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Review

Desensitized nicotinic receptors in brain

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Abstract

Desensitization is an intriguing characteristic of ligand-gated channels, whereby a decrease or loss of biological response occurs following prolonged or repetitive stimulation. Nicotinic acetylcholine receptors (nAChRs), as a member of transmitter gated ion channels family, also can be desensitized by continuous or repeated exposure to agonist. Desensitization of nicotinic receptors can occur as a result of extended nicotine exposure during smoking or prolonged acetylcholine when treatment of Alzheimer's disease (AD) with cholinesterase inhibitors, or anticholinesterase agent poisoning. Studies from our lab have shown that nAChRs desensitization is not a nonfunctional state and we proposed that desensitized nAChRs could increase sensitivity of brain muscarinic receptor to its agonists. Here, we will review the regulation of nicotinic receptor desensitization and discuss the important biological function of desensitized nicotinic receptors in light of our previous studies. These studies provide the critical information for understanding the importance of nicotinic receptors desensitization in both normal physiological processing and in various disease states.

Theme: Neurotransmitters, modulators, transporters and receptors
Topic: Acetylcholine receptors: nicotinic

Keywords: Nicotinic receptors; Desensitize; Nicotine

Contents

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1. Introduction

Desensitization is a general characteristic of ligand-gated channels, whereby a decrease or loss of biological response occurs following prolonged or repetitive stimulation [50,93,123,132,155,175]. As a member of the ligand-gated ion channel family, nicotinic acetylcholine receptor (nAChR) causes desensitization in the brain [17]. This occurs as a result of extended nicotine exposure during smoking, or via the treatment of Alzheimer's disease (AD) with cholinesterase inhibitors, or as a result of poisoning by an anticholinesterase agent.

Nicotine is a specific and highly selective ligand that binds readily to nAChRs. Repeated or chronic exposure to nicotine induces a decrease or loss of the N-like response and increases the number of nAChRs, rather than reducing their number [77]. This response may result from the ability of nicotine to desensitize or inactivate nAChRs [62,93]. Indeed, prolonged incubation with low levels of nicotine, such as that which occurs when smoking, results in little receptor activation; however, it effectively blocks the nAChRs by stabilizing the desensitized states of the receptor [30,35]. Accumulating evidence from epidemiological studies on smoking that links nAChRs and age-related neurodegenerative diseases has revealed a negative association between cigarette smoking and Parkinson's disease (PD) and AD, suggesting that, in addition to nicotine, desensitized nAChRs may be responsible for their neuroprotective actions [16,17,135]. Further, the potential clinical applications of nicotine and/or nicotinic agonists in a variety of central nervous system (CNS) diseases in general, and behavioral disorders in particular have been examined extensively [69,127,138,148,149,179,184]. In addition, nAChR desensitization also may play important roles in many biological processes including the regulation of muscarinic receptors, synapse plasticity, learning and memory [96,144]. Accordingly, it is reasonable to propose the working hypothesis that pharmacological consequences of desensitization beyond simply nicotinic receptor inactivation may contribute to the array of potential effects associated with chronic nicotine exposure.

2. Brain nicotinic receptor's structure

Brain nAChRs differ from the muscle-type heteropentameric subtypes because they contain no γ, δ or ϵ subunits, and consist of various complements of α_2–α_{10} and β_2–β_4 subunits [153]. Additionally, α_2–α_{10} and β_2–β_4 subunits have been identified and cloned from human brain [60,74,82,130]. Neuronal nAChRs subunits assemble according to a general $2\alpha3\beta$ stoichiometry [10,66,105], However, α_7, α_8 and α_9 are known to form functional homo-oligomers consisting of a single a subunit subtype [39,179]. In mammalian brain, $\alpha_4\beta_2$ and α_7 nAChRs, the two major subtypes, have different properties [36,66]. For example, α_7 nAChRs homomeric pentamers appear to bind α-bungarotoxin (α-BTX) with high affinity, rapidly and reversibly desensitize, and also have high permeability to calcium [4,67], whereas $\alpha_4\beta_2$ subtypes have a higher affinity binding for nicotine [120,166,212].

The nAChRs are extensively expressed throughout the nervous systems [186]. For instance, α_3 and β_2 subunits are expressed in cortex, thalamus, hippocampus and cerebellum, the α_4 subunit is expressed in cortex and cerebellum, but the α_7 subunit is the most widely expressed in cortex, hippocampus and thalamus [162].

The nAChRs sequence consists of (1) a large hydrophilic amino terminal domain, the long N-terminal extracellular part represents glycosylation sites, which is thought to participate in the ligand binding site, and appears to play a role in the regulation of desensitization and ion permeability [140]; (2) four hydrophobic segments

(19–27 amino) that form the transmembrane domains (TM–TM), TMII lines the ion channel [43,171,201]; (3) a small highly variable hydrophilic domain; (4) a hydrophobic C-terminal domain of approximately 20 amino acids [39,86,153]. The intracellular loop, comprised between the TM and TM segments, contains phosphorylation sites that are thought to be involved in desensitization [91]. Many drugs or activators indirectly induce the receptor desensitization by increasing intracellular calcium to phosphorylate the receptors by calcium-sensitive protein kinases [103]. Agonists binding sites are thought to locate at the interface between and subunits, and the non-competitive allosteric activators sites are located on the α subunit [103].

3. Brain nicotinic receptors function

Brain nAChRs distribute postsynaptic, as well as pre-, peri- and extra-synaptic sites, where they may modulate the neurotransmitter release, synapse action and neuronal activity by a varions of functional states [11,54,82,145], playing important roles in many physiological and pathological processes including neuron development, learning and memory, and the rewarding response induced by addictive drugs [82,90,175].

The subtypes of brain nAChR with different subunits, mediate diverse function in nervous system [4,115,120]. For example, $\alpha_3(\alpha_5)\beta_4$ subunits regulate ganglion transmission phenotype of α_3 B_2 gene deficiency is involved in decrease of ganglion transmission. nAChRs containing α_4 partially regulate DA release (the release is also regulated by six subunits), but glutamate release is mostly modulated by α_7 subunit[7]. β_2 subunits control GABA release and responses to ACh by DA neurons in mesencephalon. β_3 subunits located in striatum alter motor activity by means of modulating DA release [39]. In addition, β_2 gene knockout is responsible for cortex atrophy, neuron loss and gliacyte increase, suggesting that nAChRs are involved in neuron survival [41]. In addition, nAChRs also participate in development and maintenance of neuronal circuits [121,129,163].

4. Brain nicotinic receptors desensitization

Research about nAChRs desensitization began with the classics experiment of Katz and Thesleff in 1957 [94]. For nAChRs, desensitization can be described as a decline in response to nicotine after repetitive exposure to nicotine. The onset of desensitization is both time- and concentration-dependent [77,93]. This process reflects the time-dependent accumulation of receptors in long-lived nonconducting states [167]. Desensitization can be modified by a variety of cellular agents and exogenous signals [144]. When purified nAChRs are reconstituted in lipid bilayers, desensitization is also induced by agonist, indicating that desensitization must be intrinsic to the nAChRs [161].

Formal models of desensitization originally derived for muscle nAChRs have been extended across the entire nicotinic family. Katz and Thesleff [94] provided the first description of desensitization for muscle nAChRs. In particular, they noted: the onset of desensitization could be slower than recovery at low concentration of agonist; recovery depended on the type of agonist and its concentration, even at low concentration, ACh could induce extensive desensitization [94]. nAChRs exist in three states, resting state (R), activated state (O) and desensitization state (D) [92,107,144]. R confirmation has a relatively low affinity for agonists, with agonists only able to activate nAChRs and open ion channels at high concentration; in contrast, the affinity of D state for an agonist is 20 times higher than R, with agonists at low concentration able to induce desensitization and with more stable agonists binding to nAChRs [30,166].

Desensitization represents a classic form of allosteric protein behavior, in which the receptor is distributed among a number of discrete confirmations. Agonists will preferentially stabilize one or more of the states [35]. Following exposure to agonist, the time course of desensitization will be governed by the transition rate constants, but in general, because desensitized states have higher agonist affinities, over time all receptors will end up desensitized.

Models of desensitization that were first noted by Katz and Thesleff are indicative of multiple agonist binding steps [94]. An intermediate desensitized state (I) has been brought

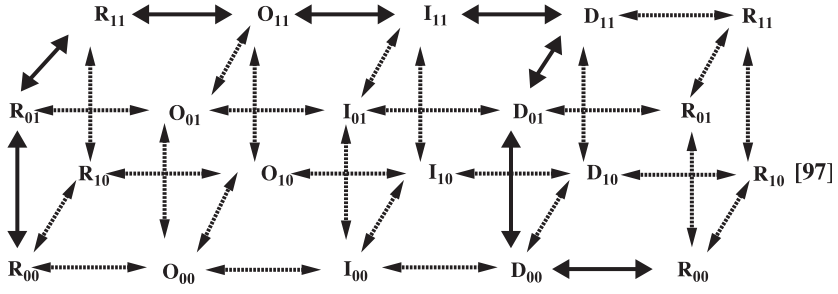

Fig. 1. Transition among the four states of nAChRs. The states of the nAChRs are resting (R), open (O), fast-onset or intermediate desensitization (I), and slow-onset or chronically desensitized state (D). It is assumed that the two agonist-binding sites are different. The subscripts indicate the state of occupation of the sites: 00, unoccupied; 01 or 10, singly occupied; 11, doubly occupied. It is assumed that the resting state and the desensitized states are directly connected by allowed transitions. Heavy arrows indicate a principal reaction pathway.

forward because it has a biexponential time course of desensitization onset and recovery [29,35,61,62]. An extended scheme that includes two sequential agonist binding steps and two desensitized states, namely intermediate (I) and high (D) affinities, has been proposed by Feltz and Trautmann. Gradually, the two-state model of desensitization and affinities of the three states (R, I and D) have been widely approved accepted [61]. Recently, a model developed by Karlin provides a good description of transition among the four states of the nAChRs (Fig. 1) [93]. Agonist binding to two sites is necessary to activate nAChRs and to open ion channels, but agonist binding to one site only could induce nAChR desensitization and close ion channels [12,56,93].

Desensitization is accompanied by an affinity shift and a structural change at the binding sites [63]. Introduction of short sequences or individual amino acids (notably lysine 152) from α_4 subunits into agonist binding loops B and C of chimeric α_7-5HT receptors conferred high affinity (α_4-like) binding, in addition to an enhancement of desensitization by low concentration nicotine [42]. In terms of the desensitization model, changes in affinity for the ligand can be accounted for by allosteric constants [25,147].

The desensitized state is associated with a closed conformation of the channel pore [12]. Mutations of a key pore-lining residue, leucine247, appear to destabilize the closed conformation of a desensitized state, making it conductive to ions, which in turn leads to a dramatic slowing of the time course of desensitization of the usually fast-desensitizing α_7 receptors [21]. Moreover, because of the high affinity of the desensitized state for agonists, the mutant α_7 receptor is now sensitive to the desensitization induced by ACh and nicotine in the concentration ranger that would cause desensitization of wild type nAChRs [164].

The β subunit plays a dominant role in desensitization kinetics. Chimeras between β_2 and β_4 subunits showed that the extracellular region, from the N-terminal to the beginning of the first transmembrane domain, controls fast and slow desensitization kinetics in β_2- and β_4-containing nAChRs [26]. Furthermore, multiple regions in the N-terminal domain are required for the slow kinetics of β_4 subunit desensitization [26], because replacement of any nonoverlapping segments of the N-terminal region produces faster, β_2-like kinetics [26]. These data suggest that slow desensitization relies on the complex interaction of several regions of the N-terminal region [13,26,144].

5. Regulation of brain nicotinic receptor desensitization

Many factors regulate the kinetics of receptor activation and desensitization, including subunits composition, temperature, membrane potential, post-translational modification, ligands, ions and interactions with the cytoskeleton [29,53,144,180].

5.1. Subunit composition

The subunits of nAChRs contribute to receptor desensitization. The properties of brain nAChR desensitization are diverse, and are temporally and spatially dependent [93]. Even on adjacent neurons in the same brain slice, nAChR activation and desensitization induced by low concentration nicotine are variable [7]. It may be that, when most nAChRs are desensitized, the minority of nAChRs are still not sensitive, i.e., when most nAChRs are in the refractory period, a minority of nAChRs continue to respond to nicotine. Because of differences in the subunit composition of nAChRs, responses to nicotine are variable between brain regions [53].

5.1.1. Homomeric α receptors

Homomeric α_7 nAChRs are 7/α-BTX and demonstrate the most rapid desensitization of any nAChRs, making it difficult to determine whether two-desensitized state exist. For example, in hippocampal cells, ciliary ganglion cells and hypothalamic neurons, the onset of desensitization appears to be a single exponential component [20]. In general, the desensitized states of α_7 nAChRs are less sensitive to agonist than heteromeric nAChRs, but α_7 nAChRs can be effectively desensitized by submicromolar doses of agonist [6].

α_7/α-BTX-sensitive receptors expressed in peripheral intracardiac ganglion and superior cervical cells desensitize very slowly [46,47], thereby challenging the prevailing notion that α_7 nAChRs desensitize quickly. These receptors desensitize with tenfold slower time constants (t=200 ms), which indicates that other factors must regulate α_7 nAChR desensitization. For instance, the rate of nAChR desensitization slows twofold when the β_2 and α_7 subunits are co-expressed in oocytes [100]. In addition, post-translational modulation such as phosphorylation alters desensitization. Following prolonged exposure to nicotine, recovery is very fast for the fast-desensitizing naïve α-BTX-sensitive nAChRs, but for some α_7 nAChRs recovery from desensitization is more complex and may depend on the species [134].

There is little known about the desensitization characteristics of other homomeric nAChRs. Desensitization of homomeric α_8 nAChRs in chick, at least in oocytes, is as same magnitude as α_7 nAChRs, but is induced at much lower concentrations of nicotine or ACh. When co-expressed with α_{10} subunit, the rate and extent of desensitization of slowly or nondesensitizing α_9 homomeric receptors in cochlear neurons are enhanced [59].

5.1.2. Heteromeric α β receptors

Although the exact number of heteromeric nAChRs remains unknown, there is a wealth of information about desensitization of the heteromeric αβ receptors. In the CNS, equilibrium binding for ^{3}H-nicotine is used to evaluate the desensitized state of $\alpha_4\beta_2$ nAChRs. Using the broad-

spectrum agonist ^{3}H-epibatidine to label CNS binding sites, nicotine displacement is biphasic, implying two sites with high and moderate affinities for this agonist. The high-affinity site represents $\alpha_4\beta_2$ nAChRs, while the lower-affinity site may include α_3-containing heteromeric nAChRs, such as the $\alpha_3\beta_4$ subtype [200,208]. Furthermore, the functional studies used to replicate the radiolabeled binding assay shows that α_4-containing nAChRs are desensitized by much lower concentrations of agonists than the α_3-containing receptors. The IC_{50} values for desensitization by nicotine or ACh are in the same range as the binding K_ds (0.1–10 and 150–400 nM) for α_4 nAChRs and α_3 nAChRs, respectively [63], further confirming the difference in the affinities of these desensitized nAChRs. In addition, the chimeric α_3/α_4 subunit co-expressed with the β_2 subunit produced receptors that have affinity similar to α_3 nAChRs, implying that the α subunit is the primary determinant of affinity [104]. However, both α and β subunits contribute to the affinity of desensitized nAChRs [79,84].

It is well known that α and β subunits jointly form binding sites for an agonist and control functional properties of nAChRs, including affinities of activated and desensitized nAChRs [40,119,152]. Additionally, distinct subunits may modulate desensitization kinetics. Many studies show that β subunits strongly influence the onset of desensitization, because the desensitizing rate of receptors containing the β_2 subunit is faster than those containing the β_4 subunit. The difference comes from the altered balance between fast and slow desensitization components; more than 90% desensitization for β_4 nAChRs is slow, while more than 90% desensitization for β_2 nAChRs is fast [26]. If the β subunit is kept constant, α_3-containing nAChRs desensitize faster than α_4-containing nAChRs [84]. Thus, in terms of the onset of desensitization, all heteromeric nAChRs can be ordered from fast to slow, as follows: $\alpha_3\beta_2 > \alpha_4\beta_2 > \alpha_3\beta_4 > \alpha_4\beta_4$ [63]. Because of the limited distribution of α_2 nAChRs in CNS, little is known about these subtypes. Naive nAChRs with heterologously expressed αβ may have a more complex molecular composition. In the CNS, $\alpha_4\beta_2$ and $\alpha_3\beta_4$ are the two major components. $\alpha_4\beta_2$ in rat medial habemula cells is desensitized with an exponential time constant (t=200 ms), but $\alpha_3\beta_4$ nAChRs in HEK cells occur within 1.2 s, which may be due to the α_5 subunit. For example, $\alpha_3\alpha_5\beta_4$ nAChRs desensitize threefold faster than $\alpha_3\beta_4$ [188]. Likewise, α_5 expressed together with $\alpha_4\beta_2$ accelerates desensitization [160,188,205]. Studies in oocytes also suggest that subunit composition contributes to recovery from desensitization.

Desensitization is influenced by subunit composition, but it is important to point out that the complex behavior of nAChRs is not simply the sum of the subunits [54].

5.2. Phosphorylation contribute to nicotinic receptors desensitization

Phosphorylation provides an important post-translational modulation of receptors via two primary mechanisms [88], namely: (1) receptors activated by binding ligands have kinase activity and can auto-phosphorylate, and (2) receptors that bind ligands alter conformation, thereby becoming substrates for some kinases/phosphatases. nAChR phosphorylation involves the latter mechanism. Phosphorylation of the nAChRs has since been shown to be regulated by the cAMP-dependent kinase (PKC), protein kinase C (PKC) and a tyrosine-specific protein kinase [88,89,176]. Almost all subunits can be phosphorylated by corresponding kinases. For example, ser353 on the γ subunit and ser361 on the δ subunit of muscle nAChRs are phosphorylated by cAMP-dependent kinase (PKA). Phosphorylation of ser361 on the δ subunit is involved in desensitization. Ser362, ser377 and ser379 on the δ subunit are substrates for PKC, and tyr355 on the β subunit, tyr364 on the γ subunit and tyr372 on the δ subunit can be phosphorylated by tyrosine kinase [66].

Phosphorylation processes may regulate the rate of desensitization of nAChRs [55,88], especially for the slow component of neuronal nAChR desensitization. For instance, calcium may play important roles in recovery from desensitization, in part via nAChR channels. As regards PKA, its activation accelerates and its inhibition slows $\alpha_4\beta_2$ recovery from desensitization [142]. In general, the rate of recovery from desensitization is governed by the balance between phosphatase and kinase activity, since inhibiting phosphatase or activating PKC would accelerate recovery from desensitization [151].

The phosphorylation sequence on nAChRs subunits lie within the intracellular loop between TM II and TM IV. In vitro, PKC or PKA may phosphorylate these sites directly [150,153] and phosphorylation has also be demonstrated in vivo, especially at ser368 on the α_4 subunit [46,201]. Phosphorylating $\alpha_4\beta_2$ nAChRs in the oocytes induced by Ca^{2+}/PKC may increase the rate of recovery from desensitization, because mutation of ser368 limits recovery [64].

Alteration of the time course of recovery from desensitization suggests that protein phosphorylation modulates allosteric changes between the activated and desensitized states [142]. The rate of recovery is governed by the movement of nAChRs: either R→I→D or D→I→R. By slowing into deep desensitization, phosphorylation promotes recovery of nAChRs because more phosphorylated nAChRs remain in the "shallow" of the intermediate desensitized state; conversely, phosphorylation accelerates recovery of desensitized $\alpha_4\beta_2$ nAChRs by altering the allosteric constant from the desensitized state towards a "shallow" intermediate state [161].

Phosphorylation reduces desensitized receptors caused by high frequency synapse stimulation [141]. In addition, phosphorylation can alter the rate of recovery from desensitization and regulate the balance between desensitized receptors and activated receptors in the absence of ligand. Thus, phosphorylation could in theory regulate the number of receptors activated by the neurotransmitter, thereby modulating synapse function without new receptor

synthesis. nAChR desensitization may be accompanied by conformational change, especially within the intracellular loop of the α_4 subunit [57]. This change prevents PKC or PKA from phosphorylating nAChRs. With reduced efficacy of protein phosphatases, this would shift the balance toward the dephosphorylated state. Different phosphatases are involved in the dephosphorylation cascade, which takes some time to complete, but it is faster than rephosphorylation. Finally, nAChRs would enter into a stable inactivated state when dephosphorylation is induced by nicotine [57]. Because the α_4 subunit has a multitude of serine phosphorylation sequences in the cytoplasmic loop [85], it is inferred that dephosphorylation leads to a conformational change, thereby blocking ion influx. Charge repulsion induced by phosphorylation causes the large loop to sit out and away from the channel pore. Conversely, dephosphorylation could cause the loop to hinder ion influx by interacting with pore components on the cytoplasmic face of receptors [57]. Slight decreases in PKC activity may change the balance in favor of dephosphorylation and deactivation of $\alpha_4\beta_2$ nAChRs [57]. However, there is no method to assess the intrinsic activity of nAChRs or PKC activity in the brain. Further work is required to determine the sites of phosphorylation that regulate nAChR activation and desensitization, which may elucidate not only the effect of nicotine in addiction, but also the mechanism of cholinergic synaptic plasticity.

5.3. Ligand contribute to nicotinic receptors desensitization

The affinity of nAChRs for endogenous agonist influences the onset of desensitization and recovery from desensitization [122,209]. For example, choline and acetylcholine have similar kinetic properties of activation and desensitization on the α_7 nicotinic receptors in rat hippocampal neurons; however, the choline-evoked currents decayed more quickly to baseline after removal of the agonist, and the recovery from desensitization was faster with choline. In addition, choline dissociates faster than ACh from the receptor. These results established that the main difference between choline and ACh is their affinity, and support the concept that the switching of endogenous agonist may change the desensitization-resensitization dynamics of α_7 [132].

The duration of nAChR exposure to agonist, as well as the concentrations of agonist modulate desensitization [52,93,122,170,174,175,187]. Prolonged exposure to low concentrations of agonist causes nAChRs to enter “deep” desensitization. The longer the exposure to ligand, the slower desensitization recovers [163,170]. In this situation, nAChRs are in the deep desensitized state. For example, acute treatment with nanomolar concentrations of nicotine can activate $\alpha_4\beta_2$ nAChRs and desensitize α_7 nAChRs; in chronic treatment, however, the two nAChR subtypes are desensitized.

5.4. Cellular environment contribute to nicotinic receptors desensitization

nAChRs are quite permeable to Ca^{2+}. Calcium ion influx through these channels could serve as a physiological trigger for regulating desensitization. Intracellular or extracellular Ca^{2+} mediates desensitization of the ion channels either directly, or indirectly, via protein kinase activation [27,96,116,133,136,210]. An increase in intracellular Ca^{2+} enhances the onset of nAChR desensitization. Indeed, desensitized nAChRs are susceptible to modulation by Ca^{2+} via second messengers such as serine/threonine kinases and calcineurin [98]. If nicotine-induced intracellular Ca^{2+} transients are limited by chelation with BAPTA, recovery from desensitization is very fast, even in the presence of PKC inhibition. Inhibition of the Ca^{2+}-dependent phosphatase calcineurin and stimulation of PKC activity by phorbol ester both promote recovery from desensitization, which implies that Ca^{2+} regulates desensitization by altering the balance of kinase/phosphatase. Thus, increasing cellular phosphorylation enhances recovery [99].

Some neuropeptides also contribute to desensitization, including substance P and calcitonin gene-related peptide (CGRP). Substance P and luteinizing hormone-releasing hormone appear to promote nAChR desensitization, which proceeds with a biphasic time course, i.e. fast and then slow components of desensitization [3,178]. Moreover, substance P can stabilize the desensitized state of the receptor, even when added subsequent to the actual desensitization and removal of agonist [178]. An amyloid peptide, βA_4 (25–35) can mimic this function of substance P [36]. The detailed process by which peptides regulate desensitization of nAChRs is not clearly understood.

Metabolites of arachidonic acid and prostaglandin D_2 serve as second messengers to promote the onset of desensitization [143], Likewise, inositol triphosphate (IP_3) and diacyl-glycerol (DAG), produced via activation of phospholipase C (PLC), can mobilize Ca^{2+} and activate PKC. Studies suggest that activation of PLC and PKC strongly influence the desensitization of nAChRs. Cholesterol, membrane lipid composition and some ions also regulate desensitizing processes by altering cell membrane fluidity or via allosteric effects [13,177].

6. Biological function of desensitized brain nAChRs

The physiological significance of nAChR desensitization has been investigated since the 1950s. Under physiological conditions, neurotransmitter release is generally not sufficient to desensitize receptors. However, for receptors that desensitize rapidly, such as the α_7 subunit nAChR, or those with high affinities for ACh that remain bound to agonists for a long time after free transmitter is cleared, desensitization may occur after repetitive stimulation. Moreover, the

selective agonist for α_7 nAChRs, choline, when used as an "ambient" transmitter, can desensitize α_7 nAChRs quickly [6,111], and the desensitized nAChRs will become an important feature of these receptors.

6.1. Up-regulating nicotinic receptors

The effects of desensitization on receptors include alterations in affinity and density [63,77,92,168]. The generally accepted view is that overstimulation induced by agonists leads to a reduction in the number of receptors. However, desensitization by long-term exposure to nicotine triggers an increase in the number of nAChRs with high affinity, and a decrease in the number of nAChRs with low affinity [33,166,185]. Up-regulation possibly relies on the fact that brain nAChRs undergo rapid desensitization and consequent inactivation after prolonged exposure to agonist [65]. The desensitization and up-regulation of nAChRs are the basis of nicotine tolerance and dependence [34,78,106,169].

The number of receptors resulting from gene transcription and post-transcriptional mechanisms determines changes in receptor expression. Most agonist effects on receptor number are accompanied by a change in mRNA levels [18,77,95,126,156,206]. However, it has been shown that chronic nicotine treatment dose not elicit any change in mRNA, which suggests that up-regulation is not due to transcription, and that a post-transcriptional mechanism is responsible for this phenomenon [78,81,126]. Possible mechanisms include the translation rate, subunit assembly and the rate of receptor turnover. Blocking protein synthesis by anisomycin or protein glycosylation by tunicamycin prevented receptor up-regulation on cortical neurons, suggesting that up-regulation in receptor number is not due to the pre-existing intracellular pool that supplies receptors to surface, but due to additional synthesis and transport to the cell surface [20].

Both the agonist and competitive antagonists up-regulated $\alpha_4\beta_2$ nAChRs [81]. However, the noncompetitive antagonist, as well as the channel blocker mecamylamine did not elicit changes in the number of $\alpha_4\beta_2$ nAChRs and also did not modify nicotine-evoked up-regulation [81]. Brain nicotinic receptor occupancy by agonist or competitive antagonists at the ACh recognition sites might be sufficient to trigger an increase in the number of nAChRs [81]. In addition, chlorisondamine and noncompetitive nicotinic antagonists did not elicit up-regulation [58], and the conformational changes induced by desensitization led to a decrease of receptor degradation, thereby causing up-regulation [58,154].

PKC/PKA and nicotine have synergistic effects on nAChR up-regulation, with PKC inhibitors not altering the up-regulation induced by nicotine, suggesting that at least two mechanisms exist: one being a direct pathway via interaction between ligand and the nAChR, with another being induced by activation of PKA or PKC [81]. It is possible that the two pathways may be linked by phosphorylation of nAChRs by PKA and PKC. Results from peripheral muscle nAChRs and electric organ nAChRs show that the alteration of cAMP levels, a process involved in desensitization, was able to phosphorylate nAChRs. Activation of PKA by cAMP can enhance the efficiency of receptor assembly and prevent receptor degradation via phosphorylation of the γ subunit, thereby leading to up-regulation of muscle nAChRs. There are multiple sequences for phosphorylation by both PKA and PKC in the intracellular loop between TMIII and TMIV of the α_4 subunit.

6.2. Facilitating mAChRs functions

The subtle regulatory relationship between desensitized nAChRs and mAChRs reveals regional characteristics in the brain. Stimulation of muscarinic receptors in the cerebral cortex and hippocampus by muscarinic agonists induces electroencephalogram seizures, behavioral convulsions, tremors, homogeneous receptor down-regulation and inhibition of spontaneous locomotor activity. All of these muscarinic effects can be enhanced by nicotinic receptor desensitization via repeated nicotine pretreatment. However, the sensitivity of muscarinic receptors in the striatum and brain stem remained unchanged when nicotinic receptors were desensitized by nicotine [189–192, 194–196,199].

Acute or chronic repetition of nicotine treatment can induce nAChR desensitization, causing hypersensitivity of mAChRs, including increasing seizure discharge to muscarinic agonists in rats. These effects can be prevented by mecamylamine. Chronic nicotine at a very low concentration that is not sufficient for inducing nAChRs desensitization cannot produce hypersensitization of mAChRs in the rabbit brain. The hypersensitivity of mAChRs will not occur after nAChR recovery from desensitization evoked by pretreatment with nicotine. Given these findings, it is reasonable to suggest that the desensitization of nAChRs is necessary for the hypersensitization of central muscarinic receptors.

The magnitude of hypersensitivity is species-dependent. The facilitated effects of desensitized nAChRs on arecoline-produced seizures in rats are similar to those in rabbits, and about 300% higher than in mice. There are also differences between acute nicotine tolerance and chronic nicotine tolerance. In rats, muscarinic agonists are more potent in acute tolerance (about 300%) than in chronic tolerance (about 200%). Similar results could also be obtained for convulsions, tremors and spontaneous locomotor activity in mice, rats and rabbits. This phenomenon exists in the cerebrum and hippocampus, but not in the striatum, thalamus or hypothalamus.

In cholinergic neurons, nAChRs and mAChRs may be localized in the same cells, or in different cells with an interactive relationship. It is well known that nicotinic and muscarinic receptors may exist in the same ganglionic cells,

and nicotinic receptors in ganglionic cells may modulate the functions of muscarinic receptors in post-ganglionic neuron-innervated targets including smooth muscle, cardiomyocytes, epithelium, and exocrine cells. The following experiments have been carried out in our laboratory to test the modulatory effects of desensitized nAChRs towards mAChRs in different cells and in the same cells.

In the parasympathetic nervous system, the modulatory effects of desensitized nAChRs towards mAChRs were investigated in different cells. In isolated ileum preparations of the guinea pig, ACh-induced contractions are characterized by two phases: an initial fast contraction for a short period, and a subsequent slow contraction for a long period that are related to parasympathetic ganglionic nAChRs and smooth muscle muscarinic receptors (m_3), respectively. After incubating the isolated ileum preparations with nicotine, acute tolerance to nicotine and ACh for initial fast contractions developed, and the hypersensitization to the muscarinic effects of ACh on smooth muscle contraction was induced. This facilitation of nicotine could be prevented by mecamylamine. These data indicate that the sensitivity of m_3 muscarinic receptors in ileum smooth muscle could be increased by parasympathetic ganglionic nicotinic receptor desensitization [193].

In mice, salivation induced by oxotremorine and arecoline, by activating m_3 muscarinic receptors of the salivary gland, was enhanced by acute repeated treatment with nicotine, which could be prevented by mecamylamine [194].

In rats, the volume of gastric acid secretion resulting from bethanechol-induced m_1 muscarinic receptor activation, and the number of m_1 muscarinic receptors in the stomach, could be increased by chronic nicotine treatment [199].

When urethane-anesthetized rats were pretreated with nicotine, acute tolerance to nicotine-induced bradycardia developed and the ability of carbachol to induce bradycardia was potentiated [190].

In another experiment, it was found that ACh-induced hypotension was potentiated by pretreatment with nicotine [195].

Together, the above results make it reasonable to suggest that, in the parasympathetic nervous system, the sensitivity to muscarinic receptors (m_1, m_2, m_3) of tissue innervated by the postganglionic cholinergic fibers was increased as a result of nAChR desensitization.

In the sympathetic nervous system, interactions between nAChRs and mAChRs have been investigated in the superior cervical ganglion using a whole-cell patch-clamp technique. An inward current was induced by nicotine via the activation of nAChRs in sympathetic neurons and nAChRs became desensitized rapidly after prolonged exposure to the nicotine. An outward current was induced by oxotremorine or pilocarpine via activation of mAChRs and it showed no desensitization on exposure to its agonists. After nAChRs were completely desensitized, the current evoked by oxotremorine or pilocarpine was increased significantly. The facilitated effect of desensitized nAChRs on mAChR activity could be prevented by mecamylamine. All these results suggest that the activity of mAChRs to their agonists could be potentiated by the desensitized nAChRs, also indicating that desensitization of nAChRs could evoke the enhancement of activity of mAChRs in the same neuron [199].

Further, investigation was also carried out to test the influence of the desensitized α_7 nAChRs on the activity of muscarinic receptors. α_7 nAChRs are the most abundant type of nicotinic receptor in the central and peripheral nervous systems. Following prolonged stimulation by choline, resulting in α_7 receptor desensitization, the inhibitory effects of pilocarpine on the amplitude of M-currents was increased, but could be prevented by á-bungarotoxin or MLA, which are highly selective α_7 receptor antagonists.

The molecular mechanism involved in modulating mAChR function by desensitized nAChRs has been investigated in the following battery of tests. Firstly, modulation of the kinetics of brain muscarinic receptors binding to their antagonists was tested in the membrane fraction from the rat cerebral cortex. The dissociation constant values for binding of a muscarinic receptor with its antagonist [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) were increased by preincubation with nicotine, but the association and dissociation kinetic processes were slowed. Unlike agonists, antagonists occupy receptors but do not influence receptor functions. Therefore, the specific binding of an antagonist with its receptor could be affected by the changes in receptor structure or conformation, not the functional states. The effects of the association and dissociation kinetics processes of muscarinic receptors with the antagonists suggest that desensitized nAChRs allosterically regulate cerebral muscarinic receptors.

Secondly, the modulatory effects on the affinity of brain muscarinic receptors for agonists and antagonists were also tested. The specific binding of the muscarinic agonist [^{3}H]oxotremorine-M with its receptor was saturable. Nicotine, at a concentration of 1.0 mol l^{-1} led to a decrease in the dissociation constant for binding of [^{3}H]oxotremorine-M without a change in maximum binding. Therefore, it was reasonable to conclude that desensitized nAChRs could increase the affinity of brain muscarinic receptors for their agonists.

The specific binding of [^{3}H]QNB with brain muscarinic receptors was also saturable. Nicotine could increase concentration-dependently the dissociation constant value for binding without affecting the maximal binding, which could be prevented by mecamylamine pretreatment. However, the effects of nicotine remained after the disulfate binding of muscarinic receptors was broken by dithiothreitol. These results reveal that nicotine could decrease the affinity of brain muscarinic receptors for their antagonists by desensitizing brain nicotinic receptors and that the decreased affinity was not influenced by the disulfate binding.

The muscarinic agonists arecoline and oxotremorine were able to displace [^{3}H]QNB to bind to muscarinic receptors, with Hill coefficients of 0.36 and 0.49, respectively. These data suggest that there is a high- and a low-affinity site.

Nicotine led to a decrease in the K_d of binding to the high-affinity muscarinic site without affecting the low-affinity binding sites. Together, these observations show that nAChR desensitization could increase the affinity of high-affinity binding sites for muscarinic agonists and promote the agonist displacement of antagonists [191,192,195].

Thirdly, modulation of the coupling relationship between muscarinic receptors and G-proteins in solubilized brain muscarinic receptor and G protein complexes was examined. The association kinetics of [^{35}S]GTP γS was significantly slowed by nicotine, and the specific binding of [^{3}H]oxotremorine-M to muscarinic receptors was decreased by GTP γS, suggesting that desensitized nAChRs could directly modulate the function of G proteins, and enhance the coupling relationship between G-proteins and muscarinic receptors.

Thus, the allosteric modulation of desensitized nAChRs on brain muscarinic receptor protein was indirect, which increased the affinity of muscarinic receptors for the agonists, but decreased affinity for the antagonist. The coupling relationship between muscarinic receptors and G-proteins could be enhanced by the presence of nicotine.

Finally, the modulatory effects on the brain muscarinic receptor–effector system were also investigated. The basic activity or stimulation by sodium fluoride 1.0 mmol l^{-1} on brain adenyl cyclase (AC) was not affected by nicotine (0.1–100 μmol l^{-1}) in the striatum, but the inhibitory effects of the muscarinic agonist carbachol were enhanced by nicotine pretreatment. These results imply that desensitized nAChRs promote m_2 or m_4 muscarinic receptors that are negatively coupled to AC, but have no direct effects on the activity of AC [197].

Inositol levels in rat cerebral cortex and hippocampus, but not striatum, were increased by acute or chronic repeated doses of nicotine; these effects were prevented by mecamylamine and reversed by LiCl. These findings suggest that desensitized nAChRs in the cerebral cortex or hippocampus can promote phosphatidylinositol turnover [197].

When the synaptosomal fraction from the rat cerebral cortex was incubated with $^{45}Ca^{2+}$, nicotine (0.01–100 μmol l^{-1}) caused a dose-dependent enhancement of synaptosomal $^{45}Ca^{2+}$ uptake, which could be prevented by mecamylamine. The effects of nicotine disappeared in the calcium-free extracellular solution. Thus, it is reasonable to suggest that desensitized nAChRs induced extracellular Ca^{2+} influx into the neurons; thereby cooperating with muscarinic receptors to increase intracellular Ca^{2+} [197].

6.3. Changing brain genes expression

Previous studies have demonstrated that nicotine administration modulated the expression of a variety of genes, including those involved in catecholamine and neuropeptide synthesis, and transcriptional activation. For example, a single injection of nicotine was reported to increase mRNA levels of tyrosine hydroxylase in NAs and VTA [35,177]. Chronic exposure to nicotine induced long-term increases in the mRNA expression of genes involved in the regulation of energy expenditure, such as neuropeptide Y (NPY), orexins and their receptors [91,113], and also induced immediate early gene expression, e.g., c-fos, jun-B, in various brain regions [87,142,160,185]. Nevertheless, comprehensive mRNA expression profiling following nicotine treatment is yet to be performed.

GeneChip arrays have been extensively used to identify gene expression profiles in various neurological disorders [86,139,157,181,182]. To begin a comprehensive survey of the gene-based molecular mechanisms that underlie tobacco addiction, we recently used the Affymetrix rat neurobiology GeneChip to analyze changes in gene expression in the whole brain of rats whose nicotinic receptors had been desensitized by chronic nicotine exposure. In nicotine-treated rats, the expression of 219 transcripts was altered. GeneChip data were confirmed for about 20% of transcripts using RT-PCR. The most striking differences in expression were found in genes involved in transmitter receptors, ion channels, as well as genes involved in signaling pathways. For example, the expression of the potassium large conductance calcium-activated channel and voltage-gated sodium channel type 1 was decreased, but expression of the voltage-gated sodium channel type 2, sodium channel β_2 and calcium channel subunit β_3 was up-regulated. Changes in the expression of these ion channels might alter nicotinic receptor function by affecting ion permeability [183].

Regarding the expression related to cell signaling, 13 transcripts were up-regulated, with seven transcripts down-regulated by chronic nicotine exposure. Transcripts related to MAPK and Ca^{2+}-dependent kinase pathways were most influenced by chronic nicotine. This included increased expression of protein kinase C, mitogen-activated protein kinase 1, protein tyrosine kinase 2 and p38 mitogen-activated protein kinase, and decreased expression of Ca^{2+}/calmodulin-dependent protein kinase 2, mitogen-activated protein kinase 3 and 6, citron, MAP kinase phosphatase and inositol 1,4,5-trisphosphate 3-kinase B [136].

In the neurotransmitter receptor system, there were nine transcripts that were up-regulated, including the NMDA receptor, dopamine D_1 receptor, GluR-B, etc.; 13 transcripts were down-regulated, such as the dopamine D_3 receptor, glutamate receptor subunit 4c and vasopressin V1b receptor. For genes related to DA transmission, dopamine D_1 receptor increased, but D_3 receptor, DA transporter and MAO(B) were decreased [136].

The expression of glutamate receptors was changed significantly by chronic nicotine administration. There are different effects of nicotine on individual subtypes. For example, GluR-K3, NMDA, mGlu3 and GluR-B were up-regulated, but GluR-δ_2, GluR-4c and GluR-D were down-regulated. In addition to DA and glutamate receptors, chronic nicotine induced expression of GABA receptors,

while expression of bradykinin receptor B1, 5-HT_3 receptor subunit and vasopressin V1b receptor were down-regulated [136].

6.4. Regulation of desensitized nAChRs on neurotransmitters and neuromodulators

6.4.1. Monoamines

The mesolimbic dopamine system is responsible for drug addiction. The effects of acute or repetitive nicotine treatment on DA release are concentration- and region-dependent [66,80,186,211], and generally involve increases in DA release [207]. However, in the medial prefrontal cortex, nicotine reduces DA release at low concentrations [159]. A 2-min exposure to micromolar levels of nicotine produces a rapid increase in ^{3}H-DA release in striatal synaptosomes [169]. With continued exposure, the response declines, apparently due to conversion of the nAChRs to a high-affinity desensitized conformation. In contrast, prolonged exposure to nanomolar concentrations of nicotine leads to a cumulative enhancement in ^{3}H-DA release. This effect is calcium-dependent and is blocked by the nicotinic antagonist, dihydro-β-erythroidine [166]. In some brain regions such as the midbrain, a nicotine challenge inhibits DA release, followed by a rebound increase. Acute nicotine treatment increases DA release in the accumbens shell and chronic repeated treatment induces sensitivity to DA overload [66].

The effect of nicotine on DA depends on concentration and animal strain. For example, a 7-day treatment with 0.03 or 0.10 mg/kg nicotine sc increased DA overload in the accumbens core of Sprague–Dawley rats and 0.30 mg/kg nicotine enhanced sensitivity to a nicotine challenge [158]. However, for Lister Hooded rats, nicotine reduced DA overload and did not affect sensitivity to nicotine challenge. When nicotine was given to mice in drinking water as their sole source of fluid, DA and its metabolites were increased. However, the subtypes of DA receptors contributing to nicotine effects remain unknown [123]. Animal and human studies suggest that chronic administration of addictive drugs might lead to impaired dopamine neurotransmission in the nucleus accumbens. There is a reduction in D_1 receptor density in the ventral striatum of human cigarette smokers relative to nonsmokers, as determined by positron emission tomography, which implies that the postsynaptic mesolimbic dopamine system might be chronically underactive in smokers. Such a hypodopaminergic state might play an important role in sustaining nicotine-seeking behavior [71].

The effects of chronic nicotine administration at two doses (3 and 12 mg/kg/day) on rat brain tissue monoamine and monoamine metabolite concentrations were studied after 21 days of treatment. Tissue concentrations of DA, NE, 5-HT and several metabolites in the striatum, hypothalamus and frontal cortex were determined by high performance liquid chromatography with electrochemical detection. Compared with a control group, nicotine treatment significantly decreased NE in the frontal cortex and increased NE in the hypothalamus at the higher dose of nicotine. The concentration of 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) was not significantly altered in any region. The 5-HT index (5-HIAA/5-HT) was significantly decreased in the hypothalamus and increased in the frontal cortex at the higher dose. Concentrations of DA and the metabolite homovanillic acid (HVA) were not significantly altered by nicotine. Nevertheless, significant decreases in the DA metabolite dihydroxyphenyl acetic acid (DOPAC) were observed in both the striatum and hypothalamus. Moreover, the DA index (DOPAC+HVA/DA) was significantly decreased in all three brain regions [104].

6.4.2. Amino acids

The effect of nicotine on amino acid transmitters is mostly focused on glutamate. For instance, subchronic nicotine (0.4 mg/kg/day) treatment for seven or 14 days resulted in a decrease followed by an increase in basal extracellular levels of striatal glutamate.

Ultrastructurally, 14-day treatment with nicotine resulted in an increase in the density of striatal glutamate immunolabeling within nerve terminals [131]. The effect of nicotine on glutamate release and uptake depends on the stage of neuronal development. Cerebellar granule and glial cells were cultured from 7-day-old rats after pre- and postnatal nicotine treatment. Ten days later, the basal release of glutamate in the granule cells prepared from the pre- and postnatal nicotine-exposed rats was higher and lower than the controls, respectively. The *N*-methyl-D-aspartate-induced release of glutamate was higher in the granule cells of postnatal nicotine treatment. However, the nicotine-induced glutamate release was either unchanged or lower in the granule cells of all nicotine-treated rats. The basal glutamate uptake was higher in the glial cells from prenatally examined cultures and lower after continuous nicotine exposure [115].

6.4.3. Opioids

Acute treatment with nicotine stimulated hypothalamic β-endorphin release. Chronic nicotine treatment desensitized β-endorphin-secreting neurons and reduced β-endorphin synthesis in the frontal cortex. Acute treatment with nicotine produced a significant increase in preproenkephalin A mRNA (PPE mRNA) in the striatum and hippocampus, but chronic treatment with nicotine caused a significant decrease in PPE mRNA in these brain regions. In both the striatum and hippocampus, there was a rebound increase in PPE mRNA 24 h after nicotine cessation [28].

In addiction, chronic treatment with nicotine (0.4 mg/kg, 14 days) induced an increase in NO metabolites in the frontal cortex, striatum, accumbens and a decrease in NOS mRNA in the hypothalamus of mice after food deprivation stress [90,199]. Moreover, NO^{2-} and NO^{3-} levels were increased by nicotine. Acute nicotine produced a significant decrease in BDNF mRNA, while chronic treatment increased BDNF mRNA [97].

The mechanisms involved in desensitized nAChR modulation of transmitter release are diverse, depending on the type of transmitter [70,203], and possibly include the following: (1) regulation of intracellular Ca^{2+}; it is a general rule that neurotransmitter release is quantal and depends on Ca^{2+}. nAChRs may modulate intracellular Ca^{2+} via the nicotinic receptor pore itself or via voltage-dependent Ca^{2+} channels on the membrane. (2) Modulation of transmitter turnover, for example, transmitter synthesis, degradation and transport. (3) Indirect modulation of one transmitter by acting on another neurotransmitter. All of these may be responsible for the diversity of effect of desensitized nAChRs on transmitters in different neurons or brain regions.

6.5. Neuroprotection

nAChRs have been shown to exert neuroprotective actions in neurodegenerative diseases, although the mechanism remains unknown. In rodents, nicotine can decrease the degeneration of neurons in the substantia nigra pars compacta [44], possibly due to a decreased incidence of PD. Pretreatment with nicotine (10^{-7}–10^{-4} mol l^{-1}) for 24 h has been shown to alleviate the toxic actions of MPP^+, indicating a neuroprotective effect of desensitized nAChRs. There are numerous model systems in which nicotine has protective effects on neurons, including excitotoxic insults of glutamate, β-amyloid or 6-OHDA exposure [45,77,114,173]. For example, exposure to nicotine for up to 24 h up-regulates α_7 nAChRs (in general, up-regulation of receptor number and binding sites are viewed as responses to desensitization) and increases PC12 cell viability after deprivation of NGF, thereby also implying that the desensitized nAChRs may be responsible for the nicotine's neuroprotective action [112]. This protective effect can be mimicked by an α_7 selective agonist and blocked by α-BTX, suggesting mediation by α_7 nAChRs subtypes [147]. The increased levels of cell surface ^{125}I-α-BTX binding sites was a further protective benefit of nicotine, also indicating that up-regulation of α_7 nAChRs may be important for neuroprotection [91]. The mechanism involved in the protection is possibly due to Ca^{2+} buffering. Based on the above results, it appears that desensitized α_7 nAChRs have a neuroprotective function through modulating signal transduction pathways [2,48,50,75,76,102]. Some studies show that $\alpha_4\beta_2$ subtypes also contribute to the protective effect of nicotine [101].

Nicotine stimulates the Src family, which activates phosphatidylinositol 3-kinase to phosphorylate Akt, and subsequently transmits the signal to up-regulate Bcl-2 and Bcl-x. Up-regulation of Bcl-2 and Bcl-x could prevent neuronal death induced by β-amyloid and glutamate [102]. These findings suggest that nAChRs have an essential protective effect in neurodegenerative disease. The results from our laboratory also showed that pretreatment with nicotine (100 μmol l^{-1}–1 mmol l^{-1}) for 24 h could prevent glutamate neurotoxicity in PC12 cells and that the protective effect was inhibited by the nAChR antagonist mecamylamine. Moreover, desensitized nAChRs induced by nicotine decreased intracellular baseline free Ca^{2+} significantly and enhanced the buffering action on Ca^{2+} overload induced by high concentrations of glutamate (5 mmol l^{-1}) in PC12 cells. Nicotine was also able to regulate mRNA and protein expression of apoptosis-related factors, including up-regulating Bcl-2 mRNA levels and Bcl-2 protein expression, as well as down-regulating Bax mRNA and protein expression [184].

The most obvious protection of desensitized nAChRs is to inhibit excessive excitation. Because of high permeability to Ca^{2+}, nAChR desensitization would decrease excitotoxicity in neurons. Mutation at the position leucine247 of the α_7 subunit significantly inhibits desensitization and inserting this mutation into mice can induce animal death [149,164]. On the contrary, replacing serine at position 248 of the α_4 subunit with phenylalanine can promote desensitization [179].

6.6. Modulating synaptic plasticity, learning and memory

Long-term potentiation (LTP) is a model of the synaptic plasticity of learning and memory. Acute or prolonged chronic nicotine treatment can decrease the threshold for LTP induction in the hippocampus CA1 [73], thereby facilitating LTP . Furthermore, nicotine can reverse the age-dependent decrease in LTP-induction [72]. The decreased threshold for LTP caused by acute nicotine treatment is mimicked by MLA and prevented by a non-α_7 nAChR blocker, suggesting that α_7 nAChR desensitization and non-α_7 nAChR activation are responsible for the effects [74]. Acute nicotine treatment more effectively decreases LTP threshold, implying that desensitized nAChRs are involved in LTP [72].

It is generally considered that both acute and chronic exposure to nicotine improves cognition in learning and memory tests, and nicotine treatment for a short period enhances working memory. Studies by Whiteaker et al. [204] showed that nicotine improved memory in rats using an active avoidance test. Nicotine also enhances memory in humans [123]. It has been affirmed that nAChRs have a critical role in the enhancement of cognition and memory by nicotine, especially the $\alpha_4\beta_2$ and α_7 subtypes in the ventral hippocampus and basolateral amygdala [50,109,110]. We recently observed the effects of repetitive nicotine treatment on memory in Wistar rats that had been treated with atropine. The results showed that the facilitatory effects of nicotine on memory disappeared when mAChRs were blocked by atropine. In contrast, memory deficits in rats treated with diazepam were ameliorated by nicotine. These studies suggest that the desensitized nAChRs induced by chronic nicotine are important for learning and memory via modulation of AChR function [117].

6.7. Regulation on synaptic signal transduction and excitatory rhythm of local neuronal circuit

Activation and desensitization of receptors is governed by the internal cellular environment, as well as agonist release and clearance. nAChR activation and desensitization can significantly modulate transmembrane signal transmission [183], and the receptors are involved in the transmission of excitation, a process ubiquitous in synaptic transmission, which is infrequent but potent.

Nicotine modulates excitation of local neuronal circuits via desensitization of nAChRs, because significant differences in desensitization may be present when comparing adjacent neurons from the same area of brain. Thus, nAChRs on a minority of neurons may remain active under conditions that can produce significant desensitization [53]. Repeated exposure to nicotine can desensitize presynaptic α_7 nAChRs rapidly and promote glutamate release [124]. Effects of nicotine on the inhibitory transmitter release are concentration-dependent. Acute nicotine administration can activate the $\alpha_4\beta_2$ receptor and desensitize the α_7 receptor to produce disinhibition. Both $\alpha_4\beta_2$ and α_7 receptors are desensitized by subsequent chronic treatment, which influences inhibitory synaptic transmission [7]. Nicotine, at a concentration of 0.5 μM, promotes synaptic responses mediated by GABA receptors, but inhibits the response at the higher concentration of 100 μM [38]. This modulatory effect of nicotine can be mimicked by nicotinic antagonists, indicating that it is due to nAChR desensitization [165]. In the CNS, there are abundant interneurons, except for pyramidal neurons and granule neurons. Most interneurons are GABAergic, mediating excitation of neuronal circuits. There is an abundance of nAChRs on interneurons in the cerebral cortex and hippocampus. Activated nAChRs can enhance GABA release to inhibit pyramidal cells; conversely, the desensitized nAChRs reduce GABA release from interneurons to disinhibit pyramidal cells, thereby regulating the excitatory rhythm of local neuronal circuits in the hippocampus and cerebral cortex [5,9]. In the CA1 layer of the hippocampus, most of the nicotinic receptor subtypes on the inhibitory interneurons are fast-desensitized α_7 subtypes, which play essential roles in modulating neuronal activities, and can modulate the local inhibitory neuronal circuit by activating endogenous GABAergic neurons [8]. α_7 desensitization not only can decrease the response to a single stimulation by an agonist, but also induce a response to repetitive agonist stimulation in a cumulative pattern [32].

7. Desensitized brain nAChRs contribute to tobacco addiction and neurodegenerative diseases

7.1. Tobacco addiction

Nicotine, as the major addictive component of tobacco, is a selective agonist of nAChRs [125]. Nicotine is well known to stimulate release of DA in the mesolimbic DA pathway, which is thought to play key roles in mediating the addictive effects of nicotine and other drugs of abuse [14,125,145]. It is proposed that the desensitization and up-regulation of nAChRs that follows chronic nicotine exposure is the basis of tolerance to nicotine displayed by smokers, and is also influential in producing withdrawal symptoms upon cessation of smoking [15,52,145,146,172].

The most prominent function of neuronal nAChRs is mediating the behavioral effects of nicotine, such as tolerance, addiction and withdrawal [19,125,145,146,202]. When smoking, nicotine obtained from tobacco arrives slowly at synapses in the brain via the blood–brain barrier, reaching concentrations of 50–600 nM, which is much lower than physiological concentrations of ACh (1 mM) evoked by a nervous pulse. Furthermore, nicotine can persist for rather a long time, because it is not degenerated by acetylcholinesterase. Thereby, the longer exposure to a low concentration of nicotine can promote nAChR desensitization after transient activation [49,108,125]. In fact, slow treatment with a low concentration of nicotine can cause some nAChR desensitization without activation via the allosteric effect of nicotine [8,125]. The desensitized nAChRs in the brain are particularly responsible for nicotine addiction [7,32,49,145,146], and the fact that chronic nicotine administration results in an increase in receptor number, coupled with a functional deactivation, suggests a mechanism for the addictive effects of nicotine [51,107].

7.2. Epilepsy

Autosomal dominant frontal lobe epilepsy (ADNFLE) results from a missense mutation in the α_4 subunit gene, replacing serine with phenylalanine at position 248 of the M_2 transmembrane domain of the subunit [23,24,128]. The mutant receptor displays reduced Ca^{2+} permeability, reduced channel opening and a faster desensitization rate [162,198]. Compared with naive receptors, the concentration of agonist inducing desensitization of these mutative receptors is 3000-fold lower [24]. In the brain of epilepsy patients, the fast desensitized α_7 nAChRs are increased enormously. The unusually high numbers of ^{125}I-α-BTX binding sites are more susceptible to seizures in response to nicotine administration in mice [31], implying that desensitized nAChRs are related to epilepsy. L250T mutation (a threonine for leucine substitution at position 250) in the α_7 nAChRs, which is known to decrease desensitization of the channel, increases the sensitivity to nicotine-induced seizures in mice [22], which also suggests an important role for desensitized α_7 nAChRs in the model of epilepsy.

7.3. Schizophrenia

The possible involvement of nAChRs in schizophrenia was suggested by the high percentage of smokers present in the schizophrenic population compared to the general

population [118]. Smoking is highly relevant to schizophrenia. Around 90% of people with schizophrenia smoke; this is approximately twice the prevalence as in people with other psychiatric disorders and three times the prevalence as in the general population. People with schizophrenia are more likely to be heavy smokers: 58.5% compared to only 0.9% of the general population. High levels of nAChR antibodies have also been observed in schizophrenic patients, which may be a contributing factor in the reduced number of nAChRs observed in schizophrenia [137]. From these observations, it was postulated that the high incidence of smoking in schizophrenics is an attempt on their part to self-medicate nicotine to overcome a deficit in nicotinic neurotransmission. The psychostimulant effects of nicotine might help the schizophrenic patients to compensate for their cognitive deficits, particularly attentional processes [69,83,184]. In this regard, nicotine has been observed to normalize psychophysiological deficits in schizophrenic patients [1,127]. The inheritance of this neuronal defect has been linked to a dinucleotide polymorphism at chromosome 15, which is also the locus for the α_7 nAChR [37,68]. The α_7 nAChR is further implicated in schizophrenia by the observation that the protein level of this subunit is significantly reduced in the frontal cortex of the schizophrenic brain compared to age-matched controls. These observations suggest that α_7 nAChRs may be extremely important in schizophrenia.

8. Conclusion

Desensitization is an intrinsic property of brain nAChRs, playing an important role in the signal transduction mediated by membrane receptors. The desensitized nAChRs can not only reduce or stop the N-like response to a repetitive agonist, but also exert important modulatory effects on brain function. These effects include homologous up-regulation of nAChRs, hypersensitization of muscarinic receptors, modulation of gene expression for ion channels, membrane receptors and signal transduction system, and also mediation of synaptic plasticity, learning and memory, and protective effects on neurons. These studies have provided a wealth of significant information for helping understand the biological basis of smoking, poisoning from anticholinesterase agents and other nAChR-related processes. Several important areas still await investigation, including (1) molecular characteristics of desensitized nAChRs; (2) the mechanism involved in the up-regulation in the number of desensitized nAChRs; (3) the effects of nAChR subtypes on desensitization and drug action; (4) the effects of desensitized nAChRs in the neuronal network; (5) the key role of desensitized nAChRs in drug addiction; and (6) the behavioral effects of nicotinic agonists and antagonists in humans, bearing in mind their possible therapeutic application in smoking addiction and brain diseases.

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