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Neuronal nicotinic receptors, important new players in brain function

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Abstract

Acetylcholine receptors are cationic channels whose opening is controlled by acetylcholine. They are key molecules in the colinergic nicotinic transmission in a number of areas of the central and perypheral nervous system. Because of the structural complexity, given by the numerous subunits that forms these receptors, they have different pharmacological and biophysical properties. Here we give a brief account of the known and consolidated data regarding neuronal nicotinic receptors, as as an introduction to the articles reported in this issue, in order to allow readers who are not familiar with the field to place the detailed information in the right context.

Keywords: Neuronal nicotinic receptor; Nicotinic receptor distribution; Nicotine; Nicotinic agent; Schizophrenia; Dementia; Ageing; Epilepsy; Tobacco addiction

1. Introduction

Nicotinic receptors are cationic channels whose opening is controlled by acetylcholine and nicotinic receptor agonists. They belong to the large family of ligand-gated ion channels that also includes the $GABA_A$, glycine and 5-HT_{3A} and 5-HT_{3B} receptors, which are formed from different homologous subunits. Nicotinic receptors are key molecules in cholinergic transmission at the neuromuscular junction of striated muscles, at the synapse in the autonomous peripheral ganglia, and in several brain areas (Sargent 1993; Gotti et al., 1997a; Changeux and Edelstein, 1998; Lindstrom, 2000). Each receptor consists of five subunits arranged in such a way as to delimit a large structure inserted into the plasma membrane with an aqueous channel in the centre (Unwin, 1993, 1996) (Fig. 1B).

Nicotinic receptors exist as a variety of subtypes, a heterogeneity that is due to the diversity of the genes encoding acetylcholine nicotinic receptor subunits. Sixteen acetylcholine nicotinic receptor subunit genes that derive from a common ancestor have so far been cloned from vertebrates (α1 to α9, β1 to β4, γ, ε and δ) (Le Novère and Changeux, 1995) (Fig. 1A). The subunits have been classified as α or ligand-binding subunits, (α1 to α9) if they contain a disulphide bond between adjacent cysteine residues analogous to Cys^{192}–$Cys^{193}$of the muscle α1 subunit, or non-α or structural subunits, if they do not contain these cysteines (β1 to β4, γ, ε and δ). The subunits have several common structural features: a large N-terminal extracellular domain, four putative transmembrane sequences (M1–M4), an intracellular loop of varying length, depending on the subunit, joining the third and fourth transmembrane domain (which is very important for the regulation of receptor function), and a short extracellular C-terminal sequence (Fig. 1C).

Analysis of the amino acid sequences of the subunits indicates that nicotinic receptors can be subdivided into three sub-families. The first consists of heteromeric muscle nicotinic receptors from skeletal muscles and fish electric organs, which have a pentameric subunit composition (α1)2β1γ1δ1 in the fetal form and (α1)2β1ε1δ1 in the mature form. These acetylcholine nicotinic receptors are selectively labelled and blocked by the antagonist α-bungarotoxin present in snake venom. The second consists of heteromeric neuronal acetylcholine nicotinic receptors that do not bind α-bungarotoxin. These latter neuronal nicotinic receptors also have a pentameric structure formed from combinations of α2, α3, α4 and α6 with either β2 or β4 subunits, and sometimes also with α5 or β3 subunits. The third subfamily consists of neuronal homoligomeric acetylcholine nicotinic receptors that bind α-bungarotoxin and are formed by the α7 or α8 or α9

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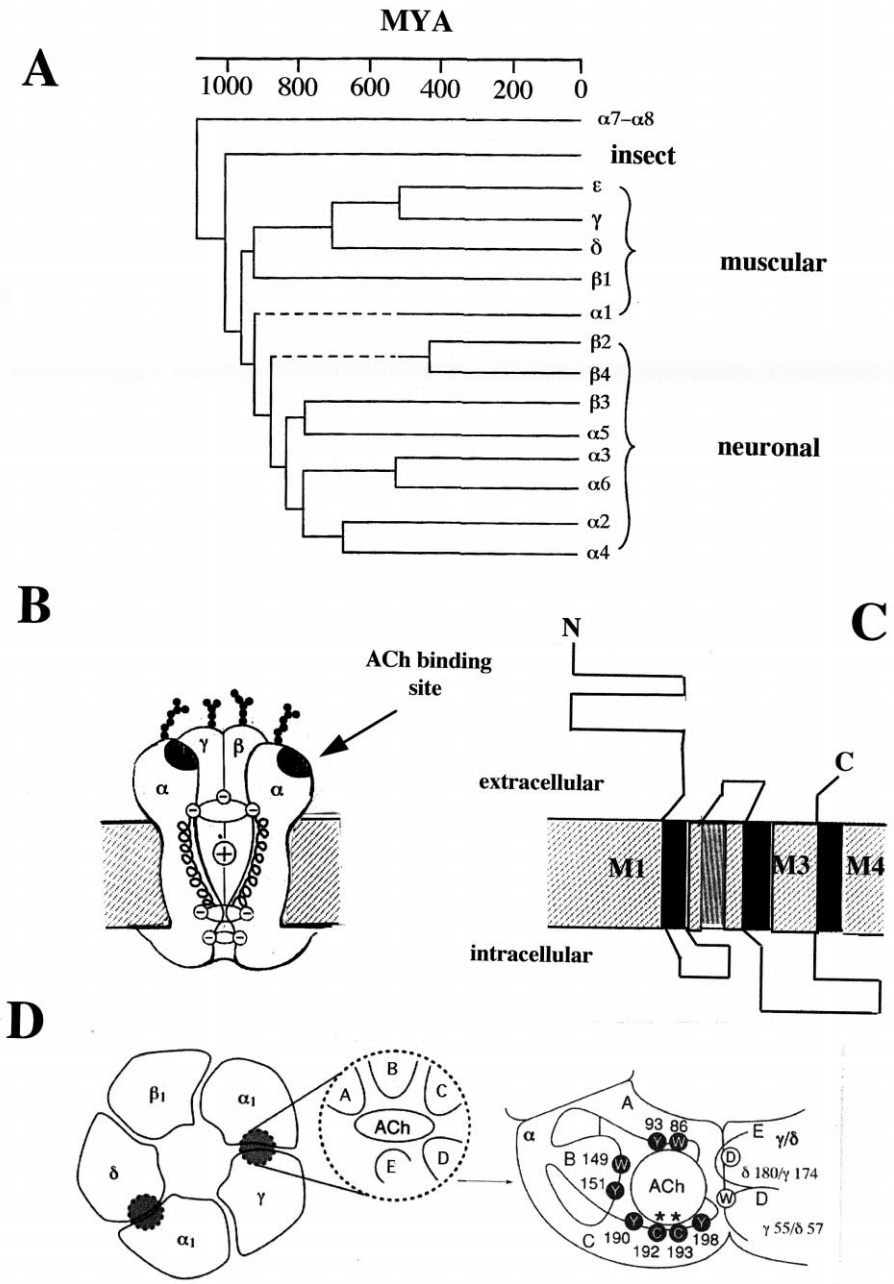

Fig. 1. (A) Schematic drawing of the putative evolutionary tree and branching of the nicotinic acetylcholine receptor family (modified from Bertrand and Changeux, 1995). MYA, million years ago. (B) Vertical section of the putative transmembrane organisation of the muscle nicotinic acetylcholine receptor. The black areas indicate the acetylcholine binding sites and rings the charged areas that control the channel permeability. (C) Putative transmembrane organisation of the nicotinic acetylcholine receptor subunit. (D) Scheme of the ACh binding sites in the muscle Nicotinic receptor. The α subunit forms the major component with the loops A, B, C, and the subunit γ or δ forms the complementary component with the loops D and E. The numbers indicate the position of the amino acids in the protein sequence. All these loops are located at the extracellular N-terminal of the subunits (modified from Changeux and Edelestein, 1998).

subunits. On the basis of the sequence homology and the fact that they form functional homomers, it has been suggested that the α7–α9 subunits represent the most primordial forms of these receptors, and that the other subunits evolved through gene duplication and subsequent divergence (Fig. 1A).

The acetylcholine binding site is located in the large extracellular N-terminal domain, at the interface between the α and non-α subunit. In muscle-type receptors, there are two non-equivalent binding sites per receptor: one located at the interface of the α and γ subunits and the other at the interface of the α and δ subunits. Studies of muscle receptors have shown that the ligand binding sites are formed by amino acid residues from both subunits and are made of at least five separated loops. The three loops (A, B, C) on the α subunit are the major components and

the other two loops (D, E), present on the γ or δ subunits, are the complementary component (Fig. 1D) (Bertrand and Changeux, 1995).

In heteromeric neuronal acetylcholine nicotinic receptors, the acetylcholine binding sites seem to be formed in a similar manner because both α and β subunits contribute to the pharmacological properties of the receptors. The homoligomeric α7 or α8 receptors have five identical acetylcholine binding sites per receptor molecule, one in each subunit (Changeux and Edelstein, 1998).

The cation channel of the muscle acetylcholine nicotinic receptor is lined by amino acids from the N-terminal third of the M1 region and those constituting the M2 region of the five subunits, and structural and functional studies have shown that these amino acids that face the lumen are critical for the properties of the channel.

Although the general architecture of nicotinic receptors is the same in the neuromuscular junction and in ganglionic or brain nerve terminals, their pharmacology and biophysical properties are very different. The basic aspects of the structure and function of the muscle nicotinic receptor are now very well known, but neuronal nicotinic receptors are much less understood and investigated.

Our knowledge of neuronal nicotinic receptors started in 1889 with the publication of the famous paper by Langley and Dickinson, who first reported that nicotine could block neuronal transmission in the superior cervical ganglion; the concept of the presence of nicotinic receptors was developed in subsequent papers published by Langley in 1905–1906. Until only a few years ago, knowledge of neuronal nicotinic receptors remained confined to the ganglia, which were seen as limitedly preserving all the qualitative peculiarities characteristic of the complicated construction of the central nervous system. The structure, function, pharmacology and distribution of brain receptors have more recently begun to be systematically investigated, and these studies have shown that nicotinic receptors are also relevant to our understanding of a number of complex brain functions and the pathogenic mechanisms of some brain pathologies. As a result, nicotinic drugs have begun to find their place as therapeutical and diagnostic tools.

2. Structure and heterogeneity of neuronal nicotinic receptors

In heterologous expression systems, α7, α8 and α9 are the subunits that can form homomeric receptors, whereas α2, α3, α4 and α6 always need to be coexpressed with β2 or β4 in order to generate functional channels. The α5 and β3 subunits have long been considered ''orphan subunits'', because they are unable to form functional channels when expressed in paired subunit combinations. Studies of the subunit composition of native nicotinic receptors have finally led to the demonstration that more than one type of α or β subunit can be incorporated in the same molecule, and that the α5 and β3 subunits can only be assembled with other functional α and β combinations (McGehee and Role, 1995; Lindstrom, 2000). The presence of the α5 subunit greatly increases Ca^{2+} permeability and changes other biophysical properties of the channels (Sivilotti et al., 1997; Gerzanich et al., 1998). Furthermore, the pharmacological responses of α3- or α4-containing receptors to nicotinic agents vary widely depending upon whether they include the β2 or β4 subunit (Luetje and Patrick, 1991; Covernton et al., 1994; Wang et al., 1996; Parker et al., 1998). Therefore, a large number of different heteromeric subtypes exist but the predominant forms are the α4β2 and α4α5β2 subtypes in the central nervous system, and the α3β4 or α3β4α5 subtypes in the peripheral nervous system (Sargent, 1993; McGehee and Role, 1995; Colquhoun and Patrick, 1997; Gotti et al., 1997b). The pharmacological response to nicotinic drugs and its characteristics (desensitisation rate, open and closure time, ion channel permeability, etc.) thus strictly depend on the subunit composition of the receptors (Papke, 1993).

The importance of understanding receptor heterogeneity is also made clear by the fact that subunit expression is regulated differently during development (Court et al., 1997), ageing (Zoli, 2000) and degenerative brain diseases (Nordberg et al., 1992; Martin-Ruiz et al., 1999), as well as by the fact that each neurone has the possibility of expressing different receptor subunits, which means that its response to nicotinic agents can be modified by changing its receptor composition according to physiological or pathological conditions (Corriveau and Berg, 1993; Poth et al., 1997; Kristufek et al., 1999). The possibility of such neuronal adaptation should be considered when nicotinic agents are used as diagnostic tools or drugs.

3. Receptor distribution

Brain cholinergic innervation comes from five major nuclei: (a) the basal forebrain, which innervates the cortex and hippocampus; (b) the diencephalus, which gives rise to local circuits and innervates the cortex; (c) the striatum, which also gives rise to local circuits; (d) the brain stem, which innervates the thalamus, the basal forebrain, the hindbrain, and the cerebellar cortex; and (e) the spinal cord, which innervates the cranial and somatic muscles and secretory glands. The system is extensively interconnected, leading to the coordinated firing of neurones and different cholinergic subsystems (Mesulam and Geula, 1988; Mesulam et al., 1989; Gotti et al., 1997a).

Nicotinic receptors are mainly located in various cortical areas, the periacqueductal grey matter, the basal ganglia, the thalamus, the hippocampus, the cerebellum, the retina, and in chickens the optic lobes (Gotti et al., 1997a).

The cortex contains α3, α4, β2, and β4 subunits, which are unequally distributed among the different layers:

the hippocampus contains α3, α4, α5, α7, β2, β3, and β4 subunits; the mesocorticolimbic system contains α2, α3, α4, α5, α6, α7, β2, β3, and β4 subunits; the auditory cortex contains α7 subunits; the optic lobe contains α2, α5, α7 and β2 subunits; and the retina contains α2, α3, α4, α6, α7, α8, β3, β4, and β2 subunits (Jones et al., 1999; Vailati et al., 1999). Receptor subtypes have a discrete pre- and/or postsynaptic localisation in the nervous system (Gotti et al., 1997a; Wonnacott, 1997), and it is possible that the nicotinic receptor subtypes in the same areas may be located in different neurone domains. Current knowledge of the gross regional localisation of receptor subtypes, which is mainly based on in situ hybridisation studies, is therefore not sufficient to define the neuronal circuits in which the nicotinic receptor subtypes are involved, and a more precise immunolocalisation of the different subunits is needed. It is also necessary to look at subunit distribution more critically because it is becoming increasingly evident that it varies in the brain of different animals species; for example, there is no α8 subunit in mammalian brain, and the α3 and α5 subunits are differently expressed and distributed in human and rodent brains (Wada et al., 1989; Rubboli et al., 1994 and Flora, personal communication).

One rather puzzling area that deserves attention is the non-neuronal distribution of neuronal nicotinic receptors which have been found in keratinocytes (Grando et al., 1995), muscle cells (Sala et al., 1996), lymphoid tissues (Nordberg et al., 1990a; Battaglioli et al., 1998), and neurosecretory cells (Chini et al., 1992). Their role in these tissues has not yet been defined, but a number of hypotheses have been put forward in relation to their involvement in pathological conditions. The pharmacological implications of these findings are that any drug aimed at the nicotinic system may have multiple effects outside the Central Nervous System.

4. Receptor functions

It has been known since the early days of pharmacology that nicotinic receptors are important in ganglionic transmission and control the function of the peripheral autonomic system (Asher et al., 1979), but their functions in the brain are still unclear. They are known to be involved in various complex cognitive functions, such as attention, learning, memory consolidation, arousal, sensory perception and in the control of locomotor activity, pain perception, body temperature regulation (Gotti et al., 1997a). Most of these data came from behavioural studies performed with nicotine or nicotinic receptor antagonists in humans and animals, and from natural models of nicotinic denervation such as degenerative brain diseases.

It is generally acknowledged that the majority of these effects are due to the presynaptic nicotinic receptors that modulate the release of a number of neurotransmitters (Wonnacott, 1997). However, postsynaptic nicotinic receptors also play important roles, the most clearly demonstrated being the control of ganglionic transmission and fast ACh-mediated synaptic transmission in the hippocampus and in the sensory cortex (Jones et al., 1999).

Experimental data indicate that different receptor subtypes are involved in different nervous system functions. Ganglionic transmission is mainly regulated by the α3(α5)β4 subtype, and the gene deletion of α3 and β2 induces a phenotype correlated with a decrease in ganglionic transmission (Xu et al., 1999). Pain control is mainly exercised through by the α4β2 subtype (Marubio et al., 1999, 2000). Dopamine release from brain dopaminergic neurones is partially controlled by an α4-containing subtype (and possibly also by an α6-containing subtype) (Le Novère and Changeux, 1996; Goldner et al., 1997; Wonnacott, 1997), whereas the release of glutamate is mainly controlled by an α7-containing subtype (Guo et al., 1998; Radcliffe et al., 1999). The β2 subtype in mice is important in the control of presynaptic GABA release (Poth et al., 1997; Lu et al., 1998) and the response to nicotine of mesencephalic dopaminergic neurones. The β3 subtype can control locomotor activity, by means of dopamine release in the striatum or other areas of the Central Nervous System in which β3-containing receptors are expressed at presynaptic or preterminal levels. Most of these data have recently been obtained by means of homologous recombination techniques with mouse stem cells, which have contributed greatly to defining the physiological role of nicotinic receptors.

Nicotinic receptors also seem to be involved in neuronal survival since aged β2 knocked out mice show region-specific changes in the cerebral cortex, with neocortical hypotrophy, the loss of hippocampal neurones, and astro- and microgliosis (a picture resembling a neuro-degenerative disease). The functional correlate of these histopathological alterations is impaired spatial learning (Picciotto et al., 1995; Xu et al., 1999; Zoli, 2000). These observations correlate well with epidemiological data showing that chronic nicotinic receptor stimulation by cigarette smoking seems to provide protection against Parkinson's disease (Baron, 1996). Moreover, in vitro studies have shown that exposure to nicotine protects neurones in cultures from drug-induced neurotoxicity (see also Quik, this issue).

A more general participation of nicotinic receptors in brain development is indicated by their precocious expression in fetal life (Court et al., 1997), and their involvement in axon guidance and directional growth (Pough and Berg, 1994) suggests that they could be involved in shaping and maintaining neuronal circuitry. Confirmation of the central role of nicotinic receptors in brain physiology is provided by the fact that the expression of the different subunits is finely regulated during development.

Nicotinic receptors have therefore become a suitable and attractive model for studying the transcriptional mech-

anisms underlying the expression of neural genes (Fornasari et al., 1999), which makes it possible to address some crucial questions concerning the generation and maintenance of distinct neural phenotypes.

5. Neuronal nicotinic receptors in pathology

Widespread interest in the biology of nicotinic receptors was really aroused by the hypothesis that they may be involved in different brain diseases. Recent studies have indicated that specific receptor subtypes are selectively implicated in certain brain diseases in which neuronal nicotinic receptors are modified. For example, nocturnal frontal epilepsy is due to a mutation in the α subunit that reduces α4β2 subtype function (Steinlein et al., 1995, 1997; Kuryatov et al., 1997) the sensory gating defect in schizophrenia seems to be correlated with an abnormal expression of the α7 subunit (Leonard et al., 1996; Freedman et al., 1997) and a typical change in Alzheimer's disease is a decrease in the number of neuronal nicotinic receptors (Nordberg et al., 1992; Hellström-Lindahl et al., 1999; Martin-Ruiz et al., 1999), with α4 being the most affected subunit (the number of receptors containing the α3 subunit is not changed and the alteration in the number of α7-containing receptors is still under debate) (Martin-Ruiz et al., 1999; Wevers et al., 1999). There is also evidence of a positive involvement of neuronal nicotinic receptors in Parkinson's disease (Baron, 1996). A very hot issue is the role of nicotinic receptors in smoking addiction, and particular attention is being given to nicotinic receptors present on the presynaptic dopaminergic terminals in the shell of the nucleus accumbens. Although some experimental and clinical data have been obtained, there is no consensus on the role of nicotine and nicotinic receptors in tobacco addiction, or on the possible mechanisms involved in this effect (see Domino, this issue).

6. Receptor pharmacology

In order to be able to control different nicotinic brain functions pharmacologically, it is very important to have drugs that selectively affect the different receptor subtypes in such a way as to maximise the desired effect and minimise the unwanted effects. The possible clinical uses of nicotinic receptor agonists and antagonists are for controlling smoking addiction, improving brain function in dementia, due to ageing or degenerative diseases, and controlling pain. They could also act as tools for the diagnosis of brain pathology by means of positron emission tomography imaging (Nordberg et al., 1990b), an approach that may also allow non-invasive monitoring of the presence and activity of receptor subtypes in selected brain areas during particular cognitive tasks in normal subjects and in patients.

The pharmacology of nicotinic receptors has always been selective, but this has previously been confined to the discrimination of muscle and ganglion receptors with the aim of obtaining curare-like drugs without ganglioplegic action and ganglioplegic agents without any activity at the neuromuscular junction. The results of this research were very positive in terms of drug development (see the series of methonium analogue (Paton and Zaimis, 1951)) and for improving our understanding of nicotinic receptor function and structure (see reviews by Zaimis, 1976 and Wess et al., 1990).

The problem of finding drugs that selectively discriminate neuronal receptor subtypes is more complex since the differences are more subtle and the number of receptor subtypes is much larger. Although a number of compounds have been synthesised and some are now in the first phases of clinical testing, very few are subtype selective (Holladay et al., 1997). This is not the place for a complete review but some examples are given below, and others can be found in the other chapters of this issue.

6.1. Homomeric receptors

The most successful results have been obtained using the α7 homomeric receptors, which very selectively bind and are blocked by nanomolar concentrations of α-bungarotoxin, methyllycaconitine and 4-oxystilbene derivatives (Palma et al., 1996; Gotti et al., 1998; McIntosh, 2000). The last two discriminate neuronal and muscle receptors, but α-bungarotoxin does not (Wonnacott et al., 1993; McIntosh, 2000). Methyllycaconitine seems to be a selective marker for the α7 receptors found in animal species, whereas the oxystilbene derivatives are α7-selective only in the case of chick receptors. The possible clinical use of α7 receptor antagonists can be foreseen in some cases of epilepsy, in tobacco addiction, and as diagnostic tools. Unfortunately, these selective compounds are not very useful clinically because of their toxicity, and the only oxystilbene derivative to have been tested in humans (MG624) is not very selective in mammals. However, they could be a starting point for future drug development (Maggi et al., 1999). The α7 receptor agonists are rather unselective, and a new compound anabaseine has similar nanomolar affinity for rat α7 and α4β2 subtypes (Kem, 1997). However, a new anabaseine derivative (GTS-21) has a one hundred (human) and 34 times (rat) lower affinity for the α7 receptor than for α4β2 subtypes (Briggs et al., 1997). This compound is now undergoing preliminary clinical testing as a drug that may be useful in patients with Alzheimer's disease. Another very promising compound is the nicotine-derived AR-R17779, which is a full agonist for the rat α7 subtype (Gordon et al., 1998). If this selectivity is maintained with human receptors, it will be a good starting point for the development of new α7 receptor-selective drugs. The recent finding that choline is a weakly selective α7 receptor agonist may also open up a new future for this old drug and explain some of its

claimed activity as a brain stimulator (Papke et al., 1997; Albuquerque et al., 1998).

6.2. Heteromeric receptors

The favourite target for selective drugs is the $\alpha 4\beta 2$ subtype, which is the most abundant neuronal nicotinic receptor subtype in the brain. Several compounds are active on these receptors, but none of them are selective for a particular subtype. Epibatidine, a substance found in the skin of the frog *Epipedobates tricolor*, activates and binds to all the receptors containing the $\alpha 2$, $\alpha 3$, $\alpha 4$ and $\alpha 7$ subunits with picomolar affinity. At higher nanomolar concentrations, it also binds to the $\alpha 7$ subtype (Gerzanich et al., 1995). It has strong analgesic properties, and this has led to research that will probably lead to useful compounds (Badio and Daly, 1994; Holladay et al., 1997). The nicotine-derived compounds (ABT-418, A-84543, RJR-2403 and SIB-1508Y) are the most selective, but their discrimination toward other receptor subtypes (especially of the ganglionic type) is in the range of only one or two orders of potency (Bencherif et al., 1996; Holladay et al., 1997; Papke et al., 1997; Lloyd et al., 1998).

It is surprising that the derivatives of cytisine (the other very active heteromeric receptor agonist) have been poorly investigated despite the fact that they could be a rich source of selective compounds.

7. Conclusions

We have briefly described what is being known about neuronal nicotinic receptors, but even this rapid overview makes it clear that there are still many open, debatable and unknown issues. In particular, we need to know more about the subunit composition, distribution and properties of native nicotinic receptors, the factors that regulate their expression and cell localisation, the significance of the developmental regulation of receptor expression, and their roles in shaping neuronal circuitry, controlling neuronal survival, and regulating cognitive functions, as well as their relevance in pathological situations and tobacco abuse. Finally, but not least importantly, we need new approaches for finding selective agonists or/and antagonists for therapeutic and diagnostic purposes. A number of the issues that we have raised here are dealt with in more detail in the accompanying papers, which interested readers will find of great value.

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