Acetylcholine (nicotinic)

Nicotinic acetylcholine (nACh) receptors are members of the Cys-loop family of ligand-gated ion channels that includes the $GABA_A$, strychnine-sensitive glycine and $5\text{-}HT_3$ receptors (Sine and Engel, 2006; Albuquerque *et al.*, 2009; Millar and Gotti, 2009). All nicotinic receptors are pentamers of 4TM subunits. Genes (Ensembl family ID ENSF00000000049) encoding a total of 17 subunits (α1–10, β1–4, δ, ε and γ) have been identified (Kalamida *et al.*, 2007). All subunits with the exception of avian α8 have been identified in mammalian species. All α subunits possess two tandem cysteine residues near to the site involved in acetylcholine binding, and subunits not named α lack these residues (Millar and Gotti, 2009). The ligand binding site is formed by residues within at least three peptide domains on the α subunit (principal component), and three on the adjacent subunit (complementary component). The high resolution crystal structure of the molluscan acetylcholine binding protein, a structural homologue of the extracellular binding domain of a nicotinic receptor pentamer, in complex with several nicotinic receptor ligands (e.g. Celie *et al.*, 2004) and the crystal structure of the extracellular domain of the α1 subunit bound to α-bungarotoxin at 1.94 Å resolution (Dellisanti *et al.*, 2007), has revealed the binding site in detail (reviewed Sine and Engel, 2006; Kalamida *et al.*, 2007; Changeux and Taly, 2008; Rucktooa *et al.*, 2009). Nicotinic receptors at the somatic neuromuscular junction of adult animals have the stoichiometry $(\alpha1)_2\beta1\varepsilon\delta$, whereas an extrajunctional $(\alpha1)_2\beta1\gamma\delta$ receptor predominates in embryonic and denervated skeletal muscle and other pathological states. Other nicotinic receptors are assembled as combinations of α(2–6) and β(2–4) subunits. For α2, α3, α4 and β2 and β4 subunits, pairwise combinations of α and β (e.g. α3β4, α4β2) are sufficient to form a functional receptor *in vitro*, but far more complex isoforms may exist *in vivo* (reviewed by Gotti *et al.*, 2006; 2009, Millar and Gotti, 2009). There is strong evidence that the pairwise assembly of some α and β subunits can occur with variable stoichiometry [e.g. $(\alpha4)_2(\beta2)_2$, or $(\alpha4)_3(\beta2)_2$] that influences the biophysical and pharmacological properties of the receptor (Millar and Gotti, 2009). α5 and β3 subunits lack function when expressed alone, or pairwise, but participate in the formation of functional hetero-oligomeric receptors when expressed as a third subunit with another α and β pair [e.g. α4α5αβ2, α4αβ2β3, α5α6β2, see Millar and Gotti (2009) for further examples]. The α6 subunit can form a functional receptor when co-expressed with β4 *in vitro*, but more efficient expression ensues from incorporation of a third partner, such as β3 (Yang *et al.*, 2009). The α7, α8 and α9 subunits form functional homo-oligomers, but can also combine with a second α subunit to constitute a hetero-oligomeric assembly (e.g. α9α10). A functional assembly of α7 and β2 subunits has additionally been reported (Khiroug *et al.*, 2002). For functional expression of the α10 subunit, co-assembly with α9 is necessary. The latter, along with the α10 subunit, appears to be largely confined to cochlear and vestibular hair cells. Comprehensive listings of nicotinic receptor subunit combinations identified from recombinant expression systems, or *in vivo*, are given in Millar and Gotti (2009).

The nicotinic receptor subcommittee of NC-IUPHAR has recommended a nomenclature and classification scheme for nACh receptors based on the subunit composition of known, naturally and/or heterologously expressed nACh receptor subtypes (Lukas *et al.*, 1999). Headings for this table reflect abbreviations designating nACh receptor subtypes based on the predominant α subunit contained in that receptor subtype. An asterisk following the indicated α subunit denotes that other subunits are known to, or may, assemble with the indicated α subunit to form the designated nACh receptor subtype(s). Where subunit stoichiometries within a specific nACh receptor subtype are known, numbers of a particular subunit larger than 1 are indicated by a subscript following the subunit (enclosed in parentheses – see also Collingridge *et al.*, 2009).

Nomenclature	α1*	α2*	α3*
Previous names	Muscle-type, muscle	–	Autonomic, ganglionic
Selective agonists	Succinylcholine [selective for $(\alpha1)_2\beta1\gamma\delta$)]	–	–
Positive allosteric modulators	–	α2β4: LY-2087101 (Broad *et al.* (2006)	–
Selective antagonists	Waglerin-1 [selective for $\alpha(1)_2\beta1\varepsilon\delta$], α-bungarotoxin, α-conotoxin GI, α-conotoxin MI, pancuronium	–	α3β2: α-conotoxin MII (also blocks α6-containing), α-conotoxin-GIC, α-conotoxin PnIA, α-conotoxin TxIA α3β4: α-conotoxin AuIB
Commonly used antagonists	$(\alpha1)_2\beta1\gamma\delta$ and $(\alpha1)_2\beta1\varepsilon\delta$: α-bungarotoxin > pancuronium > vecuronium > rocuronium > (+)-Tc (IC_{50} = 43–82 nM)	α2β2: DHβE (K_B = 0.9 μM), (+)-Tc (K_B = 1.4 μM) α2β4: DHβE (K_B = 3.6 μM), (+)-Tc (K_B = 4.2 μM)	α3β2: DHβE (K_B = 1.6 μM, IC_{50} = 2.0 μM), (+)-Tc (K_B = 2.4 μM) α3β4: DHβE (K_B = 19 μM, IC_{50} = 26 μM), (+)-Tc (K_B = 2.2 μM)
Channel blockers	$\alpha(1)_2\beta1\varepsilon\delta$ and $\alpha(1)_2\beta1\gamma\delta$: gallamine ($IC_{50}$ ~ 1 μM) $\alpha(1)_2\beta1\varepsilon\delta$: mecamylamine ($IC_{50}$ ~ 1.5 μM)	Mecamylamine, hexamethonium	α3β2: mecamylamine (IC_{50} = 7.6 μM), hexamethonium α3β4: mecamylamine (IC_{50} = 0.39 μM), hexamethonium
Radioligands (K_d)	$[^3H]/[^{125}I]$-α-bungarotoxin	$[^{3H}]/[^{125I}]$-epibatidine (hα2β4, 42 pM; rα2β2, 10–21 pM; rα2β4, 84–87 pM), $[^3H]$-cytisine	$[^3H]/[^{125}I]$-epibatidine (hα3β2, 7 pM; hα3β4, 230 pM; rα3β2, 14–34 pM, rα3β4, 290–304 pM), $[^3H]$-cytisine
Functional characteristics	$\alpha(1)_2\beta\gamma\delta$: P_{Ca}/P_{Na} = 0.16–0.2, P_f = 2.1–2.9%; $\alpha(1)_2\beta\varepsilon\delta$: P_{Ca}/P_{Na} = 0.65–1.38, P_f = 4.1–7.2%	α2β2: P_{Ca}/P_{Na} ~ 1.5	α3β2: P_{Ca}/P_{Na} = 1.5; α3β4: P_{Ca}/P_{Na} = 0.78–1.1, P_f = 2.7–4.6%

Nomenclature	α4*	α6*	α7*
Previous names	Neuronal, α-bungarotoxin-insensitive	–	Neuronal, α-bungarotoxin-sensitive
Selective agonists	α4β2: TC-2559 (Chen *et al.*, 2003), TC-2403 (RJR-2403, Papke *et al.*, 2000)	–	$(\alpha7)_5$: PNU-282987 (Bodnar *et al.*, 2005), PHA-543613 (Wishka *et al.*, 2006); PHA-709829 (Acker *et al.*, 2008), A-582941 (Bitner *et al.*, 2007), TC-5619 (Hauser *et al.*, 2009)
Positive allosteric modulators	α4β2 and α4β4: LY-2087101 (Broad *et al.* (2006)	–	$(\alpha7)_5$:Type 1: LY-2087101 (Broad *et al.*, 2006), NS1738 (Timmermann *et al.*, 2007) $(\alpha7)_5$:Type 2: PNU-120596 (Hurst *et al.*, 2005), A-867744 (Malysz *et al.*, 2009)
Selective antagonists	–	α6/α3β2β3 chimera: α-conotoxin PIA α6β2β3: α-conotoxin MII [H9A, L15A] α6β2*: α-conotoxin MII (also blocks α3β2)	$(\alpha7)_5$: α-bungarotoxin, methyllycaconitine, α-conotoxin ImI, α-conotoxin ArIB
Commonly used antagonists	α4β2: DHβE (K_B = 0.1 μM, IC_{50} = 0.08–0.9 μM), (+)-Tc (K_B = 3.2 μM, IC_{50} = 34 μM) α4β4: DHβE (K_B = 0.01 μM, IC_{50} = 0.19–1.2 μM), (+)-Tc (K_B = 0.2 μM, IC_{50} = 50 μM)	α6/α3β2β3 chimera: DHβE (IC_{50} = 1.1 μM)	$(\alpha7)_5$: DHβE (IC_{50} = 8–20 μM) $(\alpha7)_5$: (+)-Tc (IC_{50} = 3.1 μM)
Channel blockers	α4β2: mecamylamine (IC_{50} = 3.6–4.1 μM), hexamethonium (IC_{50} = 6.8–29 μM) α4β4: mecamylamine (IC_{50} = 0.33–4.9 μM), hexamethonium (IC_{50} = 91 μM)	α6/α3β2β3 chimera: mecamylamine (IC_{50} = 11 μM), hexamethonium	$(\alpha7)_5$: mecamylamine (IC_{50} = 15.6 μM)
Radioligands (K_d)	$[^3H]$/$[^{125}I]$-epibatidine (hα4β2, 10–33 pM; hα4β4, 187 pM; rα4β2, 30–46 pM; rα4β4, 85–94 pM), $[^3H]$-cytisine (hα4β2, 430–630 pM; rα4β2, 100 pM; hα4β4 100 pM), $[^3H]$-nicotine (rα4β2, 400 pM)	$[^3H]$-epibatidine (native cα6β4*, 35 pM), $[^{125}I]$-α-conotoxin MII	$[^3H]$-epibatidine ($(h\alpha7)_5$, 0.6 pM) $[^3H]$/$[^{125}I]$-α-bungarotoxin ($(h\alpha7)_5$, 0.7–5 nM), $[^3H]$-methyllycaconitine (native rα7*, 1.9 nM), $[^3H]$-A-585539 (native hα7, 70 pM; Anderson *et al.*, 2008)
Functional characteristics	α4β2: P_{Ca}/P_{Na} = 1.65, P_f = 2.6–2.9%; α4β4: P_f = 1.5–3.0 %	–	P_{Ca}/P_{Na} = 6.6–20, P_f = 8.8–11.4%

Nomenclature	α8* (avian)	α9*
Previous names	Neuronal, α-bungarotoxin-sensitive	–
Selective agonists	–	–
Selective antagonists	–	$(\alpha9)_5$: α-bungarotoxin, strychnine, nicotine, muscarine α9α10: α-contoxin RgIA, α-bungarotoxin, strychnine, nicotine, muscarine
Commonly used antagonists	$(\alpha8)_5$: α-bungarotoxin > atropine ≥ (+)-Tc ≥ strychnine	$(\alpha9)_5$: α-bungarotoxin > methyllycaconitine > strychnine ~ tropisetron > (+)-Tc α9α10: α-bungarotoxin > tropisetron = strychnine > (+)-Tc
Channel blockers	–	–
Radioligands (K_d)	$[^3H]$-epibatidine ($(\alpha8)_5$, 0.2 nM) $[^3H]$/$[^{125}I]$-α-bungarotoxin (native α8*, 5.5 nM)	$[^3H]$-methyllycaconitine (hα9α10, 7.5 nM) $[^3H]$/$[^{125}I]$-α-bungarotoxin
Functional characteristics	–	$(\alpha9)_5$: P_{Ca}/P_{Na} = 9; α9α10: P_{Ca}/P_{Na} = 9, P_f = 22%

Commonly used agonists of nACh receptors that display limited discrimination in functional assays between receptor subtypes include A-85380, cytisine, DMPP, epibatidine, nicotine and the natural transmitter, ACh. A summary of their profile across differing receptors is provided in Gotti *et al*. (2006) and quantitative data across numerous assay systems are summarized in Jensen *et al*. (2005). Quantitative data presented in the table for commonly used antagonists and channel blockers for human receptors studied under voltage-clamp are from Buisson *et al*. (1996), Chavez-Noriega *et al*. (1997), Papke *et al*. (2001; 2008), Paul *et al*. (2002) and Wu *et al*. (2006).

Abbreviations: **A-582941**, 2-methyl-5-(6-phenyl-pyridazin-3-yl)-octahydro-pyrrolo[3,4-c]pyrrole; **A-585539**, (1*S*,4*S*)-2,2-dimethyl-5-(6-phenylpyridazin-3-yl)-5-aza-2-azaniabicyclo[2.2.1]heptane; **A-867744**, 4-(5-(4-chlorophenyl)-2-methyl-3-propionyl-1*H*-pyrrol-1-yl)benzenesulfonamide; **ABT-594**, (*R*)-5-(2-azetidinylmethoxy)-2-chloropyridine; **ACh**, acetylcholine; **DHβE**, dihydro-β-erythroidine; **DMPP**, 1,1-dimethyl-4-phenylpiperazinium; **LY-2087101**, see Broad *et al*. (2006) for structure; **NS1738**, 1-(5-chloro-2-hydroxy-phenyl)-3-(2-chloro-5-trifluoromethyl-phenyl)-urea; **PHA-543613**, *N*-[(3*R*)-1-azabicyclo[2.2.2]oct-3-yl]furo[2,3-c]pyridine-5-carboxamide; **PHA-709829**, *N*-[(3*R*,5*R*)-1-azabicyclo[3.2.1]oct-3-yl]furo[2,3-c]pyridine-5-carboxamide; **PNU-120596**, 1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea; **PNU-282987**, *N*-[(3*R*)-1-azabicyclo[2.2.2]oct-3-yl]-4-chlorobenzamide hydrochloride; **PSAB-OFP**, (*R*)-(-)-5′phenylspiro[1-azabicyclo[2.2.2]octane-3,2′-(3′H)furo[2,3-b]pyridine; **TC-2403**, (RJR-2403), (E)-*N*-methyl-4-(3-pyridinyl)-3-butene-1-amine; **TC-2559**, (E)-*N*-methyl-4-[3-(5-ethoxypyridin)yl]-3-buten-1-amine; **TC-5619**, *N*-[2-(pyridin-3-ylmethyl)-1-azabicyclo[2.2.2]oct-3-yl]-1-benzofuran-2-carboxamide; **(+)-Tc**, (+)-tubocurarine

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