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Brain nicotinic acetylcholine receptors: native subtypes and their relevance

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Neuronal nicotinic acetylcholine receptors comprise a heterogeneous class of cationic channels that is present throughout the nervous system. These channels are involved both in physiological functions (including cognition, reward, motor activity and analgesia) and in pathological conditions such as Alzheimer's disease, Parkinson's disease, some forms of epilepsy, depression, autism and schizophrenia. They are also the targets of tobacco-smoking effects and addiction. Neuronal nicotinic acetylcholine receptors are pentamers of homomeric or heteromeric combinations of α (α2–α10) and β (β2–β4) subunits, which have different pharmacological and biophysical properties and locations in the brain. The lack of subtype-specific ligands and the fact that many neuronal cells express multiple subtypes initially hampered the identification of the different native nicotinic acetylcholine receptor subtypes, but the increasing knowledge of subtype composition and roles will be of considerable interest for the development of new and clinically useful nicotinic acetylcholine receptor ligands.

Introduction

Ionotropic neuronal nicotinic acetylcholine receptors are heterogeneous cationic channels that are widely distributed in both the nervous system and non-neuronal tissues, and their opening is controlled by the endogenous neurotransmitter acetylcholine (ACh) or exogenous ligands such as nicotine. They consist of homopentameric or heteropentameric subtypes that are present in various regions of the CNS; they are principally located at presynaptic or preterminal sites (where they modulate neurotransmitter release), and are sometimes found on cell bodies or dendrites (where they mediate postsynaptic effects) [1–3].

In the CNS, acetylcholine-mediated innervation acting through nicotinic acetylcholine receptors regulates processes such as transmitter release, cell excitability and neuronal integration, which are crucial for network operations and influence physiological functions such as arousal, sleep, fatigue, anxiety, the central processing of pain, food intake and several cognitive functions [1,2,4]. Nicotinic acetylcholine receptors are particularly important in two crucial periods of brain life: early pre- and perinatal circuit formation, and age-related cell degeneration. They are involved in neuronal survival because nicotinic agonists have been shown to be neuroprotective in both *in vivo* and *in vitro* models (reviewed in Ref. [5]). Furthermore, it is becoming evident that the perturbation of nicotinic acetylcholine neurotransmission can lead to various diseases during development, adulthood and aging.

Several comprehensive reviews have described the structure and function of these channels [2–4,6–12]. We therefore aim to provide a short overview of recent studies of the subunit composition, function and pharmacology of native nicotinic acetylcholine receptor subtypes.

Receptor subtype composition and ligand-binding sites

Neuronal nicotinic acetylcholine receptors form a heterogeneous family of subtypes (Figure 1) formed by five subunits arranged around a central pore that is permeable to cations. These subunits are encoded by nine α (α2–α10) and three β (β2–β4) subunit genes, which are expressed in the nervous system and in several non-neuronal tissues [2,4,7](Figure 1a). Two main subfamilies of neuronal nicotinic acetylcholine receptors subtypes have been identified so far: αbungarotoxin (αBgtx)-sensitive and αBgtx-insensitive receptors. The αBgtx-sensitive receptors can be homopentameric (α7, α8 and α9) or heteropentameric (α7α8 and α9α10), whereas αBgtx-insensitive receptors are only heteropentameric and consist of α (α2–α6) and β (β2–β4) subunits [2].

Recent crystallization and structural determinations of ACh-binding proteins (homopentameric soluble proteins with an affinity spectrum resembling that of homomeric α7 receptors) from the snails *Lymnaea stagnalis* [13] and *Bulinus truncatus* [14], and the saltwater mollusc *Aplysia californica* [15] have helped to define the molecular details of the ligand-binding sites on the receptors. It is now known that the ligand-binding sites on the homopentameric receptors are present at the interface formed by opposite sides of the same subunit, whereas those on the heteropentameric receptors are located at the interface between two adjacent subunits that carry, respectively, the primary and complementary component of the site (Figure 1b).

It is thought that the homopentameric receptors have five identical ACh-binding sites per receptor molecule (one on each subunit interface), and the heteropentameric receptors have two binding sites per receptor molecule located at the interface between an α and a β subunit (Figure 1b and reviewed in Refs [7,16]). Functional heteropentameric receptors usually comprise: (i) two 'true' α subunits carrying the principal component of the

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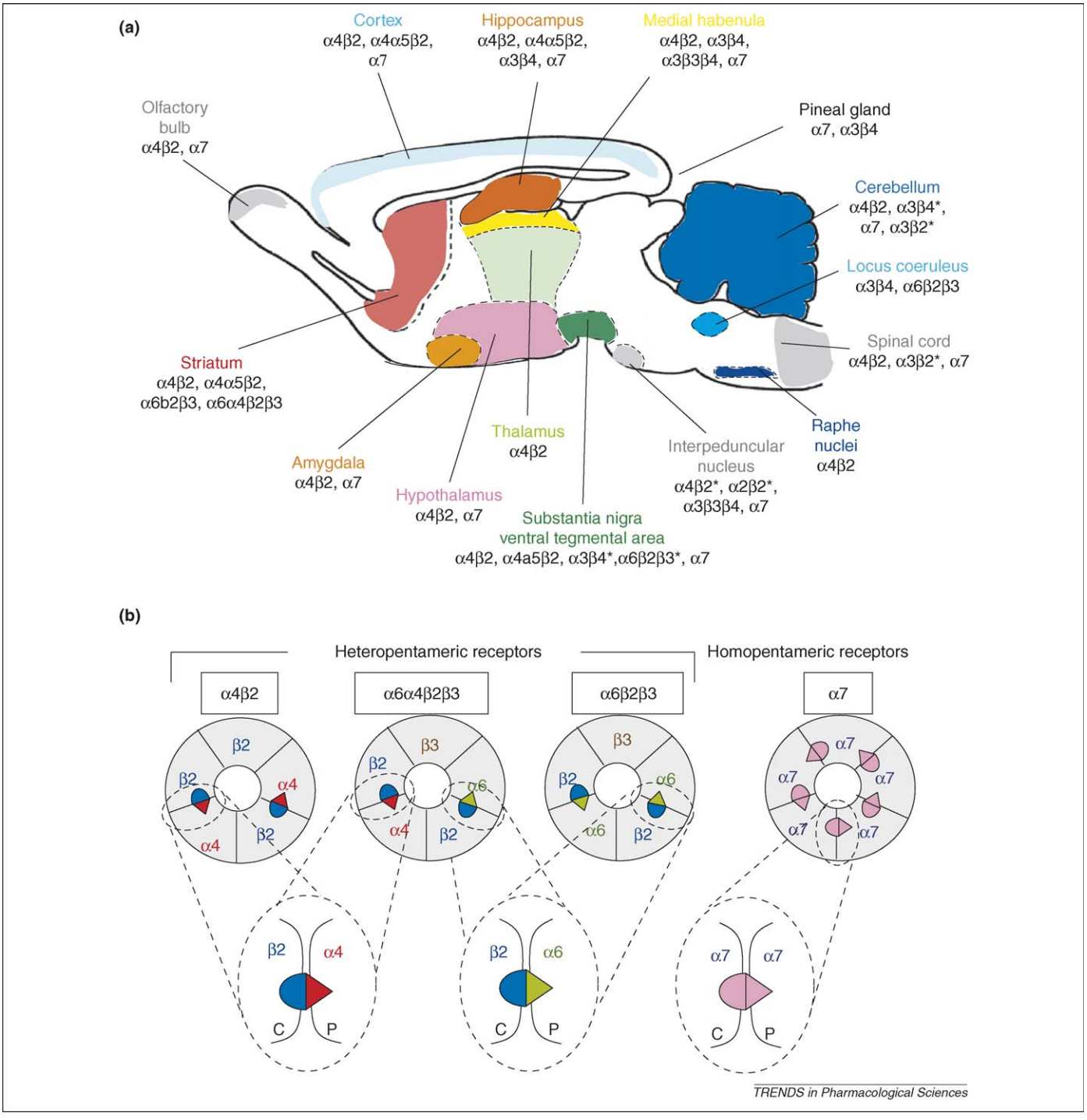

Figure 1. Regional distribution and subunit organization of the nicotinic acetylcholine receptors. **(a)** Regional distribution of the main nicotinic receptor subtypes in the rodent CNS. The subtypes present in the cortex, cerebellum, hippocampus, interpeduncular nucleus, medial habenula and pineal gland have been identified by binding, immunoprecipitation and/or immunopurification assays in tissue from rat and/or wild-type and/or receptor subunit knockout mice. The subtypes present in the amygdala, hypothalamus, locus coeruleus, olfactory bulb, raphe nuclei, spinal cord, substantia nigra–ventral tegmental area and thalamus have been deduced from *in situ* hybridization, single-cell PCR and binding studies of tissues obtained from rat and/or wild-type and/or knockout mice (reviewed in Refs [1,24,31,35]). **(b)** Organization and structure of heteropentameric and homopentameric subtypes. The pentameric arrangement of nicotinic acetylcholine receptor subunits in the heteropentameric α4β2, α4α6β2β3, α6β2β3 and homopentameric α7 subtypes and localization of the subunit interfaces of the ACh-binding site are shown. The homomeric α7 subtype has five identical ACh-binding sites per receptor molecule (one on each subunit interface). The heteropentameric α4β2 receptors have two identical binding sites per receptor molecule, located at the interface between an α4 and a β2 subunit. The heteropentameric α6α4β2β3 subtype has two different binding sites per receptor molecule, located at the interface between an α4 and a β2 subunit, and at the interface between an α6 and a β2 subunit. The heteropentameric α6β2β3 subtypes has two identical binding sites per receptor molecule, located at the interface between an α6 and a β2 subunit [1,24,31,35]. In homopentameric receptors, the interface comprises opposite sides of the same subunit; in heteropentameric receptors, the interface is located between two adjacent subunits – one carrying the primary (P) component and the other carrying the complementary (C) component of the site. Part (a) reproduced, with permission, from Ref. [88].

ACh-binding site (α2, α3, α4 or α6); (ii) two non-α subunits carrying the complementary component of the ACh-binding site (β2 or β4); and (iii) a fifth subunit (equivalent to β1 in muscle-type nicotinic acetylcholine receptors) that does not participate in ACh binding (α5, β3, but also β2 or β4).

Although it has been shown that α4β2 subtypes with different stoichiometry $(\alpha4)_2(\beta2)_3$ and $(\alpha4)_3(\beta2)_2$ can be generated in heterologous systems when the ratio of α4 to β2 subunits is changed or under different experimental conditions, no evidence of this stoichiometry has been found in brain.

Critique of methods for identifying receptor subtype expression

The identification of nicotinic acetylcholine receptor subtype composition is currently based on a combination of technical approaches and the availability of nicotinic acetylcholine receptor subunit knockout or knock-in mice. Any revision of the three generally accepted compositional rules listed above (e.g. a different receptor stoichiometry with three 'true' α subunits and two non-α subunits, see above) would require our current deductions concerning receptor composition to be changed (see Refs [17,18]).

The techniques currently used to identify nicotinic acetylcholine receptor subtypes include the regional or cellular localization of subunit mRNA (by *in situ* hybridization or single-cell PCR) or proteins (by immunoprecipitation or immunocytochemistry), receptor subtype imaging (by autoradiography, positron emission tomography or functional magnetic resonance imaging), the assessment of subtype composition and pharmacology (by binding in tissue homogenates or immunopurification), and functional assays (based on neurotransmitter release or electrophysiological techniques).

Interpreting the data obtained using these techniques requires some general caveats: namely, there is not always a correlation between mRNA levels and concentrations of the expressed subunits (see Ref. [19]); the total amount of subunit proteins can be misleading because protein subunits must assemble properly to make functional receptors; and pharmacologically identified binding sites are not always functional or located in the physiologically relevant membrane domain. More specifically, some essential technical issues should be taken into consideration.

- *In situ* hybridization using hydrolyzed riboprobes is more sensitive than *in situ* hybridization using oligoprobes, but is prone to show false positives in view of the difficulty in testing probe specificity (e.g. see Ref. [20]).
- Single-cell PCR is highly sensitive and specific but only gives all-or-none results and might therefore overestimate the relevance of some subunits: for example, compare the α2, α3 and α6 data obtained using oligoprobes, riboprobes and single-cell PCR in midbrain dopamine neurons, with those obtained in α3 and α6 knockout mice and immunoprecipitation studies [5,20–24].
- It has been recently shown that most of the available anti-subunit antibodies used in immunocytochemistry are not specific because they give similar staining patterns in tissues obtained from the respective wild-type and subunit knockout mice [25]. The specificity of the antibodies must therefore be tested first in wild-type and knockout mice.
- The anti-subunit antibodies used in immunoprecipitation and immunopurification experiments do not have the specificity limitations of those used in immunocytochemistry, possibly because of the antigen selection made by receptor binding. To quantify the receptors in native tissue, however, it is essential to determine the binding capacity of the antibodies (which is usually 70–90% of the subunit targeted by the antibodies). The spatial resolution of the immunoprecipitation technique at a regional level is of course limited.
- The main limitation of functional and binding assays is the lack of subtype-specific ligands, but the use of these assays has greatly improved with the availability of subunit-specific knockout and knock-in mice.

The techniques described above have been applied to both wild-type and, more recently, genetically engineered mice.

In terms of constitutive knockout mice, the possible existence of developmental compensations for the loss of a targeted gene must always be considered and, in principle, cannot be excluded. No evidence of such compensation has been found in β2-deficient mice because the targeted re-expression of the β2 subunit in the mesolimbic pathway of adult β2-deficient mice completely restores all of the wild-type phenotypes related to this pathway [26]. In addition, no compensation has been observed in expression of the other nicotinic acetylcholine receptor subunits or in that of nonβ2* nicotinic acetylcholine receptor subtypes [27], or in the function of the spared nicotinic acetylcholine receptor subtypes [22,24] (note that an asterisk indicates that other, unidentified subunits might also be present in the receptor subtype). By contrast, in α6 knockout mice overexpression of the α4 subunit compensates for the loss of α6* nicotinic acetylcholine receptors in striatal regions [24], and complex functional compensation might occur that must be considered when interpreting functional studies of specific subtypes.

Native subtypes

In defining the native subtypes of nicotinic acetylcholine receptor, we will follow the rules and caveats described above. Table 1 and Figure 1 show the composition, localization and number of native subtypes identified in different brain regions of the animal species studied so far. The subtypes are identified by the list of their subunits.

Localization and subunit composition

In agreement with *in situ* hybridization studies showing the widespread distribution of α4, β2 and α7 subunit mRNAs in the brain, α7 receptors and nicotinic acetylcholine receptors containing the α4 and β2 subunits are the most expressed subtypes, although their regional expression varies in different vertebrate species (Figure 1 and Table 1). In primate brain, the widespread distribution of the α2 subunit is comparable to that of the α4 subunit [28]. The other subunits of the αBgtx-insensitive subclass have a much more restricted distribution, although they might be highly expressed and have important functions in these regions (see later) [20].

The specificity of αBgtx for receptors containing the α7–α10 subunits has enabled the precise cellular and subcellular localization of αBgtx-sensitive nicotinic acetylcholine receptors to be determined by binding studies. These receptors are highly expressed in the cortex,

Table 1. Identification and quantification of nicotinic acetylcholine receptor subtypes expressed in the CNS

	Human	Monkey	Rat	Mouse	Chick
Cortex					
αBgtx-R[a]	10.6 ± 2 [45]	22.4 ± 4 [51]	48.4 ± 4 [35]	38.0 ± 3 [24]	n.d.
Epi-R[b]	12.5 ± 1 [45]	41.6 ± 4 [51]	98.4 ± 8 [35]	79.1 ± 6 [24]	107 ± 6 [52]
Subtype (% of Epi-R)[c]	α2β2* (10) [45]	α2β2* (4) [51]	α4α5β2 (15) [35]	α4β2 (65) [24]	α2α4β2* (5) [52]
	α4β2* (65) [45]	α3β2* (13) [51]	α4β2 (70) [35]	α4α5β2 (15) [24]	α4β2 (75) [52,53]
		α4β2 (35) [51]			α4α5β2 (10) [53]
		α2α4β2* (10) [51]			
Cerebellum					
αBgtx-R			n.d.		n.d.
Epi-R			45 ± 2 [32]		56 ± 8 [2]
Subtype (% of Epi-R)			α3β4 (22) [32]		
			α3α4β2 (10) [32]		
			α3α4β4 (12) [32]		
			α3β2β4 (12) [32]		
			α3α4β4 (12) [32]		
Hippocampus					
αBgtx-R			83.6 ± 2 [2]	87.3 ± 13 [2]	
Epi-R			44.5 ± 8 [2]	85.6 ± 11 [2]	
Subtype (% of Epi-R)			α4β2 (75) [2]	α4β2 (75) [2]	
			α4α5β2 (15) [2]	α4α5β2 (15) [2]	
Lateral geniculate nucleus					
αBgtx-R			65 ± 5 [37]		
Epi-R			264 ± 10 [37]		
Subtype (% of Epi-R)			α2α6β2* (4) [37]		
			α3β2* (3) [37]		
			α4α6β2β3 (9) [37]		
			α4β2 (57) [37]		
			α4α5β2 (7) [37]		
			α6β2β3 (10) [37]		
Midbrain					
αBgtx-R				50 ± 10 [31]	
Epi-R				144 ± 6 [31]	
Subtype (% of Epi-R)				α4β2* [31]	
				α3β3* [31]	
				α3β3* [31]	
				α4β2* [31]	
				α6β3* [31]	
Pineal gland					
αBgtx-R			n.d.		
Epi-R			300 [54]		
Subtype (% of Epi-R)			α3β4 (90) [54]		
Superior colliculus					
αBgtx-R			73 ± 3 [37]	n.d.	1833 ± 260 [52]
Epi-R			169 ± 3 [37]	201 ± 11 [37]	225 ± 10 [52]
Subtype (% of Epi-R)			α3β2* (10) [37]	α3β2* (11) [37]	α2α4β2 (30) [52]
			α4β2 (44) [37]	α4β2* (48) [37]	α2α4β2 (5) [52]
			α4α5β2 (6) [37]	α6β2β3* (31) [37]	α4β2 (55) [52]
			α6β2β3 (18) [37]		
			α4α6β2β3 (12) [37]		
Striatum					
αBgtx-R	n.d.	12.1 ± 2 [51]	47 ± 2 [35]	50 ± 1 [24]	
Epi-R	55.0 ± 7 [45]	55.5 ± 4 [51]	154 ± 15 [35]	72 ± 1 [24]	
Subtype (% of Epi-R)	α4β2* (76) [45]	α3β2* (14) [51]	α4β2 (42) [35]	α4β2 (46) [24]	
	α6β2* (17) [45]	α4β2* (45) [51]	α4α5β2 (19) [35]	α4α5β2 (12) [24]	
		α6β2* (10) [51]	α6α4β2β3 (8) [35]	α6α4β2β3 (11) [24]	
		α6α4β2* (10) [51]	α6β2β3 (12) [35]	α6β2β3 (19) [24]	
Retina					
αBgtx-R			44.2 ± 4 [19]		1330 ± 100 [29]
Epi-R			202 ± 17 [19]		247 ± 21 [55,56]
Subtype (% of Epi-R)			α2 and/or α3β2* (11) [19]		α4β4 (25) [55]
			α2 and/or α3β4* (3) [19]		α6β3β4* (30) [57]
			α4β2* (47) [19]		β2* (32) [56]
			α4β2β3* (15) [19]		β4* (78) [56]
			α6α4β2β3 (11) [19]		

[a]αBgtx-Rs are αbungarotoxin receptors measured by ^{125}I-labeled αbungarotoxin binding and are expressed as fmol per mg of protein.
[b]Epi-Rs are epibatidine receptors measured by [^{3}H]epibatidine binding and are expressed as fmol per mg of protein.
[c]% of Epi-R represents the percentage of the subtype over the total amount of epibatidine receptors in the tissue. Abbreviation: n.d., not determined.

hippocampus and subcortical limbic regions, and are expressed at low levels in the thalamic regions and basal ganglia. Affinity purification of these receptors from the brains of different species has confirmed that α7 receptors are pentamers of a single α7 subunit in rat and chick brain, and that chick brain also contains two additional αBgtx-sensitive subtypes: the homopentameric α8 and the heteropentameric α7α8 receptors [29].

An α7 gene that incorporates a unique 87-base-pair cassette exon has recently been identified. When expressed in oocytes, it forms channels with a slower kinetics and reversible αBgtx binding [30]. Immunolocalization studies suggest that this subtype constitutes a distinct subset of α7 receptors.

αBgtx-sensitive receptors containing the α9 and/or α10 subunits have not been found in brain, and their coexpression is limited to the cochlea and a few ganglia. The α9 subunit forms homomeric channels, whereas the α10 subunit forms functional channels only when it is coexpressed with the α9 subunit [2].

Recent studies have shown that the α7 subunit can also form functional channels with the subunits of the αBgtx-insensitive subfamily in heterologous systems, but so far there is no biochemical evidence of their existence *in vivo* (reviewed in Ref. [2]).

The α4β2 subtype was the first to be biochemically and pharmacologically characterized in total rat brain (reviewed in Ref. [29]) and has been subsequently shown to constitute the principal nicotinic acetylcholine receptor subtype in subregions such as the cortex, striatum, superior colliculus, lateral geniculate nucleus and cerebellum [5,31,32] (Table 1). Accordingly, β2 or α4 subunit knockout mice lose most of their high-affinity receptors for nicotinic agonists in the CNS [27,33].

In agreement with the data on mRNA distribution, receptor subtypes containing the α3, α6, β3 or β4 subunits have a relatively restricted distribution in the brain. In the neuronal populations in which they are expressed (e.g. α6β3* receptors in midbrain dopamine neurons, or α3β4* receptors in medial habenula neurons), however, they can constitute the main subpopulations of nicotinic acetylcholine receptors with a significant function.

The α2β2, α2β4, α3β2, α3β4, α6β2 and α6β4 nicotinic acetylcholine receptor subunits can either constitute simple subtypes or form one of the two ACh-binding interface in complex subtypes – for example, α4α6β2* in the striatum and visual pathways (Figure 1b), α3α4β2 or α3α4β4 in cerebellum [32], and α2α4β2* in retina [19,34]. Usually, when multiple α or β subunits are coexpressed, only some of the many possible subunit combinations are actually assembled: for example, in the rat midbrain almost all dopamine neurons express α4, α5, α6, β2, and β3 [22], but α6β3 and α4α5 subunits are preferentially coassembled in dopamine striatal terminals [24,35].

The factors that contribute to preferential subunit assembly are poorly understood and might include the influence of the chaperone molecules, relative subunit concentration and intrinsic affinities between pairs of subunits. These possibilities are also suggested by recent findings in β3 knockout mice that show a decrease in the expression of α6* receptors, but no change in the level of α6 mRNA [36], in dopamine cell bodies in the midbrain and, more markedly, in their terminal field in the striatum [31]. This decrease indicates that the β3 subunit is important for the formation of most α6β2* or α6α4β2* pentamers, and its loss causes defects in nicotinic acetylcholine receptor assembly, degradation and/or trafficking.

Several studies have clearly shown that the same neuronal population can express multiple subtypes of nicotinic acetylcholine receptor. The reasons for this heterogeneity are understood only partially [7]. Specific electrophysiological features of the subtypes (e.g. high versus low Ca^{2+} permeability or fast versus slow desensitization rates) might make their coexpression functionally relevant; alternatively, the various subtypes might be needed for preferential targeting to different cell compartments. *In vivo* evidence for preferential targeting of native nicotinic acetylcholine receptor subtypes is still circumstantial, but some subtypes, for example, α6β3* receptors in midbrain dopamine neurons or retinal ganglionic cells, might be targeted to the nerve terminal compartment in preference to other subtypes, such as α4* [31,35,37]. However, the precise determinants of these targeting processes are not well understood.

Pharmacology

The evidence that native αBgtx-insensitive nicotinic acetylcholine receptors are extremely heterogeneous has increased the complexity of studying their pharmacological profiles. Because a ligand-binding site is formed by the interface between a true α and a β subunit, in Table 2 we have summarized the pharmacological characteristics of α–β interfaces in heterologously expressed and native subtypes.

The agonist profile is similar for the different interfaces. The agonists epibatidine, cytisine, ACh, 1,1-dimethyl-4-phenylpiperazinium (DMPP), nicotine and the newly synthesized nicotine derivative A-85380 have limited selectivity and cannot discriminate among the different subtypes in functional assays; however, their higher affinity (particularly that of A-85380) for the αX–β2 than the αX–β4 interface can be used to characterize the various subtypes, at least in binding studies [11].

The recent discovery of *Conus* peptides in the venom of cone snails has made it possible to discriminate subtypes in both binding and functional tests. One typical example is αConotoxin MII (αCntxMII), which can block the response of ACh to heterologously expressed α6β2 and α3β2 subtypes with nanomolar affinity but is 100–10 000 times less potent towards the other subtypes [11,38,39]. The specificity of αCntxMII for native subtypes has been demonstrated by binding studies (Table 2), and its use has helped to define different interfaces in the same receptor. In the striatum, it has extremely low affinity for the α4β2 subtype, both high and low affinity for α6* receptors (α6β2β3 and α4α6β2β3 subtypes) purified from wild-type mice, and a single high affinity for the α6β2β3 subtype purified from α4 knockout mice [24]. The low-affinity binding site for αCntxMII on the wild-type α6* receptor is due to the presence of an α4–β2 interface (Figure 1b) that is absent in the striatal α6* receptors purified from

Table 2. Pharmacological characteristics of the α–β and α–α interfaces

Binding studies[a]						Functional studies[a]		
Interface[b]	Agonist profile	A-85380 K_i	ACh K_i	Antagonist profile	αCntxMII K_i	αCntx MII IC_{50}	αCntx BuIA IC_{50}	αCntx PIA IC_{50}
α2–β2	E>A*>C>A∼N>D	0.07	11	DHE>MLA>Mec	n.d.	>1000	800	>10 000
α3–β2	E>A*>D>C>N>A	0.21	56	DHE>MLA>Mec	50	0.5–2.2	5.72	74.2
α4–β2	E>A*>C>N>A>D	0.14	44	DHE>MLA>Mec	>1000	430	10 400	>10 000
α6–β2	E>C>N>A>D	0.12	2024	MLA>DHE>Mec	1.1	0.39	0.26*	0.69*
α2–β4	E>C>A*>N-A>D	18	110	DHE>MLA>Mec	n.d.	>100	121	>10000
α3–β4	E>A*>C>N>D>A	78	850	DHE>MLA>Mec	>1000	>100	27.7	518
α4–β4	E>C>A*>N>A>D	8.1	99	MLA>DHE>Mec	n.d.	>10 000	69.9	>10 000
α6–β4	E>C>N>D>A	n.d.	59	MLA>DHE>Mec	4.3	n.d.	1.54	33.5
	Agonist profile	**Nic K_i**	**ACh K_i**	**Antagonist profile**	**αBgtx K_i**	**ACh EC_{50}**	**Nic EC_{50}**	**TUB IC_{50}**
α7–α7	E>N>C>A	470	14300	MLA>TUB>DHE	1–2.4	112 000	7800	140
α8–α8	E>N>C>A	10[c]	60[c]	MLA>TUB>DHE	0.02[c]	1900	1000	600
		4500[d]	7000[d]		3.6[d]			
α7–α8	E>N>C>A	130	2600	n.d.	2.4	n.d.	n.d.	n.d.

[a]The agonist and antagonist profiles and K_i (nM) data come from binding studies [24,55,58–60], whereas IC_{50} and EC_{50} (nM) values were determined by electrophysiological experiments on transfected or native purified subtypes [24,37,38,40,41,61].

[b]For the α2–β2, α3–β2, α4–β2, α2–β4, α3–β4, α4–β4 interfaces, the data were obtained from cells transfected in pairwise combinations [58]. The K_i values of the α6–β2 interface come from experiments on native α6 receptors immunopurified from α4 knockout mice [24], whereas the αCntx BuIA and αCntx PIA IC_{50} values were obtained from electrophysiological experiments on rα6–α3β2 chimera. The values of the α6–β4 interface refer to cells transfected with the chick α6β4 subunits [55], an rα6–α3β4 chimera [41] or rα6–β4 [40]. For the α7–α7 [59], α8–α8 [60], α7–α8 [59] interfaces, the binding data were determined on immunopurified subtypes [59,60], whereas the functional data are from oocyte-expressed subtypes [61]. Abbreviations: A*, A-85380; A, acetylcholine; C, cytisine; αCntx, αconotoxin; D, DMPP; DHE, dihydro-β-erythroidine; E, epibatidine; EC_{50}, effector concentration for half maximal response; IC_{50}, inhibitor concentration for half-maximal response; Mec, mecamylamine; MLA, methyllycaconitine; N, nicotine; n.d., not determined; TUB, D-tubocurarine.

[c]High-affinity site.

[d]Low-affinity site.

α4 knockout mice, which therefore have two high-affinity binding sites.

αCntxMII has a higher affinity for the α6β2β3 receptor (inhibition constant, $K_i = 1$ nM) than for the α3β2* subtypes ($K_i = 50$ nM) [37], but this difference is not large enough to discriminate the subtypes functionally. The newly discovered αConotoxin PIA and modified αCntxMII [39] can, however, distinguish the α3β2* and α6β2* subtypes [40]. The newly discovered αConotoxin BuIA potently blocks α3* and α6* nicotinic acetylcholine receptor subtypes [41] (Table 2). This toxin binds receptors containing either β2 or β4 subunits, but its kinetics of unblocking depend on the β subunit: β4* nicotinic acetylcholine receptors have much slower off times than have the corresponding β2* receptors. These newly discovered αConotoxins have provided considerable insights into the subunit composition of brain nicotinic acetylcholine receptors and might form templates for the design of new ligands for these receptors.

In the chick CNS, some αBgtx-sensitive receptors have an α8–α8 interface [29]; this interface has a higher affinity for nicotinic agonists than the α7–α7 interface, whereas the α7–α8 interface has an intermediate affinity (Table 2).

Functional studies

Postsynaptic α7*, α4β2* and α3β4* receptor subtypes have been found in various regions of the CNS, and other postsynaptic heteropentameric subtypes might exist. There is, however, a general consensus that the nicotinic acetylcholine receptors present at presynaptic or preterminal sites are physiologically more important [10,11]. Table 3 summarizes the different nicotinic acetylcholine receptor subtypes involved in neurotransmitter release in different areas of the brain.

In *ex vivo* release experiments, the use of slices (which preserve some of their existing neuroanatomical connections) or synaptosomes (which are isolated nerve terminals) can give complementary results. For example, studies using αConotoxin AuIB have established that ∼30% of noradrenaline release from hippocampal synaptosomes is due to the α3β4* subtype [10], whereas release studies using hippocampal slices and electrophysiological recordings have shown that noradrenaline release is also indirectly modulated via an α7 receptor located in glutamate afferents and γ-amino butyric acid (GABA)-containing interneurons, thereby demonstrating that there is cross-talk among neurotransmitters in modulating noradrenaline release [42].

Studying the subtypes involved in neurotransmitter release can also give different results, depending on whether they are done in simple *ex vivo* systems or in more complex *in vivo* circuits. For example, α6* receptors mediate αCntxMII-sensitive dopamine release from striatal synaptosomes, but do not seem to be involved in the dopamine release induced by systemic nicotine *in vivo*, as demonstrated by microdialysis studies of freely moving wild-type and α6 knockout mice [36].

In contrast to glutamate release, which is directly controlled only by α7 receptors, the release of all other neurotransmitters seems to be regulated by multiple subtypes (Table 3). Moreover, owing to the complexity of neuronal networks, neurotransmitter release from a neuron is often controlled by the activation of nicotinic acetylcholine receptors located on other neurons (Table 3).

In summary, the predominantly presynaptic localization and widespread distribution of nicotinic acetylcholine receptors in several brain circuits makes it particularly difficult to characterize functionally the subtypes involved in specific behavioral or complex brain roles, although the use of genetically engineered mice should make this easier in the future.

Table 3. Nicotinic acetylcholine receptors involved in neurotransmitter release in brain

	Glu	ACh	DA	GABA	NA
Cerebellum	α7 (r) [4]				
Cortex	α7 (r)[a] [10] β2* (r) [11,62]	α4β2* (r) [11] αxβ4* (r) [11]	α3β2* (h) [63] α4β2* (r)[a] [64] α3 and/or α6β2* (r)		
Hippocampus	α7 (r)[a] [42]	α4β2* (r)[a] [10] αxβ4* (r)[a] [10]	α3β4* (r) [11,65]	α7 (r) [10] α4β2* (r) [11]	α3β2* (r) [10] α4β2* (r) [11] α3β4* (r)[a] [42] α7 (r) [11]
Interpeduncular nucleus		β4* (m)[a] [10]		αxβ2* (m) [10]	
Hypothalamus					xβ2* (r) [66]
Midbrain			α4β2* (m, r) [10]	α4β2* (m, r) [10]	
Lateral geniculate nucleus	α7 (c) [10]				
Olfactory tubercle	α7 (r) [10]		α4β2* (m)[a] [67], α3 and/or α6β2* (m) [67]		
Superior colliculus	α7 (m) [68]			α3β2* [68] and/or α6β2* (m) [68]	
Striatum	α7 (r) [10]		α4β2* (m)[a] [24,64,69] α4α5β2* (m)[a] [24,69] α6α4β2β3 (m)[a] [24,69] α6β2β3 (m)[a] [24,64,69] α7 (r) [10]		
Thalamus			α4β2* (m) [67]	α4β2* (m) [10]	

[a]Tested in synaptosomes. Abbreviations: ACh, acetylcholine; c, chick; DA, dopamine; GABA, γ-aminobutyric acid; Glu, glutamate; h, human; m, mouse; NA, noradrenaline; r, rat.

Involvement of native subtypes in pathological states

Studies of receptor subunit knockout mice have shown that brain nicotinic acetylcholine receptors are not essential for survival or for the execution of basic behaviors [2]. They are, however, important for the fine control of several more sophisticated and complex behaviors that can be evaluated only by means of appropriate tests or in particularly labile situations such as the aged brain. These findings place nicotinic acetylcholine receptors in a different, and perhaps more important, perspective because of their involvement in brain diseases and the possibility of using them as drug targets. Many pathological situations involve a lack of fine

Table 4. Involvement of nicotinic acetylcholine receptors in brain diseases

Disease	Epi binding	αBgtx binding	Effects of nicotinic drugs (Ago or Ant)	Effects of smoking	Level of heteromeric subunit
Tourette's syndrome			Both P [2,70]	P [2,71]	
Autism	Parietal cortex ↓ [72] Cerebellum ↓ [72] Thalamus ↓ [72]	Cortex → [73] Cerebellum ↑ [73]			Parietal cortex β2 ↓ [2,71] Cerebellum α4 ↓ [2,71] Thalamus β2 ↓ [2,71]
Schizophrenia	→ [74]	Hippocampus ↓ [75,76] Thalamus ↓ [76] Frontal cortex ↓ [75] Cingulate cortex ↓ [76]	P (Ago) [77]	P [72]	Hippocampus α4 → [73,78] Thalamus β2 → [76] Cortex α3 → [76]
Depression			P (Ago) [79,80]	P [2]	
Attention deficit hyperactivity disorder			P (Ago) [77,78]	P [2,77]	
Parkinson's disease	Striatum ↓ [81] SN ↓ [82] Cortex ↓ [77,83]	Cerebellum ↓ [2] Cortex ↓ [77,83]	P (Ago) [77] n.e.	P [77]	Striatum α6, β3 ↓ [45] Cortex α4, β2 ↓ [81]
Alzheimer's disease	Cortex ↓ [77] Hippocampus ↓ [77] Presubiculum ↓ [2,84] Thalamus ↓ [2,84] Striatum ↓ [2,84]	Temporal cortex → [78] Thalamus ↓ [85]		P [2]	Cortex α4, β2 ↓ [45,71] Cortex α3 → [85]
Aging	Frontal cortex ↓ [2,86] Hippocampus ↓ [2,86]	Hippocampus → [2,86] Entorhinal cortex ↓ [2,86] Thalamus ↓ [2,86]			Cortex α4, β2 ↓ [2,86] Cortex α3 → [2,86]
Tobacco dependence	Cortex ↑ [2,87]		P (Ago) [77]	P [2]	Hippocampus α4 ↑ [71] Entorhinal cortex α4 ↑ [66] Dentate gyrus α7 ↑ [87]

[a]Determined in synaptosomes. Abbreviations: ↑, increased; ↓, decreased; →, not different from control; Ago, agonists; Ant, antagonists; αBgtx, ^{125}I-labeled αbungarotoxin; Epi, [^{3}H]epibatidine; n.e., no effect; P, positive; SN, substantia nigra.

control and tuning, rather than the complete loss of a particular function, and the pharmacological restoration of appropriate tuning could have a crucial clinical effect.

Nicotinic acetylcholine receptors are involved in the pathogenesis or symptomatology of several diseases of the CNS, which can be divided into two groups: those in which a nicotinic acetylcholine receptor subunit gene is mutated and a receptor subtype function is altered, such as autosomal dominant frontal lobe epilepsy; and those involving a modification in the number of nicotinic acetylcholine receptors, such as schizophrenia, Tourette's syndrome, attention deficit hyperactivity disorder, autism, depression and anxiety, and the neurodegenerative Alzheimer's and Parkinson's diseases [1,2,4]. Nicotinic acetylcholine receptors are also the targets responsible for the behavioral effects of nicotine and tobacco dependence (Table 4).

For most diseases, the involvement of nicotinic acetylcholine receptors has been deduced on the basis of binding experiments with nonselective nicotinic ligands, clinical response to nicotinic drugs, smoking behavior, or similarities with experimental models in which nicotinic acetylcholine receptors have been modified [43,44]. Although these data are sufficient to suggest the involvement of nicotinic acetylcholine receptors, much less is known about the receptor subtypes involved and their precise role. Apart from Parkinson's disease, in which a marked reduction in the number of $\alpha6\beta3^*$ receptors in the dopamine pathway has been clearly documented, multiple receptor subtypes seem to be affected in most disorders [2,45].

Because the main localization of nicotinic acetylcholine receptors is on presynaptic structures that have a modulatory role on neurotransmission, a change in receptor number (as occurs in various diseases) does not lead to overt behavioral modifications and thus a pharmacological approach based on nicotinic agents will probably have relatively slight effects, as can be seen from the relative failure of nicotinic therapy in Alzheimer's disease, Parkinson's disease, Tourette's syndrome, schizophrenia and depression. A nicotinic approach therefore seems more appropriate as an adjuvant therapy. It is difficult to interpret nicotinic therapies, however, because it is not clear whether the beneficial effects of nicotinic agonists, as seen in Tourette's syndrome and in smoking cessation, are due to the activation or desensitization of nicotinic acetylcholine receptors [4,46].

Mutations in the channel region of the $\alpha4$ or $\beta2$ subunits have been found in some families suffering from autosomal dominant frontal lobe epilepsy. The expression of these mutations in heterologous systems has given rise to nicotinic acetylcholine receptors with gain ($\alpha4$) or loss ($\beta2$) of function but, in both cases, the nicotinic acetylcholine receptors have higher ACh sensitivity. Although the pathogenic role of the mutated receptors remains unknown, it is likely that these receptors facilitate synchronization of the spontaneous oscillations in the thalamo-cortical circuits, which are highly enriched in $\alpha4\beta2$ receptors [1,4].

Another way of demonstrating the pathogenic involvement of specific nicotinic acetylcholine receptor subtypes is to study the associations between subunit gene polymorphisms and a disease. For example, several polymorphisms in the $\alpha4$ and $\beta2$ nicotinic acetylcholine receptor subunit genes have been studied with respect to late-onset Alzheimer's disease, and a significant association has been observed for a noncoding polymorphism in *CHRNB2* (the gene encoding the $\beta2$ subunit), thereby suggesting that this gene deserves further consideration as a candidate for Alzheimer's disease [47].

Concluding remarks

The functional data obtained from heterologous systems show that a simple one- or two-subunit nicotinic acetylcholine receptor would be sufficient to assure a nicotinic response to a target cell, but studies of various tissues indicate that native nicotinic acetylcholine receptors often contain more than one type of α or β subunit, and can consist of up to four different subunits. Thus, the number of biologically relevant receptor subtypes (with their distinct biophysical and pharmacological properties) is larger than previously thought, which might have important functional implications. It must also be considered that the properties of a receptor subtype can vary depending on its molecular and cellular milieu. Knowing the subunit composition of nicotinic acetylcholine receptors in a particular neuronal pathway is therefore a prerequisite for understanding the roles of the native receptors and the rational design of new drugs.

So far, most studies of pharmacological receptor regulation have concentrated on the pharmacological and biophysical properties of nicotinic acetylcholine receptors, and little is known about receptor turnover and trafficking [48] or about how drugs can affect these receptor features and intracellular subunit associations. Recent studies on how nicotine [49,50] or nicotinic drugs [43] determine the expression of specific subtypes raise the possibility of developing novel strategies aimed at modifying the expression of nicotinic acetylcholine receptor subtypes in different brain and cell domains, thereby generating important effects on neuronal excitation and leading to further insights into this class of receptor.

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