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Review

Nicotinic receptor-based therapeutics and candidates for smoking cessation

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ABSTRACT

Tobacco dependence is the most preventable cause of death and is a chronic, relapsing disorder in which compulsive tobacco use persists despite known negative health consequences. All currently available cessation agents (nicotine, varenicline and bupropion) have limited efficacy and are associated with high relapse rates, revealing a need for more efficacious, alternative pharmacotherapies. The major alkaloid in tobacco, nicotine, activates nicotinic receptors (nAChRs) which increase brain extracellular dopamine producing nicotine reward leading to addiction. nAChRs are located primarily presynaptically and modulate synaptic activity by regulating neurotransmitter release. Subtype-selective nAChR antagonists that block reward-relevant mesocorticolimbic and nigrostriatal dopamine release induced by nicotine may offer advantages over current therapies. An innovative approach is to provide pharmacotherapies which are antagonists at nAChR subtypes mediating nicotine evoked dopamine release. In addition, providing multiple medications with a wider array of targets and mechanisms should provide more treatment options for individuals who are not responsive to the currently available pharmacotherapies. This review summarizes the currently available smoking cessation therapies and discusses emerging potential therapeutic approaches employing pharmacological agents which act as antagonists at nicotinic receptors.

Contents

Abbreviations: bPiDDB, *N,N'*-dodecane-1,12-diyl-*bis*-3-picolinium dibromide; r-bPiDDB, reduced-bPiDDB; *, indicates putative nAChR subtype assignment.

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1. nAChRs and smoking

1.1. Introduction

Tobacco dependence is the most preventable cause of death and is a chronic, relapsing disorder in which compulsive tobacco use persists despite its known negative health consequences [1–3]. Relapse typically occurs within the first month of cessation in ~80% of tobacco smokers attempting to quit, and after six months there is only a 3% success rate [4]. A strong correlation has been reported between tobacco smoking and mood disorders [4–7]. Clinically depressed individuals are more likely to smoke tobacco, to be nicotine dependent, and to have more difficulty with cessation and greater withdrawal symptoms [8–11]. In addition to depression, other neuropsychological diseases (e.g., schizophrenia and Tourette's syndrome) are known to be comorbid with tobacco dependence [12–16]. This review summarizes currently available smoking cessation therapies and novel therapeutic approaches employing pharmacological agents which act at nicotinic acetylcholine receptors (nAChRs).

1.2. Diversity of nAChRs

nAChRs are members of the Cys-loop family of ligand-gated ion channel receptors, and consist of pentameric transmembrane proteins with diverse composition [17,18]. Nicotine activates all known nAChR subtypes, with varying affinities [19–22]. Significant functional diversity is suggested by the identification of 12 genes encoding α2–α10 and β2–β4 subunits and based on results from *in situ* hybridization studies which reveal discrete, but overlapping, CNS distribution of mRNAs encoding these subunits [23–30]. Although nAChR subtype predominance does not necessarily reflect functional importance, the α4β2* subtype is predominant in the CNS and is probed by high affinity [^{3}H]nicotine binding to brain membranes [31–33]. Immunoprecipitation studies indicate that more than two different subunits assemble to form functional receptors and individual neurons elaborate multiple subtypes [19,34–38], further increasing complexity, diversity and challenges associated with the elucidation of the function of specific native nAChR subtypes. In addition to heteromeric nAChRs, homomeric nAChRs consist of α7, α8, α9 or α10 [30,31,39,40]. α7* nAChRs are the second most abundant in brain [17,30,31], and are probed by [^{3}H]methyllycaconitine binding to brain membranes [41–44]. The exact subunit composition, stoichiometry and arrangement of native nAChRs remains to be elucidated conclusively [45]. Nevertheless, evidence indicates that nAChR subunit composition has an important impact on pharmacological sensitivity, including agonist and antagonist affinity at the nAChR binding site [23,46–49].

1.3. nAChRs and neurotransmitter release

Nicotine activation of nAChRs increases brain extracellular DA which mediates, at least in part, nicotine reward and leads to nicotine addiction [50,51]. Mesocorticolimbic and nigrostriatal DA systems, including the nucleus accumbens (NAcc), medial prefrontal cortex (mPFC), striatum and associated circuitry, have been implicated in drug reward. The NAcc shell is believed to encode primary appetitive stimuli associated with unconditioned reward produced by nicotine [50,52,53]. mPFC encodes secondary conditioned stimuli associated with environmental cues paired with nicotine, and integration of motivational information from mPFC occurs in striatum leading to initiation and execution of movement in reward expectancy and detection [52,54]. In these brain regions, nAChRs are located primarily presynaptically and modulate synaptic activity by regulating neurotransmitter release [21,24,39,55–62].

Rat substantia nigra neurons express mRNA for α3, α4, α5, α6, α7, β2, β3 and β4 subunits [25,26,30,63,64] and express multiple subtypes that may be involved in nicotine-evoked striatal DA release. Studies using β2 knockout mice reveal that β2 is necessary for nicotine-evoked DA release [58,65–70]. Subtype assignment of native nAChRs mediating nicotine-evoked DA release is based largely on inhibition of agonist-induced responses by subtype-selective antagonists, defined by their inhibitory activity in cell systems expressing nAChR subunits of known composition. A major role for α6 and β3 in nicotine-evoked DA release in striatum is based on both knockout and gain-of-function studies [71,72]; these subunits are highly expressed in substantia nigra and the ventral tegmental area (VTA) [25,27,64,71,73]. α-Conotoxin MII (α-CtxMII) inhibits nicotine-evoked [^{3}H]DA release from striatal preparations [74–78]. Although α-CtxMII was thought to be a selective antagonist for α3-containing subtypes, the finding that ^{125}I-α-CtxMII binding remains in α3 knockout mice, but is abolished in α6 knockouts, provides supports that α-CtxMII is a selective antagonist at α6-containing nAChRs [79–81]. Novel α-Ctx peptides (e.g., α-CtxPIA) have higher selectivity for α6-containing over α3-containing nAChRs and inhibit nicotine-evoked [^{3}H]DA release from rat striatum [82]. Although subtype-selective α-Ctx peptide antagonists represent useful pharmacological tools, mechanistic interpretations should be made with caution. For example, specific nAChR subtypes may display higher affinity for these α-Ctx peptides; however, one cannot rule out the possibility that these molecules also inhibit other nAChRs subtypes with lower potency, as well as other subtypes that have not yet been fully elucidated but contribute to the functional response. Further, it is unlikely these neurotoxin peptides will be developed into pharmacotherapies for tobacco cessation, in part due to their poor brain bioavailability and their susceptibility to cleavage by peptidases. Thus, these molecules will likely only be useful as pharmacologic tools.

Results from a comprehensive molecular genetics study in which an individual subunit gene (α4, α5, α7, β2, β3, and β4) has been deleted suggest that 6 different subtypes, including α-CtxMII-sensitive (α6β2β3*, α4α6β2β3*, α6β2* and α4α6β2*) and α-CtxMII-insensitive (α4β2* and α4α5β2*) subtypes, mediate nicotine-evoked DA release from mouse striatal synaptosomes, whereas deletion of β4 and α7 subunits had no effect [67,83]. The α4α6β2β3* subtype constituted ~50% of α6-containing nAChRs on DA terminals of wild-type mice and has the highest sensitivity to nicotine of any native nAChR subtype [59,84], strongly implicating α4α6β2β3* in nicotine-evoked DA release. Thus, different subtypes mediate nicotine-evoked DA release, suggesting that small molecule antagonists could differentially target these sites to selectively inhibit nicotine-evoked DA release and reward.

Although DA is of major interest in nicotine addiction, evidence exists that norepinephrine (NE) also plays a role [85–87]. Nicotine evokes NE release from rat hippocampal [88–92] and cortical

[93,94] synaptosomes and slices, and releases NE in hypothalamus as shown in *in vivo* microdialysis studies [93]. Nicotine-induced NE release modulates DA function, and thereby, may contribute indirectly to nicotine addiction [95]. α3, α4, α5, α6, α7, β2, β3 and β4 subunit mRNAs are expressed in locus coeruleus, providing potential subtype diversity in NE cell body and terminal regions [26,27,30,96–98]. The nAChR subtype most associated with mediating nicotine-evoked NE release from hippocampal synaptosomes is α3β4*, based on inhibition (34%) by α-CtxAuIB, whereas α-CtxAuIB does not inhibit nicotine-evoked DA release from striatal synaptosomes [89,99]. These same studies show that α-CtxMII does not inhibit nicotine-evoked NE release from rat hippocampus, suggesting that α6β2-containing nAChRs are not involved in this response. More recently, α-CtxBuIA (which distinguishes β2 from β4) was used to evaluate nAChRs (α6α4β2β3β4 and α6α4β2β3) contributing to nicotine-evoked NE release in mouse hippocampus; interestingly these subtypes were distinct from those mediating nicotine-evoked NE release in rat hippocampus, i.e., α3β4* and αxβ4*, but not α6 [100]. These results indicate that nAChR subtypes mediating nicotine-evoked NE release include β4 in rat, whereas subtypes mediating this effect in mouse hippocampus include β2 and β4 subunits. Thus, species differences need to be taken into consideration with regards to the relative contribution of various subtypes in mediating nicotine-evoked NE release. Similarly, species differences have also been noted with respect to nicotine-evoked DA release. While α6-containing nAChRs comprise 30% of presynaptic nAChRs mediating nicotine-evoked DA release in mice, 70% of nAChRs mediating nicotine-evoked DA release are α6-containing in non-human primates [67,77,101,102]. Another issue for consideration is the proportional amount of DA release mediated by α6β2-containing nAChRs differs among brain regions. For instance, while α-CtxMII only slightly diminished DA release evoked by electrical stimulation in the dorsal striatum, DA release in the nucleus accumbens was almost completely eliminated [103,104].

1.4. Nicotine-mediated changes in nAChRs

Increases in nAChR expression as a function of repeated nicotine administration has been well described in the literature (for review see [105]). Nicotine-mediated increases in nAChR expression are subtype specific, with some nAChR subtypes resistant and some sensitive to up-regulation. Subtypes containing α2, α3 and α5 are not thought to be up-regulated by chronic nicotine administration [106,107]. Repeated activation of α4-containing nAChRs results in receptor up-regulation [108,109]. Subtypes containing β2 subunits are also up-regulated following repeated nicotine, and deletion of the β2 subunit eliminates receptor up-regulation [102]. This may be of particular importance, given that β2 subunits are thought to be present in all nAChRs that mediate nicotine-evoked DA release [67,84]. Conversely, the effect of repeated nicotine administration on α6-containing receptors is less clear, with studies showing up-regulation [22], down-regulation [110,111] and no change [72,112].

Additional regulatory response to repeated nicotine administration include altered subunit stoichiometry, specifically the stoichiometry of α4β2 nAChRs (α4(2)β2(3) and α4(3)β2(2)). The functional consequences of this altered stoichiometry have been shown to be variation in agonist (e.g., nicotine and acetylcholine) and antagonist (e.g., mecamylamine) sensitivities, rate of desensitization and calcium permeability [22,113–117]. The observation that partial deletion of the α4 and β2 subunit genes changes acetylcholine sensitivity of $^{86}Rb^{+}$ efflux in cortex and thalamus supports the conclusion that α4β2 exists in different stoichiometries in native tissues [118]. Chronic nicotine has been shown to up-regulate the high sensitivity α4β2 isoform, and may also down-regulate midbrain α6-containing nAChRs [110]. Since the composition of nAChRs is more complex than initially thought, taken together with the observation that all subunits expressed in a subtype contribute to antagonist sensitivity, there may be opportunities to take advantage of this complexity and dynamic responsiveness to set the stage for discovery of subtype-selective nAChR antagonists, particularly antagonists targeted at the specific nAChR subtypes important for treating smoking cessation.

1.5. nAChR desensitization

Nicotine both activates and desensitizes nAChRs [119–121]. Activation of nAChRs occurs when nicotine or endogenous acetylcholine binds at the interface of two α subunits or an α and β subunit, resulting in a conformational change that opens the channel pore and allows sodium and calcium influx [122,123]. Desensitization is defined as a decline in response to nicotine following repeated exposure [124]. The kinetics of both receptor activation and desensitization are subtype-dependent [120,125,126]. For example, a recent study found that α7* nAChRs had faster activation than α4β2* containing receptors, and that nAChRs sensitive to cytisine (i.e., β4* nAChRs) had faster activation than all other subtypes [127,128]. The β subunit has a strong influence on desensitization kinetics, with nAChRs that contain β2 subunits having a much faster rate of desensitization than nAChRs with β4 subunits [129]. α Subunits have also been shown to modulate desensitization kinetics with α4 subunits associated with slowly desensitizing currents [130]. Further, inclusion of α5 subunits increases the speed with which receptors desensitize [131]. Studies using *Xenopus* oocytes to express different nAChR subunits found that the speed with which heteromeric nAChRs desensitize can be rank ordered from fast to slow as α3β2 > α4β2 > α3β4 > α4β4 [132].

Interestingly, nAChR desensitization, and the resulting up-regulation that occurs as a result of diminished nicotinic functional activity, is thought to play a role in tolerance and craving [102,108,121,133]. A recent clinical trial found that typical tobacco use results in almost complete (88–95%) occupancy of α4β2* nAChRs, indicating that smokers maintain saturation of this nAChR subtype throughout the day. This study also purported that tobacco craving is only alleviated when receptor occupancy is at least 88%. Thus, when a sufficient percentage of previously desensitized nAChRs become unoccupied, and as a result recover to a responsive state, this leads to tobacco craving [134]. Several clinical studies using nicotine replacement to aide in tobacco smoking cessation support the above contention. Smokers administered a 21 mg nicotine patch 24 h/day had significantly lower craving during the first 2 weeks following cessation than smokers administered a 15 mg nicotine patch for only 16 h/day [135]. Moreover, smokers administered a 35 mg nicotine patch 24 h/day exhibited reductions in both withdrawal symptoms and craving and a significantly reduced risk of lapse [136]. Since the patch provides a constant supply of nicotine to an individual, it is highly likely that these results are mediated, at least in part, through continuous nAChR desensitization. Thus, repeated nicotine administration may act as a functional antagonist by inactivating nAChRs [105,137], implying that antagonist-induced inactivation of nAChRs has therapeutic potential for smoking cessation since the functional outcome is the same as agonist-induced desensitization.

2. nAChR partial agonists as smoking cessation treatments

2.1. Introduction

Currently available cessation agents have been shown to have limited efficacy and are associated with high relapse rates

[4,138–140], revealing a need for alternative more efficacious pharmacotherapies. In targeting nAChRs mediating nicotine-evoked DA and NE release for medication development, either subtype-selective agonists or antagonists can be developed. Each strategy has potential advantages and limitations. nAChR agonists (partial or full) are generally well-tolerated and produce good patient compliance, as they substitute for the reinforcing effect of tobacco use [4,138–140]. Partial and full agonists at nAChRs also provide relief from withdrawal symptoms that typify abstinence [141]. While nicotine replacement therapy has been the mainstay of smoking cessation therapeutics, several new and potential smoking cessation therapeutics are nAChR partial agonists. Partial agonists mimic nicotine replacement therapy by alleviating withdrawal symptoms and craving resulting from smoking cessation, while simultaneously reducing both nicotine reinforcement and the repetitive nicotine-induced phasic DA release mediated by nAChRs [142,143]. Compared with the full agonist, partial agonists may have lower abuse liability due to the less than maximal response with respect to neurotransmitter release [144]. However, the beneficial effects of agonist replacement therapy are less than optimal [145]. A potential disadvantage of nAChR agonist replacement therapy is that continued stimulation of nAChRs maintains the dependence induced by tobacco use. Thus, in the event of a relapse to tobacco use following nicotine replacement therapy, the reinforcing effect of nicotine self-administration may be reinstated rapidly.

2.2. Varenicline

Originally developed by Pfizer, Inc. in 1997 [146], varenicline (Chantix) is structurally related to the plant alkaloid cytisine (discussed below), and one of only three smoking cessation therapeutics currently approved by the United States Food and Drug Administration (FDA). Initial *in vivo* binding studies found that varenicline has high affinity for the $\alpha4\beta2$ nAChR subtype with little affinity for other subtypes [146]. Further, in rat brain slices, varenicline was found to release lower concentrations of DA (40–60% of that released by nicotine) [147]. Collectively, these findings suggested that varenicline is a partial agonist at $\alpha4\beta2^*$ nAChRs [146]. However, studies report that varenicline also is expressed as a full agonist at $\alpha7$ nAChRs in cell systems [148]. In humans, maximal absorption of varenicline occurs within 3–4 h of oral administration, and the drug has an elimination half-life of ~24 h [149], primarily through renal excretion [150]. Further, steady-state conditions are established within 4 days of oral administration in healthy adults [149].

Varenicline fully substitutes for nicotine in preclinical drug discrimination studies and blocks nicotine self-administration in rats [147]. According to several recent reviews that summarize the results from Phases 2 and 3 clinical trials, varenicline generally increases the chances of a successful quit attempt 2–3-fold greater than placebo [151,152]. A recent multicenter, randomized, double-blind placebo-controlled study found continuous abstinence rates of 44% for varenicline during 9–12 weeks after quitting, which was higher than the abstinence rates for patients treated with bupropion (30%) or placebo (18%) [153]. Further, while the majority of patients, regardless of therapeutic intervention, returned to a regular pattern of smoking during the 9–52 weeks follow-up period, abstinence rates among patients that received varenicline (22%) were still higher than those for patients receiving bupropion (16%) or placebo (8% [153]). In February, 2008, a public health advisory note was issued by the FDA, stating that patients taking varenicline experience serious neuropsychiatric symptoms, including behavior, agitation, depressed mood, suicidal ideation, and attempted and completed suicide [154]. However, the number of patients experiencing these behavioral changes is small, and an analysis of neuropsychiatric adverse events in the patients that participated in all nine completed, placebo-controlled clinical trials is currently being performed [143,153]. Results from these analyses will be important for evaluating the continued use of varenicline as a smoking cessation therapeutic.

2.3. Cytisine

As was discussed above, varenicline is a structural analog of cytisine, an alkaloid present in several plant species, including *Cytisus laburnum* [146]. Cytisine was originally characterized as a selective, partial agonist with high affinity for $\alpha4\beta2^*$ nAChRs [155]. In support of this, cytisine has been found to evoke [^{3}H]DA release from both striatal slices and synaptosomes *in vitro*, with a maximum effect ~50% of that found for nicotine [156,157]. The partial agonist properties of cytisine have also been demonstrated *in vivo*, where it increases the rate of DA turnover in the nucleus accumbens with a maximum effect ~40% of that found for nicotine [146]. However, similar to varenicline, cytisine interacts with additional nAChR subtypes, including $\alpha4\beta4$- and $\alpha6$-containing subtypes [158,159]. Further, the efficacy of cytisine appears to vary depending on the species and experimental system used [160].

Behaviorally, cytisine decreases locomotor activity in drug naïve rats, with a maximum effect lower than that produced by nicotine [161]. Cytisine had no effect on locomotor activity in rats that received repeated nicotine administration (0.4 mg/kg/day for 21 days) [161], indicating the development of cross-tolerance. In drug discrimination studies, cytisine substituted for nicotine [162]. In a limited number of studies, cytisine has been shown to have reinforcing properties. Drug naïve mice self-administer cytisine intravenously [163], providing evidence for its reinforcing properties. In other studies, cytisine has been shown to produce conditioned place preference in rats [164]. A limited number of clinical trials assessing the usefulness of cytisine in smoking cessation have been reported. A recent uncontrolled clinical trial reported smoking cessation efficacy rates (~14%) for cytisine comparable with that obtained with nicotine replacement therapy [165].

2.4. Dianicline

Another partial agonist currently in development by Sanofi Aventis, Inc. for use as a smoking cessation therapeutic is dianicline. Dianicline (SSR591813) is similar in structure to both varenicline and cytisine, although mechanistic information is somewhat limited [166]. Dianicline has a high affinity for human $\alpha4\beta2$ nAChRs and a low affinity for other nAChR subtypes expressed in *Xenopus* oocytes, HEK 293 cells and IMR-32 cells [166]. In electrophysiology studies, dianicline exhibited an E_{max} of 19% of the response to acetylcholine, indicating its action as a partial agonist [166]. In light of the recent studies demonstrating that varenicline and cytisine are not selective for $\alpha4\beta2^*$ nAChRs, it is likely that dianicline will also lack selectivity for $\alpha4\beta2^*$ due to its structural similarity with these two drugs. In drug discrimination studies, dianicline substituted for nicotine, although at doses that decreased the rate of responding [166]. In clinical trials, dianicline had a 16% success rate compared to 8% for placebo [167], however, in February, 2008, Sanofi Aventis announced that the development of dianicline was terminated [168].

2.5. Sazetidine-A

Sazetidine-A is a novel nAChR ligand reported to have high affinity and selectivity for $\alpha4\beta2$ nAChRs [169,170]. Affinity for $\alpha4\beta2$ was 4-orders of magnitude higher than for $\alpha3\beta4$ nAChR

subtypes expressed in cell systems [169,170], and prolonged incubation with this novel compound increased the density of α4β2 binding sites [169]. However, initial studies determined that sazetidine-A did not stimulate $^{86}Rb^{+}$ efflux from cells stably expressing α4β2 nAChRs, suggesting that this compound may not activate nAChRs [169]. Interestingly, preincubation with sazetidine-A reduced nicotine-evoked $^{86}Rb^{+}$ efflux that was not readily reversible [169], indicating that receptor desensitization had occurred. Thus, while sazetidine-A did not activate α4β2* nAChRs in this cell expression system, these receptors were up-regulated and desensitized. This mechanism of action has been termed "silent desensitization", since desensitization occurs without receptor activation. Interestingly, co-incubation of sazetidine-A with nicotine did not result in inhibition of $^{86}Rb^{+}$ efflux, whereas preincubation with sazetidine-A prior to incubation with nicotine inhibited completely the effect of nicotine. These results have been interpreted to indicate that sazetidine-A has low affinity for α4β2* nAChRs in the resting state, but high affinity in the desensitized state. Further, the results suggest sazetidine-A binds with high affinity to nAChRs that spontaneously convert to the desensitized state, trapping the receptors in this desensitized state and resulting in inhibition of channel function [169,137].

Recent studies using more sensitive voltage clamp techniques reported that sazetidine-A induces current amplitudes in cells expressing α4β2 nAChRs similar to amplitudes induced by acetylcholine [170], suggesting that this novel nAChR ligand acts as a agonist. In this study, sazetidine-A was also found to potently evoke [^{3}H]DA release from rat striatal slices and produced an E_{max} about 90% of that produced by nicotine. Sazetidine-A-evoked [^{3}H]DA release was inhibited completely by both mecamylamine and dihydro-β-erythroidine (DHβE [170]), indicating that the response is nAChR mediated. Further, the α6 nAChR selective antagonist α-CtxMII also inhibited sazetidine-A evoked [^{3}H]DA release with an I_{max} of 50%. Collectively, these findings suggest that both α4β2* and α6* nAChRs mediate sazetidine-A evoked [^{3}H]DA release. At 1000-fold higher concentrations, sazetidine-A also evoked [^{3}H]NE release from rat hippocampal slices, with an E_{max} of ~50% of the maximal response to nicotine, suggesting a partial agonist action. Similarly, the response to sazetidine-A was inhibited completely by both mecamylamine and DHβE [170]. Furthermore, sazetidine-A substitutes completely for nicotine in the drug discrimination assay [171], also consistent with a nAChR agonist action. Thus, in contrast to the initial results obtained using the $^{86}Rb^{+}$ efflux assay, sazetidine-A acts as a partial agonist at nAChRs in hippocampous and as a full agonist at nAChRs in striatum. As such, sazetidine-A may have utility as a smoking cessation agent similar to other nAChR agonists.

3. nAChR antagonists as smoking cessation treatments

3.1. Introduction

Given the availability of replacement therapies using either full or partial agonists or therapies that indirectly stimulate reward-relevant DA receptors, an innovative alternative approach is to provide pharmacotherapies which are antagonists at nAChR subtypes mediating neurotransmitter release associated with the reward produced by tobacco smoking. In this regard, subtype-selective nAChR antagonists that block reward-relevant mesolimbic DA release induced by nicotine may offer an advantage. Blockade of nAChRs mediating nicotine-evoked NE release may also be a viable target for preventing relapse, as NE neurotransmitter systems are implicated in nicotine seeking behavior [172,173]. Since relapse rates are high among smokers primarily due to the environmental cues that surround the experience of tobacco use [174,175], it may be inevitable that many tobacco users will lapse (e.g., smoke one cigarette) during a quit attempt. In this regard, it may be advantageous to use a medication that effectively blocks the pleasurable effect of the initial lapse, thus discouraging a full-scale relapse back to a pattern of regular smoking. In addition, providing multiple medications with a wider array of mechanistic targets should enhance the clinical utility among individuals who are not responsive to the currently available agonist pharmacotherapies. For example, mecamylamine has been shown to have clinical efficacy in double-blind, placebo controlled studies [3,176,177], but its nonselective inhibition of peripheral nAChR has produced untoward peripheral side effects that have precluded its clinical development. The significance of this alternative approach is that it will lead to the development of subtype selective antagonists, which retain and/or enhance the efficacy of mecamylamine, while exhibiting reduced and/or negligible peripheral side effects, thus breaking the impasse for developing a clinically useful nAChR antagonist.

3.2. Mecamylamine

Mecamylamine dose-dependently decreases nicotine self-administration, a behavioral task used to measure reward associated with drug administration in laboratory animals [178–186]. Pretreatment with mecamylamine also blocks performance in a progressive ratio (PR) model of nicotine self-administration [187]. Furthermore, cue-induced reinstatement of nicotine-seeking behavior, whereby re-introduction of an environmental cue associated with nicotine delivery reinstates extinguished nicotine seeking, is also blocked in rats by pretreatment with mecamylamine [188,189].

Building upon the findings in the preclinical literature, several clinical studies have investigated the therapeutic potential of mecamylamine in regards to tobacco use cessation [190]. Mecamylamine, a noncompetitive antagonist at all known central and peripheral nAChRs, reverses both positive and negative subjective effects of intravenous nicotine in smokers [191]. Mecamylamine alone was reported also to be beneficial in reducing smoking satisfaction [192]. In a randomized, double-blind placebo-controlled study, mecamylamine combined with a nicotine transdermal patch was shown to improve smoking cessation outcome for up to one year compared to nicotine alone [177]. Since mecamylamine is an open channel blocker, these results suggest that the presence of the agonist augments access of mecamylamine to its binding site within the receptor channel pore. Due to the lack of selectivity at nAChRs, including inhibition of peripheral nAChRs, the clinical utility of mecamylamine is limited by its anticholinergic side effects (e.g., constipation, hypotension [193]). These studies provide precedence for the use of nAChR antagonists as tobacco use cessation agents. A selective drug, which is targeted at central nAChRs that specifically mediate nicotine-evoked DA release, would be predicted to retain the beneficial therapeutic effects of a nAChR antagonist while averting the peripherally mediated side effects. Such an antagonist treatment may be especially useful for highly motivated individuals attempting to quit tobacco use.

3.3. Bupropion

The antidepressant, bupropion, has demonstrated benefit as a tobacco use cessation agent [139,140,172,194–198]. In addition to its antidepressant activity, which presumably derives from its ability to inhibit DA and NE transport into the presynaptic nerve terminal, bupropion is an effective and well-tolerated tobacco use cessation agent [196,199]. Similarly, reboxetine, a selective NE transporter (NET) inhibitor and effective antidepressant [200–202], decreases nicotine self-administration in rats [85] and

inhibits nicotine-evoked [^{3}H]NE release from superfused brain slices [203]. Taken together with the findings from the above studies with mecamylamine, these results provide rationale for determining if antagonists that selectively inhibit central nAChRs mediating nicotine-evoked DA and/or NE release will decrease nicotine self-administration and relapse.

Bupropion inhibits the function of both the DA transporter (DAT) and the NET, which likely contributes to its efficacy as a tobacco use cessation agent. Bupropion inhibits [^{3}H]DA uptake (IC_{50} = 2 μM) into striatal synaptosomes and [^{3}H]NE uptake (IC_{50} = 5 μM) into hypothalamic synaptosomes [3,176,177,204,205]. Increased extracellular DA and NE concentrations may substitute for nicotine-evoked neurotransmitter release as a result of tobacco smoking. However, nicotine reinforcement has been associated primarily with increased DA release [3,50,51,176,177,206]. Bupropion dose-dependently increases presynaptic vesicular DA uptake and redistributes vesicular monoamine transporter protein [207]. Important from the current perspective, bupropion acts as a nAChR antagonist, inhibiting (IC_{50} = 11 μM) nAChR agonist-induced $^{86}Rb^+$ efflux from cells expressing α3β4 ganglionic nAChRs, from human clonal cells expressing muscle-type nAChRs (IC_{50} = 1.5 μM [208], and from *Xenopus* oocytes expressing rat α3β2 (IC_{50} = 1.3 μM), α4β2 (IC_{50} = 8 μM) and α7 (IC_{50} = 60 μM) nAChRs [209]. $^{86}Rb^+$ efflux models K^+ efflux and is a functional assay for nAChRs [210–215]. Bupropion inhibition of nAChR function is not surmounted by increasing agonist concentration, and bupropion does not displace [^{3}H]nicotine binding to native nAChRs, consistent with allosteric inhibition [208,209]. Bupropion metabolites also inhibit nAChR function [216]. We evaluated the ability of bupropion to specifically inhibit native nAChRs mediating nicotine-evoked [^{3}H]DA and [^{3}H]NE release from rat striatal and hippocampal slices [217]. Bupropion inhibited nicotine-evoked [^{3}H]DA and [^{3}H]NE release (IC_{50} = 1.3 and 0.32 μM, respectively), indicating that bupropion acts as a nAChR antagonist at subtypes mediating this response. DAT and NET were not mediating the bupropion-induced inhibition of nicotine-evoked neurotransmitter release since the superfusion buffer included saturating concentrations of nomifensine or desipramine. Bupropion concentrations that inhibit DAT and NET did not inhibit field stimulation-evoked [^{3}H]DA release, suggesting mediation by nAChRs. Bupropion-induced decreases in smoking may result from one or both mechanisms (i.e., nAChR antagonism and DAT/NET inhibition), both of which may contribute to its smoking cessation and antidepressant efficacy.

3.4. UCI-30002

Another novel antagonist currently being examined for potential use as a smoking cessation therapeutic is UCI-30002 [218]. UCI-30002 is a positive allosteric modulator of $GABA_A$ receptors [219]; however, since $GABA_A$ and nAChRs are both members of the ligand-gated ion channel superfamily, it was hypothesized that this novel compound may have efficacy as an allosteric modulator at α4β2* nAChRs [218]. UCI-30002 inhibited nicotine-evoked currents in *Xenopus* oocytes expressing neuronal α4β2, α7 and α3β4 nAChR subtypes [218]. Further, UCI-30002 also inhibited nicotine-evoked currents in oocytes expressing muscle-type nAChRs. Thus, UCI-30002 may act as a negative allosteric modulator of nAChRs similar to its action at $GABA_A$ receptors [218], although additional evidence is needed to support this mechanism of action.

With regards to behavioral effects, UCI-30002 inhibited high-dose, nicotine-induced seizures [218]. More importantly, UCI-30002 decreased nicotine self-administration on both a fixed ratio 5 (FR5) and a PR schedule [218], suggesting that this compound decreases nicotine reward. Further, UCI-30002 did not alter food-maintained responding, indicating that it specifically decreased responding for nicotine [218]. While additional studies are needed, these results are promising and suggest that UCI-30002 may have potential as a smoking cessation therapeutic. Thus, negative allosteric modulators of nAChRs may constitute an unexplored target for the development of novel therapeutics to treat nicotine addiction.

3.5. bPiDDB and r-bPiDDB

Development of antagonists selective for nAChRs mediating nicotine-evoked DA and NE release should retain therapeutic efficacy as smoking cessation agents without producing peripheral side effects. Unfortunately, no compounds are available for clinical use that have this profile. In this regard, *N-n*-alkylnicotinium analogs with C_7-C_{12} *N-n*-alkyl groups potently inhibit nicotine-evoked [^{3}H]DA release from rat striatal slices in an orthosteric manner and inhibit high affinity [^{3}H]nicotine binding to rat brain membranes [220,221]. Structurally related *N-n*-pyridinium analogs with C_{10}–C_{20} *N-n*-alkyl groups were also potent inhibitors of nicotine-evoked [^{3}H]DA release, and the longer chain analogs (C_{15} and C_{20}) showed incomplete maximal inhibition (I_{max} = 50%), supporting the involvement of more than one nAChR subtype in this effect of nicotine [222]. These pyridinium analogs had little affinity for the [^{3}H]nicotine binding site [222], indicating enhanced selectivity for nAChRs mediating nicotine-evoked DA release.

The related *bis*-quaternary ammonium compounds, hexamethonium and decamethonium, which are considered to be simplified analogs of *d*-tubocurarine, have been used to differentiate muscle and ganglionic nAChRs [223–225]. The *bis*-quaternary ammonium structural framework was utilized to enhance nAChR subtype selectivity and afford a new class of *N,N′*-alkane-diyl-*bis*-3-picolinium (bAPi) analogs [226–228]. These polar and charged analogs were predicted to have poor brain bioavailability following systemic administration. However, previous work with structurally related polar *mono*-nicotinium analogs revealed good affinity for the blood–brain barrier (BBB) choline transporter, and active transport into brain [229]. A lead analog, *N,N′*-dodecane-1,12-diyl-*bis*-3-picolinium dibromide (C_{12}, bPiDDB; Fig. 1), was evaluated for inhibition of nicotine-evoked DA release and for its ability to inhibit the discriminative stimulus and/or locomotor stimulant centrally mediated effects of nicotine. bPiDDB exhibited little affinity for α4β2* and α7* high affinity ligand binding sites, nor for nAChRs modulating DA transporter function, but potently inhibited nicotine-evoked [^{3}H]DA release (IC_{50} = 2 nM; I_{max} = 64% [228]), bPiDDB did not inhibit electrically evoked [^{3}H]DA release, suggesting specific nAChR inhibitory effects and a lack of toxicity to DA neurons. Schild analysis suggested that bPiDDB interacts in an orthosteric manner at nAChRs mediating nicotine-evoked [^{3}H]DA release. To determine if bPiDDB interacted with α-CtxMII-sensitive α6β2-containing

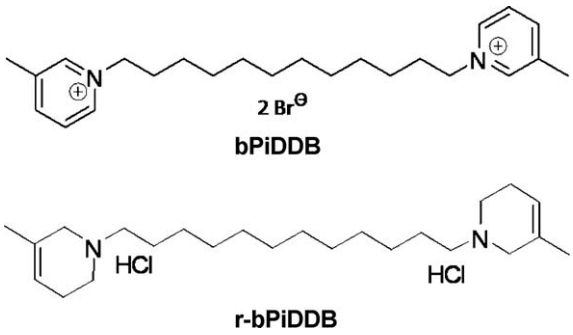

Fig. 1. The structures of *N,N*-dodecane-1,12-diyl-*bis*-picolinium dibromide (bPiDDB; top) and reduced-bPiDDB (r-bPiDDB; bottom).

nAChRs, slices were exposed concomitantly to maximally effective concentrations of bPiDDB (10 nM) and α-CtxMII (1 nM). Inhibition of nicotine-evoked [^{3}H]DA release was not different with the combination compared with either antagonist alone, suggesting that bPiDDB interacts with α6β2-containing nAChRs. These results support the interpretation that similar to α-CtxMII, the lead analog bPiDDB is a selective high potency antagonist at a subset of nAChR subtypes containing α6 and β2, and likely inhibits α6β2*, α6β2β3*, α4α6β2* and/or α4α6β2β3*. Furthermore, bPiDDB exhibited high affinity for the blood–brain barrier choline transporter in vivo and [^{14}C]bPiDDB was a substrate for the choline transporter [230], indicating brain bioavailability. In microdialysis studies using rats, bPiDDB (SC) decreased extracellular DA levels in nucleus accumbens following systemic nicotine [231] and decreased intravenous nicotine self-administration, but not sucrose maintained responding [232]. Surprisingly, in contrast to mecamylamine and DHβE, bPiDDB did not block the discriminative stimulus effect of nicotine [228]. Since mecamylamine and DHBE block the full complement of β2-containing nAChRs mediating nicotine-evoked DA release, whereas bPiDDB blocks only a subset of β2-containing nAChRs (i.e., those also containing α6) and only partially inhibits nicotine-evoked [^{3}H]DA release, these results suggest that inhibition of all β2-containing nAChRs and/or complete inhibition of nicotine-evoked DA release may be required to block the nicotine cue.

In contrast to the discriminative stimulus effect of nicotine, DA systems are critically involved in mediating the locomotor stimulant effect of nicotine. Locomotor sensitization produced by repeated nicotine administration is also associated with increased nicotine-evoked DA release in NAcc [233,234]. Both mecamylamine and DHβE block the nicotine-induced hyperactivity in nicotine-sensitized rats. Similarly, bPiDDB decreased nicotine-induced hyperactivity [228]. Since bPiDDB did not reduce locomotor activity when co-administered with saline in nicotine-sensitized rats, the bPiDDB-induced decrease in nicotine-induced hyperactivity was not likely due to nonspecific motor impairment. The bPiDDB-induced decrease in nicotine-induced hyperactivity likely reflects a specific blockade of nAChRs mediating the nicotine-evoked DA release. Future experiments should determine whether bPiDDB would be useful for preventing cue-dependent relapse to tobacco smoking in animal models and clinical populations.

The bPiDDB molecule is able to access brain via the blood–brain barrier choline transporter, even though it is a *bis*-quaternary ammonium salt with insignificant lipophilic character, and thus, is unable to permeate cell membranes by passive diffusion. Pharmacokinetic analysis in the rat indicates that bPiDDB has good brain bioavailability when administered via the subcutaneous route and reaches behaviorally relevant concentrations in brain with no indication of toxicity [235]. Nevertheless, when given by the oral route, bPiDDB has poor plasma and brain bioavailability, and thus, may be categorized as a poorly drugable molecule. As part of a structural optimization program to improve the drugability of *bis*-quaternary ammonium analogs that act as subtype-selective nAChR antagonists, we identified a structural analog of bPiDDB in which the two quaternary ammonium groupings (3-picolinium headgroups) were converted into tertiary amino moieties (3-methyl-1,2,5,6-tetrahydropyridyl) through a simple chemical reduction procedure, affording r-bPiDDB (Fig. 1), a highly lipophilic molecule with greatly improved drugability.

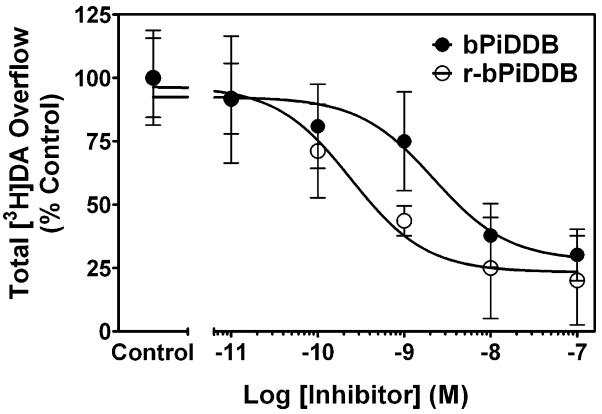

Fig. 2. Concentration dependence of bPiDDB and r-bPiDDB inhibition of nicotine-evoked [^{3}H]DA overflow from superfused rat striatal slices. Superfusion buffer contained nomifensine (10 μM) and pargyline (10 μM) throughout the experiment. Striatal slices were superfused in the absence (control) or presence of bPiDDB or r-bPiDDB for 36 min and then for an additional 36 min with nicotine (10 μM) added to the buffer. Control represents [^{3}H]DA overflow in response to nicotine (total [^{3}H]DA overflow as a percentage of tissue-^{3}H content; mean ± S.E.M.). The concentration response curves were generated by nonlinear regression. Data are expressed as percentage of control; n = 6 rats/group.

r-bPiDDB has physicochemical properties that predict good bioavailability by the oral route and it is a potent inhibitor of nicotine-evoked [^{3}H]DA release from superfused rat striatal slices (IC_{50} = 0.29 nM, I_{max} = 74%), and thus, had 10-fold greater potency than bPiDDB (Fig. 2). These results suggest that the two quaternary ammonium head groups in the bPiDDB molecule may not be a

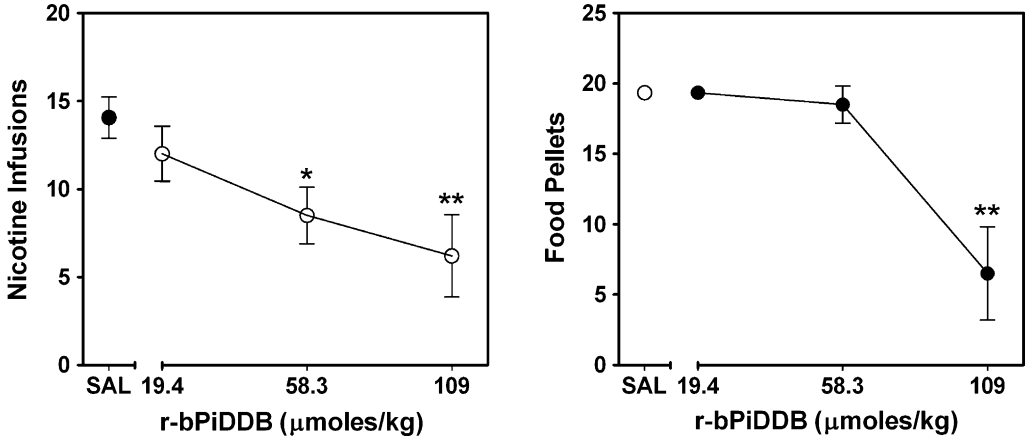

Fig. 3. Nicotine self-administration (left) and food-maintained responding (right) in rats following acute treatment with varying doses of r-bPiDDB. Results are expressed as the number of nicotine infusions or sucrose pellets earned (mean ± S.E.M.) during a 60-min operant conditioning session. n = 4–10/dose. Nicotine self-administration was decreased dose-dependently by r-bPiDDB [$F_{3,24}$ = 5.17, $p < 0.01$]. r-bPiDDB did not decrease food-maintained responding significantly, except for the highest dose of r-bPiDDB [$F_{3,24}$ = 5.17, $p < 0.01$]. *Represents a significant difference compared to saline (SAL) control group, $^*p < 0.05$, $^{**}p < 0.01$.

structural requirement for nAChR antagonism and are replaceable with more lipophilic, non-quaternary tertiary amino headgroups that can be protonated at physiological pH, allowing more efficient partitioning through biological membranes.

Similar to bPiDDB, which was effective in decreasing nicotine self-administration in the rat [232] r-bPiDDB, over a broad dose range, also exhibited effectiveness in the nicotine self-administration model (Fig. 3). Importantly, some specificity of effect was obtained, as a dose of r-bPiDDB (58.3 μmol/kg) that significantly decreased nicotine self-administration did not alter responding for food reinforcement (Fig. 3). Further, the ability of r-bPiDDB to decrease nicotine self-administration was retained without any loss of effect following 7 repeated daily treatments (data not shown). Additional structurally related *bis*-tertiary amino analogs are being evaluated to optimize the specificity of effect on nicotine self-administration.

Taken together, these findings suggest that the two quaternary ammonium head groups in the *bis*-quaternary ammonium series of analogs are not a structural requirement for nAChR antagonism, and that neurochemical and behavioral activity can be retained and improved by simple conversion of the quaternary ammonium headgroups in bPiDDB to their chemically reduced *bis*-tertiary amino equivalents, as in r-bPiDDB. The two tertiary amino groups in r-bPiDDB can be predominantly protonated at physiological pH, since r-bPiDDB is predicted to have pK_a values in the range 9–9.5, providing cationic moieties at physiological pH that may interact with the nAChR binding site in a similar manner to the azaaromatic quaternary ammonium headgroups in the bPiDDB molecule. Thus, this approach may result in better lead candidates for drug development, since the reduced *bis*-tertiary amino equivalent analogs likely will be potent, behaviorally active, and orally bioavailable due to their physicochemical properties.

Acknowledgments

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The University of Kentucky holds patents on bPiDDB and r-bPiDDB. A potential royalty stream to L.P.D. and P.A.C. may occur consistent with University of Kentucky policy.

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