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Invited review

Expression of cloned α6* nicotinic acetylcholine receptors

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ABSTRACT

Nicotinic acetylcholine receptors (AChRs) are ACh-gated ion channels formed from five homologous subunits in subtypes defined by their subunit composition and stoichiometry. Some subtypes readily produce functional AChRs in *Xenopus* oocytes and transfected cell lines. α6β2β3* AChRs (subtypes formed from these subunits and perhaps others) are not easily expressed. This may be because the types of neurons in which they are expressed (typically dopaminergic neurons) have unique chaperones for assembling α6β2β3* AChRs, especially in the presence of the other AChR subtypes. Because these relatively minor brain AChR subtypes are of major importance in addiction to nicotine, it is important for drug development as well as investigation of their functional properties to be able to efficiently express human α6β2β3* AChRs. We review the issues and progress in expressing α6* AChRs.

This article is part of the Special Issue entitled 'The Nicotinic Acetylcholine Receptor: From Molecular Biology to Cognition'.

1. Introduction

α6β2β3* AChRs are of special neuropharmacological interest for several reasons. α6, α4, and β2 subunits are required to form AChRs critical for addiction to nicotine, because knockout of any of these subunits prevents nicotine self-administration in mice (Pons et al., 2008). AChRs assembled from these subunits (i.e., α6α4β2* subtypes) are identified by immune-isolation and study of knockout mice in midbrain dopaminergic neurons, which are critical for nicotine reward and addiction (Champtiaux et al., 2003; Drenan et al., 2010; Gotti et al., 2010; De Biasi and Dani, 2011). β3 subunits are adjacent to α6 subunits in the genome and they are usually co-expressed (Han et al., 2000; Quik et al., 2000; Cui et al., 2003). This suggests that the complex (α6β2)(α4β2)β3 AChR subtype is important for nicotine addiction. This subtype in dopaminergic nerve endings promotes release of dopamine (Salminen et al., 2007; Drenan et al., 2010; Exley et al., 2011; Liu et al., 2012). It is the subtype controlling dopamine release that is most sensitive to activation by nicotine (Salminen et al., 2007; Kuryatov and Lindstrom, 2011). Loss of α6* AChRs is an early sign of dopaminergic neuron loss in Parkinson's disease (Gotti et al., 2006; Quik et al., 2011; Srinivasan et al., 2014). Both (α6β2)(α4β2)β3 and $(\alpha6\beta2)_2\beta3$ subtypes of AChRs are vulnerable to nigrostriatal damage in an animal model of Parkinson's disease (Quik et al., 2005). Transgenic mice expressing a hypersensitive form of α6 subunit exhibit enhanced dopaminergic neuron activity and locomotor hyperactivity (Drenan et al., 2008, 2010).

Each AChR has five homologous subunits: α6 and β2 form one acetylcholine (ACh) binding site, α4 and β2 form another ACh binding site, and β3 is the accessory subunit (Millar and Gotti, 2009; Hurst et al., 2013). Having two types of ACh binding sites in (α6β2)(α4β2)β3 AChRs suggests that they have unusual neuropharmacological properties. α-Conotoxin MII (α-CtxMII) is an antagonist for ACh binding sites formed at the interface of α6 and β2 subunits, and a critical tool for localizing and identifying the function of α6β2* AChRs (Whiteaker et al., 2000; Champtiaux et al., 2003; McIntosh et al., 2004). α-CtxMII is an antagonist for both α6β2* and α3β2* AChRs, but is often used for detecting α6β2* AChRs because in brain the α3 subunit is expressed almost exclusively in the medial habenula (Cartier et al., 1996; Kuryatov et al., 2000; Han et al., 2000; Whiteaker et al., 2002; McIntosh et al., 2004). Several α-conotoxin variants that are more selective for α6β2* AChRs were subsequently discovered (Dowell et al., 2003; McIntosh et al., 2004; Azam et al., 2010). As peptides, these α-conotoxins cannot cross the blood–brain barrier, or lead to development of small molecule therapeutics targeting α6* AChR related neurological disorders.

Abbreviations: ACh, acetylcholine; AChR, nicotinic acetylcholine receptor; α-Ctx, α-conotoxin; eGFP, enhanced green fluorescent protein; ER, endoplasmic reticulum; GABA, gamma-aminobutyric acid; HEK, human embryonic kidney; N-2a, Neuroblastoma 2a; SNc, substantia nigra pars compacta; VTA, ventral tegmental area.

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In order to study the neuropharmacology of α6* AChRs, understand their pharmacology, and develop bioavailable drugs specific for them, it is necessary to express specific human subtypes of these AChRs. Neuronal cell lines for this purpose are not available. Expressing cloned AChRs has challenges. Although functional expression of AChRs, including α6β4* AChR subtypes, is generally easy to achieve in the *Xenopus* oocyte system (Gerzanich et al., 1997; Kuryatov et al., 2000; Broadbent et al., 2006; Dash et al., 2011a), expression of α6 and β2 forms many (α6β2) ACh binding sites that can be labeled with ^{3}H-epibatidine but not mature functional AChRs on the oocyte surface (Gerzanich et al., 1997; Kuryatov et al., 2000). Expressing α6β2* AChRs in cultured cell lines is even more difficult. Human α6* AChRs can be expressed in transfected HEK cells, and β3 can increase their sensitivity to up-regulation by nicotine, but the level of expression for both α6β2β3 and α6β4β3 AChRs is too low for assaying AChR function even after nicotine up-regulation (Tumkosit et al., 2006). $(\alpha4\beta2)_2\beta3$ AChRs assemble very efficiently in transfected HEK cells to form functional AChRs (Kuryatov et al., 2008). However, transfection of an α4β2 HEK cell line with α6 does not result in efficient assembly of α6α4β2 AChRs (Kuryatov et al., unpublished). Another issue of expressing heteromeric AChRs in both oocytes and cells is the potential of forming multiple stoichiometries with distinct properties, such as $(\alpha4\beta2)_2\beta2$ and $(\alpha4\beta2)_2\alpha4$ (Zwart and Vijverberg, 1998; Nelson et al., 2003; Kuryatov et al., 2005; Sallette et al., 2005; Harpsøe et al., 2011; Mazzaferro et al., 2011). These challenges are being overcome through the use of mutants, chimeras, and concatamers (Kuryatov et al., 2000, 2011; Broadbent et al., 2006; Capelli et al., 2011; Jensen et al., 2013, 2014; Henderson et al., 2014; Ley et al., 2014). This review focuses primarily on the significance and expression of α6β2* AChRs, but compares them with what is known about α6β4* AChRs.

2. Neuropharmacological properties of α6β2β3* AChRs

One of the gold standards to locate and distinguish the neuropharmacological properties of various AChR subtypes is the use of selective ligands, such as α-bungarotoxin for α7 and muscle type AChRs or DhβE or epibatidine for β2-containing AChRs (Hurst et al., 2013). A 16 amino acid peptide, α-conotoxin MII (α-CtxMII) from the marine cone snail *Conus magus*, was initially found to be a highly selective antagonist for α3β2* AChRs, and later identified to also have high affinity for α6β2* AChRs, but very low affinity for α2* and α4* AChRs (Cartier et al., 1996; Kuryatov et al., 2000; McIntosh et al., 2004). This toxin subsequently become a useful tool for identifying α6β2* AChRs and evaluating their importance in the effects of nicotine both in brain and in heterologous systems as described here and in Sections 3.1.1 and 4.1.

In 2000, Whiteaker et al. developed a radioactive version of this toxin, ^{125}I α-CtxMII, which allowed locating α6* AChR subtypes in brain tissue. Homozygous null mutant (α6−/−) mice showed complete loss of brain [^{125}I] α-CtxMII binding sites (Champtiaux et al., 2002), and α3 knockout mice showed no significant loss of [^{125}I] α-CtxMII binding sites, except in the habenulo-interpeduncular nuclei (Whiteaker et al., 2002). This suggests that α6, rather than α3, is critical for dopamine release in brain. The percentage and sensitivity of α6β2* AChR subtypes are obtained by assessing the portion of agonist-stimulated release of dopamine which is sensitive to α-CtxMII block, as discussed in Section 3.1.1 and 3.2 (Table 1).

Subsequently, various α-conotoxins and their mutants were developed with equal or better selectivity for α6 versus α3 than that of α-CtxMII, and used to identify α6* subtypes and their physiological importance in aminergic neurons (Dowell et al., 2003; McIntosh et al., 2004; Azam et al., 2005, 2010; Luo et al., 2013). Via analyzing sequences interacting with AChRs, a mutant form of

Table 1
Functional characterization of α6β2* AChRs in dopaminergic neurons.

Subtypes	Species	Location	EC_{50} (μM)	α6* AChRs (% of total response)	Reference
α6β2*	Mouse	Striatal synaptosomes	0.77 ± 0.27	33	Salminen et al., 2004
		Striatal synaptosomes	0.81 ± 0.12	26	Cui et al., 2003
		Striatal synaptosomes	0.11 ± 0.04	26	Drenan et al., 2008
		Olfactory tubercle	0.082 ± 0.037	21	
		Striatal synaptosomes	0.62 ± 0.19	29	Salminen et al., 2007
		Striatum	0.099 ± 0.026	15	Marks et al., 2014
		Olfactory tubercle	0.110 ± 0.024	15	
		Dorsal striatum	0.031 ± 0.017	17	Drenan et al., 2010
		Olfactory tubercle	0.075 ± 0.025	24	
	Monkey	Caudate	0.31 ± 0.10	68	McCallum et al., 2005
		Putamen	0.32 ± 0.10	70	
		Nucleus accumbens	0.58 ± 0.24	80	
		Nucleus accumbens	0.33 ± 0.16	75	McCallum et al., 2006a
		Striatal synaptosomes	0.93 ± 0.13	80	Perez et al., 2012
α6L9′Sβ2*	Mouse	Striatal synaptosomes	0.047 ± 0.011	58	Drenan et al., 2008
		Olfactory tubercle	0.025 ± 0.004	65	
		Dorsal striatum	0.016 ± 0.006	46	Drenan et al., 2010
		Olfactory tubercle	0.015 ± 0.004	67	
α6(non α4)β2*	Mouse	Dorsal striatum	0.88 ± 0.16		Drenan et al., 2010
		Olfactory tubercle	0.97 ± 0.15		
		Striatal synaptosomes	0.103 ± 0.031		Champtiaux et al., 2003
α6L9′S(non α4)β2*	Mouse	Dorsal striatum	0.25 ± 0.02		Drenan et al., 2010
		Olfactory tubercle	0.43 ± 0.09		
α6(non β3)*	Mouse	Striatal synaptosomes	0.42 ± 0.83	5.1	Cui et al., 2003
α6(non α4 or β3)*	Mouse	Striatal synaptosomes	21.24 ± 3.21	4.6	Salminen et al., 2007
α6α4β2β3	Mouse	Striatal synaptosomes	0.23 ± 0.08		Salminen et al., 2007
α6β2β3			1.52 ± 0.19		

Function was assayed by measuring [^{3}H]-dopamine release induced by nicotine. Percent of total response contributed by α6* AChRs was assayed using the α6-selective antagonist, α-Ctx MII. α6L9′S: α6 gain-of-function mutant.