked appropriate tools to assess them adequately [3]. As the field progressed, multiple tools were developed, including molecular biology techniques, selective agonists and antagonists, radioligands, antibodies, heterologous expression systems and transgenic mice, which enabled research on specific nicotinic subtypes in the CNS. Two neuronal nAChR subtypes have been examined extensively: those containing α7 subunits and those containing α4 and β2 subunits. The respective roles of these subtypes have been sufficiently studied in various disease states to support the clinical development of therapeutics for smoking cessation, depression and cognitive disorders [4]. Research around other nAChR subtypes, including those containing the α6 subunit, is less advanced. With further work, it is anticipated that a better understanding of these subtypes will also lead to novel medications.

nAChRs encompass a family of ligand-gated ion channels consisting of a variety of subtypes. Each receptor subtype is formed from 5 separate protein subunits that co-assemble to form a pore permeable to cations such as Ca^{2+}, Na^{+} and K^{+}. There have been seventeen vertebrate (sixteen mammalian) nAChR subunits cloned (α1–10, β1–4, γ, δ, ε), providing the potential for a large number of subunit combinations [5]. However, the assembly of nAChRs appears to be a highly regulated process, with certain subunit combinations favored based on subunit expression patterns, subunit interactions, post-translational modification and other cellular processes [2,6,7].

The pharmacology of each nAChR subtype is defined by the α and β subunits that make up the receptor. Each subunit protein is characterized by an N-terminal extracellular agonist-binding domain, four transmembrane spanning regions, which contribute to the channel and traverse the cell membrane, and a substantial intracellular domain composed of the loop between the third and fourth transmembrane sequences [2]. The subunit composition of each nicotinic receptor subtype determines the pharmacological characteristics of the ligand binding sites and the cation preference of the channel. Both homomeric combinations, such as $(\alpha7)_5$, and heteromeric combinations, such as $(\alpha4\beta2)_2\alpha4$ and $(\alpha4\beta2)_2\beta2$, have been described. For homomeric subtypes, there are five putative competitive ligand binding sites, one between each α7–α7 extracellular domain interface [8]. In the heteromeric combinations, there are two putative receptor binding interfaces that contribute to competitive ligand binding sites, each residing at the interface between the extracellular domains of neighboring α and β subunits. The fifth subunit is generally considered an accessory subunit and not a component of the orthosteric ligand binding sites. All five subunits in the pentameric complex contribute to channel kinetics, such as activation, inactivation, desensitization, channel open times, ion conductance and selectivity [2]. The receptor binding and functional properties of each subtype are unique, but overlap sufficiently to make distinguishing between them challenging with existing pharmacological agents. This is especially true for subtypes that have subunits in common or where differing subunits share a high degree of homology.

The diversity of nAChR subtypes is physiologically relevant, as it allows for a broad range of cellular roles. Changes in nAChR subunit expression in development [9], specificity of expression in different organs, across brain regions [10] and cellular compartments [11] and even changes in subunit composition in response to drug treatment [12,13] have been reported. This permits a wide variety of functional roles in normal and disease states and provides opportunities for pharmacological manipulation and drug design. Such is the case for receptors containing the α6 subunit, which have generated much interest in the research and pharmaceutical communities. The α6 subunit has a restricted expression pattern, being predominantly confined to dopaminergic neurons implicated in the reward pathway and motor behavior, but also found in noradrenergic neurons, the visual system and a few other regions. This makes α6-containing nAChRs relevant for a number of CNS disorders such as drug addiction, Parkinson's disease (PD) [14] and potentially others [15].

Research over the last decade has revealed that there is a subfamily of closely related nAChR subtypes containing the α6 subunit, which vary by the composition of other subunits in the pentameric complex. Subtle pharmacological differences between α6* subtypes (the asterisk indicates the presence of other subunit types in the pentamer) have created specific challenges to research. Therefore, separate *in vitro* expression systems are needed for each likely α6* receptor combination to fully explore differences in the pharmacology. Unlike α4β2 and α7 subtypes, however, it has been difficult to generate heterologous cell lines that express α6* receptors. Recent breakthroughs have been made in creating model systems for working with α6* subtypes, but there are still gaps in the tool set available. In addition, highly selective agonists and antagonists for the various α6* subtypes have yet to be identified and are critically needed for manipulating α6* receptor populations *in vivo*, *ex vivo* and *in vitro*. Radioligands selective for the various α6* subtypes are also needed to discriminate α6* subtypes containing closely related subunit combinations. These challenges have limited the study of receptor binding and functional properties of α6* nAChRs with *in vitro* expression systems and *in vivo* models.

2. Progress to date

Incremental progress has been made in the study of α6* nAChRs. Initial *in situ* hybridization studies identified the regional expression of α6 subunit mRNA. Early work to characterize α6* subtypes relied on a combination of relatively non-selective radioligand binding and/or functional assays that did not differentiate adequately from other nAChR subtypes. Eventually, more selective approaches were used such as subunit-null transgenic animals and peptide ligands, often in combination with immunoprecipitation assays using antibodies targeting nAChR subunits. These studies have provided valuable insights into the stoichiometry of various α6* nAChRs and their physiological roles.

2.1. From cloning to transgenic animals

Cloning first identified the α6 subunit gene sequence [16], which has a high degree of homology to that of the α3 subunit. Once the sequence was determined, highly specific antisense oligonucleotide probes could be designed to measure mRNA expression in various brain regions. Le Novere et al. [17] determined that α6 subunit mRNA expression was restricted to a few areas of the rat brain. The greatest density of α6 mRNA was measured in catecholamingeric areas, such as dopaminergic nuclei (substantia nigra pars compacta, SNc; and ventral tegmental area, VTA) and noradrenergic nuclei (locus coeruleus; LC). In these areas, α6 mRNA density was higher than other nicotinic subunits. Further, α6 and β3 mRNA were typically co-expressed, suggesting that these two subunits may co-assemble in the same receptor complex. α6 mRNA expression was also found in the reticular thalamic nucleus, the supramammillary nucleus, the mesencephalic V nucleus, medial habenula and interpeduncular nucleus. This study yielded the first clues to the potential role of α6-containing receptors and the authors speculated involvement of