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Mini Review
A Review on the Receptor-ligand Molecular Interactions in the Nicotinic Receptor Signaling Systems

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Abstract

Nicotine is regarded as the main active addictive ingredient in tobacco products driving continued tobacco abuse behavior (smoking) to the addiction behavior, whereas nicotinic acetylcholine receptors (nAChR) is the crucial effective apparatus or molecular effector of nicotine and acetylcholine and other similar ligands. Many nAChR subunits have been revealed to bind to either neurotransmitters or exogenous ligands, such as nicotine and acetylcholine, being involved in the nicotinic receptor signal transduction. Therefore, the nicotinic receptor signalling molecules and the receptor-ligand molecular interactions between nAChRs and their ligands are universally regarded as crucial mediators of cellular functions and drug targets in medical treatment and clinical diagnosis. Given numerous endeavours have been made in defining the roles of nAChRs in response to nicotine and other addictive drugs, this review focuses on studies and reports in recent years on the receptor-ligand interactions between nAChR receptors and ligands, including lipid-nAChR and protein-nAChR molecular interactions, relevant signal transduction pathways and their molecular mechanisms in the nicotinic receptor signalling systems. All the references were carefully retrieved from the PubMed database by searching key words "nicotine", "acetylcholine", "nicotinic acetylcholine receptor(s)", "nAChR*", "protein and nAChR", "lipid and nAChR", "smok*" and "tobacco". All the relevant referred papers and reports retrieved were fully reviewed for manual inspection. This effort intend to get a quick insight and understanding of the nicotinic receptor signalling and their molecular interactions mechanisms. Understanding the cellular receptor-ligand interactions and molecular mechanisms between nAChRs and ligands will lead to a better translational and therapeutic operations and outcomes for the prevention and treatment of nicotine addiction and other chronic drug addictions in the brain's reward circuitry.

Key words: Nicotinic receptor signalling, nicotine, nicotinic acetylcholine, nicotinic acetylcholine receptor, receptor-ligand interaction

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INTRODUCTION

Smoking is the well-documented cause of preventable death in many countries and areas and nicotine is recognized as the main ingredient of used tobacco products and one of the most active addictive ingredients in abused to bacco and other addictive drugs driving continued tobacco abuse or smoking behavior to an addiction behavior despite individual knowledge of the possible harmful consequences[1-3].It is a chemical tertiary amine alkaloid and in particular in its charged status, it binds to diverse subunits and/or subtypes of nicotinic acetylcholine receptors (nAChRs) that are integral allosteric membrane proteins with average molecular mass of 290 kDA in the nicotinic cholinergic systems of the central nervous system[2, 3]. nAChRs form a Cys-loop family of ligand-gated ion channels (LGICs) mediating the effects of neurotransmitter acetylcholine (ACh) and these receptors are among the most well understood allosteric membrane proteins from a structural and functional perspective[4]. In terms of the molecular structures, the nAChRs belong to a superfamily of membrane-bound neurotransmitter receptors mediating the transformation of a chemical signal released from one neuron to an electrical signal at the next cell. Similar to the case of the cholinergic neurotransmitter system, nAChRs are also widely distributed and they participate in cholinergic signaling in nearly every neural area. There area lot of nicotinic acetylcholine receptors in human and animal nicotinic cholinergic system due to the structure diversity. Many nAChR subunits have been revealed to bind to either neurotransmitters or exogenous ligands, such as nicotine and acetylcholine, being responsible for the nicotinic receptor signal transduction. Therefore, the nicotinic receptor signalling molecules and the receptor-ligand molecular interactions between nAChRs and their ligands are universally regarded as crucial mediators of cellular functions and drug targets to in medical treatment and clinical diagnosis and pathophysiology research. Up today, too many efforts have been made in defining a role and risk or risk potential of nAChRs in response to nicotine and other addictive drugs in the development of various diseases and reports of our group have also summarized the genes and genomics and pathways associated with the genetic regulation and functional aspects of nicotine addiction or smoking and other drug addiction[2,5-12]. Meanwhile, there are currently few reviews on the receptor-ligand molecular interactions between nAChRs and ligands[13], although some studies and research reports on the lipid-nAChR and protein-nAChR molecular interactions and other nAChR's receptor-ligand interactions. By searching the PubMed database with key words "nicotine", "acetylcholine", "nicotinic acetylcholine receptor(s)", "nAChR*", "protein and nAChR", "lipid and nAChR", "smok*" and "tobacco", all the relevant review papers and research reports were carefully retrieved and manual inspection. All the retrieved review papers and research reports were fully reviewed. Furthermore, we also manually checked the references individually for additional studies not indexed by the PubMed database. Given our group has made many efforts in the research of functional genes and pathways of nAChRs associated with nicotine addiction or nicotine dependence[5-12], this review focuses on recent studies and reports on the receptor-ligand interactions between nAChR receptors and ligands, including lipid-nAChR and protein-nAChR molecular interactions, relevant signal transduction pathways and their molecular mechanisms in the nicotinic receptor signalling systems. This review intends to get a quick insight and understanding of the nicotinic receptor signaling and their molecular interactions mechanisms.

STRUCTURAL INFORMATION OF nAChRs

Up to date, 17 homologous nAChR subunits encoded by 17 unique genes have been identified, consisting of subunits alpha1-alpha10 (α1-α10), beta1-beta4 (β1-β4), gamma (γ), delta (δ) and epsilon (ε), whereas there are totally five muscular types of nAChR subunits and twelve nervous types of nAChR subunits identified in human and animals[4]. Meanwhile, these subunits are also the members of the Cys-loop family of LGICs besides those receptors for serotonin or 5-hydroxytryptamine (5-HT), dopamine, gamma-aminobutyric acid (GABA) and glycine[8-10,14]. nAChRs are initially found expressed in muscle, nerve and sensory cells where they play important roles in the neuronal chemical signal transmission and molecular modulation in the central nervous system (CNS) and the peripheral nervous system (PNS)[15]. Besides skeletal muscle, they are also expressed in epithelial and immune cells and in the sympathetic and parasympathetic ganglia, where they are responsible for the fast synaptic transmission. They are involved in the rapid transmitting effects of ACh widely expressed throughout the CNS areas. However, the nAChRs can respond to low ACh concentrations too and they are also the target of regionally and systemically released ACh and widely applied pharmacological agents like nicotine. In recent years, nAChRs have been widely studied and well regarded as implicated in a number of neuromuscular, neurological and psychiatric disorders, such as lung cancer[16]. Particularly, activation of the brain nAChRs can result in an enhanced release of various neurotransmitters, including dopamine, 5-HT, glutamate and GABA, whereas nAChRs are excitatory

receptors for the neurotransmitter ACh expressed in the brain in both pre- and post-synaptic locations[17]. Therefore, the neuronal nAChRs have been recently identified as important targets for therapeutic drug discovery in connection with those chronic disorders.

The overall structure of the neuronal nAChR is a homo-pentamer or hetero-pentamer composed from the 16 subunits identified in mammalian species[18]. They are hetero-pentameric or homo-pentameric integral membrane proteins with a fivefold axis of pseudosymmetry perpendicular to the membrane and each subunit can be subdivided into two principal domains, i.e. the extracellular domains (ECDs) and transmembrane domains (TMDs)[19]. Each subunit of nAChR possesses an amino-terminal extracellular domain (ligand binding domain) and four helices of trans membrane domains (TMD1-TMD4), an intracellular loop and an extracellular C-terminus. The pore of the channel is lined by TMD2 from five co-assembled subunits[20]. The extracellular domain (ECD) carries the acetylcholine (ACh) binding site at the boundary between subunits and the ion-pore transmembrane domain (TMD) delineates an axial cation-specific channel[21]. These topologically distinct domains are coupled allosterically to each other[21,22]. Therefore, nAChRs possess the structural elements necessary to convert the chemical signal of neurotransmitters, typically a local increased concentration of extracellular ACh, into an electrical signal generated by the opening of the ion channel. Furthermore, the acute effect of ACh consists of the fast opening (microsecond to millisecond) of a cationic channel of nAChRs that is permeable to Na^+, K^+ and sometimes Ca^{2+} ions. There is a considerable interest in modulating nAChRs to treat various nervous system related disorders, such as nicotine addiction, alcohol dependence, lung cancer, schizophrenia, depression, Alzheimer's disease and Parkinson's disease[2,5-12]. Structurally, each subunit of nAChRs contains a beta-sheet-rich N-terminal extracellular portion of approximately 200 residues, a 4-alpha-helical transmembrane segment of about 150 residues and a variable C-terminal intracellular segment that forms contacts with the cytoskeleton[19,23]. Residues in the intracellular domain (ICD) have been linked to gating kinetics and assembly or trafficking[23]. The receptor spans approximately 150 Å along its longest axis, with 60-70 Å being attributed to the extracellular domain (ECD) and about 40 Å attributed to the transmembrane domain (TMD)[24]. The ion conduction and channel gating function of the nAChR is localized to TMD, whereas the five subunits form a donut structure around a central pore that conducts ions in the open state and each subunit contains four alpha-helical segments labeled M1-M4[23,24].

The nicotine molecule is also membrane permeable in its uncharged form. In other words, nicotine can influence intracellular processes indirectly and directly through nAChRs by entering the cytoplasm, whereas the nicotine molecule diffuses readily into brain tissue in which it binds to nAChRs. At the same time, recent reports show that nicotine-induced reward, addiction and withdrawal involve a wide range of nAChR subtypes expressed in diverse neural systems, an some in vitro or in vivo studies have indicated that nicotine activates the mesolimbic-dopamine system and elevates the synaptic release of dopamine in the ventral striatum, which partially mediates the rewarding effects of nicotine and other similar drugs of abuse. Accordingly, their dysfunction has been implicated in many neurological disease states. In particular, the activity of any particular synapse is dependent on the identity and quantity of the neurotransmitters at that synapse and on the specificity of the post-synaptic receptors. After chronic use of nicotine drugs, removal of nicotine produces a withdrawal syndrome that can be relieved by nicotine replacement therapy, although the withdrawal syndrome is not mediated exactly by the same mechanisms or by the same neural circuits that initiate addiction or dependency. Therefore, nAChRs are regarded as potential molecular targets for the abuse of nicotine or tobacco, alcohol, cocaine and other drugs.

LIPID-nAChR INTERACTION

Lipid-nAChR interaction and lipid-dependent uncoupled conformation of nAChRs: Since the early research showed that a motional restricted shell of annular lipids surrounds the membrane-bound nAChRs, the experimental evidence has supported the notion that the interface between protein moiety and adjacent lipid molecules is the locus of a variety of pharmacologically relevant processes. The first interest in lipid-nAChR interactions arose from early attempts to isolate functional *Torpedo* nAChRs from their native membranes. Since the nAChR is an integral membrane protein, initial attempts led to receptors that bound agonist, but did not undergo agonist-induced conformational transitions[13,25]. Functional nAChR samples were finally obtained only after it was established that lipids are required throughout solubilization and purification in order to stabilize nAChR structure and function[26]. In lipid membranes lacking activating lipids, the nAChR adopts an uncoupled conformation binding ligand, but it does not transition into an open conformation[27]. However, any functional changes in nAChRs can have profound effects on synaptic behaviour which can be readily

seen even at the clinical level. Several mutations have been found in one subunit of the high affinity neuronal type nAChRs that increase the activity of the receptor and lead to a genetically transmissible form of epilepsy[28] and lowering the lipid to protein ration below 45:1 (mol:mol) during purification resulted in irreversible inactivation of the nAChR[28]. In addition, the ability of the nAChR to undergo conformational transitions is highly dependent on the specific lipid species in the reconstituted membrane environment. Anionic and neutral lipids optimally support receptor function, though the nAChR appears to have loose requirements for the specific type of anionic or neutral lipid[29]. Both anionic lipids and neutral lipids are abundant in native *Torpedo* membranes (~15 and ~35% molar ratio, respectively), suggesting that membrane compositions that support receptor function likely mimic the native membrane environment[13,30]. On the other hand, the ability of the nAChR to undergo conformational transitions is highly sensitive to lipids. Therefore, understanding the conformational selection of nAChRs and mechanism of lipid-dependent uncoupling is essential to understanding lipid-nAChR interactions, which may be implicated in pathological conditions such as nicotine additive. Excitingly, there are currently some reports on proposed uncoupling methods to elucidate the mechanism of lipid-dependent uncoupling.

Neurotransmission at chemical synapses is fundamental to the propagation of electrical signals within the nervous system and central to this process is the ability of Cys-loop receptors to convert a chemical input into an electrical output by conducting ions across the synaptic membrane in response to neurotransmitter binding[30,31]. At the molecular level, this not only requires the ability to bind agonist and conduct ions, but also the ability to effectively translate agonist binding into ion channel opening/gating. The agonist-binding sites, which are located on the extra-membranous surface of the receptor, are thus allosterically coupled to the distant transmembrane ion pore. Factors that affect the ability of Cys-loop receptors to bind agonist, conduct ions and/or couple agonist binding to ion channel gating have the potential to modulate the synaptic response and thus influence the transmission of electrical signals. One factor that is known to affect the activity of several Cysloop receptors is the lipid composition of the membrane in which they are embedded. The structural and functional properties of the AChRs are strongly dependent on the lipids in the vicinal microenvironment. The influence on receptor properties is mainly exerted by the AChR-vicinal ("shell" or "annular") lipids, which occur in the liquid-ordered phase as opposed to the more disordered and "fluid" bulk membrane lipids. Fluorescence studies have identified discrete sites for fatty acids, phospholipids and cholesterol on the AChR protein and electron-spin resonance spectroscopy has enabled the establishment of the stoichiometry and selectivity of the shell lipid for the AChR and the disclosure of lipid sites in the AChR transmembrane region. Experimental evidence supports the notion that the interface between the protein moiety and the adjacent lipid shell is the locus of a variety of pharmacologically relevant processes, including the action of steroids and other lipids. It was surmised that the outermost ring of M4 helices constitutes the boundary interface, most suitable to convey the signals from the lipid microenvironment to the rest of the transmembrane region and to the channel inner ring in particular[32]. Lipid sensitivity of the nAChRs from *Torpedo* has been known since initial studies showed that to preserve a fully functional nAChRand the receptor must be purified in the presence of exogenous lipid and then reconstituted into a membrane with a particular lipid composition[13,33]. Subsequent studies have focused on defining the roles that individual lipid species play roles in supporting nAChR function. The consensus is that both cholesterol and anionic lipids (e.g. phosphatidic acid or PA) are required in a reconstituted phosphatidylcholine (PC) membrane to provide an optimal environment. Cholesterol and PA both increase the proportion of nAChRs stabilized in an agonist-activatable conformation[34]. As the chemical labelling pattern of the nonactivatable PC-nAChR is similar to that of the desensitized nAChR, it has been suggested that lipids modulate the natural equilibrium between resting and desensitized receptors[35]. However, other studies indicated that PC-nAChR is not desensitized[36]. It was reported that lipid-dependent inactivation was not related to agonist-induced desensitization. Instead, the reporters observed that PC-nAChR adopted a novel conformation in which allosteric coupling between the agonist-binding sites and transmembrane pore was lost[37]. Furthermore, they revealed that uncoupling led to a substantial increase in solvent accessibility with minimal effects on nAChR secondary structure and thermal stability[38]. Those data showed that the lipid environment surrounding the nAChR transmembrane domain influences communication between the intact agonist binding and transmembrane pore domains and they also suggested that the lipid-exposed transmembrane M4 helix acts as a lipid-sensor modulating interactions at a coupling interface between the two domains[38]. The existence of this uncoupled conformation might explain how membrane-soluble allosteric modulators (including lipids) influence Cys-loop receptor function.

Lipids influence the ability of Cys-loop receptors to gate open in response to neurotransmitter binding, whereas the

functional activity of the Torpedo nAChRs to undergo agonist-induced channel gating is sensitive to lipids[39]. The observed stabilization of Cys-loop receptor structure induced by cholesterol depends upon the different conditions used to form reconstituted vesicles by detergent dialysis procedures[40]. The increasing levels of both cholesterol and anionic lipids in PC membranes stabilize the increasing proportions of agonist-responsive nAChRs[41] and M4 trans membrane helix plays a key role as a lipid-sensor in translating bilayer properties into altered nAChR function[42]. It was shown that the lipid inactivated nAChR was not desensitized but instead, it adopts a novel conformation where the allosteric coupling between its neurotransmitter-binding sites and transmembrane pore is lost, while the uncoupling is accompanied by an unmasking of previously buried residues, suggesting weakened association between structurally intact agonist-binding and trans membrane domains[43]. In the absence of activating lipids, the nAChR adopts an uncoupled conformation that binds agonist but does not usually undergo agonist-induced conformational transitions[44]. Defining the mechanisms underlying lipid-dependent uncoupling of agonist-binding and channel-gating is central to understanding nAChR-lipid interactions. Such studies may have broader implications because neuronal nAChRs that are functionally uncoupled have been observed in heterologous expression systems and may play a role in the response to nicotine[45]. Lipid-dependent mechanisms for "awakening" uncoupled nAChRs have been identified and could play a role modulating synaptic communication[46]. The lipid-dependent uncoupled conformation may also be germane to the interpretation of crystal structures of detergent-solubilized pLGICs[47]. In addition, among other contributions, the reconstitution into liposome of defined lipid composition has provided an excellent model system in which to characterize the lipid dependence of functional aspects of the receptor. In particular, the presence of cholesterol at high concentrations in the reconstituted vesicles is widely accepted as a requirement for optimal receptor cationgating activity upon agonist binding and also for protection of agonist-induced affinity state transitions[48].

Lipid-nAChR interactions are likely complex and have been proposed to occur through several different mechanisms. Most anionic and/or neutral lipids are able to support nAChR function to some extent, making it unlikely that lipids act on the nAChR through a highly specific mechanism - i.e. through specific lipid binding sites[43]. Studies using spin-labeled lipids show that the receptor discriminates in favour of both anionic and neutral lipids. Specifically, the nAChR interacts with lipids in its vicinity, slowing their rotational and translational movements[49]. A proportion of these lipids were found to be 'immobilized', leading to the suggestion that the receptor is encircled by a ring, or 'annulus,' of lipids that have the highest affinity for the lipid/receptor interface. Phosphatidic acid and cholesterol, which are particularly effective at stabilizing functional nAChR, have the highest degree of immobilization, suggesting that the nAChR selectively associates with lipids that promote its function[50]. Data from fluorescence quenching studies and molecular dynamics simulations propose that non-annular sites within the nAChR TMD may also exist for the specific binding of cholesterol, although there is no direct experimental evidence for this[51]. As phosphatidic acid and cholesterol both increase ordering of the lipid membrane, it was proposed that nAChR function may also be affected by membrane bulk properties[52]. Although membrane fluidity has been shown to have only minor effects on nAChR function[53], recent data showed that altering the thickness of phosphatidylcholine (PC) membranes shifts the conformational equilibrium of the nAChR between functional and non-functional states, providing evidence that membrane bulk properties also have an effect on nAChR function[54]. Previous studies show that the nAChR can influence its own lipid micro environment and the introduction of nAChR to lipid membranes increases membrane order, which may feed back into receptor function[55]. This effect is strikingly higher for membranes containing anionic lipids than for those lacking them. In addition, the Torpedo nAChR co-purifies with phospholipase C activity, suggesting that the receptor or a closely associated factor is able to modify the identity of lipid species in its immediate vicinity[56]. Recent experiments show that signaling by synthetic diacylglycerol derivatives downstream of phospholipase C activity increase nAChR surface expression[57].

As for the "uncoupled" protein conformation or uncoupling of nAChRs, the nAChR subunits reconstituted into PC membranes (PC-nAChR) in the absence of anionic lipids and cholesterol adopts a non-functional conformation. The conformational equilibria of the "uncoupled" nAChR and the influence of lipids on nAChR state transitions had been extensively studied[58]. It was discovered that *Torpedo* nAChR reconstituted into phosphatidylcholine membranes lacking cholesterol and anionic lipids adopts a conformation in which agonist binding is uncoupled from channel gating[59]. Further more insight into the "uncoupled conformation" of the nAChR and its functional implications can be found in another review[60]. Cryo-EM images and Fourier-transform infrared (FTIR) spectra both indicate that PC-nAChR adopts a native-like pentameric fold with similar thermostability to the nAChR

in functional membranes, consistent with previous observations that the lipid membrane has little effect on overall receptor structure[61]. The lipid environment therefore likely affects nAChR function by influencing receptor conformation dynamics. Characterization of PC-nAChR in terms of the classical resting-open-desensitized conformational equilibrium, however, initially yielded conflicting results. Photolabeling studies using the hydrophobic probe 3-trifluoromethyl-3-(m-[125I] iodophenyl) diazirine (i.e. [125I] TID), which preferentially labels lipid-exposed residues, revealed similar labeling patterns for PC-nAChR as for desensitized nAChR. These studies suggested that the non-functional PC-nAChR is not stabilized in a resting conformation and may be non-functional because it is stabilized in the desensitized conformation[62]. In fact, other biophysical tools have shown that the nAChR reconstituted into a mixture of PC and phosphatidylserine is stabilized primarily in the desensitized state[63]. The desensitized nAChR, however, is characterized by high affinity binding for both agonists, such as acetylcholine and channel blockers, such as ethidium bromide[64]. Subsequent studies revealed that PC-nAChR binds acetylcholine with low, resting-state-like affinity and it is unclear whether it binds ethidium at all[65]. It was thus proposed that PC-nAChR adopts a conformation distinct from the classical resting, open and desensitized conformations. As PC-nAChR is able to bind agonist, but does not flux cations, it was suggested that there is a functional uncoupling of ligand binding from gating-nAChR thus adopts a novel "uncoupled" conformation. Furthermore, the M4 lipid-sensor model of lipid-dependent uncoupling was proposed[66]. A key question that arises from the observation that lipids affect nAChR function is how the receptor senses and changes its conformation in response to its lipid environment. Researchers have proposed that the lipid sensitivity of the nAChR involves the M4 alpha-helix in the TMD, on account of its highly lipid-exposed position on the periphery of the receptor[67]. Mutations of the lipid-facing residues in M4 in Torpedo and mammalian muscle-type nAChRs affect its channel-opening response to agonist, despite being distant from both the ligand binding site and the channel pore[68]. Molecular dynamics simulations also suggest that the orientation of M4 is at least partially dependent on bulk membrane properties[59]. Lipids in the nAChR's vicinity (annular lipids) may therefore preferentially stabilize certain orientations of M4 to affect the conformational equilibrium and gating. The sensing and uncoupling of nAChR with lipid are implicated that changes in lipid metabolism are increasingly linked to human diseases, which could lead to altered nAChR function[69]. Actually, alterations in the functional activity of nAChRs have been implicated in nearly all the neuronal diseases and syndromes, including lung cancer, schizophrenia, Alzheimer's disease and Parkinson's disease[53]. There is thus a real and pressing need for a greater understanding of nAChR-lipid interactions and their role in nAChR biology.

In addition, recently increasing use of computational simulation tools, especially molecular docking in the field of nicotinic receptors, also led to the discovery and subsequent publication of several models of ligand-receptor interactions. These computational models and molecular docking are all based on the crystal structure at 2.7 A° resolution of a protein related to the extracellular N-terminus of nAChRs. In the absence of any X-ray or NMR information on specific nAChRs, this new structure has provided a reliable alternative to explore the structures and sub-structures of nAChRs and dock several nAChR agonists and antagonists[70].

FUNCTIONAL EFFECTS OF STEROIDS ON nAChRs IN LIPID-nAChR INTERACTION

Anionic lipids and sterol (such as cholesterol) are often critical membrane lipid components to ensure ion channel stability and functionality, whereas their importance to the channel functions is often explained via indirect and direct mechanisms. The indirect mechanism proposes that anionic lipids and cholesterol alter lipid packing and fluidity that ultimately affect the internal protein dynamics and functions[71]. In fact, the stabilization of Cys-loop receptor structure induced by cholesterol was observed[72]. However, these membrane lipids are potent modulators of the nicotinic acetylcholine receptor (nAChR) from Torpedo. Lipids influence nAChR function by conformational selection and kinetic mechanisms, stabilizing varying proportions of activatable versus non-activatable conformations, as well as influencing the transitions between these conformational states[73]. Some membranes stabilize an electrically silent uncoupled conformation that binds agonist but does not undergo agonist-induced conformational transitions, while the uncoupled nAChR, however, does transition to activatable conformations in relatively thick lipid bilayers, such as those found in lipid rafts[74]. Furthermore, anionic lipids and cholesterols are also crucial to the function of nicotinic acetylcholine receptors (nAChR). Cholesterol and other sterols or water soluble analogues affect both the ligand recognition ability and the ion channel functions of these proteins. Meanwhile they also modify the equilibrium between the agonist-dependent functional states of the nAChR[75]. Besides that of cholesterol, the effects of steroids and various water

soluble analogues have been characterized in detail on both muscle-type and neuronal type nAChRs[76]. In the central nervous system, steroids and neurosteroids proved to be modulating nAChRs. The neurosteroid 17beta-estradiol potentiates alpha4beta2 nAChRs, but not rat alpha4beta2 nAChRs[77]. Glucocorticoids are also modulators of nAChR channel function and an allosteric mechanism was proposed to account for the mechanism of action of these ligands[78]. Using an alanine-substituted quadruple mutant of four putative lipid-exposed residues in TMD4 (Leu411, Met415, Cys418 and Thr422).The effect of hydrocortisone into four Ala-substituted receptors was dissected and it was found that the Thr422 (a residue located close to ECD membrane in the alpha-subunit TM4) had direct bearing on the inhibition exerted by hydrocortisone on the receptor[74]. In the meantime, endogenous steroids can modulate the activity of transmitter-gated channels by directly interacting with the receptor. Jin and Steinbach[79] found a portable site for 17beta-estradiol binding on any subunit of a nicotinic alpha4beta2 receptor using concatemers of subunits and chimeric subunits[79].

In particular, nAChRs require proper lipid compositions in order to function properly. Normally, agonist binding to the extracellular (EC) domains could trigger opening of the ion channels and stimulate the cation permeation. However, without the presence of anionic phosphatidic acid (PA) and/or cholesterol in the membrane, reconstituted Torpedo californica nAChR would not be able to undergo an agonist-induced conformation change to retain the channel gating activity[80]. It has been demonstrated that membranes containing phosphatidylcholine (PC)/PA/cholesterol in 3:1:1 are particularly effective at maintaining the stability of functional reconstituted nAChR[81]. Cheng *et al.*[82] investigated their interactions with an open- and closed-channel alpha4beta2 nAChR by over 10-ns molecular dynamics simulations in a ternary lipid mixture of 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC), 1-palmitoyl-2-oleoyl phosphatidic acid (POPA) and cholesterol with a ratio of 3:1:1[82]. They found that, on average, there were 65 and 74 interfacial lipids around the closed- and open-channel alpha4beta2 nAChR, respectively, in the equilibrated simulation systems, while 42% of the interfacial POPA in the open-channel system had acyl chains partially inserted into intra- or inter-subunit cavities, as compared to only 7% in the closed-channel alpha4beta2[83]. No cholesterol was found in cavities within single subunits, though some cholesterol infiltrated into the gaps between subunits. Because of its smaller head group, POPA could access some non-annular sites where POPC could not easily reach due to steric exclusion. Furthermore, POPA not only acted as an acceptor for hydrogen bonding (H-bonding) as POPC did, but also as a donor through its hydroxyl group for H-bonding with the backbone of the protein. The charged head group of POPA allowed the lipid to form stable salt bridges with conserved Arg and Lys residues at the interfaces of the transmembrane (TM) and extracellular (EC) or intracellular (IC) domains of the alpha4beta2. A higher number of salt bridges and hydrogen bonds (H-bonds) between POPA and the alpha4beta2 nAChR were found in the open system than in the closed system, suggesting a potential role of POPA in the equilibrium between different channel states. Most interfacial POPA molecules showed lower order parameters than the bulk POPA due to the mixed effect of gauche defects, hydrophobic mismatch and the lipid orientations near the magic angle. These unique properties enable the interfacial POPA to achieve what POPC cannot with regard to specific interactions with the protein, thereby making POPA essential for the function of nAChR.

PROTEIN-nAChR INTERACTION

Interaction between G protein and nAChR: Historically neurotransmitter receptor classes have been divided into two categories: Ionotropic and metabotropic. A number of ionotropic receptors have been shown to interact with G proteins, contributing to the signal transduction or signalling pathways, such as the AMPA(alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor which has been shown to interact with Galphai subunits for the purpose of regulating silent synapses, the glycine receptor which has been shown to interact with beta-gamma G protein subunits, thereby regulating channel opening and kinetics and nAChRs alpha4beta2 and alpha3beta4alpha5, which have been shown to interact with both the Galphao and beta gamma subunits. Structurally, nAChRs are a pentameric class of ionotropic receptors found widely expressed throughout the brain. Each subunit is composed of four transmembrane domains with a long intracellular loop formed between the 3rd and 4th transmembrane domain. This domain is where the majority of protein interactions with the receptor is hypothesized to occur and is the most variable, least conserved part of each nAChR subunit, conferring special properties to each receptor. Alpha7 is unique among the nAChRs in that it possesses five identical alpha subunits and can conduct calcium at levels roughly ten times that of the other common nAChRs found throughout the brain. Alpha7has also been implicated as a regulator of neural

development. A number of studies have demonstrated that alpha7 expression is critical for establishing the inhibitory GABA signal necessary for proper circuit formation. It has also been shown that alpha7 expression is paramount for glutamatergic synaptogenesis within the hippocampus. Less studied however is the role of alpha7 in regulating axon growth, perhaps the key starting point for neural development. It was demonstrated that alpha7 interacts with G proteins to regulate calcium influx and the cytoskeletal machinery underlying growth[84]. The corresponding subsequent work reveals that alpha7, acting in concert with the G proteins Galphao and Galphaq, regulate axon growth via intracellular calcium release from internal stores[85]. Previous studies demonstrated a first ever alpha7 mediated calcium signal within the growth cone, an interaction with G proteins and a mechanism by which alpha7 can regulate the cytoskeletal machinery in the developing neurite[86]. These studies aimed at understanding the critical role of ionotropic neurotransmitter receptor interactions with G proteins and particularly the metabotropic functions of alpha7 receptor in proper brain development.

On the other hand, neuronal development is marked by important phases in growth involving structural remodeling of the soma and an outgrowth of neurites culminating in the formation of functional axons and dendrites[87] and various neurotransmitter receptors and the receptor mediated signal transduction are known to contribute to neuronal development[88]. Among the signal transduction pathways mediated by these receptors, signalling via the second messenger family of heterotrimeric GTP binding protein (G protein) is one crucial mechanism for structural remodeling during neuronal development[89]. Inhibitory G proteins such as Galphai/o are enriched in developmental structures such as axonal growth cones during neurite navigation[90]. In addition to being activated by membrane spanning G protein coupled receptors (GPCRs), Galphai/o can be activated by intracellular calcium[91]. Meanwhile, calcium signalling is also important during cellular development where it has been shown to play a role in the growth and navigation of newly formed neurites[87]. Calcium-conducting ligand-gated ion channels, such as the nAChRs, are abundant during nervous system development and have been found to modulate the growth of neurons in the hippocampus and cortex[92]. In differentiated neurons, nAChRs contribute to structural remodeling within presynaptic terminals and dendritic spines[93]. The alpha-bungarotoxin (Bgtx) sensitive alpha7 receptor is homopentameric and conducts mainly calcium upon activation[94]. Previous studies indicate a role for alpha7 receptors in the growth of neurons and the formation of synapses[95]. Deactivation of alpha7 by ligands such as Bgtx as well as endogenous transmitters such as kynurenic acid (KYNA) has been found to impact neuronal growth[96]. GPCRs control a wide range of physiological processes and are the target for many clinically used drugs, whereas the activation of single GPCR underlies multiple important aspects of lung physiology and pathophysiology. Best known is the control of bronchoconstriction and bronchodilation through modulation of airway smooth muscle (ASM) contraction, which forms the cornerstone of current standard of care for chronic lung diseases like asthma and COPD[97]. GPCRs are also important in lung immunology such as cellular responses to inflammatory cytokines, chemokines, prostaglandins and leukotrienes[98].

It is crucial for developing new drugs to explore and understand the specific pathways in which G proteins and their coupled receptors bind agonists and antagonists, their organisation in the membrane, while their regulation after agonist binding are important properties too. One way to achieve this knowledge is through the study of interactions between G protein and nAChRs. In most cell types, co-expression of ionotropic nAChRs as well as metabotropic muscarinic receptors ensures a fast and slow acetylcholine signaling response, respectively, whereas the nAChRs mediate communication between neurons by conversion of chemical neurotransmitter signals into a transmembrane flux of ions[99]. Presently, at least nine different nAChR subunits are expressed in the mammalian brain. In the hippocampus and cortex homomeric alpha7 and heteromeric alpha4beta2 nAChRs have been shown to contribute to neurotransmitter release and dendritic plasticity[100]. Upon ligand activation, alpha7 nAChRs conduct cations into the cell[101]. However, because the receptor channel desensitizes within milliseconds[102], it is possible that ligand binding can also set into motion longer lived downstream signaling events.

Furthermore, neuronal nAChRs are mainly expressed during brain development and contribute to neurogenesis, neurite outgrowth and synaptic maturation[103]. Although nAChRs are ligand-gated ion channels enabling a rapid depolarizing current across the plasma membrane[104], recent evidence indicates that these receptors activate metabotropic responses by directly coupling to intracellular signaling proteins[105]. This process appears to be driven by the ability of the alpha7 nAChR to directly bind to large heterotrimeric GTP binding proteins (G proteins) [106]. The alpha7 acetylcholine nicotinic receptor (alpha7) is an important mediator of cholinergic transmission during brain development. Signaling via the second messenger family of heterotrimeric GTP binding proteins (G proteins) is one mechanism for structural remodeling during neuronal development[107]. Functional

interactions between small monomeric G proteins and various nAChRs have also been observed[108]. Experiments in keratinocytes indicate that alpha7 nAChR signaling via Rho GTPases enables mechanical plasticity during wound healing[109]. The activation of alpha9 nAChRs in epithelial cells is shown to activate Rac and Rho GTPases leading to the regulation of focal adhesions and cellular morphology[110]. Conversely, Rho/Rac GTPase activity can regulate the targeting and expression of nAChRs at the cell surface[111]. In fact, small monomeric Rho family GTPases are key regulators of the cytoskeleton directing cell migration, axon guidance and protein trafficking, while Rho GTPase activity can be modulated by many large G proteins[112]. Receptor mediated signal transduction appears crucial in regulating the assembly, disassembly and reorganization of the cytoskeleton during growth and previous study has demonstrated that G protein coupling is necessary for alpha7 nAChR-mediated activation of RhoA and the regulation of cytoskeletal growth at the growth cone[113].

In fact, nAChRs couple to a myriad of signaling, scaffolding and trafficking proteins in neural and immune cells[114]. All the nAChRs maintain an M3-M4 loop, which varies in length and sequence identity between different subunits[115]. The M3-M4 loop contributes to the trafficking and clustering of the nAChR via an association with the cellular cytoskeleton[116]. nAChRs are also found to associate with several types of G proteins, which can contribute to cross-talk between nAChRs and G protein-coupled receptors[117]. Studies on the mechanisms of G protein binding to the glycine receptor 1 (GlyR1) reveal the existence of a G protein-binding cluster (GPBC) within the M3-M4 loop of Cys-loop receptors[118]. A mutation at the GPBC is sufficient to abolish G protein binding in HEK 293 cells[119] and PC12/N2a cells[120]. Actually, a protein sequence alignment of the M3-M4 loop reveals a conservation of the GPBC in the alpha7 nAChR. A mutation of these residues in alpha7 nAChRs attenuates interaction with G proteins and reduces the capacity of the receptor to activate phospholipase C (PLC) and inositol triphosphate (IP_3) calcium release in response to choline. These findings show a role for G protein coupling in alpha7 nAChR signalling[121]. In physiology, alpha7 nicotinic acetylcholine receptors (nAChRs) play an important role in synaptic transmission and inflammation. In response to ligands, this receptor channel opens to conduct cations into the cell but desensitizes rapidly. Recent studies showed that alpha7 nAChRs bind signaling proteins such as heterotrimeric G proteins. It was demonstrated that direct coupling of alpha7 nAChRs to G proteins enables a downstream calcium signaling response that can persist beyond the expected time course of channel activation. This process depends on a GPBC in the M3-M4 loop of the receptor[122]. Particularly, a mutation of the GPBC in the alpha7 nAChR (alpha$7_{345-348A}$) abolishes interaction with $G\alpha_q$ as well as $G\beta\gamma$ having no effect on receptor synthesis, cell-surface trafficking, or alpha-bungarotoxin binding, whereas expression of alpha$7_{345-348A}$ did significantly attenuate the alpha7 nAChR-induced $Galpha_q$ calcium signaling response as evidenced by a decrease in PLC-beta activation and IP_3R-mediated calcium store release in the presence of the alpha7 selective agonist choline[123]. Taken together, these reports provided new evidence for the existence of a GPBC in nAChRs serving to promote intracellular signaling.

INTERACTIONS BETWEEN OTHER PROTEINS AND nAChRs

Besides the interactions between nAChR and G protein and GPCR protein, there are also a few reports of interactions between nAChRs and other proteins. For instance, the interactions between beta-amyloid protein and alpha7 nAChR in the rat neuronal septum-diagonal band complex rat[124]. The beta-amyloid protein is much concerned because of its relevance or correlation to Alzheimer's disease (AD). AD is a neurodegenerative disorder or neurological dementia in elderly persons that averagely affects 20-30 million individuals in the worldwide statistics[125]. The brains of AD patients manifest two characteristic lesions, i.e. extracellular amyloid and intracellular neurofibrillary tangles of hyperphosphorylated tau protein[126]. The amyloid hypothesis states that the formation of beta-amyloid proteins (Aβ) by neurons is identified as the primary trigger of the pathogenesis of AD[127]. The effects of intracerebral injection of the beta-amyloid protein (Aβ1-40) on the alpha7 subtype of nAChR in neurons of the septum-diagonal band (MS-nDBB) complex were studied in rats[128].

Another interesting case is the interaction between alpha3beta4nAChR and PrP(C) which is the cellular isoform of prion protein widely expressed in most tissues involvement[129]. PrP (C) has been shown to interact with many synaptic proteins, including nAChRs and intracellular proteins involved in nicotinic receptor signaling pathways and/or systems and it was suggested that PrP(C) was a member of a multi-protein membrane complex participating in the formation and function ofalpha3beta4 nAChR[130].

In additional, there was a co-existence of muscarinic and nicotinic receptors and their functional interaction in mouse Beta-TC6 cells possessing nicotinic receptor also expressed muscarinic receptors[131]. It was reported that carbamylcholine (a mixed agonist for muscarinic and nicotinic receptor) induced a smaller extent of insulin secretion than

oxotremorine M (a selective muscarinic agonist), which suggested that concomitant stimulation of muscarinic and nicotinic receptors by carbamylcholine resulted in the negative type of the receptor interaction[132-134]. Furthermore, modulating the activity of some complex integral membrane proteins with small molecule drugs has been successful and it demonstrated that the signalling properties and ion conductance can be modified[133,135,136]. These classes of membrane proteins are linked to the pathophysiology of a number of respiratory diseases, whereas targeting these proteins with monoclonal antibodies represents a significant opportunity to add new therapeutics to treat respiratory diseases[137,138]. Additionally, there are a number of studies reported the multiple roles and possible effects of α7 nicotinic receptor activation on developing hippocampal neurons or in modulating glutamatergic systems in the nervous system[139-147]. Moreover, nicotinic receptor related antibodies promise to be of benefit including specificity, different mechanisms of physiological actions, such as inhibition, agonist, internalisation, cell depletion, improved safety profile and a longer duration of action. The generation of functional antibodies against these target classes remains technically challenging as a result of difficulties in the over-expression of membrane proteins. And there are also issues with protein solubilisation, purification and stability and the limited availability of epitopes to target for some proteins due to the limited exposure of extracellular loops at the cell membrane. However, the opportunities to target these membrane proteins with biologics has led to very significant activities in the biotechnology sector and successes are emerging in isolating monoclonal antibodies against these targets.

CONCLUSION

All in all, understanding the cellular receptor-ligand interactions and molecular mechanisms above between nAChRs and ligands will lead to a better translational and therapeutic operations and outcomes for the prevention and treatment of nicotine addiction and other chronic drug addictions. That is, the molecular signalling and relevant signal transduction pathways of nAChRs canregulate the behavioural and physiological response to nicotine and other addictive drugs. In contrast, exposure to addictive drugs may alter the secreted level of nicotine and functional activity of nAChRs in the brain's reward circuitry.

SIGNIFICANCE STATEMENT

By searching the PubMed database with key words "nicotine", "acetylcholine", "nicotinic acetylcholine receptor(s)", "nAChR*", "protein and nAChR", "lipid and nAChR", "smok*" and "tobacco", all the relevant review papers and research reports were carefully retrieved and manual inspection. All the retrieved review papers and research reports were fully reviewed. The study sought to fill up the gap to summarize the nAChR's receptor-ligand interactions and to reveal the possible mechanisms of those molecular interactions in the nicotinic receptor signalling systems. This review aimed to get an insight into some specific functional aspects of nAChRs in recent research for the future important drug design and medicinal development in medical treatment and clinical diagnosis and pathophysiology research, this review focuses on recent studies and reports on the receptor-ligand interactions between nAChR receptors and ligands, including lipid-nAChR and protein-nAChR molecular interactions, relevant signal transduction pathways and their molecular mechanisms in the nicotinic receptor signalling systems. This review intends to get a quick insight and understanding of the nicotinic receptor signaling and their molecular interactions mechanisms.

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REFERENCES

1. Garrett, B.E., C.A. Rose and J.E. Henningfield, 2001. Tobacco addiction and pharmacological interventions. Expert Opin. Pharmacother., 2: 1545-1555.
2. Li, M.D. and M. Burmeister, 2009. New insights into the genetics of addiction. Nat. Rev. Genet., 10: 225-231.
3. Ma, Y., L. Wen, W. Cui, W. Yuan and Z. Yang *et al*., 2017. Prevalence of cigarette smoking and nicotine dependence in men and women residing in two provinces in China. Front. Psychiatry, Vol. 8. 10.3389/fpsyt.2017.00254.
4. Benowitz, N.L., 2009. Pharmacology of nicotine: Addiction, smoking-induced disease and therapeutics. Annu. Rev. Pharmacol. Toxicol., 49: 57-71.
5. Wang, J. and M.D. Li, 2010. Common and unique biological pathways associated with smoking initiation/progression, nicotine dependence and smoking cessation. Neuropsychopharmacology, 35: 702-719.

6. Wang, J., W.Y. Cui, J. Wei, D. Sun, R. Gutala, J. Gu and M.D. Li, 2011. Genome-wide expression analysis reveals diverse effects of acute nicotine exposure on neuronal function-related genes and pathways. Front. Psychiatry, Vol. 2. 10.3389/fpsyt.2011.00005.
7. Wang, J., W. Yuan and M.D. Li, 2011. Genes and pathways co-associated with the exposure to multiple drugs of abuse, including alcohol, amphetamine/methamphetamine, cocaine, marijuana, morphine and/or nicotine: A review of proteomics analyses. Mol. Neurobiol., 44: 269-286.
8. Cui, W.Y., C. Seneviratne, J. Gu and M.D. Li, 2012. Genetics of GABAergic signaling in nicotine and alcohol dependence. Hum. Genet., 131: 843-855.
9. Ma, Y., M. Wang, W. Yuan, K. Su and M.D. Li, 2015. The significant association of Taq1A genotypes in DRD2/ANKK1 with smoking cessation in a large-scale meta-analysis of Caucasian populations. Transl. Psychiatry, Vol. 5, No. 12. 10.1038/tp.2015.176.
10. Wen, L., H. Han, Q. Liu, K. Su and Z. Yang *et al*., 2017. Significant association of the CHRNB3-CHRNA6 gene cluster with nicotine dependence in the Chinese han population. Scient. Rep., Vol. 7, No. 1. 10.1038/s41598-017-09492-8
11. Jiang, K., Z. Yang, W. Cui, K. Su, J.Z. Ma, T.J. Payne and M.D. Li, 2017. An exome-wide association study identifies new susceptibility loci for age of smoking initiation in African-and European-American populations. Nicotine Tob. Res., Vol. 1. 10.1093/ntr/ntx262.
12. Liu, Q., H. Han, M. Wang, Y. Yao and L. Wen *et al*., 2018. Association and cis-mQTL analysis of variants in CHRNA3-A5, CHRNA7, CHRNB2 and CHRNB4 in relation to nicotine dependence in a Chinese han population. Transl. Psychiatry, Vol. 8, No. 1. 10.1038/s41398-018-0130-x.
13. Arias, H.R., 2012. Molecular interactions between ligands and nicotinic acetylcholine receptors revealed by studies with acetylcholine binding proteins. J. Thermodyn. Cat., Vol. 3.
14. Balfour, D.J.K., 2015. The Role of Mesoaccumbens Dopamine in Nicotine Dependence. In: The Neuropharmacology of Nicotine Dependence. Current Topics in Behavioral Neurosciences, Vol. 24, Balfour, D.J.K. and M. Munafo (Eds.)., Springer, Cham, pp: 55-98.
15. Rose, J.E., 2007. Multiple brain pathways and receptors underlying tobacco addiction. Biochem. Pharmacol., 74: 1263-1270.
16. Dineley, K.T., A.A. Pandya and J.L. Yakel, 2015. Nicotinic ACh receptors as therapeutic targets in CNS disorders. Trends Pharmacol. Sci., 36: 96-108.
17. Pandya, A.A. and J.L. Yakel, 2013. Effects of neuronal nicotinic acetylcholine receptor allosteric modulators in animal behavior studies. Biochem. Pharmacol., 86: 1054-1062.
18. Changeux, J.P. and S.J. Edelstein, 2001. Allosteric mechanisms in normal and pathological nicotinic acetylcholine receptors. Curr. Opin. Neurobiol., 11: 369-377.
19. Changeux, J.P. and S.J. Edelstein, 2005. Allosteric mechanisms of signal transduction. Science, 308: 1424-1428.
20. Corringer, P.J., N.L. Novere and J.P. Changeux, 2000. Nicotinic receptors at the amino acid level. Annu. Rev. Pharmacol. Toxicol., 40: 431-458.
21. Karlin, A., 2002. Ion channel structure: Emerging structure of the nicotinic acetylcholine receptors. Nat. Rev. Neurosci., 3: 102-114.
22. Chen, L., 2010. In pursuit of the high-resolution structure of nicotinic acetylcholine receptors. J. Physiol., 588: 557-564.
23. Corringer, P.J., F. Poitevin, M.S. Prevost, L. Sauguet, M. Delarue and J.P. Changeux, 2012. Structure and pharmacology of pentameric receptor channels: From bacteria to brain. Structure, 20: 941-956.
24. Galzi, J.L., F. Revah, A. Bessis and J.P. Changeux, 1991. Functional architecture of the nicotinic acetylcholine receptor: From electric organ to brain. Annu. Rev. Pharmacol. Toxicol., 31: 37-72.
25. McGehee, D.S., 1999. Molecular diversity of neuronal nicotinic acetylcholine receptors. Ann. N. Y. Acad. Sci., 868: 565-577.
26. Stokes, C., M. Treinin and R.L. Papke, 2015. Looking below the surface of nicotinic acetylcholine receptors. Trends Pharmacol. Sci., 36: 514-523.
27. Corringer, P.J., S. Bertrand, S. Bohler, S.J. Edelstein, J.P. Changeux and D. Bertrand, 1998. Critical elements determining diversity in agonist binding and desensitization of neuronal nicotinic acetylcholine receptors. J. Neurosci., 18: 648-657.
28. Leslie, F.M., C.Y. Mojica and D.D. Reynaga, 2013. Nicotinic receptors in addiction pathways. Mol. Pharmacol., 83: 753-758.
29. Dani, J.A. and D. Bertrand, 2007. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu. Rev. Pharmacol. Toxicol., 47: 699-729.
30. Matsunaga, K., T.W. Klein, H. Friedman and Y. Yamamoto, 2001. Involvement of nicotinic acetylcholine receptors in suppression of antimicrobial activity and cytokine responses of alveolar macrophages to *Legionella pneumophila* infection by nicotine. J. Immunol., 167: 6518-6524.
31. Heeschen, C., J.J. Jang, M. Weis, A. Pathak and S. Kaji *et al*., 2001. Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. Nat. Med., 7: 833-839.
32. Wang, H., M. Yu, C.A. Amella, M. Ochani and M. Tanovic *et al*., 2003. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. Nature, 421: 384-388.
33. Wallace, T.L. and D. Bertrand, 2013. Alpha7 neuronal nicotinic receptors as a drug target in schizophrenia. Expert Opin. Ther. Targets, 17: 139-155.
34. Lombardo, S. and U. Maskos, 2015. Role of the nicotinic acetylcholine receptor in Alzheimer's disease pathology and treatment. Neuropharmacology, 96: 255-262.
35. Improgo, M.R., A.R. Tapper and P.D. Gardner, 2011. Nicotinic acetylcholine receptor-mediated mechanisms in lung cancer. Biochem. Pharmacol., 82: 1015-1021.

36. Gotti, C., F. Clementi, A. Fornari, A. Gaimarri and S. Guiducci *et al*., 2009. Structural and functional diversity of native brain neuronal nicotinic receptors. Biochem. Pharmacol., 78: 703-711.
37. Wu, J. and R.J. Lukas, 2011. Naturally-expressed nicotinic acetylcholine receptor subtypes. Biochem. Pharmacol., 82: 800-807.
38. Sgard, F., E. Charpantier, S. Bertrand, N. Walker and D. Caput *et al*., 2002. A novel human nicotinic receptor subunit, α10, that confers functionality to the α9-subunit. Mol. Pharmacol., 61: 150-159.
39. Wilson, G.G. and A. Karlin, 2001. Acetylcholine receptor channel structure in the resting, open and desensitized states probed with the substituted-cysteine-accessibility method. Proc. Natl. Acad. Sci. USA., 98: 1241-1248.
40. Unwin, N., 2005. Refined structure of the nicotinic acetylcholine receptor at 4 A resolution. J. Mol. Biol., 346: 967-989.
41. Mordvintsev, D.Y., Y.L. Polyak, O.V. Levtsova, Y.V. Tourleigh and I.E. Kasheverov *et al*., 2005. A model for short α-neurotoxin bound to nicotinic acetylcholine receptor from *Torpedo californica*: Comparison with long-chain α-neurotoxins and α-conotoxins. Comput. Biol. Chem., 29: 398-411.
42. Brannigan, G., J. Henin, R. Law, R. Eckenhoff and M.L. Klein, 2008. Embedded cholesterol in the nicotinic acetylcholine receptor. Proc. Natl. Acad. Sci. USA., 105: 14418-14423.
43. Taly, A., P.J. Corringer, D. Guedin, P. Lestage and J.P. Changeux, 2009. Nicotinic receptors: Allosteric transitions and therapeutic targets in the nervous system. Nat. Rev. Drug Discov., 8: 733-750.
44. Arneric, S.P., M. Holladay and M. Williams, 2007. Neuronal nicotinic receptors: A perspective on two decades of drug discovery research. Biochem. Pharmacol., 74: 1092-1101.
45. Levin, E.D. and A.H. Rezvani, 2007. Nicotinic interactions with antipsychotic drugs, models of schizophrenia and impacts on cognitive function. Biochem. Pharmacol., 74: 1182-1191.
46. Romanelli, M.N., P. Gratteri, L. Guandalini, E. Martini, C. Bonaccini and F. Gualtieri, 2007. Central nicotinic receptors: Structure, function, ligands and therapeutic potential. ChemMedChem., 2: 746-767.
47. Gotti, C., M. Moretti, A. Gaimarri, A. Zanardi, F. Clementi and M. Zoli, 2007. Heterogeneity and complexity of native brain nicotinic receptors. Biochem. Pharmacol., 74: 1102-1111.
48. Grady, S.R., M. Moretti, M. Zoli, M.J. Marks and A. Zanardi *et al*., 2009. Rodent habenulo-interpeduncular pathway expresses a large variety of uncommon nAChR subtypes, but only the α3β4 and α3β3β4 subtypes mediate acetylcholine release. J. Neurosci., 29: 2272-2282.
49. Kracun, S., P.C. Harkness, A.J. Gibb and N.S. Millar, 2008. Influence of the M3-M4 intracellular domain upon nicotinic acetylcholine receptor assembly, targeting and function. Br. J. Pharmacol., 153: 1474-1484.
50. Marsh, D. and F.J. Barrantes, 1978. Immobilized lipid in acetylcholine receptor-rich membranes from *Torpedo marmorata*. Proc. Natl. Acad. Sci. USA., 75: 4329-4333.
51. Lindstrom, J., R. Anholt, B. Einarson, A. Engel, M. Osame and M. Montal, 1980. Purification of acetylcholine receptors, reconstitution into lipid vesicles and study of agonist-induced cation channel regulation. J. Biol. Chem., 255: 8340-8350.
52. Criado, M., H. Eibl and F.J. Barrantes, 1984. Functional properties of the acetylcholine receptor incorporated in model lipid membranes. Differential effects of chain length and head group of phospholipids on receptor affinity states and receptor-mediated ion translocation. J. Biol. Chem., 259: 9188-9198.
53. Kilian, P.L., C.R. Dunlap, P. Mueller, M.A. Schell, R.L. Huganir and E. Racker, 1980. Reconstitution of acetylcholine receptor from *Torpedo californica* with highly purified phospholipids: Effect of α-tocopherol, phylloquinone and other terpenoid quinones. Biochem. Biophys. Res. Commun., 93: 409-414.
54. Huganir, R.L., M.A. Schell and E. Racker, 1979. Reconstitution of the purified acetylcholine receptor from *Torpedo californica*. FEBS Lett., 108: 155-160.
55. Epstein, M. and E. Racker, 1978. Reconstitution of carbamylcholine-dependent sodium ion flux and desensitization of the acetylcholine receptor from *Torpedo californica*. J. Biol. Chem., 253: 6660-6662.
56. Bertrand, D., 2002. Neuronal nicotinic acetylcholine receptors and epilepsy. Epilepsy Curr., 2: 191-193.
57. Jones, O.T., J.H. Eubanks, J.P. Earnest and M.G. McNamee, 1988. A minimum number of lipids are required to support the functional properties of the nicotinic acetylcholine receptor. Biochemistry, 27: 3733-3742.
58. Fong, T.M. and M.G. McNamee, 1986. Correlation between acetylcholine receptor function and structural properties of membranes. Biochemistry, 25: 830-840.
59. Ochoa, E.L.M., A.W. Dalziel and M.G. McNamee, 1983. Reconstitution of acetylcholine receptor function in lipid vesicles of defined composition. Biochim. Biophys. Acta (BBA)-Biomembr., 727: 151-162.
60. Sunshine, C. and M.G. McNamee, 1992. Lipid modulation of nicotinic acetylcholine receptor function: The role of neutral and negatively charged lipids. Biochim. Biophys. Acta (BBA)-Biomembr., 1108: 240-246.
61. Addona, G.H., H. Sandermann Jr., M.A. Kloczewiak, S.S. Husain and K.W. Miller, 1998. Where does cholesterol act during activation of the nicotinic acetylcholine receptor? Biochim. Biophys. Acta (BBA)-Biomembr., 1370: 299-309.
62. Baenziger, J.E., S.E. Ryan, M.M. Goodreid, N.Q. Vuong, R.M. Sturgeon and J.B. Corrie, 2008. Lipid composition alters drug action at the nicotinic acetylcholine receptor. Mol. Pharmacol., 73: 880-890.

63. Schiebler, W. and F. Hucho, 1978. Membranes rich in acetylcholine receptor: Characterization and reconstitution to excitable membranes from exogenous lipids. FEBS J., 85: 55-63.
64. Popot, J.L., R.A. Demel, A. Sobel, L.L. Deenen and J.P. Changeux, 1978. Interaction of the acetylcholine (Nicotinic) receptor protein from *Torpedo marmorata* electric organ with monolayers of pure lipids. FEBS J., 85: 27-42.
65. Sine, S.M. and A.G. Engel, 2006. Recent advances in Cys-loop receptor structure and function. Nature, 440: 448-455.
66. Barrantes, F.J., 2004. Structural basis for lipid modulation of nicotinic acetylcholine receptor function. Brain Res. Rev., 47: 71-95.
67. Barrantes, F.J., V. Bermudez, M.V. Borroni, S.S. Antollini and M.F. Pediconi *et al*., 2010. Boundary lipids in the nicotinic acetylcholine receptor microenvironment. J. Mol. Neurosci., 40: 87-90.
68. Baenziger, J.E., C.M. Henault, J.P.D. Therien and J. Sun, 2015. Nicotinic acetylcholine receptor-lipid interactions: Mechanistic insight and biological function. Biochim. Biophys. Acta (BBA)-Biomembr., 1848: 1806-1817.
69. Barrantes, F.J., 1992. Structural and functional crosstalk between acetylcholine receptor and its membrane environment. Mol. Neurobiol., 6: 463-482.
70. Rankin, S.E., G.H. Addona, M.A. Kloczewiak, B. Bugge and K.W. Miller, 1997. The cholesterol dependence of activation and fast desensitization of the nicotinic acetylcholine receptor. Biophys. J., 73: 2446-2455.
71. DaCosta, C.J.B., A.A. Ogrel, E.A. McCardy, M.P. Blanton and J.E. Baenziger, 2002. Lipid-protein interactions at the nicotinic acetylcholine receptor: A functional coupling between nicotinic receptors and phosphatidic acid-containing lipid bilayers. J. Biol. Chem., 277: 201-208.
72. Hamouda, A.K., M. Sanghvi, D. Sauls, T.K. Machu and M.P. Blanton, 2006. Assessing the lipid requirements of the *Torpedo californica* nicotinic acetylcholine receptor. Biochemistry, 45: 4327-4337.
73. Baenziger, J.E., M. Morris, T.E. Darsaut and S.E. Ryan, 2000. Effect of membrane lipid composition on the conformational equilibria of the nicotinic acetylcholine receptor. J. Biol. Chem., 275: 777-784.
74. Criado, M., H. Eibl and F.J. Barrantes, 1982. Effects of lipids on acetylcholine receptor. Essential need of cholesterol for maintenance of agonist-induced state transitions in lipid vesicles. Biochemistry, 21: 3622-3629.
75. Barrantes, F.J., 2002. Lipid matters: Nicotinic acetylcholine receptor-lipid interactions (Review). Mol. Membrane Biol., 19: 277-284.
76. Da Costa, C.J.B. and J.E. Baenziger, 2009. A lipid-dependent uncoupled conformation of the acetylcholine receptor. J. Biol. Chem., 284: 17819-17825.
77. Artigues, A., M.T. Villar, A.M. Fernandez, J.A. Ferragut and J.M. Gonzalez-Ros, 1989. Cholesterol stabilizes the structure of the nicotinic acetylcholine receptor reconstituted in lipid vesicles. Biochim. Biophys. Acta (BBA)-Biomembr., 985: 325-330.
78. Baenziger, J.E. and P.J. Corringer, 2011. 3D structure and allosteric modulation of the transmembrane domain of pentameric ligand-gated ion channels. Neuropharmacology, 60: 116-125.
79. Jin, X. and J.H. Steinbach, 2011. A portable site: A binding element for 17β-estradiol can be placed on any subunit of a nicotinic α4β2 receptor. J. Neurosci., 31: 5045-5054.
80. Corrie, J.B., S.A. Medaglia, N. Lavigne, S. Wang, C.L. Carswell and J.E. Baenziger, 2009. Anionic lipids allosterically modulate multiple nicotinic acetylcholine receptor conformational equilibria. J. Biol. Chem., 284: 33841-33849.
81. Vallejo, Y.F., B. Buisson, D. Bertrand and W.N. Green, 2005. Chronic nicotine exposure upregulates nicotinic receptors by a novel mechanism. J. Neurosci., 25: 5563-5572.
82. Cheng, M.H., Y. Xu and P. Tang, 2009. Anionic lipid and cholesterol interactions with alpha4beta2 nAChR: insights from MD simulations. J. Phys. Chem. B, 113: 6964-6970.
83. Da Costa, C.J.B., L. Dey, J.P.D. Therien and J.E. Baenziger, 2013. A distinct mechanism for activating uncoupled nicotinic acetylcholine receptors. Nat. Chem. Biol., 9: 701-707.
84. Da Costa, C.J.B. and J.E. Baenziger, 2013. Gating of pentameric ligand-gated ion channels: Structural insights and ambiguities. Structure, 21: 1271-1283.
85. De Planque, M.R., D.T. Rijkers, R.M. Liskamp and F. Separovic, 2004. The αM1 transmembrane segment of the nicotinic acetylcholine receptor interacts strongly with model membranes. Magnet. Resonance Chem., 42: 148-154.
86. Marsh, D., A. Watts and F.J. Barrantes, 1981. Phospholipid chain immobilization and steriod rotational immobilization in acetylcholine receptor-rich membranes from *Torpedo marmorata*. Biochim. Biophys. Acta (BBA)-Biomembr., 645: 97-101.
87. Ellena, J.F., M.A. Blazing and M.G. McNamee, 1983. Lipid-protein interactions in reconstituted membranes containing acetylcholine receptor. Biochemistry, 22: 5523-5535.
88. Arias, H.R., P. Bhumireddy and C. Bouzat, 2006. Molecular mechanisms and binding site locations for noncompetitive antagonists of nicotinic acetylcholine receptors. Int. J. Biochem. Cell Biol., 38: 1254-1276.
89. Dreger, M., M. Krauss, A. Herrmann and F. Hucho, 1997. Interactions of the nicotinic acetylcholine receptor transmembrane segments with the lipid bilayer in native receptor-rich membranes. Biochemistry, 36: 839-847.
90. Sunshine, C. and M.G. McNamee, 1994. Lipid modulation of nicotinic acetylcholine receptor function: The role of membrane lipid composition and fluidity. Biochim. Biophys. Acta (BBA)-Biomembr., 1191: 59-64.

91. Labriola, J.M., J.B. Corrie, S. Wang, D. Figeys, J.C. Smith, R.M. Sturgeon and J.E. Baenziger, 2010. Phospholipase C activity affinity purifies with the *Torpedo nicotinic* acetylcholine receptor. J. Biol. Chem., 285: 10337-10343.
92. Kamerbeek, C.B., M.V. Mateos, A.S. Valles, M.F. Pediconi, F.J. Barrantes and V. Borroni, 2016. Diacylglycerol levels modulate the cellular distribution of the nicotinic acetylcholine receptor. Int. J. Biochem. Cell Biol., 74: 1-11.
93. Bhushan, A. and M.G. McNamee, 1990. Differential scanning calorimetry and Fourier transform infrared analysis of lipid-protein interactions involving the nicotinic acetylcholine receptor. Biochim. Biophys. Acta (BBA)-Biomembr., 1027: 93-101.
94. Carswell, C.L., J. Sun and J.E. Baenziger, 2015. Intramembrane aromatic interactions influence the lipid sensitivities of pentameric ligand-gated ion channels. J. Biol. Chem., 290: 2496-2507.
95. Methot, N., C.N. Demers and J.E. Baenziger, 1995. Structure of both the ligand-and lipid-dependent channel-inactive states of the nicotinic acetylcholine receptor probed by FTIR spectroscopy and hydrogen exchange. Biochemistry, 34: 15142-15149.
96. Katz, B. and S. Thesleff, 1957. A study of the desensitization produced by acetylcholine at the motor end-plate. J. Physiol., 138: 63-80.
97. Boyd, N.D. and J.B. Cohen, 1980. Kinetics of binding of [3H] acetylcholine and [3H] carbamoylcholine to *Torpedo postsynaptic* membranes: Slow conformational transitions of the cholinergic receptor. Biochemistry, 19: 5344-5353.
98. Arias, H.R., 1997. Topology of ligand binding sites on the nicotinic acetylcholine receptor. Brain Res. Rev., 25: 133-191.
99. Herz, J.M., D.A. Johnson and P. Taylor, 1987. Interaction of noncompetitive inhibitors with the acetylcholine receptor. The site specificity and spectroscopic properties of ethidium binding. J. Biol. Chem., 262: 7238-7247.
100. Jozwiak, K., S. Ravichandran, J.R. Collins, R. Moaddel and I.W. Wainer, 2007. Interaction of noncompetitive inhibitors with the α3β2 nicotinic acetylcholine receptor investigated by affinity chromatography and molecular docking. J. Med. Chem., 50: 6279-6283.
101. Jozwiak, K., S. Ravichandran, J.R. Collins and I.W. Wainer, 2004. Interaction of Noncompetitive inhibitors with an immobilized α3β4 nicotinic acetylcholine receptor investigated by affinity chromatography, quantitative-structure activity relationship analysis and molecular docking. J. Med. Chem., 47: 4008-4021.
102. Henault, C.M., J. Sun, J.D. Therien, J.B. Corrie and C.L. Carswell *et al*., 2015. The role of the M4 lipid-sensor in the folding, trafficking and allosteric modulation of nicotinic acetylcholine receptors. Neuropharmacology, B96: 157-168.
103. Antollini, S.S., Y. Xu, H. Jiang and F.J. Barrantes, 2005. Fluorescence and molecular dynamics studies of the acetylcholine receptor γM4 transmembrane peptide in reconstituted systems. Mol. Membr. Biol., 22: 471-483.
104. Xu, Y., F.J. Barrantes, X. Luo, K. Chen, J. Shen and H. Jiang, 2005. Conformational dynamics of the nicotinic acetylcholine receptor channel: A 35-ns molecular dynamics simulation study. J. Am. Chem. Soc., 127: 1291-1299.
105. Xu, Y., F.J. Barrantes, X. Luo, K. Chen, J. Shen and H. Jiang, 2015. Correction to Conformational dynamics of the nicotinic acetylcholine receptor channel: A 35-ns molecular dynamics simulation study. J Am Chem Soc., 137: 3992-3992.
106. Liu, X., Y. Xu, H. Li, X. Wang, H. Jiang and F.J. Barrantes, 2008. Mechanics of channel gating of the nicotinic acetylcholine receptor. PLoS Comput. Biol., Vol. 4, No. 1. 10.1371/journal.pcbi.0040019.
107. Xu, Y., X. Luo, J. Shen, W. Zhu, K. Chen and H. Jiang, 2006. Molecular dynamics of nicotinic acetylcholine receptor correlating biological functions. Curr. Protein Peptide Sci., 7: 195-200.
108. Auerbach, A., 2015. Activation of endplate nicotinic acetylcholine receptors by agonists. Biochem. Pharmacol., 97: 601-606.
109. Bouzat, C., A.M. Roccamo, I. Garbus and F.J. Barrantes, 1998. Mutations at lipid-exposed residues of the acetylcholine receptor affect its gating kinetics. Mol. Pharmacol., 54: 146-153.
110. Lasalde, J.A., S. Tamamizu, D.H. Butler, C.R.T. Vibat, B. Hung and M.G. McNamee, 1996. Tryptophan substitutions at the lipid-exposed transmembrane segment M4 of *Torpedo californica* acetylcholine receptor govern channel gating. Biochemistry, 35: 14139-14148.
111. Tamamizu, S., Y.H. Lee, B. Hung, M.G. McNamee and J.A. Lasalde-Dominicci, 1999. Alteration in ion channel function of mouse nicotinic acetylcholine receptor by mutations in the M4 transmembrane domain. J. Membr. Biol., 170: 157-164.
112. Cruz-Martin, A., J.L. Mercado, L.V. Rojas, M.G. McNamee and J.A. Lasalde-Dominicci, 2001. Tryptophan substitutions at lipid-exposed positions of the gamma M3 transmembrane domain increase the macroscopic ionic current response of the *Torpedo californica* nicotinic acetylcholine receptor. J. Membr. Biol., 183: 61-70.
113. Tamamizu, S., G.R. Guzman, J. Santiago, L.V. Rojas, M.G. McNamee and J.A. Lasalde-Dominicci, 2000. Functional effects of periodic tryptophan substitutions in the αM4 transmembrane domain of the *Torpedo californica* nicotinic acetylcholine receptor. Biochemistry, 39: 4666-4673.
114. Shen, X.M., F. Deymeer, S.M. Sine and A.G. Engel, 2006. Slow-channel mutation in acetylcholine receptor αM4 domain and its efficient knockdown. Ann. Neurol., 60: 128-136.

115. Lee, Y.H., L. Li, J. Lasalde, L. Rojas, M. Mcnamee, S.I. Ortiz-Miranda and P. Pappone, 1994. Mutations in the M4 domain of *Torpedo californica* acetylcholine receptor dramatically alter ion channel function. Biophys. J., 66: 646-653.
116. Santiago, J., G.R. Guzman, K. Torruellas, L.V. Rojas and J.A. Lasalde-Dominicci, 2004. Tryptophan scanning mutagenesis in the TM3 domain of the *Torpedo californica* acetylcholine receptor beta subunit reveals an α-helical structure. Biochemistry, 43: 10064-10070.
117. Castillo, R.I., L.E. Rojo, M. Henriquez-Henriquez, H. Silva and A. Maturana *et al*., 2016. From molecules to the clinic: Linking schizophrenia and metabolic syndrome through sphingolipids metabolism. Front. Neurosci., Vol. 10. 10.3389/fnins.2016.00488.
118. Monteiro-Cardoso, V.F., M.M. Oliveira, T. Melo, M.R. Domingues and P.I. Moreira *et al*., 2015. Cardiolipin profile changes are associated to the early synaptic mitochondrial dysfunction in Alzheimer's disease. J. Alzheimer's Dis., 43: 1375-1392.
119. Mahley, R.W., 2016. Central nervous system lipoproteins highlights: ApoE and regulation of cholesterol metabolism. Arterioscler. Thromb. Vasc. Biol., 36: 1305-1315.
120. Notarangelo, F.M. and A. Pocivavsek, 2017. Elevated kynurenine pathway metabolism during neurodevelopment: Implications for brain and behavior. Neuropharmacology, 112: 275-285.
121. Posadas, I., B. Lopez-Hernandez and V. Cena, 2013. Nicotinic receptors in neurodegeneration. Curr. Neuropharmacol., 11: 298-314.
122. Martin, L.F. and R. Freedman, 2007. Schizophrenia and the α7 nicotinic acetylcholine receptor. Int. Rev. Neurobiol., 78: 225-246.
123. Schaaf, C.P., 2014. Nicotinic acetylcholine receptors in human genetic disease. Genet. Med., 16: 649-656.
124. Dutertre, S. and R.J. Lewis, 2004. Computational approaches to understand α-conotoxin interactions at neuronal nicotinic receptors. FEBS J., 271: 2327-2334.
125. Yu, R., D.J. Craik and Q. Kaas, 2011. Blockade of neuronal α7-nAChR by α-Conotoxin ImI explained by computational scanning and energy calculations. PloS Comput. Biol., Vol. 7, No. 3. 10.1371/journal.pcbi.1002011.
126. Lechleiter, J., M. Wells and R. Gruener, 1986. Halothane-induced changes in acetylcholine receptor channel kinetics are attenuated by cholesterol. Biochim. Biophys. Acta (BBA)-Biomembr., 856: 640-645.
127. Santiago, J., G.R. Guzman, L.V. Rojas, R. Marti and G.A. Asmar-Rovira *et al*., 2001. Probing the effects of Membrane cholesterol in the *Torpedo californica* acetylcholine receptor and the novel lipid-exposed mutation αC418W in *Xenopus* Oocytes. J. Biol. Chem., 276: 46523-46532.
128. Fernandez-Ballester, G., J. Castresana, A.M. Fernandez, J.L.R. Arrondo, J.A. Ferragut and J.M. Gonzalez-Ros, 1994. A role for cholesterol as a structural effector of the nicotinic acetylcholine receptor. Biochemistry, 33: 4065-4071.
129. Fernandez-Ballester, G., J. Castresana, A.M. Fernandez, J.L.R. Arrondo, J.A. Ferragut and J.M. Gonzalez-Ros, 1994. Role of cholesterol as a structural and functional effector of the nicotinic acetylcholine receptor. Biochem. Soc. Trans., 22: 776-780.
130. Arias, H.R., M.B. Sankaram, D. Marsh and F.J. Barrantes, 1990. Effect of local anaesthetics on steroid-nicotinic acetylcholine receptor interactions in native membranes of Torpedo marmorata electric organ. Biochim. Biophys. Acta (BBA)-Biomembr., 1027: 287-294.
131. Bouzat, C. and F.J. Barrantes, 1993. Hydrocortisone and 11-desoxycortisone modify acetylcholine receptor channel gating. Neuroreport, 4: 143-146.
132. Barrantes, F.J., S.S. Antollini, C.B. Bouzat, I. Garbus and R.H. Massol, 2000. Nongenomic effects of steroids on the nicotinic acetylcholine receptor. Kidney Int., 57: 1382-1389.
133. Fernandez, A.M., G. Fernandez-Ballester, J.A. Ferragut and J.M. Gonzales-Ros, 1993. Labeling of the nicotinic acetylcholine receptor by a photoactivatable steroid probe: Effects of cholesterol and cholinergic ligands. Biochim. Biophys. Acta (BBA)-Biomembr., 1149: 135-144.
134. Berg, D.K., W.G. Conroy, Z. Liu and W.M. Zago, 2006. Nicotinic signal transduction machinery. J. Mol. Neurosci., 30: 149-152.
135. Rudiger, T. and J. Bolz, 2008. Acetylcholine influences growth cone motility and morphology of developing thalamic axons. Cell Adhesion Migration, 2: 30-37.
136. Halff, A.W., D. Gomez-Varela, D. John and D.K. Berg, 2014. A novel mechanism for nicotinic potentiation of glutamatergic synapses. J. Neurosci., 34: 2051-2064.
137. Nordman, J.C., P. Muldoon, S. Clark, M.I. Damaj and N. Kabbani, 2014. The α4 nicotinic receptor promotes CD4+ T-cell proliferation and a helper T-cell immune response. Mol. Pharmacol., 85: 50-61.
138. Stone, T.W. and L.G. Darlington, 2002. Endogenous kynurenines as targets for drug discovery and development. Nat. Rev. Drug Discov., 1: 609-620.
139. Douthwaite, J.A., D.K. Finch, T. Mustelin and T.C.I. Wilkinson, 2017. Development of therapeutic antibodies to G protein-coupled receptors and ion channels: Opportunities, challenges and their therapeutic potential in respiratory diseases. Pharmacol. Ther., 169: 113-123.
140. Liu, Q.S. and D.K. Berg, 1999. Actin filaments and the opposing actions of CaM kinase II and calcineurin in regulating α7-containing nicotinic receptors on chick ciliary ganglion neurons. J. Neurosci., 19: 10280-10288.

141. Campbell, N.R., C.C. Fernandes, A.W. Halff and D.K. Berg, 2010. Endogenous signaling through α7-containing nicotinic receptors promotes maturation and integration of adult-born neurons in the hippocampus. J. Neurosci., 30: 8734-8744.
142. Nordman, J.C., W.S. Phillips, N. Kodama, S.G. Clark, C.A. Del Negro and N. Kabbani, 2014. Axon targeting of the α7 nicotinic receptor in developing hippocampal neurons by Gprin1 regulates growth. J. Neurochem., 129: 649-662.
143. Nordman, J.C. and N. Kabbani, 2014. Microtubule dynamics at the growth cone are mediated by α7 nicotinic receptor activation of a Gαq and IP3 receptor pathway. FASEB J., 28: 2995-3006.
144. Cheng, Q. and J.L. Yakel, 2015. The effect of α7 nicotinic receptor activation on glutamatergic transmission in the hippocampus. Biochem. Pharmacol., 97: 439-444.
145. Koukouli, F. and U. Maskos, 2015. The multiple roles of the α7 nicotinic acetylcholine receptor in modulating glutamatergic systems in the normal and diseased nervous system. Biochem. Pharmacol., 97: 378-387.
146. Beinat, C., S.D. Banister, M. Herrera, V. Law and M. Kassiou, 2015. The therapeutic potential of α7 nicotinic acetylcholine receptor (α7 nAChR) agonists for the treatment of the cognitive deficits associated with schizophrenia. CNS Drugs, 29: 529-542.
147. Nordman, J.C. and N. Kabbani, 2012. An interaction between α7 nicotinic receptors and a G-protein pathway complex regulates neurite growth in neural cells. J. Cell Sci., 125: 5502-5513.