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Desensitization of nicotinic ACh receptors: shaping cholinergic signaling

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Nicotinic ACh receptors (nAChRs) can undergo desensitization, a reversible reduction in response during sustained agonist application. Although the mechanism of desensitization remains incompletely understood, recent investigations have elucidated new properties underlying desensitization, indicating that it might be important to control synaptic efficacy, responses to cholinergic agents, and certain nAChR-related disease states. Thus, studying how different nAChR subunits contribute to desensitization might help to explain variations in responsiveness to drugs, and might thus improve their therapeutic applications. Agonist-specific desensitization, desensitization arising from resting receptors, natural mutations dramatically altering desensitization, and the possibility that recovery from desensitization is an important process for modulating receptor function, together provide a new framework for considering desensitization as a target to shape cholinergic signaling.

Introduction

Nicotinic ACh receptors (nAChRs) are ligand-gated ion channels assembled as pentamers of diverse subunits [1,2]. Usually, nAChRs are fast transducers of signals activated by the transmitter ACh, or by the drug of abuse nicotine [1,2]. Nevertheless, when ACh, nicotine or related agonists are continuously applied, nAChRs become 'desensitized' (i.e. temporarily inactive) [3,4]. Desensitization can therefore be seen as a use-dependent, readily reversible form of signal plasticity that might shape synaptic efficacy in various brain regions, or even protect cells from uncontrolled excitation [5–8]. Although the role of nAChR desensitization in normal cholinergic transmission remains unclear, its potential to control cholinergic activity and induce adaptive changes is considerable. In the short period of seconds to minutes, nAChR desensitization underlies the brief skeletal muscle paralysis caused by agents such as succinylcholine during general anesthesia [9]. Over a longer time-frame, nAChR desensitization might be important in understanding the therapeutic efficacy of various nicotinic drugs now used to treat the cholinergic dysfunction associated with neurodegenerative disease [10,11]. Furthermore, nAChR desensitization might even lead to chronic modulation of nAChRs in the brain of tobacco smokers [5–7]. The aim of this review is to clarify the molecular mechanisms involved in nAChR desensitization, the compensatory changes triggered by it, the phenotypes resulting from its alteration, and new strategies for its modulation with the ultimate goal of fine-tuning cholinergic function in health and disease.

Desensitization depends on nAChR subunits

In the mammalian nervous system, nAChRs can be broadly classified as either α7-containing nAChRs that desensitize rapidly (in milliseconds) or non-α7 receptors that desensitize slowly (in seconds) and are made up of various combinations of α and β subunits [1,2,4]. Among non-α7 receptors, the two most common subtypes are α3β4 (mainly expressed by autonomic neurons and moderately susceptible to desensitization) and α4β2 receptors (widely found in the brain and very prone to desensitization) [4] (Table 1). At the vertebrate neuromuscular junction, nAChRs are composed of two α subunits, one β subunit, one δ subunit, and either a γ or an ε subunit [12]. On skeletal muscle fibers, desensitization is slow and might not have a significant role in shaping single endplate currents, which have a fast time-course [13]. However, because the recovery from desensitization of muscle-type nAChRs takes time, the numbers of desensitized receptors might significantly increase in relation to the length of their repeated activation [14,15].

Desensitization kinetic properties

When a medium to high (μM to mM) concentration of agonist is applied, nAChRs are first activated and can then desensitize with subsequent recovery after agonist removal. This process will be referred to here as 'classical desensitization' (Figure 1; see Box 1a for kinetic scheme) that develops usually in the range of tens of milliseconds, although different subtypes of nAChRs have differential susceptibility to desensitization (Table 1). Furthermore, nicotine and ACh have differential ability to desensitize

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Table 1. Relationship between activation[a] and desensitization-induced inhibition[b] of nAChRs

nAChR subtype	Agonist	EC_{50}[a] (μM)	IC_{50}[b] (μM)	Ratio EC_{50}/IC_{50}	Refs
Rat α3β4	Nicotine	65	1.2	54	[72]
	ACh	14	10	1.4	[34]
Rat α4β2	Nicotine	14	<0.01	>1400	[16]
	Nicotine	15	0.06	250	[72]
Human α7	Nicotine	91	0.7	130	[73]
	ACh	177	2.3	77	[73]
	ACh	173	>10 000	<0.017	[74]
Rat α7	Nicotine	90	1.3	69	[72]
	ACh	513	>10 000	<0.05	[74]

[a]Activation is expressed as the EC_{50} value, which is the concentration of agonist producing half-maximal response amplitude.
[b]Inhibition is expressed as the IC_{50} value, which is the concentration of agonist reducing the amplitude of the test response by 50%.

nAChRs. For example, for the same agonist concentration producing equivalent receptor activation, desensitization of α4β2 receptors can be complete for nicotine but only partial for ACh (Figure 2a). This mechanism involves changes in the receptor molecular structure, which will later be discussed in more detail.

Low agonist concentrations can induce desensitization even without apparent receptor activation, a process that will be referred to here as 'high-affinity desensitization' (HAD) [16] (Figure 1). HAD is a slow process (taking seconds to minutes), and therefore is more likely than classical desensitization to be generated during chronic exposure of agonist. HAD is also receptor-subtype specific, affecting preferentially α4β2 rather than α7 or α3β4 nAChRs (Table 1). This selectivity can be functionally important because, as will be discussed, the nicotine-induced stimulation of midbrain dopaminergic neurons via α7-containing nAChRs might be due to the preferential desensitization of non-α7 nAChRs via HAD [7]. Together with desensitization, long (hours to days) exposure to an agonist (e.g. nicotine) can produce sustained changes in receptor sensitivity owing to upregulation of both α4β2 and α7-containing neuronal nAChRs via HAD [17] (Figure 1).

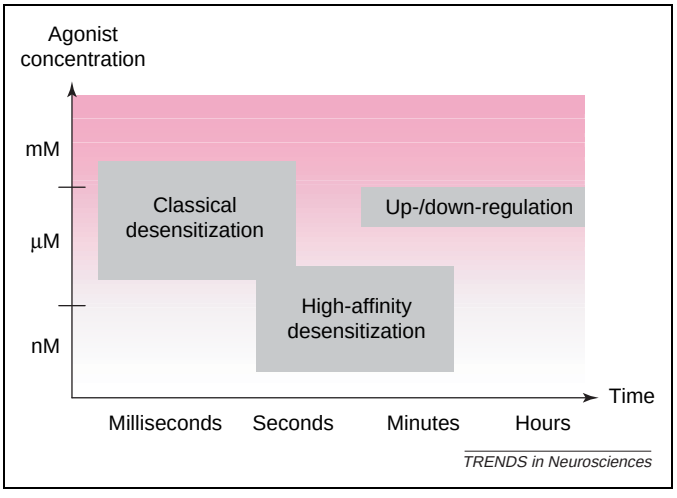

Figure 1. Distinct mechanisms participate in agonist-induced modulation of responsiveness to transmitter. 'Classical desensitization' induced by relatively high (micromolar to millimolar) agonist concentrations proceeds from the open receptor state in milliseconds (e.g. for α7 nAChRs) or seconds (e.g. for α4β2 nAChRs). 'High-affinity desensitization' (HAD) induced by low agonist concentrations proceeds from the agonist-bound closed state before channel opening (i.e. without receptor activation) with slow kinetics (seconds to minutes). For α4β2 nAChRs, HAD could be evoked by very low concentrations of nicotine (~1000 times less than those required for channel activation) [16], whereas for other nAChR subtypes, HAD can overlap to a different degree with classical desensitization. Kinetic models corresponding to HAD and classical desensitization are shown in Box 1. The upregulation or downregulation of nAChRs is usually observed with long-lasting application of agonist [17].

The receptor model put forward by Katz and Thesleff in 1957 [3] is described in Box 1(a). In this scheme, desensitization represents a classic form of allosteric protein behavior, in which the receptor is distributed (in the absence of ligand) between several discrete conformations [2], and the agonist simply increases the probability of receptor transitions between states [18]. An update of the model involves the sequential occupation of two agonist-binding sites on the same receptor to produce activation (Box 1b); accordingly, HAD might be a transition of the receptor bound to one ligand molecule from a closed state (AR) to a desensitized state (AD). Although our understanding of receptor desensitization has been validated by kinetic modeling for muscle-type nAChRs [3,19] and α7 nAChRs [20,21], detailed formalisms to explain desensitization of non-α7 receptors are lacking.

Agonist-specific recovery from desensitization

In the classical receptor model [3], the recovery from desensitization should be independent of the type of agonist used. Although the recovery from desensitization depends on the nAChR subtype [4], recent findings suggest a novel, agonist-specific rate of recovery for α7 [20], α3β4 [22], α4β2 [16] and muscle-type nAChRs [23]. When the onset and extent of desensitization are similar, the recovery of α4β2 receptors after the removal of nicotine requires much more time than recovery after the removal of ACh [16] (Figure 2b). The agonist-dependent recovery from desensitization suggests an agonist-specific rate of dissociation from the AD state [22]. Indeed, α7 receptor function can be modulated by interweaving applications of different agonists (e.g. ACh and choline) with distinctive recovery rates from desensitization [20]. The revised receptor scheme (Box 1b) implies that the transition rates of the desensitized receptor bound with two ligand molecules (A_2D), to singly bound (AD) and to unbound (D) are responsible for the agonist dependence of the recovery process [16].

Desensitization and agonist trapping

As originally considered by Katz and Thesleff [3], high affinity of desensitized receptors for agonist indicates that they might be potential traps for unbound agonist molecules [14,15]. Whereas non-α7 receptors possess two agonist-binding sites [24,25], α7 receptors might theoretically contain up to five binding sites [21,26], making them

Box 1. Kinetic models of desensitization

(a) Cyclic kinetic model of desensitization (modified from [3])

$$\begin{array}{ccc} R + A & \leftrightarrow & AR^* \\ \updownarrow & & \updownarrow \\ D + A & \leftrightarrow & AD \end{array}$$

In this model, A is the agonist; R, R* and D are the receptor in the resting, open and desensitized states, respectively. According to Katz and Thesleff [3], desensitization onset is agonist-dependent, recovery from desensitization is agonist-independent, desensitization corresponds to a high-affinity receptor state, and a fraction of receptors can exist in desensitized state. In this model, following agonist (A) binding to the resting receptor (R) and subsequent receptor activation (AR*), desensitization arises (when experimental conditions allow it) as agonist-bound desensitized (AD) and agonist-free desensitized (D) states. Single-channel recordings from muscle nAChRs confirm the existence of multiple desensitized states [75]. At equilibrium, a fraction of receptors are found in the desensitized states (AD or D) [4]. It is assumed that AD is characterized by its high affinity for the agonist, whereas R has a relatively low affinity, and AR* an intermediate one [2].

(b) Kinetic model including high-affinity desensitization from the agonist-bound closed state (modified from [16,19])

$$\begin{array}{ccccccc} R + A & \leftrightarrow & AR + A & \leftrightarrow & A_2R & \leftrightarrow & A_2R^* \\ \updownarrow & & \updownarrow & & & & \updownarrow \\ D + A & \leftrightarrow & AD + A & & \longleftrightarrow & & A_2D \end{array}$$

This model suggests binding of two agonist molecules and transition into the desensitized state from both the agonist-bound closed state (AR) and from the open receptor state (A_2R^*). The former has a high-affinity for agonist because it can be evoked by low (nanomolar) concentrations of agonist, and it could represent the receptor conformation responsible for generating high-affinity desensitization (HAD) because no activation is involved.

especially suited for trapping ACh. At neuromuscular junctions, the 'trapping' of released ACh by desensitized receptors can shorten the decay of cholinergic postsynaptic currents (with relatively little decrease in amplitude), restoring the efficiency of neuromuscular transmission impaired by pharmacological inhibition of acetylcholinesterase activity [14,15]. During high-frequency stimulation, such an effect is expected to preserve the large safety factor that depends on the duration of single endplate potentials [15].

Molecular determinants of desensitization

The structure of muscle-type nAChRs has been elucidated in electron-microscope studies, under conditions to minimize desensitization (<5 ms agonist application) [25]. Desensitized receptors show a structural transition in which the γ and δ subunits switch to a less-symmetrical configuration [27]. Although identification of the molecular structures underlying desensitization is still incomplete, site-directed mutagenesis within receptor subunits has revealed that replacement of certain elements can transform the desensitization properties of the receptor when expressed in heterologous systems. However, if mutant receptors display altered desensitization, this does not necessarily prove that the mutated sites are the structural motifs responsible for this process. In fact, because desensitization is a function of agonist binding and receptor activation, any mutant expressing changes in these parameters could show changes in the properties of desensitization. Furthermore, receptor properties might be influenced by the expression system itself.

Notwithstanding these considerations, significant examples of structures controlling nAChR desensitization are shown in Figure 3. A single-point mutation (S284L) in the second transmembrane domain (M2) of the α4 subunit strongly increases the rate of onset of classical desensitization without effect on agonist affinity [28] (Figure 3a,i). Other M2 mutations in the α4 subunit can produce similar effects whereas, as will be discussed later, mutations in M2 of the β2 subunit slow desensitization (Figure 3). Single-point mutations in M2 of α7 receptors can greatly decrease the desensitization onset and strongly increase agonist affinity [29,30] (Figure 3a,ii). The dissociation between changes in agonist affinity and desensitization onset suggest that there are independent sites for receptor activation and desensitization [31], an issue which can be explored using receptor modeling [32,33]. In addition to single amino acids, larger subunit domains have a role in desensitization. In fact, large regions of the extracellular N-terminal domain of the β2 subunit (when combined with the α3 subunit) are important to ensure the fast onset of classical desensitization [34] (Figure 3a,iii), whereas the N-terminal domain of the α4 subunit, along with the first three transmembrane domains, appears responsible for HAD [35] (Figure 3a,i).

Interestingly, subunit splice variants or different subunit assembly can also produce changes in desensitization properties. For instance, α7 receptors of autonomic ganglion neurons desensitize more slowly than those of central neurons [36], probably because of cell-dependent expression of an α7 subunit isoform [37]. Co-expression of α7 and β2 subunits (an assembly that might also be found *in vivo* [38]) produces receptors with slower desensitization than homomeric α7 receptors [39] (Figure 3b,ii). In addition, presence of the α5 subunit confers stronger desensitization properties to the slowly desensitizing α3β4 receptor [40] (Figure 3b,i). Recent data from recombinant α4β2 receptors shows that, even with the same subunit composition, desensitization is largely enhanced when the ratio of β to α subunits increases [31] (Figure 3b,ii). Thus, the α:β subunit ratio can determine the degree of desensitization.

Modulation of desensitization

Desensitization is a plastic process, rapidly adapting to changes in neuronal activity through modulation by local factors. One of these is the endogenous peptide substance P, which powerfully facilitates desensitization by binding to an allosteric site distinct from the ACh-recognition sites [41]. Because substance P colocalizes with ACh in the splanchnic nerve terminals in the adrenal gland from which substance P is released in response to stress [41],

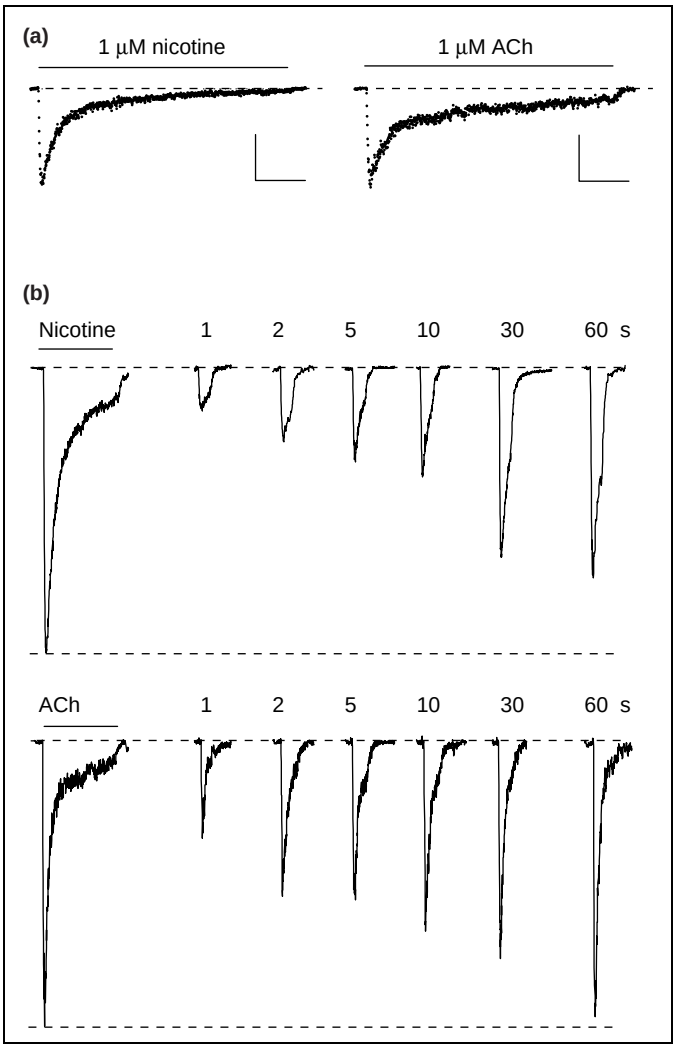

Figure 2. Differential onset and recovery from desensitization of α4β2 nAChRs activated by nicotine or ACh. **(a)** Inward current responses of human embryonic kidney cell-line (HEK) cells expressing rat α4β2 nAChRs to nicotine or ACh. Scale bar, 1 s and 110 pA. **(b)** The rate of recovery from desensitization is measured by brief (i.e. 2 s) application of either 10 μM nicotine or 100 μM ACh, followed by a second application at the time intervals indicated (in seconds) above the responses. Note that after the ACh-induced desensitization, the peak amplitude of the test current returns to the control level (lower broken line) sooner than when nicotine is used to desensitize the receptors. Although nicotine (unlike ACh) can readily cross cell membranes and accumulate intracellularly, it is unlikely that the slow recovery rate from nicotine-evoked desensitization reflects the gradual release of this drug from a cell pool because the rate constant of nicotine uptake is $\sim 4 \times 10^{-3}$ min^{-1} [71], thus largely in excess of the brief application time of nicotine shown here. Adapted, with permission of Blackwell Publishing Ltd, from [16].

this peptide might thus attenuate cholinergic responses *in vivo*. Other modulators are intracellular messengers (e.g. Ca^{2+}) that primarily target the recovery from, rather than the onset of, desensitization [4,42,43] (Figure 4). For α7-containing nAChRs in rat hippocampal interneurons [43] and for native nAChRs in chromaffin cells [42,44], the recovery from desensitization is delayed by high intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). This is probably because Ca^{2+} catalyzes the activity of enzymes such as protein kinase C (PKC) or calcineurin, whose dynamic balance controls the recovery process [4] (Figure 4). Conversely, for α4β2 nAChRs expressed in *Xenopus* oocytes, the rise in $[Ca^{2+}]_i$ and subsequent activation of PKC promotes recovery from desensitization [4,45].

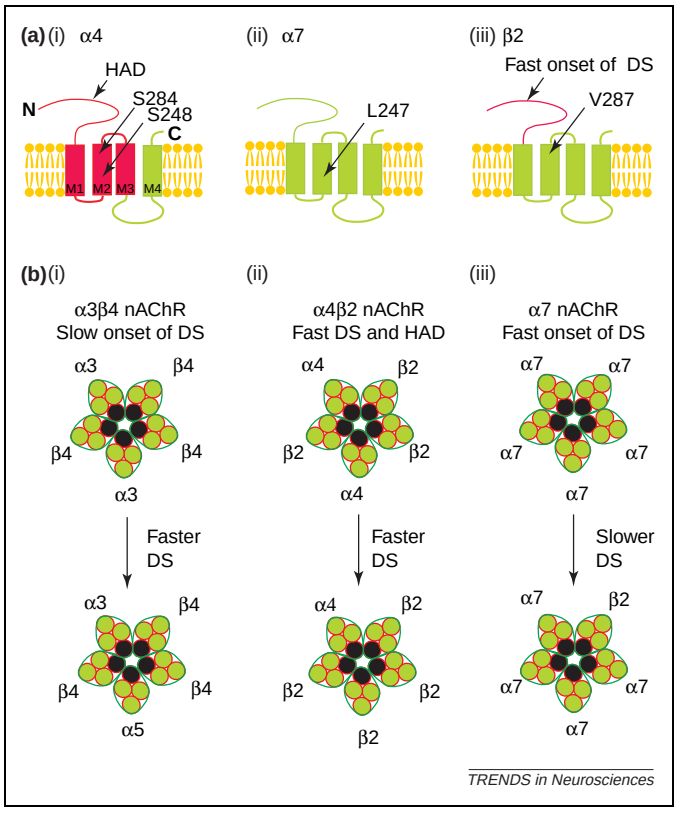

Figure 3. Schematic examples of structural determinants controlling desensitization of nAChRs. **(a)** (i) The extracellular N-terminal and the M1–M3 transmembrane domains (red) control 'high-affinity desensitization' (HAD) [35], whereas single amino acid residues (S284 and S248 in the M2 domain) are reported to control classical desensitization of α4-containing nAChRs [28,63]. (ii) A single amino acid residue (L247 in M2) can control fast desensitization of α7 nAChRs [29], although other residues might also be involved [30]. (iii) The extracellular N-terminal of the β2 subunit (red) is involved in the onset of classical desensitization (DS) [34]. The V287 site of M2 also controls desensitization [61,64]. **(b)** Bird's-eye view of various pentameric assemblies of nAChR subunits with differing desensitization properties; black regions (belonging to M2) surround the central pore. (i) Inclusion of the α5 subunit confers faster desensitization to α3β4 receptors [40]; (ii) higher numbers of β subunits speed-up desensitization of α4β2 receptors [31]; (iii) coexpression of α7 and β2 subunits slows-down desensitization of α7 receptors [39].

nAChR desensitization at cholinergic synapses

In the mammalian CNS, excitatory cholinergic transmission mediated by either α7 or non-α7 nAChRs has been observed in GABAergic interneurons of the hippocampus [46], in hypothalamic supraoptic neurons [47], in developing visual cortex neurons [48], in Renshaw cells [49] and in deep interneurons in the spinal cord [50]. Whether the endogenous transmitter ACh can desensitize these receptors has not been systematically investigated. However, hypothalamic nAChRs show modest desensitization, even when presynaptic inputs are repeatedly activated [47]. Likewise, in the peripheral nervous system, nAChR-mediated synaptic transmission onto cervical ganglion [51] or adrenal chromaffin [52] cells shows no desensitization, even when presynaptic afferents are stimulated at 20–60 Hz. By contrast, at similar stimulation frequencies, nAChRs at the frog neuromuscular junction can show cumulative desensitization, although responses to single stimuli possess only minimal desensitization [53]. Because the time-course of nAChR-mediated synaptic current decay is in the 5–10 ms range, receptor deactivation plus rapid ACh hydrolysis (rather than desensitization) seem to terminate the transmitter action of ACh at most

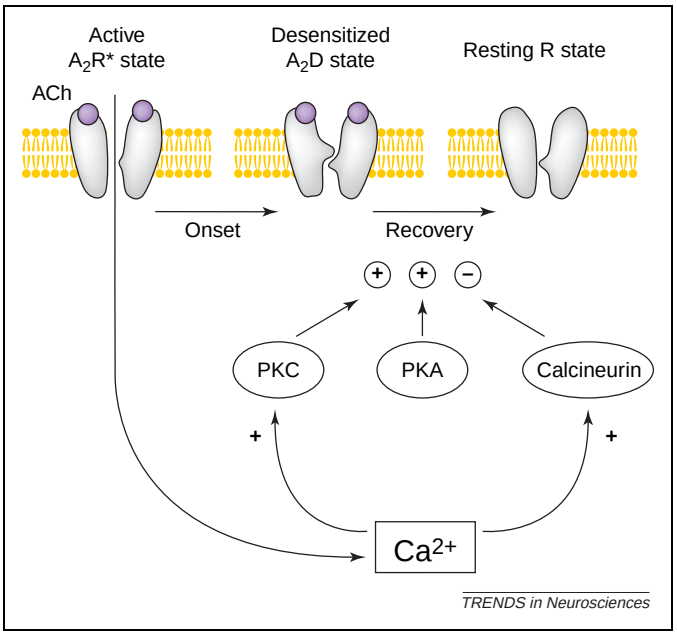

Figure 4. Recovery from desensitization of α3β4 nAChRs in adrenal chromaffin cells is modulated by phosphorylation. After the application of agonists at high concentrations, activated Ca^{2+}-permeable receptors (A_2R^*) [16] proceed into the desensitized (A_2D) state, followed by transition into the resting (R) state. Note that two agonist molecules remain bound to the desensitized receptor. Recovery from the desensitized to the resting state can be accelerated by phosphorylation involving protein kinase C (PKC) or cAMP-dependent protein kinase (PKA), whereas dephosphorylation by calcineurin delays recovery [42]. Intracellular Ca^{2+} thus fine-tunes the recovery process by shifting the balance between PKC and/or calcineurin activity. For the sake of brevity, the AD and D desensitized states (Box 1b) [16] have been omitted from this scheme, although they also represent potential targets for modulation by phosphorylation.

cholinergic synapses [5]. Nevertheless, desensitization could have an important role in limiting cholinergic transmission, for example whenever the action of acetylcholinesterase is reduced owing to a genetic enzyme deficit [54] or is pharmacologically blocked [14,15,53].

nAChR desensitization controls the main action of brain nAChRs to facilitate transmitter release

Although postsynaptic nAChRs can mediate fast cholinergic transmission in the brain, historically nAChRs had been thought to act primarily by controlling the release of other neurotransmitters (e.g. glutamate, GABA and dopamine) from nerve terminals [55,56], and in this way to regulate synaptic efficacy, synaptic plasticity and cognition, and also nicotine addiction [5,6]. Thus, nAChRs can be viewed as gain-setters of excitatory and inhibitory signals mediated by other transmitters. This effect can be achieved through the differential distribution and desensitization of nAChRs, as shown by their complex interactions within the midbrain dopaminergic system. This circuit is involved in nicotine addiction via dopaminergic and GABAergic neurons in the ventral tegmental area (VTA) and substantia nigra compacta (SNc). In particular, in the VTA the cell bodies of dopaminergic neurons are excited by activation of their non-α7 nAChRs [6,57]. However, the cell bodies of GABAergic neurons, which synapse onto and inhibit dopaminergic neurons, also have excitatory non-α7 receptors [6,57]. Therefore, the relative balance of excitation by the non-α7 receptors of dopaminergic and of GABAergic neurons is important in expressing the network dopamine-mediated output. In addition, dopaminergic neurons are activated by glutamatergic afferents (expressing facilitatory presynaptic α7 receptors) from prefrontal cortex neurons impinging onto dopaminergic neurons [6,57].

How the midbrain dopaminergic system will operate when exposed to nicotine is dictated by the fine equilibrium among transmitter systems. For example, a relatively low dose of nicotine (as delivered by tobacco smoking) stimulates the midbrain dopaminergic neurons to release dopamine into the nucleus accumbens (NAc) [6,7,57,58]. In this case, it appears that low doses of nicotine preferentially (although not completely) desensitize the non-α7 nAChRs of dopaminergic and GABAergic neurons [7], probably via HAD mechanisms (Table 1). At the same time, the nicotine-induced activation of α7-containing receptors (which show much less HAD than non-α7 receptors) enables them to enhance glutamate-mediated excitatory inputs to the dopaminergic neurons, thus facilitating the release of dopamine onto NAc neurons [6]. In addition, because brainstem cholinergic neurons innervate GABAergic rather than dopaminergic neurons in the VTA [6], endogenous ACh and nicotine could combine to amplify the desensitization of nAChRs on GABAergic neurons, to enhance dopamine-mediated transmission further. *In vivo* studies using acetylcholinesterase inhibitors support the role of disinhibition of VTA dopaminergic neurons by the build-up of endogenous cholinergic activity [6].

Analogous considerations can be applied to interpret the analgesic action of nicotinic drugs [59]. For example, in the deep dorsal horn area of the spinal cord, where pain sensation is processed before being transferred to brain regions, inhibitory and excitatory interneurons express different sets of nAChR subunits (α2, α4, α6, β2, and α3, α7, β2, respectively [60]). This suggests that in the future, subunit-selective nAChR agonists might produce better analgesia by exploiting the activation and desensitization of restricted nAChR subtypes.

Pathophysiology of nAChR signaling: potential role of desensitization

Epilepsy and seizures

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is a rare form of epilepsy characterized by brief seizures during the night [61]. Several mutations have been found in genes encoding the α4 and β2 neuronal nAChR subunits (although several affected families do not possess any mutations in these genes), many of which reside in or adjacent to M2 of these subunits. Although these mutations produce similar clinical symptoms [62], they have various effects on channel function, including a change in receptor desensitization [10,28]. For example, α4-S248F mutant receptors express faster desensitization kinetics, and slower recovery from desensitization, than wild-type α4 nAChR [63]. Conversely, the β2-V287L mutation significantly slows the rate of desensitization [61,64]. Although it is still unclear how mutations might cause the seizures associated with ADNFLE, the functional properties of the mutated nAChR in ADNFLE generally indicate that a mutant gain of function is

responsible for the neuronal network dysfunction [10]. If the mutant nAChRs were part of an inhibitory circuit, either regulating the release of GABA or the overall excitability of GABAergic interneurons [55,65], impaired GABA-mediated transmission would produce epileptic hyperexcitability [63].

Congenital myasthenic syndromes

Congenital myasthenic syndromes (CMS) are a heterogeneous group of disorders caused by genetic defects affecting neuromuscular transmission [12]. Various mutations in muscle nAChR genes can alter either the expression or the kinetics of these receptors. Mutations involving changes in the kinetics of nAChRs include slow-channel mutations (i.e. causing abnormally slow synaptic potential decay) and fast-channel mutations (i.e. causing abnormally fast synaptic response decay) [12]. One particular slow-channel mutation, α-V249F (a mutation in M2 of the α subunit), shows enhanced activation and agonist-binding affinity, plus increased desensitization – effects that are thought to compromise neuromuscular transmission [66]. In a fast-channel CMS with attenuated postsynaptic response, one mutation (ε-D175N) in the ε subunit preferentially affects the desensitized state of receptors because it decreases their closed-state affinity for ACh by 17-fold, but decreases the desensitized-state affinity by 800-fold [67]. Although there are many CMS-related mutations in proteins other than nAChRs, the common outcome is abnormal synaptic communication to skeletal muscles, including weakness, severe disability, difficulty in breathing, and death.

Protean actions of nAChRs

The activation and/or regulation of nAChRs, particularly of those containing the α7 subunit, are paradoxically linked to both neuroprotection and neurotoxicity *in vivo* and *in vitro*. For example, the neuroprotective action of nicotine in cultured PC12 cells has been suggested to be due to upregulation of α7-containing nAChRs following their persistent desensitization [11]. Such neuroprotection might have clinical implications because some nicotinic ligands appear to minimize the cognitive deficits associated with Alzheimer's disease [11]. The mechanisms underlying this effect are complex. For instance, on mouse cortical neurons, nicotine activates α7-containing and β2-containing nAChRs, to decrease glutamate-mediated Ca^{2+} influx via a Ca^{2+}-dependent signal transduction cascade that involves calcineurin and depression of L-type voltage-gated Ca^{2+} channels [68]. Although nAChRs are rapidly desensitized after nicotine treatment, a small response to nicotine remains to provide a residual level of Ca^{2+} entry adequate to activate a high-affinity Ca^{2+} sensor such as calcineurin. Meanwhile, low-affinity Ca^{2+} sensors (such as the Ca^{2+}-dependent kinases that can evoke neurotoxicity [68]) are not activated because they require a much stronger Ca^{2+} signal. Furthermore, the rapid desensitization of α7-containing nAChRs might also help to prevent Ca^{2+} overload and neurotoxicity, because expression of a mutant form of this channel that dramatically reduces desensitization also enhances neurotoxicity [8]. Hence, the extent of nAChR activation and its coupling to $[Ca^{2+}]_i$ and intracellular enzymes can set the balance between excitotoxicity (due to the strong release of glutamate) and neuroprotection (due to the inhibition of Ca^{2+} influx).

The large cholinergic deficit observed in Alzheimer's dementia is associated with loss of nAChRs, predominantly affecting the readily-desensitizing α4-containing receptors [69]. Symptomatic relief of the cognitive impairment by chronic administration of nicotinic agents might therefore rely on surviving nAChR subtypes less susceptible to desensitization, and on the use of allosteric modulator drugs that do not act as agonists on nAChRs, and therefore cannot generate their widespread desensitization [70].

Concluding remarks

nAChR desensitization should represent a major target for the upregulation and/or downregulation of nAChR function. By exploiting its dependence on the nAChR subtype, it might be possible in the future to obtain differential regulation of cholinergic signals within the same circuit expressing heterogeneous nAChRs. Furthermore, because desensitization can occur even at agonist concentrations insufficient to activate receptors, it might be possible to minimize drug doses in the interest of safety.

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