Diverse strategies targeting alpha7 homomeric and alpha6beta2* heteromeric nicotinic acetylcholine receptors for smoking cessation

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Abstract

Preclinical studies suggest that a diversity of nicotinic acetylcholine receptors (nAChRs) with different sensitivities to nicotine may contribute to tobacco addiction. Using rodent intravenous nicotine self-administration as a preclinical model with good predictive validity for therapeutic efficacy for tobacco cessation, investigators have identified heteromeric $\alpha_6\beta_2^*$ and homomeric $\alpha_7$ nAChRs as promising novel therapeutic targets to promote smoking abstinence ($^*$denotes the possible assembly with other subunits). The data suggest that diverse strategies that target these subclasses of nAChRs, namely inhibition of $\alpha_6\beta_2^*$ nAChRs and stimulation of $\alpha_7$ nAChRs, will support tobacco cessation. $\alpha_6\beta_2^*$ nAChRs, members of the high-affinity family of $\beta_2^*$ nAChRs, function similarly to $\alpha_4\beta_2^*$ nAChRs, the primary target of the FDA-approved drug varenicline, but have a much more selective neuroanatomical pattern of expression in catecholaminergic nuclei. Whereas activation of $\beta_2^*$ nAChRs facilitates nicotine self-administration, stimulation of $\alpha_7$ nAChRs appears to negatively modulate both nicotine reinforcement and $\beta_2^*$ nAChR function in the mesolimbic dopamine system. Although challenges and caveats must be considered in the development of therapeutics which target these nAChR sub-populations, an accumulation of data suggest that $\alpha_7$ nAChR agonists, partial agonists or positive allosteric modulators and $\alpha_6\beta_2^*$ nAChR antagonists, partial agonists or negative allosteric modulators may prove effective therapeutics for tobacco cessation.

Keywords

Smoking cessation; drug development; nicotinic; alpha7; alpha6; nicotine dependence

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Part 1: Introduction

Reductions in tobacco smoking have reached a plateau in developed countries where a majority of smokers report wanting to quit.\(^1\) Many of these individuals are non-responsive to currently available therapeutics for tobacco cessation. When nicotine replacement is delivered via routes of administration considered safer than smoking, it greatly increases abstinence in comparison to smokers who use no therapy, but still only assists a small minority of smokers in quitting.\(^2\) Nicotine in tobacco exerts its psychoactive effects at a diversity of nicotinic acetylcholine receptors (nAChRs) where it both stimulates these receptors and inactivates them via desensitization. Although most preclinical studies suggest that activation of \(\beta_2\) subunit containing nicotinic receptors (\(\beta_2^*\) nAChRs; *denotes possible assembly with other subunits), is required for nicotine reinforcement and reward,\(^3\)-\(^12\) it is remains unclear from a therapeutic perspective whether activation or inhibition of diverse nicotinic receptor subtypes may best support tobacco cessation.\(^13\) Bupropion, an FDA-approved smoking cessation drug which acts as a dopamine (DA) and norepinephrine (NE) transporter blocker, also has antagonist properties at nAChRs.\(^14\) Mixed agonist/antagonist strategies have been proposed to support tobacco cessation.\(^15\)-\(^17\) Considerable increases in therapeutic efficacy are observed with varenicline and the natural derivative of the golden rain plant, cytisine\(^18\) which, as partial agonists of \(\alpha_4\beta_2^*\) nAChRs, have mixed agonist and antagonist properties. Given the diversity of nAChRs and their functions, it is possible that the effectiveness of varenicline may also be attributed in part to its selective nAChR targeting of the \(\beta_2^*\) nAChRs, including \(\alpha_4\beta_2^*\) nAChRs and \(\alpha_6\beta_2^*\) nAChRs. Although with lower affinity, varenicline is also a full agonist at \(\alpha_7\) nAChRs and \(\alpha_3\beta_4^*\) nAChRs\(^19\),\(^20\) which preclinical data suggest may make important contributions to tobacco dependence phenotype.\(^21\)-\(^25\)

\(\alpha_4\beta_2^*\) nAChRs are well-studied for their role in nicotine addiction phenotype.\(^4\),\(^7\)-\(^11\),\(^26\) Based on rodent intravenous self-administration of nicotine, a preclinical model with good predictive validity for therapeutics for smoking cessation, this review will focus on two novel nAChR targets for tobacco cessation, the \(\alpha_7\) homomeric nAChRs and the high sensitivity, heteromeric nAChRs containing \(\alpha_6\) and \(\beta_2\) subunits (\(\alpha_6\beta_2^*\) nAChRs). See\(^21\),\(^27\),\(^28\) for a more comprehensive review of nAChR localization and contributions to tobacco addiction. When justified, this review will specify a more complete representation of subunits, but otherwise will more conservatively use the * nomenclature to indicate that precise subunit composition is yet to be determined. Part 2 of this review will provide the reader with an overview of nAChR composition and function, highlighting differences between the \(\alpha_7\) and \(\alpha_6\beta_2^*\) nAChRs. These diverse receptor subtypes do not only respond differently to nicotinic agonists such as ACh and nicotine, but Part 3 of this review of the literature will show that the \(\alpha_7\) and \(\alpha_6\beta_2^*\) nAChR subtypes also differentially regulate nicotine addiction behavior. Of import from a therapeutic perspective, activation of \(\alpha_6\beta_2^*\) nAChRs, like \(\alpha_4\beta_2^*\) nAChRs, supports the reinforcing efficacy of systemically administered nicotine;\(^3\),\(^5\),\(^9\),\(^10\),\(^29\),\(^30\) in contrast, inhibition of \(\alpha_7\) nAChRs facilitates rodent motivation to self-administer nicotine.\(^22\) Thus, with nicotine self-administration as a behavioral endpoint, it appears that \(\alpha_7\) nAChRs work in opposition to \(\beta_2^*\) nAChRs. These preclinical data suggest that novel therapeutic strategies for smoking cessation include

Ann N Y Acad Sci. Author manuscript; available in PMC 2015 October 01.
inhibition of α6β2* nAChRs and activation of α7 nAChRs. Due to their unique neuroanatomical expression profiles, therapeutics targeting α7 nAChRs and α6β2* nAChRs may benefit different populations of smokers than have quit with existing therapies. The rather selective expression of α6β2* nAChRs in catecholaminergic nuclei including the mesolimbic DA pathway suggests that targeted treatment of this nAChR subtype could benefit smokers while having reduced potential for side effects (but see Part 4 for considerations). The expression of α7 nAChRs has overlap with α6β2* and α4β2* nAChRs within the mesolimbic circuitry but is enriched in areas complementary to α4β2* nAChRs that support cognition and arousal. However, it should be noted that while overlapping on the tissue level, the expression of α7 and α6β2* nAChR are distinctly different on the cellular and subcellular levels in these tissues. Part 4 of this review will focus on drug development strategies and caveats for pharmacological stimulation of α7 nAChRs and inhibition of α6β2* nAChRs.

Part 2: Nicotinic receptor composition, function and expression

2.1 Nicotinic receptor diversity

The first studies of synaptic physiology, a necessary prelude to understanding brain function, were conducted at the neuromuscular junction, where the neuronal release of acetylcholine (ACh) leads to the activation of ion channels and, ultimately, muscle contraction. Although classified as nicotinic, the synaptic receptors of the mammalian neuromuscular junction are only weakly activated by nicotine and are physiologically distinct from the neuronal nAChRs in regard to their subunit composition. This facilitates selective targeting of nAChRs in brain where nicotine reinforcement takes place. The diverse composition and neuroanatomical location of neuronal nAChRs lends to their unique roles in addiction, attention, emotion and other behaviors, making selective targeting of subclasses of nAChRs possible for the treatment of tobacco addiction.

Like nAChRs at the neuromuscular junction, neuronal nAChRs are ligand-gated ion channels made up of 5 subunits. Consideration of the subunit composition of muscle-type receptors led to the assumption that there were two agonist-binding sites in each receptor pentamer, at the interfaces between the α (in current nomenclature, α1) subunits and non-alpha subunits. The non-alpha subunits contributing to agonist binding sites were identified as the δ and either γ or ε subunits at muscle nAChRs. These sites for binding ACh and other agonists are referred to as the “orthosteric” binding sites to distinguish them from various allosteric sites where other ligands or proteins may bind to regulate the receptors properties in ways that can either increase or decrease the likelihood of channel activation. The muscle β subunit (in current nomenclature, β1) does not directly bind agonist. Subunits filling this fifth position in a pentamer have come to be known as structural or accessory subunits which do not necessarily participate in binding. Heteromeric neuronal nAChRs contain a combination of α and β subunits with binding believed to take place at the interface of α and its neighboring subunit. Models of receptor subunit composition based on the muscle receptor have been useful in understanding the functionality of the first nAChR subunit genes cloned from neuronal tissues. Some subunits were identified as alpha analogs based on the presence of vicinal cysteines in the ligand binding domain; however, it was unclear...
how to classify the neuronal non-alpha subunits. The observation that they could only substitute for β1 subunits led to the classification of all neuronal non-alpha subunits as beta analogs. In total nine additional “alpha-type” subunits (α2–α10) and three additional “beta” subunits (β2–β4) have been cloned from neuronal tissue, although α8 has not yet been reported to be expressed in mammals. The model for a heteromeric receptor complex applied well to the first neuronal genes cloned. The α2, α3, and α4 genes formed functional receptors when expressed in Xenopus oocytes with either β2 or β4, each pair apparently able to form functional ligand binding domains with distinct properties. Pairwise expression of these subunits, however, results in mixed receptor populations, as either an α or a β can take the accessory subunit position, resulting in receptors with distinct functional and pharmacological properties. Two subunits, α5 and β3, do not appear to participate in functional agonist binding sites, but can co-assemble with other subunits, serving as accessory subunits. Although such accessory subunits do not contribute to the primary agonist binding sites, they nonetheless have important impact on the function and pharmacology of the receptor subunit complexes.

The characterization of the heteromeric neuronal nAChR (summarized in Figure 1) also provided insight into early autoradiographic characterization of nicotine binding sites in brain. The ubiquitous pattern of high-affinity binding of nicotine corresponded to the overlapping expression pattern for α4 and β2 subunits, which are now known to constitute the main high-affinity nicotine receptors in rodent brain. α4β2* receptors (receptors containing two α4β2 agonist binding dimers and a fifth subunit, most often α4, β2, or α5) are the most abundant class of heteromeric nAChR in rodent brain. A phenylalanine residue in which is present in the β2 subunit is thought to contribute to the high affinity of β2* nAChRs. This high-affinity class of nAChRs also includes the α-conotoxin MII-sensitive subclass of receptors, α6β2* and α3β2*, which may or may not co-express with the α4 subunit. With the exception of the medial habenula and the fasciculus retroflexis, where α-conotoxin MII binding is primarily attributed to α3β2* nAChRs, this high-affinity class of nAChRs also includes the α-conotoxin MII-sensitive subclass of receptors, α6β2* and α3β2*, which may or may not co-express with the α4 subunit. With the exception of the medial habenula and the fasciculus retroflexis, where α-conotoxin MII binding is primarily attributed to α3β2* nAChRs, and the VTA and interpeduncular nucleus where α3β2* and α6β2* nAChRs are co-expressed, most α-conotoxin MII binding in brain is at the α6β2* nAChRs. In contrast to α4β2* nAChRs that do not express α3 or α6, the α-conotoxin MII-sensitive nAChRs have a more restricted expression profile in catecholaminergic nuclei in the brain. Of relevance for their role in tobacco addiction, as will be discussed later in this review, the α6β2* nAChRs are greatly enriched in ventral tegmental area (VTA) dopamine neurons.

For a number of years a mystery remained concerning a putative class of nAChR in brain which did not bind nicotine or ACh with high affinity, but did bind the snake toxin, α-bungarotoxin, which had proven useful in isolating the muscle nAChR. Understanding these binding sites came only with the discovery of a second family of nAChR subunits, α7 – α10, which could function as homomeric, or sometimes heteromeric, complexes without requiring co-assembly with β subunits. Unique properties of these homomeric receptors in contrast to the β2* nAChRs will be given special consideration in this review.
2.2 Nicotinic receptor function

Nicotinic acetylcholine receptors are allosteric proteins that have multiple conformational states, with the equilibria among these states regulated by ligand binding. The simplest models allow for the existence of distinct states, including resting, activated and desensitized as illustrated in Figure 2.\textsuperscript{56, 57} Positioned on neuronal dendrites, soma and terminals, neuronal nAChRs are expressed widely throughout the nervous system on cells that release diverse neurotransmitters. Since agonist binding results in both activation and desensitization, nAChRs promote nicotine- and ACh-associated modulation of neuronal function including stimulation or inhibition of neurotransmitter release.\textsuperscript{58, 59} In the brain ACh is released in a relatively diffuse manner, and functions primarily as a modulator of neuronal function and subsequently affects the release of various neurotransmitters, including glutamate, GABA, norepinephrine, serotonin, ACh itself, and DA. Binding of endogenous ACh or exogenous agonist compounds (e.g. nicotine) at the orthosteric binding sites results in an increase in the probability of opening of the nAChR ion channel, permits passage of cations across the membrane of the neurons and leads to depolarization of the cell which can facilitate neuronal firing.\textsuperscript{60-62} The ligand activity at allosteric binding sites which can positively or negatively modulate the function of agonist-bound nAChRs will be considered further in Part IV of this review. The activated state of the receptor is intrinsically unstable and further conformational changes are likely to occur, associated with nonconducting desensitized states. It is generally believed that the receptor activation achieved with cigarette delivery of nicotine, albeit transient, is important for the immediate reinforcing properties of the drug and that this reinforcement is associated with a parallel increase in mesolimbic DA release. After a period of excitation, which varies widely across the heteromeric and homomeric receptor subtypes, the heteromeric nAChRs thermodynamically favor a desensitized state in which the channels are closed with the agonist still bound to the receptor.\textsuperscript{63} In this highest affinity state, the desensitized heteromeric nAChRs are typically unavailable for ligand-gated activation; however, evidence suggests that an intermediate level of desensitization can occur where both the β2* nAChRs and the α7 nAChRs equilibrate between activated and desensitized states resulting in low levels of steady-state activation.\textsuperscript{64, 65} Following a period of desensitization, upon removal or metabolism of agonist, the receptors typically revert back to the inactive, unbound state. It is widely accepted that activation of the β2* nAChRs, including α4β2 and α6β2* nAChRs, is both necessary and sufficient for nicotine self-administration and reward.\textsuperscript{3, 5, 8-11} Recent evidence suggests that a variety of nicotine addiction behaviors, including nicotine reinforcement, are supported by inhibition of diverse nAChR subtypes.\textsuperscript{13, 66-68}

2.3 Heteromeric α6β2* nAChRs

The α6β2* nAChRs (including α6β2, α3α6β2, α6β2β3, α3α6β2β3, α4α6β2, and α4α6β2β3) are members of an α-conotoxin MII-sensitive subclass of β2* nAChRs that have a high affinity for nicotine and ACh. Genetically modified mice engineered to be hypersensitive to agonist compounds\textsuperscript{11, 69} or in which each of these subunits, alone or in combination, have been deleted,\textsuperscript{7, 9, 32, 47, 50, 70} have further contributed to our knowledge of the expression and function of these nAChRs. The α6 subunit was initially identified via cloning and is most homologous to the α3 subunit,\textsuperscript{71} perhaps explaining their shared affinity for α-
conotoxin MII when coupled with β2. The development of more refined conotoxins for study of α6* nAChRs subtypes and studies in traditional heterologous systems indicate that α6 subunits may also co-assemble with α3 and β4 without a β2 subunit in the retina and the dorsal root ganglion.72-76

Genetic null mutations of α3 and α6 subunits in mice show that, with the exception of the interpeduncular nucleus and the VTA where α3β2* and α3α6β2* nAChRs are co-expressed with α6β2* nAChRs (minus α3), most α-conotoxin MII binding is to α6* nAChRs50, 52 so this ligand selectively binds and antagonizes α6β2* nAChRs in VTA DA terminal projection areas. α6 and β3 mRNA are co-expressed in catecholaminergic nuclei and pull-down assays show that α6β2* nAChRs have a high incidence of co-expression with β3 as an accessory element.40, 48, 70 A number of studies suggest that α4 may also co-express with α6 and β2 and that VTA α4α6β2* nAChRs are uniquely more sensitive to nicotine than β2* nAChRs that do not assemble with both of these subunits. Functional ex vivo studies suggest that, minus an α3 subunit, the full complement of α6β2* nAChRs promotes DA release at the terminals.48-50

Understanding the function of α6β2* nAChRs presented a special challenge since α6 and β2 subunits do not readily form receptors in cell lines or Xenopus oocyte. Molecular pharmacology studies using α3/α6 chimeras and molecular concatamers, which clone the precise subunit composition and organization of the nAChRs, confirm that α6β2β3 nAChRs are permeable to calcium and bound by compounds such as varenicline and di-hydrobeta-erythroidine (DHβE) that also bind to the α4β2* nAChRs.19, 64, 77 Although it would appear that the α4 subunit contributes largely to this shared affinity,19, 77 it has proven difficult with pharmacology alone to distinguish contributions of α4β2* nAChRs and α6β2* nAChRs and α4α6β2* nAChRs. Ex vivo electrophysiology of VTA DA neurons of wild type, α6 and α4 subunit knockout mice shows that 300 nM concentrations of nicotine, which preferentially desensitize α4β2* nAChRs without an α6 subunit, results in stimulation of α4α6β2* nAChRs.48, 78-80 These data reveal that mesolimbic α4α6β2* nAChRs are uniquely sensitive to activation in response to physiologically relevant concentrations of nicotine that are achieved during smoking as demonstrated in Figure 3A. In addition, nicotine amplifies the action of endogenously released ACh in modulating dopamine release via these receptors,81, 82 though perhaps through a different mechanism. Gain-of-function mice created by mutating a Ser to Leu residue in the M2 domain of the α6 subunit have further enhanced electrophysiological and neurochemical studies of α6* nAChRs to show that stimulation of α6* nAChRs is sufficient to enhance dopamine neuron firing in response to nicotine or ACh.69

Whereas β2* nAChRs are ubiquitously expressed throughout the brain, α-conotoxin MII binding studies show that α6β2* nAChRs have a more selective expression profile. Numerous studies have demonstrated functional α6 nAChRs in CNS catecholamine neurons.83 As an accumulation of data suggests that nicotine addiction behavior is regulated in large part by the mesocorticolimbic DA pathway which projects from the VTA to the nucleus accumbens (NAc), anterior cingulate cortex, hippocampus, and amygdala; hence it is of relevance to their putative role in tobacco addiction phenotype that α6β2* nAChRs are highly enriched in the dopamine neurons of the VTA. The α6β2* nAChRs are co-expressed

Referenced: Ann N Y Acad Sci. Author manuscript; available in PMC 2015 October 01.
with α3β2* nAChRs in the VTA but are preferentially expressed on DA terminals in the nucleus accumbens. Although a combination of β2* nAChRs contribute to nicotine stimulated DA release in the NAc,48-50 cyclic voltammetry studies suggest that α6β2* nAChRs support 80% of DA release at terminals,51 α6β2* nAChRs are much less involved in dorsal striatum DA release,81, 84 but are likely to contribute to motoric behavior at the level of the substantia nigra where decreases in α6β2* nAChRs expression are associated with DA Parkinson’s disease phenotype in humans and animal models.85, 86 The development of increasingly more selective α-conotoxin MII compounds has enabled the study of α6 in isolation of α3 where these receptors are co-expressed, e.g.52, 87 More recently, the presence of α6β2* nAChRs has also been reported in presynaptic GABAergic boutons,88 in noradrenergic terminals in the hippocampus89 and on retinal glutamatergic projection neurons and GABAergic soma in the superior colliculus of the visual system.90 α6* nAChRs are located postsynaptically on the cell-body where they regulate cell firing and mediate direct postsynaptic effects. α6* nAChRs are also located presynaptically on nerve terminals where they serve to modulate the release of neurotransmitters including dopamine,49 norepinephrine91 and GABA.92 In the periphery of mammals, the adrenal gland participates in release of catecholamines, primarily adrenalin, into the bloodstream. In humans, this release is modulated in part by α6* nAChRs,75 suggesting that α6* nAChRs contribute to systems that regulate arousal.

### 2.4 Homomeric α7 nAChRs

Alpha7 nAChR are distinguished from other nAChR by a number of unique physiological and pharmacological properties, including a high permeability to calcium (PCa:PNa of ≥0), and rapid and reversible desensitization.55 Even under optimized conditions, the maximal probability for any single α7 receptor within a large population to participate in a synchronized transient current is two orders of magnitude lower than that of a typical heteromeric receptor. Under optimal steady-state conditions the probability of any single α7 receptor being open is less than one in a million.93 This low probability of being open, in part, reflects the extreme instability of the open conformation under control conditions, when rarely occurring isolated channel openings are typically less than 100 μs in duration. α7 nAChRs are expressed in the inderpeduncular nucleus, the nucleus accumbens shell and the ventral tegmental area (VTA) of the mesolimbic DA pathway where they regulate nicotine stimulated DA neuron activity (discussed in greater detail below).34, 94 The α7 subunit is also expressed at high levels in the hippocampus, the hypothalamus,55, 95 and α-bungarotoxin binding is enriched in deep layers of cortex.44 In addition to brain areas that regulating cognition, attention and arousal, α7 nAChRs have also been shown to have functionally important expression in non-neuronal tissues, such as cells of the immune system.96 Phylogenetic data indicate that the α7 gene represents a sort of ancestral nAChR, a protein that may have evolved in organisms that did not rely on fast chemical neurotransmission. This is consistent with the presence of α7 in numerous non-neuronal cell types97-99 and the fact that α7 receptors are not strictly receptors for acetylcholine but respond also to choline,100, 101 the ubiquitous precursor to ACh. This feature suggests that α7 nAChRs are adapted to respond following enzymatic breakdown of ACh and propose a possible mechanism by which α7 nAChRs may oppose the activity of the β2* nAChRs. Recent evidence supports a modulatory role for α7 nAChRs in the VTA to decrease β2*
nAChR function via stimulation of intracellular signaling cascades that inhibit β2* nAChRs.94

The functional properties of α7 receptors have been studied in Xenopus oocytes,65, 102 cultured hippocampal neurons,102-106 mammalian cell lines93 and native neuronal tissues.107 One consistent finding is that the kinetic properties of α7 receptors cannot be adequately described by the models of heteromeric nAChR discussed above.

For a quiescent population of heteromeric nAChR in the absence of agonist, the rapid application of a concentration of ACh sufficiently high to saturate the agonist binding sites produces maximal synchronous transient activation. When a similar application of agonist is made to a population of α7 receptors, the maximal synchronous transient activation occurs when only a fraction of the agonist binding sites are occupied.93, 102, 108-110 This observation suggests that, as with the case of heteromeric receptors, the allosteric effects of binding to just one or two of the five possible binding sites may promote channel opening but that at higher levels of binding, the receptor is most likely to adopt nonconducting conformations as illustrated in Figure 3C. Recently, data suggest that in addition to functioning, rather inefficiently as a ligand-gated ion channel, α7 nAChRs may also mediate channel-independent signal transduction.111, 112 However, it is perhaps more likely that the alpha7 ion channel activity is essential for modulating reward in the dopamine pathways of the brain, as indicated for the cognitive effects of α7 agonists.113 As will be discussed in more detail in Part 4 below, pharmacological compounds which have their action at an allosteric binding site of the α7 nAChRs can both promote activation and destabilize desensitization of the α7 nAChRs.

Part 3: Preclinical evidence to support inhibition of α6β2* and stimulation of α7 nAChRs as therapeutic strategies for tobacco cessation

3.1 Selective inhibition of α6* nAChRs impairs nicotine use

Since the first null mutation of the β2 nAChR subunit demonstrated that β2 knockout mice would not self-administer nicotine,9 it has become widely accepted that activation of the β2* nAChRs supports nicotine reinforcement and reward. It had been presumed that this was due to β2 nAChR subunit assembly with α4, more recent studies have expanded upon this data to suggest that mesolimbic β2* nAChRs that assemble with an α6 and/or α4 subunit play a critical role in both the initiation10 and maintenance of nicotine self-administration.3, 5 Mice with a null mutation of the α4, α6, or β2 nAChR subunit gene do not acquire nicotine self-administration. Initiation of nicotine self-administration is rescued in these mice, however, with selective neuroanatomical re-expression of each of these subunits in the VTA region,10 suggesting that α4α6β2* nAChRs in DA neurons are critical for initiation of nicotine reinforcement as depicted in Figure 3. A combination of pharmacological and genetic studies have identified a class of DA neurons in the VTA that is highly sensitive and persistently activated by nicotine requires both the α4 and α6 subunits.78 More chronic nicotine self-administration studies reveal that activation of mesolimbic α6β2* nAChRs in the VTA5 as well as on DA terminals in the NAc shell3, 48 are critical for maintenance of nicotine self-administration and nicotine-stimulated DA release. Intra-VTA infusion of
DHβE, an antagonist of α4β2* and α6β2* nAChRs or a selective α6β2* nAChR antagonist, α-conotoxin PIA blocks systemic nicotine self-administration in well-trained rats, but see studies on intra-VTA nicotine self-administration which suggest a dichotomous role for these receptors in the VTA. Selective antagonism of α6β2* nAChRs in the NAc shell also significantly attenuated the degree to which animals were willing to work for an intravenous nicotine infusion, suggesting that α6β2* nAChRs are exerting their effects on the terminals of VTA DA receptors as well as on the soma. In this latter study rats were reinforced on a progressive ratio schedule of reinforcement, which is thought to measure motivation for drug reinforcers.

Local delivery of drugs into the brain provides internal experimental control that is not afforded by systemic administration and lends neuroanatomical interpretation of the data, but from a therapeutic perspective, it is important to test whether global inhibition of α6β2* nAChRs will decrease the reinforcing efficacy of nicotine without having unintended effects on other behaviors. This research has been limited by the fact that the selective α6β2* nAChR peptide antagonists described above do not cross the blood brain barrier. Recent development of a putative selective antagonist of α6β2* nAChRs, BPiDI, has revealed that this novel compound reduces nicotine-induced DA overflow in striatal slices. Similar to local infusions of selective α6β2* nAChR antagonists into the accumbens shell or VTA, subcutaneous injection of BPiDI is effective at reducing nicotine self-administration, suggesting that this compound crosses the blood brain barrier. Further studies are needed to determine the selectivity of this drug to curb nicotine use as systemic administration of BPiDI also had some effect to inhibit food self-administration. Preclinical studies using ethanol as a primary reinforcer suggest that α6β2* nAChRs in the VTA may broadly regulate drug reward and the conditioned reinforcing properties of cues associated with drug reinforcers. Given the expression of α6β2* nAChRs in the visual system and substantia nigra, systemic effects of compounds that reduce α6β2* nAChR activity should also be assessed for potential visual and motor side-effects prior to advancement to clinical trials. Off target effects of drugs may be subverted using negative allosteric modulators or partial agonist drugs. Varenicline, which is a partial agonist at α6β2* nAChRs as well as α4β2* nAChRs, is purported to have its beneficial effect on smoking cessation via its partial agonist properties at β2* nAChRs. Varenicline’s full agonist properties at α7 nAChRs may also prove relevant for its efficacy in smoking cessation as is discussed below. Incomplete inhibition of β2* nAChRs with the partial agonist rather than a full antagonist is intended to circumvent precipitation of withdrawal while blunting nicotine reinforcement. Studies in mice, however, show that global CNS delivery of a selective α6β2* nAChR antagonist peptide reduces “affective” withdrawal and has no effect on somatic withdrawal behavior in mice. Together these behavioral and pharmacological and genetic data suggest that inhibition of α6β2* nAChRs may be an effective strategy to promote tobacco cessation in human subjects, but there is a need for further development and study of selective α6β2* nAChR antagonists, partial agonists and negative allosteric modulators that cross the blood brain barrier.
3.2 Selective activation of α7* nAChRs decreases motivation for nicotine use

Compared to β2* nAChRs, it has proved more difficult to identify contributions of α7 nAChRs to nicotine reinforcement. Early studies in α7 null mutant mice suggested that α7 nAChRs were not critical for nicotine discrimination and that a lack of α7 nAChRs had little impact on nicotine place conditioning or initiation of oral nicotine self-administration. Studies testing nicotine self-administration in rats have returned mixed results using methyllycaconitine (MLA), an α7 nAChR antagonist with poor penetration of the blood brain barrier. Moreover, since MLA significantly blocks α-conotoxin MII-sensitive release of dopamine and binding, it is not clear if reductions of intravenous nicotine self-administration observed following MLA exposure are due to α7 nAChR antagonism or due to blockade of α6β2* nAChRs. Using a highly selective α7 nAChR antagonist, ArIB, local inhibition of α7 nAChRs in anterior cingulate cortex or the NAc shell results in a 3 fold increase in rat responding for nicotine under progressive ratio schedules. Given that the low doses of nicotine achieved under this stringent schedule of reinforcement is not likely to result in a net stimulation of α7 nAChRs, this increased motivation for nicotine is likely due to blockade of endogenous ACh or choline signal at these receptors rather than a blockade of nicotine signal. Knockout mice lacking their α7 nAChRs have reported reductions in oral preference for nicotine compared to their wild type counterparts, but it is not clear from the two bottle choice paradigm if α7 null mutant mice “like” nicotine less if they are titrating their doses because they are more sensitive to nicotine. The latter hypothesis is supported by recent data showing that nicotine conditioned place preference is shifted to the left in α7 nAChR knockout mice at exceptionally low nicotine doses. This preclinical evidence in rodents may provide some insight into the heavy smoking behavior of individuals with schizophrenia, who post mortem studies show have a 50% decrease in expression of α7 nAChRs. Evidence from a preclinical animal model of smoking cessation efficacy shows that stimulation of α7 nAChRs decreases nicotine self-administration. In contrast to antagonist studies, local infusion of a highly selective α7 nAChR agonist into the NAc shell, like antagonism of α6β2* nAChRs, results in significant decreases in motivation to self-administer nicotine as measured by reduced responding under progressive ratio schedules. In support of rat self-administration which suggests that stimulation of α7 nAChRs reduces nicotine reinforcement, administration of a selective α7 nAChR agonist blocked nicotine reward behavior in the place conditioning paradigm and α7 gain of function mice failed to demonstrate nicotine reward in this paradigm at a wide range of doses.

It would appear from these studies that rather than its activation being required for nicotine reinforcement, stimulation of α7 nAChRs modulates self-administration behavior. Selective removal of the α7 nAChR function removes this modulation, leaving the α6β2* and other heteromeric β2* nAChRs unopposed as depicted in the model shown in Figure 3. Unlike β2 subunit null mutant mice which fail to show nicotine-stimulated DA release, nicotine stimulated DA release is significantly elevated in the NAc of α7 nAChR knockout mice. Whether α7 nAChRs directly or indirectly decrease α6β2* nAChR function in the NAc shell or if the two subtypes of nAChRs have their primary effects via a common downstream effector is unclear. Within the VTA, however, recent studies indicate that inhibition of α7 nAChRs on DA neurons leads to elevated β2* nAChR...
activity on these neurons and that activation of $\alpha_7$ nAChRs, via changes in peroxisome proliferator-activated receptors type-$\alpha$ signaling, leads to subsequent decreases in $\beta_2^*$ nAChR function. $^{94}$ Independent of mechanism, together these findings support stimulation of $\alpha_7$ nAChRs as a novel means by which to curb tobacco use. Further studies assessing systemic administration of compounds that stimulate $\alpha_7$ nAChRs on nicotine addiction phenotype are needed. Due to potential benefits for cognition and memory, drug development on this front has received more focus than compounds targeting $\alpha_6^* \beta_2^* $ nAChRs. A number of selective agonists and positive allosteric modulators of $\alpha_7$ nAChRs are being developed for other uses and are already in clinical trials.$^{142}$ Preclinical and clinical studies of these ligands may reveal a novel therapeutic for smoking cessation.

**Part 4: Pharmacological tools for inhibition of $\alpha_6^* \beta_2^*$ and activation of $\alpha_7$ nAChRs: implications for drug development**

### 4.1 Targeting $\alpha_6^* \beta_2^*$ nAChRs and $\alpha_7$ nAChRs for tobacco cessation

With the relatively rapid delivery of nicotine achieved with cigarettes, a phase of high affinity receptor activation is typically followed by the onset of equilibrium desensitization of nAChRs. As detailed above, activation of mesolimbic $\alpha_6^* \beta_2^*$ nAChRs occurs at lower concentrations than for $\alpha_4^* \beta_2^*$ nAChRs that do not express $\alpha_6$ $^{78}$ and several studies suggest that populations of $\alpha_6^* \beta_2^*$ nAChRs also show a slower decay rate, suggesting that $\alpha_6^* \beta_2^*$ nAChRs may have longer periods of net activation than other subclasses of nAChRs.$^{77, 78, 143, 144}$ An accumulation of preclinical data showing that inhibition of $\alpha_6^* \beta_2^*$ nAChRs reduces nicotine-stimulated DA release, nicotine reward and intravenous nicotine self-administration,$^{3, 5, 9, 10, 47, 48, 115}$ supports inhibition of $\alpha_6^* \beta_2^*$ nAChRs as a strategy for tobacco cessation. In contrast, the $\alpha_7$ nAChRs are less likely than heteromeric nAChRs to either be activated or stabilized in desensitized states by levels of nicotine achieved in the brains of smokers, but these receptors are responsive to both ACh and its metabolic precursor and breakdown product, choline.$^{100, 101}$ Rather than their activation being required to support nicotine self-administration, $\alpha_7$ nAChRs appear to act as modulators of nicotine reinforcement that work in opposition to the $\alpha_6^* \beta_2^*$ and $\alpha_4^* \beta_2^*$ nACHRs. In contrast to findings with $\alpha_6^* \beta_2^*$ nAChRs, selective genetic and pharmacological inhibition of the $\alpha_7$ nAChRs increases motivation to intravenously self-administer nicotine and results in elevated nicotine-stimulated DA release.$^{22, 141}$ Rather, as selective agonism of accumbens $\alpha_7$ nAChRs decreases motivation for nicotine self-administration, the data support stimulation of $\alpha_7$ nAChRs as a strategy to promote tobacco cessation as depicted in Figure 4.$^{22}$

### 4.2 Route of administration

Consideration should be given to route of administration when developing therapeutics for tobacco cessation. Therapeutics in their most common forms are slow delivery products. When a potentially activating molecule like nicotine is delivered slowly, as with systemic administration via a patch or pill, there is likely to be relatively little synchronized receptor activation, and, instead, receptors equilibrate amongst multiple conformational states.$^{145, 146}$ This equilibrium predominantly favors desensitization and thus also blunts receptor-mediated responses to endogenous cholinergic stimuli. This may explain why most forms of
nicotine replacement are effective in a small minority of smokers who are motivated to quit. Once deemed safe, faster delivery platforms, such as e-cigarettes, could provide an effective delivery system for compounds intended to stimulate nAChRs. Route of administration should not be a concern with delivery of antagonist and partial agonists intended to inhibit receptor activity as would be the strategy with α6β2* nAChRs, but the typical oral and cutaneous routes of drug administration could preclude sufficient delivery of an agonist that could selectively stimulate α7 nAChRs. As described briefly above and in detail elsewhere, however, the concentration-dependent form of α7 nAChR desensitization renders the α7 nAChRs uniquely sensitive to a class of highly selective positive allosteric modulators (PAMs) which can destabilize one or more desensitized states and thereby greatly increase the probability of channel opening. Also, as noted above, the desensitized states of α7 receptors can be further distinguished from those of heteromeric nAChR because they do not show significantly increased affinity for agonists compared to the resting state of the receptor. As a consequence, while the prolonged presence of a low concentration of ACh, or a drug like nicotine, will induce high levels of heteromeric receptor desensitization, α7 receptors will be largely unaffected and remain responsive to fluctuating stimuli generated from endogenous ACh signals. α7 nAChR PAMs may increase the sensitivity, efficacy and duration of these ACh signals.

4.3 Ligands that inhibit α6β2* nAChRs

There are two major challenges in designing therapeutics that selectively antagonize α6β2* nAChRs. The first has been to develop drugs that bind the α6 subunit without having appreciable affinity for α3* and α4* nAChRs in the absence of the α6 subunit. The second has been to develop compounds with high selectivity for α6β2* nAChRs that cross the blood brain barrier. The first of these challenges can be achieved by screening molecules against libraries of α6/α4 or α6/α3 chimeras in functional assays. This strategy has led to the development of peptide compounds with significant sensitivity for the α6 subunit over α3* nAChRs, which are prevalent in the autonomic ganglia and over α4* nAChRs, which are ubiquitously expressed throughout the brain. Recent studies utilized α-conotoxin BuIA, a compound with known binding selectivity for β2 over β4 and a 50,000 fold higher potency for α6β2* compared to α4β2* nAChRs to determine the sites of the α subunit interface that confer binding affinity and potency. These studies revealed that replacing residues 59-207 of the α4 subunit with the α6 results in >7000 fold increase in IC50 efficacy compared to its action at α4 with critical sights present on the C-Loop between residues 184 and 207 of α6. Cone snail toxins, which bind to vertebrate and mammalian α6* nAChRs, have provided a valuable, naturally-occurring template from which to develop compounds that selectively target α6β2* nAChRs; these large molecule peptides, however, do not readily penetrate the blood brain barrier. Adapting the size and lipophilicity of these peptides to encourage passive diffusion is challenging and the compounds may still have difficulty accessing the target receptors. Recent developments suggest that the saturable transport systems may prove to be a more efficient means of providing drug delivery to the CNS. The addition of structures intended to shepherd the α6β2* nAChR antagonists across transporters in the endothelial layer or choroid plexus could increase the bioavailability of α6β2* nAChR ligands, but should be carefully screened to assure that they do not alter compound affinity, selectivity or efficacy. To date there are no selective α6β2*
nAChR compounds in clinical trials, but the field is rife with scientific tools to support their development.

Partial agonists and negative allosteric modulators (NAMs) provide additional strategies to promote inhibition of α6β2* nAChRs. Although much more selective in their neuroanatomical expression than α4β2* nAChRs, a prevalence of α6β2* nAChRs in the nigra-striatal pathway and the visual system may call for therapeutic approaches that inhibit, rather than block neurotransmission at these receptors. Negative allosteric modulation of the α6β2* nAChRs could prove sufficient to reduce activity of mesolimbic α6β2* nAChRs without adversely impacting the function of these receptors in the visual and motor systems. The pharmacology of the partial agonist varenicline has a significant effect at α6β2* nAChRs. Recent development of novel bispidine β2* nAChRs partial agonist compounds with a lower affinity than varenicline for ganglionic α3β4 nAChRs may also promote smoking cessation while reducing side effects, but these drugs need to be tested for their affinity to α6β2* nAChRs and for their capability to reduce nicotine self-administration. As we have come to understand that α6β2* nAChRs are a critical subclass of β2* nAChRs that support nicotine reinforcement, a more selective α6β2* nAChR partial agonist may prove to have greater efficacy or reduced side effects in smokers. Since varenicline is a full agonist at α7 nAChRs, albeit with lower affinity than for its action at β2* nAChRs, it would be of further interest to discover if there is synergy in a combined therapeutic approach that supports inhibition of α6β2* nAChRs and stimulation of α7 nAChRs.

4.4 Ligands that stimulate α7 nAChRs

Selective stimulation of α7 nAChRs is an innovative strategy for smoking cessation treatment. A number of highly selective α7 nAChR agonists and PAMs of diverse structure have been developed in recent years. Full and partial α7 nAChR agonist drugs mimic the effects of cholinergic and nicotine stimulation, but unlike global nAChR stimulation, preclinical studies show that selective agonism of α7 nAChRs reduces motivation for nicotine use. Agonist drugs lead to rapid desensitization of nAChRs, but as mentioned above, the desensitized state of α7 nAChRs is not as stable as that of the high affinity heteromeric nAChRs.

Another means of achieving stimulation of α7 nAChRs is via positive allosteric modulation of these receptors. As opposed to a full agonist, which mimics the effects of ACh, choline and nicotine to activate the α7 nAChRs, a nAChR PAM exerts no nAChR activity on its own, but enhances the efficacy of full agonist via binding at an alternate, allosteric, site. Type I PAMs may lower the activation threshold of nAChRs to agonist ligands. Type II PAMs additionally increase nAChR responsiveness to agonist ligands, enhance channel current amplitudes and slow the decay of channel current activity, suggesting that Type II PAMs destabilize nAChR desensitization to increase equilibrium current (e.g. Figure 4C). Although PAMs which have intrinsic allosteric agonist activity have been described, a true α7 nAChR PAM does not activate α7 nAChRs on its own, but in binding to an allosteric site (different from the orthosteric nicotine/ACh binding site) selectively enhances nicotine and ACh activity at the α7 nAChRs. Hence, administration of
PAMs might be expected to decrease motivation for tobacco when individuals are smoking cigarettes or could increase the efficacy of nicotine replacement therapies. Because they have no effect on their own, nAChR PAMs may be less likely than agonist compounds to result in undesirable side effects, but careful screening of these compounds is warranted to ensure that exaggerated receptor stimulation does not cause toxicity, particularly if used in combination with nicotinic agonists. In individuals who have reduced expression of α7 nAChRs, such as those with schizophrenia diagnosis, in vitro α7 nAChR expression studies using the truncated, dominant negative α7 duplicate gene suggest that treatment with a Type II α7 PAM may promote stimulation of these receptors. Both α7 nAChR full agonist and α7 PAM dosing may need to be adjusted for these individuals, although preclinical studies suggest that PAMs may be less likely than a full agonist to lead to changes in α7 nAChRs that could affect dosing after repeated exposure. A number of selective α7 nAChR agonists and PAMs have been developed for treatment of cognitive and attention deficits that are evident with CNS disorders, many of which have advanced to clinical trials. With development of clinical trials on tobacco cessation, there is promise that some of these compounds may be repurposed or prescribed off label to help individuals quit smoking.

Part 5: Summary

Smoking continues to be a major public health concern. Although existing therapeutics for smoking cessation have appreciated some success in assisting smokers to quit, a number of individuals have been unresponsive or intolerant to existing treatments. As individuals have a diversity of reasons for smoking, they may also benefit from different cessation strategies for quitting. An accumulation of recent studies identify the heteromeric α6β2* and homomeric α7 nAChRs as promising novel therapeutic targets to promote tobacco abstinence. A combination of electrophysiological, pharmacological and genetic studies have begun to suggest that the efficacy of drugs which inhibit the α4β2* nAChRs may indeed be targeting α4α6β2* nAChRs. Given their more selective neuroanatomical distribution, drugs which more selectively inhibit α6β2* nAChR function could appreciate therapeutic success with fewer side effects. The broad assumption that activation of nAChRs promotes nicotine reward and reinforcement had resulted in the conclusion from early studies in α7 nAChR knockout mice that these low affinity receptors were not critical for nicotine addiction. An accumulation of recent data has begun to identify that unlike β2* nAChRs, an inverse relationship exists between α7 nAChR function and nicotine reinforcement and reward. Reductions in α7 nAChR function achieved by genetic or pharmacological manipulation promotes nicotine addiction phenotype whereas selective stimulation of α7 nAChRs reduces nicotine reward and motivation to self-administer nicotine. Together these studies identify novel classes of nAChRs and unique strategies for treatment of tobacco dependence.

Acknowledgments

The authors thank Dr. Nicole Horenstein for helpful comments. The authors of this review are supported by the following grant support: Virginia Foundation for Healthy Youth grant 8520893 and NIH grants R01 DA031289 and UH2 TR000958 to DHB, R01 GM103801 and R01 GM48677 to JMM, and R01 GM57481 to RLP. Virginia Commonwealth University holds a provisional patent application, Serial No.:61/835,872 "Alpha7 nicotinic
acetylcholine receptor (nAChR) stimulation to promote smoking cessation” with D.H. Brunzell as an inventor. The University of Utah holds patents on α-conotoxins with J.M. McIntosh as an inventor. The University of Florida has a pending patent application, U.S. Patent Application Docket No. UF-222106-8770, “Compositions, methods of use, and methods of treatment for nicotine dependence in high risk patients” with R.L. Papke as an inventor.

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Ann N Y Acad Sci. Author manuscript; available in PMC 2015 October 01.


Figure 1.
Classes of nicotinic acetylcholine receptors (nAChRs) and contributing subunits. Competitive agonists bind to a site formed by the interface of α and non-α subunits. In neuronal nAChRs, ligand binding occurs at the α-β subunit interface. In muscle nAChRs, binding occurs at α-δ, α-γ and α-ε subunit interfaces. Because the muscle nAChR β1 subunit does not bind agonist, therapeutic compounds for smoking cessation can selectively target neuronal nAChRs without producing adverse off-target effects at the muscle receptors. The

<table>
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<th>Heteromeric nAChR</th>
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<tr>
<td>Ligand-binding dimers</td>
<td>Structural</td>
</tr>
<tr>
<td>α1 δ</td>
<td>α1 γ</td>
</tr>
<tr>
<td>Embryonic only</td>
<td>Adult only</td>
</tr>
</tbody>
</table>

**Embryonic**

- δ
- α1
- γ
- β1

**Adult**

- δ
- α1
- ε
- β1

---

**“Neuronal”-types**

<table>
<thead>
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<th>Ligand-binding alphas</th>
<th>Ligand-binding betas</th>
<th>Structural</th>
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<tbody>
<tr>
<td>α2</td>
<td>α3</td>
<td>α4</td>
</tr>
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</table>

**“Homomeric” nAChR**

- α7 (α8)
- α9
- α10
accessory subunits, noted here for their structural contributions, can also contribute to ligand binding affinity and the channel properties of the receptor when bound by agonist.
Figure 2.
Models for the activation and desensitization of heteromeric nAChRs. Cartoons depict a cross section of heteromeric nAChRs, viewed as if the structural subunit were removed. The characteristic conformational states of the nAChRs highlight the transmembrane topology and key elements associated with ligand binding and conformational change. In the unbound receptor (resting state) the C-loop of each subunit, which is located over the presumptive agonist binding site, extends away from the surface in the “Apo-conformation”, and the Cys-loop is in proximity to the short extracellular loop (ECL) between transmembrane domains TM2 and TM3. When an agonist diffuses into the binding pocket, a series of conformational changes are energetically favored that lead to a reconfiguration of the C-loop closing in on the ligand, and the dissolution of point-to-point amino acid interactions that are stable in the resting state. This begins a wave of conformational change that is transmitted through the protein and promotes an effect at the Cys-loop-ECL interface which causes the conformational change to encompass the formation of the ion channel, resulting in the activated state. It is important to note that stimulation of nAChRs does not typically occur in isolation but en mass. In moving from the unbound resting state to the fully bound resting state, pools of nAChRs will initially show synchronized behavior with many channels open all at once for a brief period of time. Subsequent to an initial period, when many channels will be open with high probability, channels will begin to accumulate in the desensitized state and remain there with greatest probability until levels of agonist occupancy are reduced following the diffusion or metabolism of transmitter.
Figure 3.
A model of nicotinic receptor subtype contributions to nicotine reinforcement and DA release as might be expected with increasing concentrations of nicotine (depicted in gray). Nicotine and endogenous ACh are depicted as blue triangles. A) Unbound α7 nicotinic acetylcholine receptors (nAChRs) are inactive and at rest at low concentrations of nicotine. 300 nM nicotine that is sub-threshold to stimulate α7 nAChRs preferentially desensitizes α4β2* nAChRs. In the VTA, α4α6β2* nAChRs on DA neurons are persistently activated by this physiologically relevant concentration of nicotine believed to be achieved in the brains of smokers. B) Higher concentrations of 1-3 μM nicotine activate α4β2* as well as α6β2* nAChRs. After activation, these receptors stabilize in a desensitized state, but fast application of nicotine activates sufficient subpopulations of these receptors to support nicotine reinforcement. Genetic and pharmacological manipulations that block activation of α6β2* or α4β2* nAChRs, lead to reductions in nicotine self-administration and nicotine stimulated DA release, hence, activation of β2* nAChRs appears necessary for nicotine reinforcement. C) With higher concentrations of nicotine (10 μM), nicotine reinforcement would be satiated or reduced in smokers. The high affinity α4β2* and α6β2* nAChRs would be shifted to the desensitized state and hence, block reinforcing efficacy of subsequent applications of the drug. Higher concentrations of nicotine sufficient to stimulate populations of α7 nAChRs would reduce motivation to self-administer nicotine. Stimulation of α7 nAChRs by endogenous ACh would have a similar behavioral effect and stimulation of α7 nAChRs would have an indirect effect to inhibit nicotine reinforcement via stimulation of signaling pathways that inhibit β2* nAChR function on VTA DA neurons. Individuals who have blunted α7 nAChRs, such as those with schizophrenia, may smoke more heavily than individuals with a full complement of α7 nAChRs. α7 nAChR agonist ligand bound to more than two binding sites favors the desensitized state of these receptors. *Denotes possible assembly with other subunits. β3 is the typical accessory subunit that
assembles with α6β2* nAChRs. The high sensitivity α4β2* nAChRs, those with β2 rather than α4 or α5 at the accessory site, are depicted above.
Figure 4.
A functional model for α7 and α6β2* nAChRs as therapeutic targets for smoking cessation. Nicotine and endogenous ACh are depicted as blue triangles; increasing concentrations of nicotine are shown in gray. A) Low concentrations of nicotine favor activation of α6β2* nAChRs that support nicotine reinforcement. Based upon preclinical research, which reveals that selective stimulation of α7 nAChRs reduces motivation to self-administer nicotine and blunts β2* nAChR function, stimulation of α7 nAChRs with a full agonist (red triangle) or a positive allosteric modulator (red plus) ought to reduce nicotine reinforcement and motivation to smoke cigarettes. α7 Type I PAMs increase sensitivity of the α7 nAChRs so that lower than normal concentrations of nicotine and ACh result in a net activation of α7 nAChRs. B) As has been shown with nicotine self-administration in rodents, an animal model with good predictive validity for smoking cessation efficacy, direct inhibition of α6β2* nAChRs with an α6β2* nAChR full or partial antagonist (black X) ought to be sufficient to reduce smoking urges. C) Type II PAMs prevent desensitization as well as promote stimulation of nAChRs. An α7 nAChR PAM ought to decrease nicotine’s reinforcing efficacy, perhaps via indirect inhibition of high sensitivity α4β2* and α6β2* nAChRs.