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Allosteric nicotinic receptors, human pathologies

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Abstract — Nicotinic acetylcholine receptors are ligand-gated ion channels present in muscle and brain. These allosteric oligomers may exist in several conformational states which include a resting state, an open-channel state, and a desensitized refractory state. Recent work has shown that point mutations in the nicotinic receptor may, altogether, abolish desensitization, increase apparent affinity for agonists and convert the effect of a competitive antagonist into an agonist response. These pleiotropic effects are interpreted in terms of the allosteric model. This paper reviews recent evidence that such mutations occur spontaneously in humans and may cause diseases such as congenital myasthenia or familial frontal lobe epilepsy. In addition, nicotinic receptors are involved in tobacco smoking. Accumulating evidence, including experiments with knock-out animals, indicates that addiction to nicotine is linked to the activation of β2-subunit containing nicotinic receptors in the dopaminergic mesolimbic neurons which are part of the reward systems in the brain. Current research also indicates that nicotinic agonists might serve as therapeutic agents for Alzheimer's disease and Tourette's syndrome, as well as for schizophrenia. This paper extends and updates a recently published review. (©Elsevier, Paris)

Résumé — Récepteurs nicotiniques allosteriques et pathologies humaines. Les récepteurs nicotiniques sont des canaux activés par un neurotransmetteur présents dans le muscle et dans le cerveau. Ces oligomères allostériques peuvent exister dans différentes conformations telles qu'un état de repos, un état avec le canal ouvert et un état désensibilisé, réfractaire à l'activation. Des travaux récents ont montré que des mutations ponctuelles dans les récepteurs nicotiniques peuvent, d'un même coup, supprimer la désensibilisation, augmenter l'affinité apparente des agonistes et convertir l'effet des antagonistes compétitifs en réponse à un agoniste. Ces effets pléiotropiques sont interprétés à l'aide du modèle allostérique. Le présent article résume la découverte récente de l'occurence spontanée de telles mutations chez l'humain et des pathologies qu'elles causent telles que des myasthénies congénitales et des épilepsies du lobe frontal. De plus, les récepteurs nicotiniques sont impliqués dans la tabagie. Un nombre croissant de travaux, notamment faisant usage d'animaux recombinants, démontre que la dépendance à la nicotine est liée à l'activation de récepteurs nicotiniques contenant la sous-unité β2, dans les neurones dopaminergiques mésolimbiques qui participent aux systèmes de récompense dans le cerveau. Les travaux contemporains indiquent également que les agonistes nicotiniques pourraient remplir le rôle de médicament pour le traitement de la maladie d'Alzheimer, du syndrome de Gilles de la Tourette, et de la schizophrénie. Cet article est une mise à jour étendue d'une revue publiée antérieurement. (©Elsevier, Paris)

nicotinic acetylcholine receptors / allosteric proteins / congenital myasthenia / frontal lobe epilepsy / Alzheimer's disease / schizophrenia / nicotine addiction

1. Introduction

The nicotinic acetylcholine receptor (nAChR) was the first neurotransmitter receptor biochemically and functionally identified [2, 3], in part because high amounts of the receptor protein were available in the fish electric organ [4] and also because snake venom α-toxins had been identified as highly selective markers of nAChRs [5]. Recombinant DNA technologies further led to the demonstration that the structural and functional properties of this membrane allosteric protein from fish are, to a large extent, paralleled by those of brain nicotinic receptors thus opening the field to human brain pathologies [6, 7].

In this review, two aspects of nAChRs relevant to medicine are presented. First, point mutations in muscle and brain nAChRs may produce congenital myasthenia and familial epilepsies. The phenotypes of the mutated nAChRs are interpreted in terms of changes in the properties of the allosteric transitions. Second, nicotinic drugs, despite their addictive properties, could potentially alleviate neurologic and psychiatric disorders.

2. Nicotinic receptors as allosteric membrane proteins

The nAChRs compose a family of ligand-gated ion channels differentially expressed in skeletal muscle and nerve cells (reviewed in [8–10]). They form 300 kDa transmembrane hetero- (or homo-) pentamers from a repertoire of 16 known different types of subunits referred to as α1–α9, β1-β4, γ, δ, and ε. The subunits are regularly distributed around an axis of quasi-symmetry delineating the ion channel (*figure 1a, d*). Each subunit contains a large N-terminal hydrophilic domain exposed to the synaptic cleft, followed by three transmembrane segments

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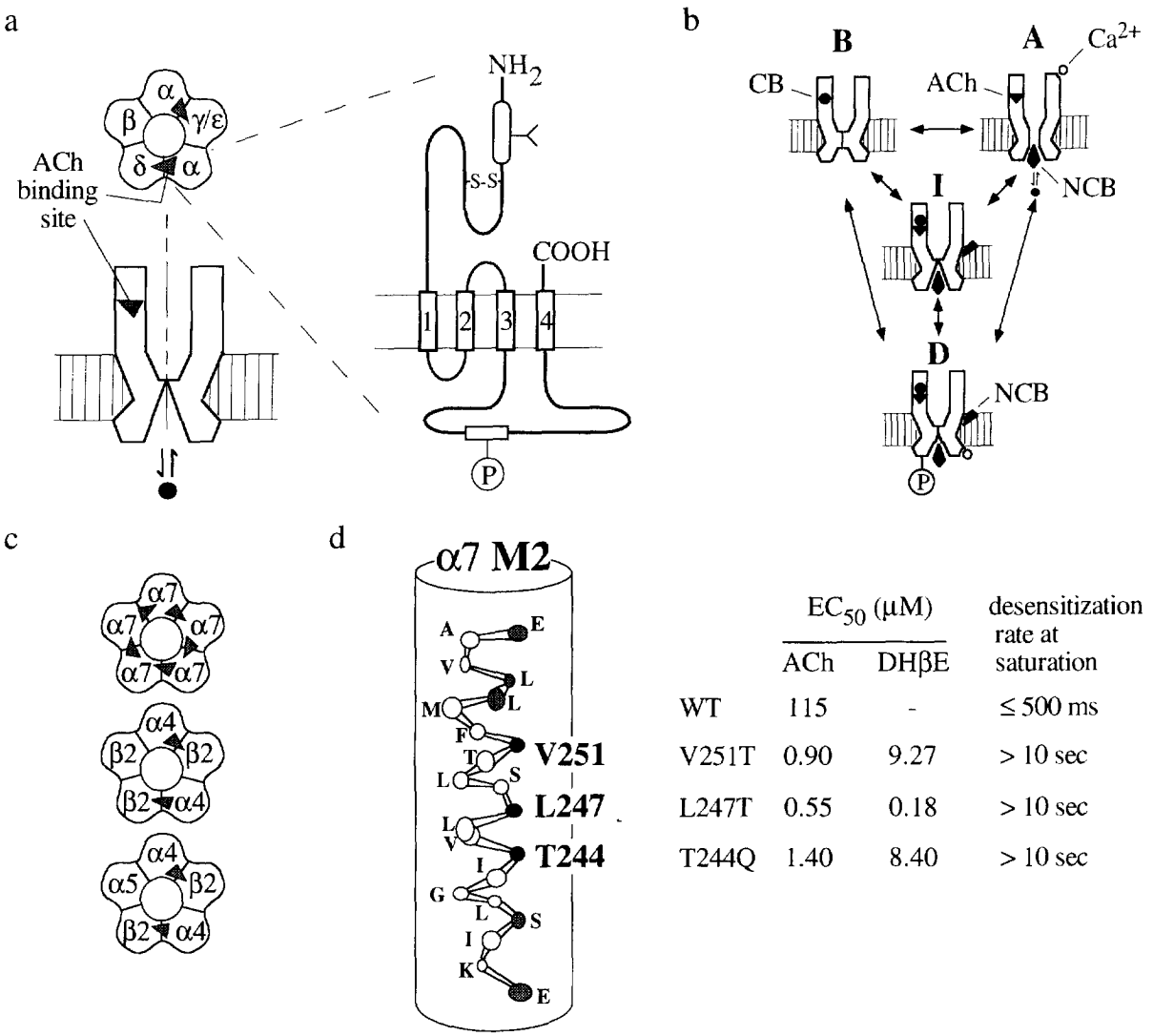

	EC_{50} (μM)		desensitization rate at saturation
	ACh	DHβE	
WT	115	-	≤ 500 ms
V251T	0.90	9.27	> 10 sec
L247T	0.55	0.18	> 10 sec
T244Q	1.40	8.40	> 10 sec

Figure 1. Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels with allosteric properties [30]. **a.** Muscle and *Torpedo* nAChRs are pentameric oligomers. The five homologous subunits are organized around an axis of quasi-symmetry perpendicular to plane of the plasma membrane, that delineates the ion channel pore. Each subunit exhibits a similar transmembrane organization sketched on the right. The binding sites are located at the interface of the extracellular N-terminal domains of the subunits, and the ion channel is lined by the M2 transmembrane segment. **b.** The nAChRs undergo allosteric transitions between a small number of states: resting (B), active (A) and desensitized (I, D) states [30, 31]. Various ligands preferentially bind to different states as indicated. CB, competitive blockers; NCB, non-competitive blockers; ACh, acetylcholine. **c.** Putative organization of three different types of neuronal nAChRs: homopentamers of α7 subunit, heteropentamers of α4 and β2, heteropentamers of α4, β2 and α5. **d.** The M2 transmembrane segment is putatively organized in an α-helix. Mutations of residues in M2 facing the channel pore increase the apparent affinity for nicotine, convert the antagonist dihydro-β-erythroidine into an agonist and drastically slow the desensitization rate of mutated α7-nAChRs (adapted from [41]). Adapted from [1].

(M1–M3), a large intracellular loop and a C-terminal transmembrane segment (M4). Acetylcholine binding sites are located at the interface between α and non-α subunits in the N-terminal regions [8, 11–13]. They include a principal component of three loops A, B, and C and a complementary component of at least three loops, D, E and F, on the non-α subunit. In homo-oligomeric receptors, the two components are carried by identical subunits [11, 14]. A wide diversity of binding properties in hetero-oligomers

results from the combinatorial diversity of the active site structure (e.g., [14]). The ion channel is lined by the M2 segment from each of the five subunits [15–21]. Neuronal nAChRs are more permeable to calcium ions than muscle nAChRs (neuronal nAChRs: pCa/pNa from 15 to 0.5 depending on the subunit composition; muscle nAChRs: pCa/pNa of about 0.2) [8, 9, 22].

Muscle nAChRs have a fixed composition $[\alpha 1]_2[\beta 1][\delta][\gamma \text{ or } \varepsilon]$ in vertebrates. Neuronal nicotinic receptors are composed of neuron-specific subunits homologous to the muscle subunits. To date, ten neuronal subunits have been identified in mammals ($\alpha 2$–$\alpha 7$, $\alpha 9$, $\beta 2$–4) (e.g., [10, 23]). Of the more than 20 000 possible combinations of subunits, only a few yield functional receptors. The $\alpha 7$ and $\alpha 9$ subunits form functional homo-oligomers when expressed in *Xenopus* oocytes, while the $\alpha 2$–4, $\alpha 6$ subunits produce hetero-oligomers with the $\beta 2$ or the $\beta 4$ subunit (*figure 1c*, reviewed in [24, 25]). The $\alpha 5$ subunit can associate with $\alpha 3\beta 2/4$ and $\alpha 4\beta 2$ subunits and thus form hetero-oligomers with three different subunits (*figure 1c*; [26, 27]). The sequence homology of $\beta 3$ with the $\alpha 5$ subunit suggests that $\beta 3$ possesses a similar function [28], and is also integrated into functional nAChRs [29].

Upon application of nicotinic agonists, both muscle and neuronal nAChRs undergo fast activation leading to an open-channel state, and a slow desensitization reaction leading to a closed-channel state refractory to activation. Activation and desensitization of muscle and brain nAChRs correspond to transitions between a small number of discrete structural states with distinct binding properties and ion channel conductance [30]. Consistent with the allosteric MWC model and its extension to membrane receptors [30, 31], the different conformational states may spontaneously exist in the absence of ligands, and nicotinic effectors cooperatively modify the equilibrium and kinetic constants for the transitions between the states (*figure 1b*). The pharmacological and kinetic characteristics of these states depend upon the subunit composition. Indeed, the two main subtypes of brain nAChRs strikingly differ: the human $\alpha 4\beta 2$ and $\alpha 7$ have respectively a low and a high EC_{50} for nicotine (0.3–5 μM versus 40–110 μM); at saturation, they desensitize respectively in the 10-s and in the 10–100 ms range (or less) [32–36]. The kinetic constants governing the ligand binding and the transitions between the different states (14 independent rate constants for a four state model) have been estimated for muscle nAChR [31] and the analysis extended to neuronal nAChR mutants [31, 37, 38].

Site-directed mutagenesis of affinity-labeled residues in the channel and active site domains [39–43] revealed that mutations of single amino acids can modify multiple functions of the nAChR. For instance, mutations in the channel lining region M2 (e.g., $\alpha 7$T244Q, $\alpha 7$L247T, $\alpha 7$V251T) produce a 100-fold increase in apparent affinity for agonists, a loss of desensitization and a conversion of competitive antagonists to agonists (*figure 1d*) [39, 41–43] (reviewed in [8, 37]). The allosteric model accounts for these pleiotropic phenotypes. Different classes of phenotypes may be associated with selective changes either in the binding properties (K phenotype), in the biological activity of the ion channel (γ-phenotype) or in the isomerization constants between receptor conformations (L-phenotype) and possibly in both [37, 44].

The neuronal nicotinic receptor subunits are expressed differentially in the mammalian brain. In situ hybridization in the rat brain shows that $\alpha 4$, $\beta 2$ and $\alpha 7$ are widely expressed, that $\alpha 3$ and $\alpha 5$ are less ubiquitous and that $\alpha 6$, $\beta 3$, $\beta 4$ and $\alpha 2$ are only expressed in a few brain structures (*table I*). In contrast, $\alpha 3$ and $\beta 4$ are the most abundant nAChR subunits in the autonomic peripheral nervous system [45]. The distribution of nAChRs has also been studied with radiolabeled nicotinic ligands. At least four different types of nicotinic-ligand binding activity with different distributions have been distinguished: the high affinity binding sites for nicotine that correspond to $\beta 2$-containing nAChRs [46, 47] are found throughout the brain (e.g., [48]) and are also labeled by three other nicotinic ligands: acetylcholine (in the presence of atropine to block muscarinic ligands), cytisine and epibatidine. The high-affinity binding sites for epibatidine (but not for nicotine) have a much restricted localization limited mostly to the habenulo-interpeduncular system and the dorsal medulla oblongata; these sites may be separated into two families, since only a subset of them binds cytisine with a high affinity [47] (see also [49]). They correspond presumably to $\beta 4$-containing nAChRs [4]. The α-bungarotoxin binding sites that correspond to $\alpha 7$-containing nAChRs [50] are distributed throughout the brain with a prevalent localization in the limbic system. As a consequence of such diversity in function and distribution, nAChRs may contribute to a wide array of brain functions [51]. Conversely, dysfunction of a single nAChR subunit may produce diverse deficits.

3. Congenital myasthenia and familial epilepsies result from nAChR point mutation

Genetic analysis of several human (and animal) pathologies has revealed nAChR mutations yielding pleiotropic phenotypes (*figure 2*). The mutations are

Table I. Differential distribution of nAChR subunit mRNA in the rat brain ([28] and references therein). Recent work suggests a broader expression pattern of the subunits α3 and β4 observed by in situ hybridization [112]. However such observations do not seem to be consistent with recent binding experiments in β2-knockout mice [47]. The expression of α7 in the dopaminergic nuclei (DA nuclei) was recently demonstrated [91]. NTS, nucleus of the tractus solitarius. Adapted from [1].

	α2	α3	α4	α5	α6	α7	β2	β3	β4
Telencephalon									
olfactory bulb	+	++	+	++	-	++	++	-	+
isocortex									
layer II-III	-	-	+	+	-	+	++	-	-
layer IV	-	+	+	+	-	+	++	-	-
layer V	-	-	++	+	-	++	++	-	-
layer VI	-	-	++	++	-	++	++	-	-
hippocampal formation	(+)	(+)	+	+	-	+++	++	-	-
striatum	-	-	-	-	-	-	+	-	-
septum	-	-	+	-	-	+	+	-	-
hypothalamus	-	-	+	-	-	-	+	-	-
supraoptic n.	-	-	+	-	-	+++	+	-	-
Diencephalon									
pineal gland	-	+++	-	+	-	-	+	-	+++
habenula	-	+++	++	+	(+)	(+)	++	++	+++
thalamus	-	+	+++	-	+	-	+++	+	-
Mesencephalon									
DA nuclei	-	(+)	++	++	+++	(+)	++	+++	-
mesenc V nucleus	-	-	+	-	+	-	++	+++	-
interpeduncular nucleus	++	+	+	++	+	(+)	++	+	+
Rhombencephalon									
vestibular nuclei	-	-	+	+	-	++	+	-	-
cerebellum	-	+	-	+	-	-	+	+	+
locus coeruleus	-	(+)	-	-	+++	-	++	+++	(+)
motor nuclei	-	+	+	+	-	-	++	-	-
NTS	-	++	(+)	+	-	-	++	-	++
area postrema	-	++	++	+	-	-	++	-	+

homologous or even identical to those initially studied in reconstituted α7 homo-oligomers and their phenotype may be also interpreted in terms of the allosteric model.

A mutation in the *deg-3* gene coding for a putative nAChR subunit of the nematode *C. elegans* results in neurodegeneration [52]. This *deg3*-I293N mutation is likely to cause an 'increase-of-function' similar to that initially found with the vertebrate α7V251T mutation [41]. The neurotoxicity could plausibly arise from a large toxic influx of calcium associated with a non-desensitizing and/or spontaneously open nAChR channel [39, 41].

In humans, myasthenia gravis is a sporadic disease caused by an auto-immune reaction directed against muscle nAChRs. However, some congenital myasthenic syndromes are associated with point mutations in the muscle α1, β1 or ε subunits. Mutations reducing channel opening transitions (e.g., εP121L, ε1254ins18), or affecting nAChR assembly (εR147L) cause myasthenic symptoms only when combined with a null mutation of the other allele [53–55]. Null mutations [56] result in symptoms only when expressed on both alleles. In accordance, animal models with a knock-out of the subunit ε express obvious myasthenic symptoms only in a homozygous genotype [57]. In these myasthenic patients and animal models, neurotransmission is partially rescued at the neuromuscular junction by the persistence of expression of the fetal nAChR γ subunit.

Mutations increasing the time spent by nAChRs in the open state also produce myasthenic syndromes, even as heterozygous mutations [58–63] (but see [54]). Mutations causing such 'increase-of-function' phenotypes occur near the ligand binding region (α1G153S, α1V156M), in the transmembrane M2 segment delineating the ion channel (α1T254I,

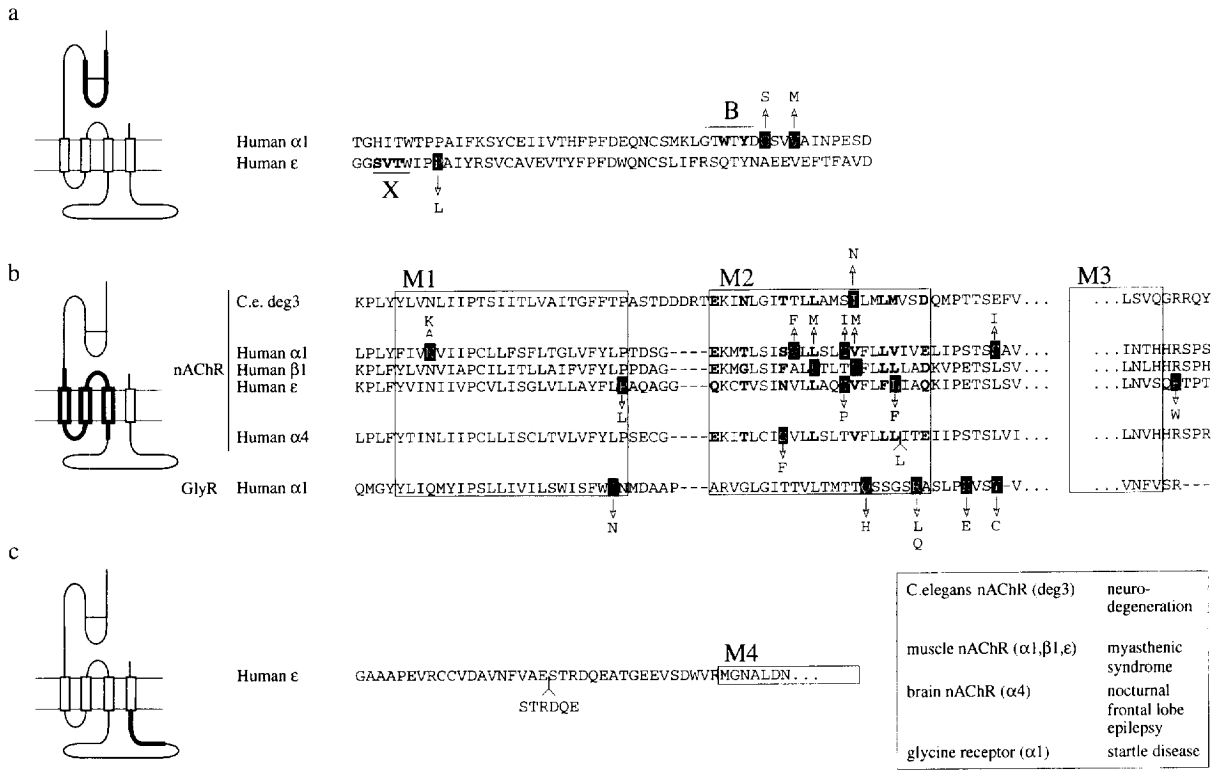

Figure 2. Pathogenic mutations affecting allosteric transitions in the muscle and neuronal nicotinic receptors and in the homologous glycine receptor. Each disease is caused by a single mutation among the mutations indicated. The drawings on the left indicate the protein domain concerned. The mutations are indicated by the amino acid symbol above/under the sequence with an arrow pointing from the wild-type highlighted residue. Null mutations are not presented here. **a.** Mutations near the ligand binding regions. B, B-loop in the principal component of the binding site. X, unnamed region in the complementary component of the binding site [12]. The mutations drawn are: εP121L [53], α1G153S [59, 62] and α1V156M [62]. **b.** Mutations in/near the transmembrane domains. Note the large number of mutations in the M2 region. The amino acids facing the channel pore are indicated in bold. The mutations in nAChR subunit gene are *deg3*I293N [52], α1N217K [60], α1V249F [63], α1T254I [62], α1S269I [62], β1L262M[61], β1V266M [60], εP245L [54], εT264P[58], εL269F [60, 111], εR311W [54], α4S248F [66], α4-776 (ins3) [69]. The mutations in the α1 glycine receptor gene are: GlyRα1-I244N, GlyRα1-Q266H, GlyRα1-R271Q, GlyRα1-R271L, GlyRα1-K276E, GlyRα1-Y279C (references in [70]). Alignment was performed with the program Clustalw of DG Higgins and PM Sharp. **c.** The mutation in the intracellular loop between the M3 and M4 segments is ε1254ins18 [55]. Adapted from [1].

α1V249F, β1V266M, β1L262M, εL269F) or in adjacent regions (α1N217K, α1S269I, εP245L) (*figure 2*). They may affect both intrinsic ligand binding (K phenotype) and opening transition/desensitization (L phenotype) processes or both [37, 38, 44]. Neighboring mutations may produce different phenotypes; for instance, α1G153S slows agonist dissociation while α1V156M decreases the rate of channel closing [59, 62]. Some mutations (α1V249F, β1V266M, εT264P, εL269F) produce a high rate of spontaneous openings in the absence of ligand [58, 60, 63], a phenotype consistent with the allosteric model on the basis of a shift of the allosteric equilibrium in favor of the open state [37, 38, 64]. The confirmation that such 'increase-of-function' mutations in muscle nAChRs are pathogenic was recently obtained in an animal model of transgenic mice carrying the εL269F mutation [65].

Recently, some familial epilepsies have been linked to mutations in the α4 nAChR subunit. In an Australian family, an α4S248F mutation was found to produce autosomal dominant frontal lobe epilepsy [66]. The mutated serine faces the channel pore, as initially demonstrated by chlorpromazine labeling in

Torpedo nAChRs (references in [8]). The mutation of the homologous residue in brain α7 nAChRs (α7T244, *figure 1d*) causes drastic changes in the affinity for ACh and the desensitization properties of the nAChRs [41]. In the human α4 gene, α4S248F produces a limited change in the apparent affinity for acetylcholine and a five-fold increase in the desensitization rate of α4β2 nAChRs [67], a decrease in the mean single channel conductance and a loss of calcium permeability [68]. Furthermore, this mutant exhibited an 'use-dependent potentiation' of the electrophysiological response to nicotinic agonists [68]. In a Norwegian family, the same epileptic syndrome was linked to the insertion of a GCT triplet at nucleotide 776, resulting in the insertion of a leucine at codon 260 [69]. In oocyte experiments, this insertion causes a 12-fold increase in the apparent affinity for ACh of human α4β2. As the insertion is adjacent to a pair of leucines previously identified as a critical element for calcium permeability [22], it might also reduce calcium permeability, though definitive evidence is lacking for this mutant. It is unclear for both mutations α4S248F, α4 (776ins3) whether the phenotype is due to a 'loss of function' (increased desensitization rate and loss of calcium permeability) or to an 'increase of function' (increase in apparent affinity, 'use-dependent' potentiation).

Point mutations that change allosteric properties occur in other ligand-gated ion channels, such as the glycine receptor α1 subunit. Human hereditary hyperekplexia is caused by mutations in the M1–M2 and M2–M3 loops that lead to a dramatic reduction of efficacy of the agonist ([70] and references therein). Overall, these results show that point mutations can cause either a loss of function or an apparent 'increase of function' by altering the allosteric transitions of the nAChRs. Increase-of-function mutations occur frequently and may be as pathogenic as null mutations. Since each allosteric state of the nAChR possesses a distinct pharmacological profile, one may anticipate the development of novel pharmacological agents targeted not only to a particular combination of subunits but to each of the diverse conformations spontaneously accessible by the various receptor oligomers.

4. Null mutation of neuronal nAChRs, Alzheimer's disease and memory

The role of defined nAChR subunits in brain function has been examined using knock-out mice. Mice lacking the most widely expressed β2 subunit survive, feed and mate normally [46]. Their brains are of a normal size and morphology. The high affinity nicotine binding sites (classically attributed to α4β2 nAChRs) completely disappear from the brain of homozygous mutant mice, whereas the α-bungarotoxin sites (corresponding to the α7-containing nAChRs) persist. Electrophysiological responses to nicotine are no longer recorded in the thalamus but persist in a few structures expressing the β4 nAChR subunit (such as the medial habenula) [46, 47]. Further analysis of the β2 mutant mice has shown that the β2-containing nAChRs are expressed both in the somato-dendritic compartment, and in the axonal compartment of neurons as presynaptic nAChRs [71]. The absence of the β2 subunit affects the performance in associative memory (passive avoidance) tests of mutant animals and suppresses the improvement of the performance by nicotine [46]. Activation of β2-containing nAChRs by endogenous ACh is thus likely to take place in the course of these memory tasks.

Preliminary results indicate that the knock-out of the α7 subunit yields animals that survive normally but display an anomalous synchronisation during EEG recordings [72].

Nicotine enhancement of memory processes has motivated clinical trials of nicotinic treatment in the Alzheimer's disease (AD). The severity of symptoms in AD is well correlated with a reduction in cortical acetycholine ([73] and references therein) and AD patients exhibit a marked reduction in the number of high affinity nicotine binding sites [74]. Nicotine treatment partially relieves the cognitive deficits of AD [75, 76]. The site of this beneficial action of nicotine is not yet clearly established. For instance, nicotine may increase the levels of ACh in the cortex by recruiting presynaptic nAChRs on ACh terminals in the cortex (e.g., [77]). As the use of nicotine presents a number of side effects linked to the activation of peripheral nAChRs, attempts are made to find nicotinic drugs specific for brain subtypes such as ABT418 [78], SIB-1508Y [79] and RJR 2403 [80].

5. Nicotinic receptors in the reward system and tobacco abuse

The nAChRs subunits are abundantly expressed in the mesencephalic dopaminergic nuclei ([28] and references therein). These nuclei are part of the mesostriatal reward system. Theoretical work has underlined the critical function of reward systems in learning of behavioral rules by selection [81, 82]. Dysfunction or anomalous chemical stimulation of these systems strongly affects brain function. Indeed, the mesostriatal dopaminergic system is a common target of many addictive drugs (reviewed in [51, 83]).

Accumulating data suggest that both tobacco smoking in humans and nicotine self-administration in animals are associated with an increase in dopamine release following nicotinic actions on the mesencephalic dopaminergic neurons (reviewed in [84]). Self-administration of nicotine shares common mechanisms with that of other addictive drugs. Minimal doses of nicotine, comparable to those causing self-administration behavior, trigger a specific increase of metabolism and the release of dopamine in the nucleus accumbens, as observed with strongly addictive drugs such as cocaine or amphetamines [85]. Nicotine and cocaine self-administration activates a number of common brain structures, as visualized with c-Fos immunoreactivity, notably the terminal fields of the mesencephalic dopaminergic neurons [86]. It should be noted that tobacco smoking is not only associated with nicotine intake but also with respiratory sensations of smoke intake (review in [84]); nicotine action could also be amplified by changes in dopamine metabolism, since smokers display a 40% reduction of monoamine oxidase B compared to former smokers or non-smokers [87].

What is the composition of the nAChRs involved in the self-administration of nicotine? The concentration of nicotine in the plasma of smokers is in the 100–500 nM range [88]. Oocyte experiments with human nAChRs reveal which combinations of subunits may respond to such low concentration of agonists in vivo. EC_{50} values below 10 μM have been observed for α4β2, α4β4 α3β2 ([33] but see [36]) and α3β2α5 [27]. In situ hybridization experiments (references in [28]) indicate that the nAChR subunits forming these combinations (except β4) are expressed in the mesencephalic nuclei. These nuclei also contain high amounts of the α6 and β3 subunit mRNAs, suggesting the contribution of an α6β3β2 subtype [28]. Recent work on the β2-knock-out mice show that the β2-mutant animals no longer self-administer nicotine after priming by cocaine, indicating that β2 is part of the nAChRs involved in nicotine reinforcement [89]. Furthermore, in the mutant animals low concentrations of nicotine fail to stimulate the mesencephalic dopaminergic neurons recorded on slices in vitro and systemic nicotine injection no longer enhances the striatal release of dopamine [89].

The chronic intake of nicotine results in a so-called 'neuronal adaptation'. This includes desensitization of nAChRs (reviewed in [90]) [91]. Desensitization of neuronal nicotinic receptors may recover only in tens of minutes (e.g., [91–94]) and thus likely contributes to short term adaptation to nicotine. Furthermore, nAChRs may directly contribute to endogenous reward processes. Indeed, they contribute to cholinergic synaptic transmission between the posterior cholinergic nuclei (latero-dorsal tegmental nucleus, pedonculopontine nucleus) and the mesencephalic dopaminergic neurons (e.g., [95, 96]). Chronic nicotine treatment thus likely affects the brain reward mechanisms as shown with self-stimulation protocols [97] or nicotine withdrawal experiments [98].

6. The nicotinic receptors in psychiatric and neurological disorders

The high prevalence of tobacco-smoking in schizophrenic patients suggests that nicotine intake by cigarette consumption may be a form of self-medication. By stimulating the mesencephalic dopaminergic system (see above), and more specifically by increasing the burst firing of dopaminergic neurons to burst activity, nicotine might compensate for the hypofrontality observed in schizophrenia [9]. Nicotine has been found to reverse the cognitive deficits produced by haloperidol in schizophrenics [100]. A synergy between nicotine and dopaminergic neuroleptics also exists in the treatment of Tourette's syndrome [101]. Nicotine has been proposed as an alternative to drugs increasing the brain levels of dopamine in the treatment of attention deficit/hyperactivity disorder [102]. Finally, nicotine and various nicotinic agonists might help to compensate the deficit in striatal dopamine in Parkinson's disease patients and might, in some instances, relieve the symptom of the disease ([103] but see [104]). The interaction of nicotine with the mesencephalic dopaminergic system might thus explain its action on psychiatric and neurological disorders.

Schizophrenic patients often exhibit, among diverse symptoms, a diminished habituation to auditory stimulation (reviewed in [105]). Experiments in the rodent have shown that auditory gating is impaired by antagonists of α7 nAChRs. Furthermore, the number of α-bungarotoxin sites is reduced in post-mortem brains of schizophrenics. The deficit of sensory gating in schizophrenics might thus be due to a reduction, or a loss, of α7 nAChR function. Consistent with this hypothesis, genetic analysis in nuclear families with at least two cases of schizophrenia has shown that the deficit in auditory gating is significantly linked to a genetic marker neighboring the locus of the α7 gene [106]. A study of the relatives of schizophrenics sharing the deficit in auditory gating revealed that nicotine could reverse the deficit, presumably by activating α7 nAChRs (references in [105]). This result is quite unexpected, since human α7 nAChRs exhibit a low sensitivity to nicotine (see *Introduction*). However, recent experiments in the chick [107] and rat [108] have

shown that low doses of nicotine can activate α-bungarotoxin sensitive nAChRs in glutamatergic nerve terminals. The α7 nAChR subunit, possibly together with still unidentified subunit(s) (see however, [109]), may thus form another relevant target for nicotinic therapies of psychiatric disorders.

7. Conclusion

Over 25 years after the identification and purification of the *Torpedo* nAChR (see [30]), the accumulating knowledge on the nAChRs in vertebrates has led to the demonstration that alterations of these receptors are responsible for a variety of familial disorders of the central and peripheral nervous system. Conversely, these receptors are now considered as relevant targets for nicotinic therapies of brain disorders.

Previous experiments combining photoaffinity labeling and site-directed mutagenesis had shown that changes of critical amino acids, in the nAChR channel or ligand binding site, may markedly affect its function in a pleiotropic manner. They may for instance, either reduce or increase channel opening in the presence and sometimes in the absence of acetylcholine by altering the allosteric properties of the protein. Interestingly, analogous, if not identical, point mutations in human nAChR genes (and glycine receptor genes) have been shown to cause pathologies either by a loss or by an 'increase-of-function'. Mutations causing pathologies via changes in allosteric properties have also been described for G-protein linked receptors [110]. Development of novel nicotinic therapies with pharmacological agents targeted to these diverse 'allosteric' phenotypes may thus be anticipated.

While the strategic location of nAChRs in the dopaminergic reward system renders nicotine an addictive drug, it also underlies potential beneficial effects of nicotine in the treatment of psychiatric disorders. Furthermore, nAChRs may relieve symptoms of AD or schizophrenia via pathways different from the dopaminergic system; nicotinic agents which specifically activate nAChR subtypes absent from the dopaminergic system, and thus with no (or diminished) addictive properties should, therefore, be looked for.

Fundamental research on properties of nAChRs in normal and pathological situations opens many new strategies to design drug therapies targeted not only to specific nAChRs in defined brain circuits but also to specific allosteric transitions impaired by nAChR gene mutations in humans.

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