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Review

Development of Novel Pharmacotherapeutics for Tobacco Dependence: Progress and Future Directions

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Abstract

Introduction: The vast majority of tobacco smokers seeking to quit will relapse within the first month of abstinence. Currently available smoking cessation agents have limited utility in increasing rates of smoking cessation and in some cases there are notable safety concerns related to their use. Hence, there is a pressing need to develop safer and more efficacious smoking cessation medications.

Methods: Here, we provide an overview of current efforts to develop new pharmacotherapeutic agents to facilitate smoking cessation, identified from ongoing clinical trials and published reports.

Results: Nicotine is considered the major addictive agent in tobacco smoke, and the vast majority of currently available smoking cessation agents act by modulating nicotinic acetylcholine receptor (nAChR) signaling. Accordingly, there is much effort directed toward developing novel small molecule therapeutics and biological agents such as nicotine vaccines for smoking cessation that act by modulating nAChR activity. Our increasing knowledge of the neurobiology of nicotine addiction has revealed new targets for novel smoking cessation therapeutics. Indeed, we highlight many examples of novel small molecule drug development around non-nAChR targets. Finally, there is a growing appreciation that medications already approved for other disease indications could show promise as smoking cessation agents, and we consider examples of such repurposing efforts.

Conclusion: Ongoing clinical assessment of potential smoking cessation agents offers the promise of new effective medications. Nevertheless, much of our current knowledge of molecular mechanisms of nicotine addiction derived from preclinical studies has not yet been leveraged for medications development.

Introduction

The negative impact of tobacco dependence on society is staggering. It is predicted that ~0.6 billion current smokers worldwide will die from smoking-related illnesses, such as chronic obstructive pulmonary disorder (COPD), lung cancer, and cardiovascular disease (Besson et al., 2012; Coe et al., 2005; Doll, Peto, Boreham, & Sutherland, 2004; Ezzati & Lopez, 2003; Mathers & Loncar, 2006). If current trends in tobacco use persist, by 2020 smoking will become the largest single health problem worldwide, causing ~8.4 million deaths annually (Murray & Lopez, 1997). The World Bank estimates that in high-income countries, smoking-related healthcare accounts for between 6% and 15% of all healthcare costs, ~$160 billion annually. Smokers who quit before the onset of tobacco-related illness can largely avoid the increased mortality risk (Doll, Peto, Wheatley, Gray, & Sutherland, 1994; Peto et al., 2000). Nevertheless, ~80% of smokers attempting to quit will relapse within the first month of abstinence (Benowitz, 2009). The development of efficacious smoking-cessation aids is perhaps the single most cost-effective intervention possible within a healthcare system (Knight, Howard, Baker, & Marton, 2009). Currently, nicotine replacement products such as gum and patch, Chantix (varenicline) and Zyban (bupropion) are the only medications for smoking cessation approved by the Food and Drug Administration (FDA). The antihypertensive medication clonidine, an α2 adrenergic agonist, and the tricyclic antidepressant nortriptyline are sometimes used as second-line agents for smoking cessation (Corelli & Hudmon, 2002), but their use in this context has not been approved by the FDA. Although current smoking-cessation agents facilitate cessation efforts, they have limited effectiveness. In smokers attempting to quit, ~23% treated with varenicline and ~16% treated with bupropion remain abstinent after 1 year, compared with just ~9% of those treated with placebo (Knight et al., 2009). Pharmacotherapy is therefore an effective strategy to promote abstinence from smoking, but there remains

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considerable risk of release even when treated with the most efficacious medications currently available. Moreover, Chantix and Zyban must now carry "black box" warnings on their labeling because of reported serious mental-health events associated with their use, with issues related to tolerability and compliance also representing limitations in their use (Catz et al., 2011; Hays & Ebbert, 2010; Oncken et al., 2006; Zhao, Schwam, Fullerton, O'Gorman, & Burstein, 2011). This highlights the pressing need to better understand the neurobiology of tobacco dependence and thereby develop safer, more effective therapeutics with good tolerability and compliance profiles.

Dimensions of Tobacco Dependence and Smoking-Cessation Pharmacotherapeutics

Nicotine is considered the major component of tobacco smoke responsible for addiction (Stolerman & Jarvis, 1995). In the context of medications development to aid smoking cessation, the current issue of the Diagnostic and Statistical Manual of Mental Disorders (DSM–IV) addresses two major conditions related to tobacco use: nicotine-use disorder and nicotine-induced disorder. Nicotine-use disorder is characterized by the gradual development of tolerance to many of the physiological effects of nicotine; the use of tobacco products in larger amounts or over a longer period than intended; persistent desire to smoke or unsuccessful attempts to cut down on tobacco; a great deal of time spent in obtaining or using tobacco; social, occupational, or recreational activities being reduced because of tobacco use; and tobacco use continuing despite physical or psychological problems caused or exacerbated by tobacco. Cessation of tobacco use triggers the expression of an aversive nicotine withdrawal syndrome (Kenny & Markou, 2001). Nicotine-induced disorder (withdrawal) is defined by the DSM-IV as a condition manifested in an individual after cessation of tobacco consumption after a period of at least several weeks of daily nicotine use, followed within 24 h after abrupt cessation or reduction of use. Symptoms of nicotine withdrawal include dysphoric or depressed mood; insomnia; irritability; frustration or anger; anxiety; difficulty concentrating; restlessness; decreased heart rate; and increased appetite or weight gain.

All current FDA-approved medications for tobacco dependence are approved for the indication "aid to smoking cessation," with the primary endpoint in clinical trials generally being 4 weeks of continuous abstinence from tobacco consumption. All approved smoking-cessation medications have been shown to reduce nicotine consumption in preclinical or clinical assessments, reflecting diminished reinforcing properties of nicotine that likely explains the clinical efficacy of these compounds (Aubin et al., 2008; George, Lloyd, Carroll, Damaj, & Koob, 2011; Levin et al., 2012). Hence, we have focused our attentions in this article on the mechanisms of nicotine reinforcement in the context of medications development for smoking cessation and have identified opportunities for novel future development of pharmacological agents that may reduce nicotine reinforcement. Nevertheless, it is important to point out that this focus on nicotine reinforcement represents a limitation in the scope of the article when wider aspects of nicotine-use disorder and nicotine-induced disorder are considered. Indeed, the development of medications designed to alleviate specific aspects of the broad spectrum of symptoms associated with nicotine use or withdrawal will likely serve to facilitate cessation efforts in human smokers without necessarily impacting the reinforcing properties of nicotine. For example, medications designed to limit weight gain or reduce anxiety, restlessness, or irritability during withdrawal may facilitate abstinence from tobacco smoking (Kenny & Markou, 2001). Also, it should be noted that the highest efficacy is seen when pharmacotherapy is combined with behavioral interventions, and this approach is recommended in clinical practice guidelines in the United States (Fiore et al., 2008). Hence, although not considered in detail here, it is nonetheless important to recognize the beneficial effects of behavioral interventions on smoking cessation, and efforts to understand how nonpharmacological aids to smoking cessation may facilitate to develop improved treatment strategies to facilitate long-term abstinence.

Nicotinic Acetylcholine Receptors and Nicotine Reinforcement

Understanding how and where nicotine contained in tobacco smoke acts in the brain to trigger its addiction-related actions is likely to provide valuable insights into the neurobiology of the nicotine habit in smokers that can help guide drug development efforts. The addiction-relevant actions of nicotine are derived from its stimulatory actions on neuronal nicotinic acetylcholine receptors (nAChRs) in the central nervous system (CNS). As such, nAChRs are key targets for the development of therapeutic agents for smoking cessation. Nicotinic receptors are composed of five distinct membrane-spanning subunits (α and β subunits) (Albuquerque et al., 1995; Lena & Changeux, 1998). The neuronal α subunit exists in nine isoforms (α2–α10), whereas the neuronal β subunit exists in three isoforms (β2–β4) (Elgoyhen, Johnson, Boulter, Vetter, & Heinemann, 1994; Elgoyhen et al., 2001; Le Novere, Corringer, & Changeux, 2002). Nicotinic receptors containing α4 and β2 subunits (denoted as α4β2* nAChRs) are the most prevalent in the CNS (Flores, Rogers, Pabreza, Wolfe, & Kellar, 1992) and are considered the major subtype involved in regulating the addiction-relevant actions of nicotine (Buisson & Bertrand, 2002). Indeed, nicotine-replacement therapy (NRT), such as nicotine gum and patch, is believed to act primarily at high-affinity α4β2* nAChRs (Kenny & Markou, 2001), and varenicline was developed as an α4β2* nAChR partial agonist (see below). Hence, there is much effort devoted to developing nAChR-based therapeutics for smoking cessation.

There is now considerable evidence that the reinforcing actions of nicotine are related to its stimulatory effects on mesoaccumbens dopamine transmission, which comprises dopamine-containing neurons that arise in the ventral tegmental area (VTA) and project to the nucleus accumbens (NAcc). In particular, the actions of nicotine at α4β2* nAChRs in the VTA are believed to play a key role in the reinforcing effects of the drug that motivate the establishment and maintenance of the tobacco habit. Indeed, genetic ablation of β2 subunits in mice, resulting in the elimination of high-affinity nAChRs in the

brain, abolishes sensitivity to the reinforcing effects of nicotine (Picciotto et al., 1998). Conversely, mice expressing mutant α4 nAChR subunits (α4 knock-in mice) that are hyper-responsive to nicotine display enhanced sensitivity to the rewarding effects of the drug even at very low doses (Tapper et al., 2004). Consistent with a role for mesoaccumbens dopamine transmission in these effects, viral-mediated re-expression of β2 subunits in the VTA of β2 knockout (KO) mice rescued their sensitivity to nicotine reinforcement (Maskos et al., 2005; Pons et al., 2008). Also, VTA dopamine neurons are hyper-responsive to nicotine in the α4 knock-in mice (Tapper et al., 2004). From a treatment perspective, it is noteworthy that all currently available smoking-cessation therapeutics have at least some action at α4β2* nAChRs. As noted above, varenicline is a partial agonist at α4β2* nAChRs, and its therapeutic actions are related at least in part to a stimulatory effect on midbrain dopamine transmission. Varenicline increases mesoaccumbens dopamine transmission in wildtype mice but not in mice with null mutation in β2 nAChR subunits (Reperant et al., 2010). Moreover, virus-mediated re-expression of functional β2 nAChR subunits in the mesoaccumbens pathway of the KO mice "rescues" the stimulatory effects of varenicline on dopamine transmission (Reperant et al., 2010). These data are consistent with an important role for α4β2* nAChRs in the VTA in the therapeutic actions of varenicline. Bupropion and its metabolites have also been shown to have antagonist actions at α4β2* and other subtypes of nAChRs (Damaj et al., 2010; Pandhare et al., 2012; Slemmer, Martin, & Damaj, 2000). The clinical utility of nicotine NRT is believed to reflect an action at high-affinity α4β2* nAChRs by the nicotine in these products, thereby substituting for at least some of the actions derived from nicotine in tobacco smoke. These findings suggest that modulation of midbrain dopamine systems may, to some degree at least, represents a common underlying mechanism of currently available smoking-cessation agents.

Although α4β2* nAChRs are undoubtedly involved in nicotine reinforcement, there is growing evidence for contributions from other subtypes of nAChRs also. In particular, α6* nAChRs are emerging as an important class of nAChRs in nicotine reinforcement. There is particularly dense expression of α6 subunit mRNA in the VTA and NAc (Azam, Winzer-Serhan, Chen, & Leslie, 2002; Champtiaux et al., 2002; Le Novere, Zoli, & Changeux, 1996; Quik et al., 2000), which plays a major role in supporting the positive reinforcing actions of nicotine. In the NAc and other domains of the striatum, dopaminergic terminals express α6β2β3* and α6α4β2β3* nAChR subtypes (Champtiaux et al., 2003; Salminen et al., 2004; Zoli et al., 2002), with considerable evidence suggesting that α6β3* nAChRs regulate the stimulatory effects of nicotine on dopamine release in this region (Champtiaux et al., 2003). The α6α4β2β3* nAChR subtype enriched in the NAc and striatum has the highest sensitivity to nicotine of any native nAChR so far identified (Grady et al., 2007). Gotti et al. (2010) reported that infusion of α-conotoxin MII (CntxMII), a α3/α6β2* selective antagonist, or α-conotoxin PIA (CntxPIA), a α6β2* nAChR selective antagonist, directly into the VTA decreased nicotine-evoked increases in midbrain dopamine levels. Intra-VTA infusion of CntxMII also decreased responding for intravenous nicotine self-administration in rats (Gotti et al., 2010). Similarly, Brunzell and colleagues reported that intra-NAc infusion of CntxMII decreased the motivation of rats to self-administer nicotine, as measured using a progressive ratio schedule of reinforcement (Brunzell, Boschen, Hendrick, Beardsley, & McIntosh, 2010). More recently, it was shown that KO mice lacking protein kinase C epsilon (PKCε KO mice) self-administered less nicotine and had attenuated place conditioning for nicotine than their wildtype counterparts (Lee & Messing, 2011). The PKCε KO mice also demonstrated decreased levels of α6 and β3 nAChR subunit mRNA transcripts in the midbrain and striatum, and deficits in cholinergic modulation of dopamine release in the NAc (Lee & Messing, 2011). Hence, it was hypothesized that PKCε may regulate α6 (and also β3) nAChR signaling and thereby influence nicotine reinforcement (Lee & Messing, 2011). Using an "acute" nicotine self-administration procedure in which mice are restrained but can nose-poke for intravenous nicotine infusions via a catheter in the tail vein during a single session, it was shown that α6 subunit KO mice had attenuated levels of nicotine intake compared with wildtype mice (Pons et al., 2008). Taken together, these findings suggest that α6* nAChRs may be promising targets for medications development for tobacco dependence. However, it is important to note that α6 KO mice were recently shown to respond for nicotine infusions in a manner similar to wildtype mice, as measured using a self-administration procedure in which nicotine infusions were delivered directly into the VTA (Exley et al., 2011). Using this same procedure, α4 KO mice had a marked deficit in responding for the drug (Exley et al., 2011). It was proposed that this discrepancy in the contribution of α6*nAChRs to regulating systemically versus intra-VTA delivered nicotine infusions may reflect distinct contributions of α6* nAChRs in the soma (VTA) compared with axon terminals (NAc) to nicotine reinforcement (Exley et al., 2011).

α4β2 nAChRs can exist in high- and low-sensitivity states, a property attributed to the fact that distinct stoichiometries of the receptor are possible due to alternative subunit assembly (Nelson, Kuryatov, Choi, Zhou, & Lindstrom, 2003; Zwart & Vijverberg, 1998). Specifically, it has been proposed that the receptor can exit as a $(\alpha4)_2(\beta2)_3$ pentamer that has high sensitivity to agonist stimulation, or a $(\alpha4)_3(\beta2)_2$ pentamer that has low-sensitivity low-agonist stimulation. Interestingly, varenicline and nicotine can activate both the high and low stoichiometries of the α4β2 nAChR, whereas a wide range of other putatively selective α4β2 nAChR ligands such as AZD3480, ABT-089, and dianicline only activated the high-sensitivity stoichiometry (Anderson et al., 2009). The relevance of this finding to the therapeutic effects of nicotine and varenicline is unclear, but it is an intriguing possibility that molecules that modify the low-sensitivity state of the α4β2*nAChR may be particularly beneficial for smoking cessation. As noted above, α6* nAChRs are also believed to contribute to the stimulatory effects of nicotine on striatal dopamine transmission (Grady et al., 2007). Interestingly, varenicline-induced increases in dopamine release from striatal or prefrontal cortical tissues was unaffected by the α3/α6β2* selective antagonist CntxMII (Anderson et al., 2009), consistent with the largely α4β2 nAChR-specific actions of varenicline and suggesting that α6* nAChRs are unlikely to play a major role in the therapeutic actions of varenicline.

In addition to α4β2* and α6* nAChRs, there is evidence that α7 nAChRs may also play a role in regulating nicotine intake. Brunzell and McIntosh reported that antagonism of α7 nAChRs in the NAc or anterior cingulate cortex (ACC), achieved by local infusion of the α7 nAChR-selective antagonist α-conotoxin ArIB [V11L,V16D] (ArIB), increased responding for nicotine in

rats (Brunzell & McIntosh, 2012). Conversely, NAc infusion of the α7 nAChR agonist, PNU 282987, decreased the motivation to consume nicotine (Brunzell & McIntosh, 2012). It has also been shown that the stimulatory effects of nicotine on mesoaccumbens dopamine transmission are potentiated in α7 nAChR KO mice (Besson et al., 2012). Moreover, α7 KO mice respond at lower levels for intra-VTA infusions of nicotine, an effect interpreted as increased sensitivity to the reinforcing properties of the drug necessitating lower quantities being required to achieve the desired hedonic state (Besson et al., 2012). Together, these findings suggest that increasing α7 nAChR signaling in the brains of smokers may decrease the motivation to smoke. It is therefore interesting to note that EnVivo Pharmaceuticals is currently assessing the effects of EVP-6124, a selective α7 nAChR agonist, on smoking cessation (Table 1). Nevertheless, there are inconsistencies in the literature with regard to the role for α7 nAChRs in nicotine reinforcement, with a number of reports suggesting that the α7 subunit KO mice, or rodents treated with α7 nAChR antagonists, demonstrate either unaltered or reduced nicotine consumption (Grottick et al., 2000; Levin et al., 2009; Markou & Paterson, 2001). Hence, the precise role for α7 nAChRs in nicotine reinforcement remains unclear.

The above findings demonstrate that α4β2* nAChRs play a key role in regulating the stimulatory effects of nicotine on midbrain dopamine systems and thereby control the reinforcing properties of nicotine. As such, α4β2* nAChRs are key targets for medications development for smoking cessation and continued effects to develop molecules that modulate the activity of this nAChR subtype are likely to yield new effective smoking-cessation agents. Moreover, other subtypes of nAChRs are also involved in regulating the stimulatory effects of nicotine on midbrain dopamine systems, particularly α6* and α7 nAChRs, and as such may serve as novel targets for medications development.

Nicotinic Acetylcholine Receptors and Smoking Cessation

To date, varenicline is the only FDA-approved smoking-cessation agent that was rationally designed through traditional drug discovery processes based on its action as an α4β2* nAChR partial agonist (Coe et al., 2005; Dwoskin et al., 2009; Lerman et al., 2007; Reus et al., 2007). It is important to note, however, that varenicline is also a full agonist at α7 nAChRs (Mihalak, Carroll, & Luetje, 2006). The rationale for developing a α4β2* nAChR partial agonist for smoking cessation was twofold: First, it was hypothesized that a partial agonist may competitively bind to α4β2* nAChRs and thereby attenuate the stimulatory effects of nicotine obtained from tobacco smoke on mesoaccumbens dopamine transmission (Coe et al., 2005; Reperant et al., 2010). The stimulatory effect of nicotine on midbrain dopamine transmission is considered central to its reinforcing properties that contribute to the development and maintenance of the tobacco habit (Exley et al., 2011; Maskos et al., 2005; Pons et al., 2008). Hence, attenuation of this effect by varenicline may decrease the reinforcing effects of nicotine, thereby aiding smoking cessation efforts. Second, it was hypothesized that the intrinsic low levels of activation of α4β2* nAChRs by a partial agonist may substitute for the stimulatory effects of nicotine on mesoaccumbens dopamine transmission during abstinence, eliciting a moderate and sustained increase in dopamine levels (Coe et al., 2005). In this manner, α4β2* nAChR partial agonists may counteract decreases in mesoaccumbens dopamine transmission believed to occur during abstinence from tobacco smoking (Kenny & Markou, 2001), thereby again facilitating smoking-cessation efforts (Coe et al., 2005).

Chemically, varenicline is a derivative of the nAChR partial agonist (−)-cytisine; a natural product derived from *Cytisus laburnum* and other plant species (Coe et al., 2005). In electrophysiological assays using *Xenopus* oocytes, varenicline partially activates α4β2* nAChRs when tested alone (~68% of the maximal response elicited by 10 μM nicotine) (Coe et al., 2005) and partially blocks the effects of nicotine (~34% inhibition) (Coe et al., 2005). The partial agonist properties of varenicline have also been confirmed *in vivo*. For example, varenicline attenuated the stimulatory effects of nicotine on mesoaccumbens dopamine turnover (Coe et al., 2005). However, when varenicline was administered alone, it stimulated midbrain dopamine turnover to a lesser degree than nicotine (Coe et al., 2005; Reperant et al., 2010), consistent with a partial agonist activity. In early smoking-cessation studies, cytisine failed to exhibit efficacy (Benndorf, Scharfenberg, Kempe, Wendekamm, & Winkelvoss, 1970; Scharfenberg, Benndorf, & Kempe, 1971), but more recent clinical trials in eastern Europe suggest that cytisine (Tabex) may indeed promote smoking cessation (Etter, 2006). The reduced therapeutic efficacy of cytisine compared with varenicline may be attributed to its poorer absorption and brain penetration properties (Barlow & McLeod, 1969; Reavill, Walther, Stolerman, & Testa, 1990). Similarly, the α4β2* nAChR partial agonist dianicline (SSR-591,813), developed by Sanofi-Aventis for smoking cessation, is less efficacious than varenicline likely because of its poorer brain penetration properties (Rollema et al., 2010). These findings demonstrate the feasibility

Table 1. Novel Small Molecule Therapeutic Agents in Clinical Testing for Smoking Cessation

Compound	Mechanism of action	Phase	Sponsor	Status
EVP-6124	α7 nAChR agonist	II	EnVivo Pharmaceuticals	Not yet recruiting
GSK598809	Dopamine D3 receptor antagonist	II	GlaxoSmithKline	Recruiting
LIBERTAL	Reported as a phosphatidic acid-enriched phospholipid	II	Kaplan Medical Center	Completed
Surinabant	CB1 receptor antagonist	II	Sanofi-Aventis	Completed

of rationally designing small-molecule drugs that modulate nAChR signaling and that demonstrate clinical utility for smoking cessation. Thus, a major challenge for future drug development will be to better understand the nAChR subtypes that play a role in tobacco dependence. In this manner, new nAChR-based smoking cessation agents, possessing suitable drug-like physiochemical and brain penetration properties, and also favorable compliance and tolerability profiles, may be developed.

Nicotinic Acetylcholine Receptors and the Genetics of Smoking

Recent findings from human genome-wide association studies (GWAS) suggest that nAChR subtypes other than α4β2* (and α6* or α7) nAChRs may contribute to tobacco dependence and have provided important insights into novel nAChR subtypes that may regulate smoking behavior and thereby represent important targets for medications development. Allelic variation in the *CHRNA5-CHRNA3-CHRNB4* subunit gene cluster located in chromosome region 15q25, which encodes the α5, α3, β4 nAChR subunits, respectively, significantly increases risk of tobacco addiction (Berrettini et al., 2008; Bierut et al., 2008; Hung et al., 2008; Lips et al., 2009; Saccone et al., 2007; Thorgeirsson et al., 2008). In particular, a nonsynonymous single-nucleotide polymorphism (SNP) in C*HRNA5* (rs16969968) that results in an aspartic acid to asparagine substitution at amino acid reside 398 (D398N), which decreases the function of α5* nAChRs incorporating this risk allele, is associated with tobacco dependence (Bierut et al., 2008). The D398N allele is common in those of European descent, with an occurrence in approximately 35% of such individuals, and increases the risk of tobacco dependence by ~30% in individuals carrying a single copy of the variant and more than doubles the risk in those carrying two risk alleles (Bierut et al., 2008; Wang et al., 2009), a finding replicated extensively (Berrettini et al., 2008; Bierut et al., 2008; Grucza et al., 2008; Stevens et al., 2008). The D398N allele is far less prevalent in African-Americans compared with those of European descent, with a frequency of ~5% (Saccone, Wang, et al., 2009). Nevertheless, it still represents an important risk factor for tobacco dependence in African-American subjects, as a single copy of the allele has been shown to more than double the risk of tobacco dependence (Saccone, Wang, et al., 2009). In addition to tobacco dependence, the D398N major risk allele is associated with heavy smoking (Berrettini et al., 2008; Bierut et al., 2008; Grucza et al., 2008; Stevens et al., 2008), early onset of smoking behavior (Weiss et al., 2008) and with "pleasurable buzz" from tobacco (Sherva et al., 2008). The D398N allele is also a major risk factor for lung cancer and COPD in smokers (Amos et al., 2008; Hung et al., 2008; Wang, Broderick, Matakidou, Eisen, & Houlston, 2010), likely reflecting higher levels of tobacco dependence in individuals carrying risk alleles and consequently greater exposure to carcinogens contained in tobacco smoke (Le Marchand et al., 2008; Thorgeirsson et al., 2008). Similar to the rs16969968 SNP in the α5 subunit gene, there is also an increase in risk for tobacco dependence in individuals carrying the rs6495308, rs578776, or rs1051730 SNPs in the α3 subunit gene (Saccone, Saccone, et al., 2009) and rs1948 in the β4 subunit gene (Schlaepfer et al., 2008). Finally, it was recently shown that genetic ablation of α5 or β4 nAChR subunits in mutant mice results far greater consumption of nicotine compared with wildtype littermates (Fowler, Lu, Johnson, Marks, & Kenny, 2011; Frahm et al., 2011), consistent with an important role for nAChRs containing these subunits in regulating nicotine intake.

These findings suggest that α5*, α3* and/or β4* nAChRs regulate many addiction-related actions of nicotine contained in tobacco smoke and therefore may serve as novel targets for smoking-cessation therapeutics. Hence, the mechanisms through which genetic variation in *CHRNA5* increases vulnerability to tobacco addiction is an important question, as understanding this process may reveal opportunities to develop novel smoking-cessation therapeutics. As noted above, the D398N SNP associated with tobacco dependence decreases the function of α5* nAChRs incorporating this risk allele, as measured by nicotine-evoked calcium influx in human embryonic kidney, 293 cells expressing α4β2 nAChRs in combination with the wildtype (D398) or risk variant (N398) α5 nAChR subunit (Bierut et al., 2008). It is therefore likely that deficient α5* nAChR signaling increases vulnerability to tobacco dependence. Hence, novel pharmacological agents that boost α5* nAChR signaling, such as α5* nAChR positive allosteric modulators (PAMs), may have clinical efficacy as smoking-cessation aids. A more detailed consideration of the mechanisms by which α5*, α3* and/or β4* nAChRs may regulate nicotine intake is provided below. In addition to the *CHRNA5-CHRNA3-CHRNB4* gene cluster, polymorphisms in the *CHRNA6-CHRNB3* gene cluster, encoding the α6 and β3 nAChR subunits, also increase vulnerability to tobacco smoking (Hoft et al., 2009; Thorgeirsson et al., 2010; Zeiger et al., 2008). Considering the preclinical pharmacology studies described above, these human genetics findings further support the notion that α6*and β3* nAChRs may serve as targets for the development of smoking-cessation therapeutics. Finally, GWAS has identified genomic loci not involved in coding for nAChR subunits that may play a role in risk of tobacco dependence and as such may be suitable for the development of novel therapeutics. For example, polymorphisms in the galanin 1 receptor (Lori et al., 2011), 5-HT2A and 2C receptors (Iordanidou et al., 2010; Polina, Contini, Hutz, & Bau, 2009; White, Young, Morris, & Lawford, 2011), neuropeptide Y (NPY), Y2 receptor (Sato et al., 2010), catechol-O-methyltransferase (COMT) (Nedic et al., 2010), Rho GTPases (Chen et al., 2007; Lind et al., 2010), muscarinic receptors 2 and 5 (Anney et al., 2007; Mobascher et al., 2010), brain-derived neurotropic factor and its receptor (TrkB) (Amos, Spitz, & Cinciripini, 2010; "Genome-wide meta-analyses identify multiple loci associated with smoking behavior," 2010; Li, Lou, Chen, Ma, & Elston, 2008; Vink et al., 2009), neuroexin-1 (Nussbaum et al., 2008), CYP2A6 and CYP2B6 (Nakajima, 2007; Ring et al., 2007; Sellers, Tyndale, & Fernandes, 2003; Thorgeirsson et al., 2010), β-arrestin 1 and 2 (Sun, Ma, Payne, & Li, 2008), phosphatase and tensin homolog gene (Zhang, Kendler, & Chen, 2006), and GABA-B receptors (Li et al., 2009) are all associated with nicotine dependence. These findings suggest that observations made in the human genetics literature identifying genes influencing vulnerability to tobacco dependence may be leveraged for future medications development. Perhaps the most promising targets in this regard are the nAChRs containing α5, α3 and/or β4 subunits, genetic variation in the genes for which increases vulnerability to tobacco dependence.

Targets for Smoking-Cessation Downstream of Nicotinic Acetylcholine Receptors

nAChRs are predominately located on presynaptic terminals where they can modulate the release of various neurotransmitters (McGehee, Heath, Gelber, Devay, & Role, 1995). As such, activation of nAChRs by nicotine stimulates the activation of a range of signaling cascades within brain reward circuitries. The precise sequence of neurobiological events, including the neuronal populations, intracellular signaling cascades, and induced genes, that contribute to the development and persistence of the tobacco habit are unclear but under intense investigation (Kenny, Chartoff, Roberto, Carlezon, & Markou, 2009; Markou & Paterson, 2009; Picciotto & Corrigall, 2002; Stolerman, Mirza, & Shoaib, 1995; Wonnacott, Sidhpura, & Balfour, 2005). These investigations have implicated a wide range of non-nAChR targets as candidates for the development of novel therapeutics for tobacco dependence. Indeed, as shown in Table 1, a review of ongoing and completed clinical trials for smoking-cessation agents shows that non-nAChR-acting agents are in development for smoking cessation (www.clinicaltrials.gov). For example, GlaxoSmithKline in collaboration with the National Institute on Drug Abuse and McLean Hospital are conducting a phase II clinical trial to determine the effectiveness of GSK598809, a dopamine D3 receptor antagonist, to facilitate abstinence in smokers (Table 1). Sanofi-Aventis have recently completed testing the effectiveness of surinabant, a cannabinoid 1 receptor antagonist, in promoting abstinence from smoking. Disappointingly, surinabant did not improve rates of abstinence compared with placebo during a 4-week testing period (Tonstad & Aubin, 2012). However, surinabant significantly attenuated the amount of weight gained in smokers attempting to quit (Tonstad & Aubin, 2012). This suggests that surinabant could be a useful adjunctive treatment to other smoking-cessation aids or could possibly be useful in the treatment of overeating and obesity. However, considerable caution will have to be exercised should surinabant be advanced as an adjunctive for smoking cessation based on the health concerns related to previous CB1 receptor antagonists. Specifically, the cannabinoid CB1 receptor antagonist SR141716 (rimonabant) underwent clinical testing in the United States for smoking cessation and was previously approved in Europe as an adjunct for the treatment of obesity that also decreased cigarette consumption in smokers (Fagerstrom & Balfour, 2006; Fernandez & Allison, 2004; Reid, Quinlan, Riley, & Pipe, 2007). However, concerns related to depression and suicidal ideation in those treated with rimonabant (Cahill & Ussher, 2007) prompted the suspension of its use in Europe, and it has not been approved for use in the United States. Finally, the effects of LIBERTAL, described as a phosphatidic acid-enriched phospholipid, has been tested in a phase II clinical trial on smoking cessation. Phosphatidic acid may presumably modulate nAChR function by modifying the lipid environment of the plasma membrane. Alternatively, phosphatidic acid is known to be degraded into diacylglycerol by lipid phosphate phosphohydrolases (Brindley & Waggoner, 1998), an intracellular signaling molecule that can directly and indirectly modulate nAChR function (Andoh et al., 2004; Butt, Hutton, Marks, & Collins, 2002). However, the efficacy of LIBERTAL has not yet been reported (Table 1).

Vaccines and Smoking Cessation

A novel non-drug-based strategy to facilitate smoking cessation is nicotine vaccines. Nicotine-based vaccines can prime the immune system to recognize nicotine as foreign and to mount an immune response against the drug. In doing so, vaccines may reduce the amounts of nicotine penetrating into the brain (Carrera et al., 2004). A number of organizations have developed vaccines for smoking cessation (Table 2), with NicVAX developed by Nabi Biopharmaceuticals being perhaps the best known. Nabi recently completed a phase III clinical trial for smoking cessation with the vaccine NicVAX. In a previous phase II trial of 63 smokers, 33% of subjects in the NicVax group had stopped smoking for at least 30 consecutive days versus 9% in the placebo group. However, in the larger phase III trial, which included 1,000 smokers seeking to quit, NicVax did not improve abstinence rates above those seen in the control individuals (Pollock, 2011), with approximately 11% of smokers remaining abstinence in both the vaccine and control groups (Pollock, 2011). This disappointing outcome has raised doubts about the prospects of developing vaccines as smoking-cessation agents (Pollock, 2011). Conceptually, a potential drawback related to the use of vaccines to treat tobacco dependence is the fact that smokers will often compensate for decreases in the actions of nicotine, as would be expected when a vaccine decreases concentrations of nicotine penetrating into brain tissues, by increasing their tobacco consumption to overcome this effect (Ashton, Stepney, & Thompson, 1979; Gritz, Baer-Weiss, & Jarvik, 1976; Scherer, 1999). Other potential issues related to the successful use of vaccines include difficulties achieving sufficiently high antibody titers, the fact that vaccines are generally short lived, and significant interindividual variation in response to the vaccine typically observed (Fagerstrom & Balfour, 2006).

Repurposing of FDA-Approved Medications as Smoking-Cessation Aids

Bupropion (Zyban) is the only FDA-approved smoking-cessation aid that does not target nAChRs as its primary mode of action. Instead, bupropion is an atypical antidepressant (Wellbutrin) used for the treatment of depression. Nevertheless, it is important to note that bupropion and its metabolites can

Table 2. Novel Biological or Vaccine Agents in Clinical Testing for Smoking Cessation

Vaccine	Phase	Sponsor	Status
NIC002	II	Duke University	Recruiting
NIC002	II	Novartis	Completed
NicVax	I	Maastricht University Medical Center	Recruiting
NicVax	III	Nabi Biopharmaceuticals	Completed
SEL-068	I	Selecta Biosciences	Recruiting

act as nAChR antagonists (Damaj et al., 2010; Pandhare et al., 2012; Slemmer et al., 2000). The ability of bupropion to facilitate smoking cessation was discovered serendipitously when it was shown to decrease cigarette consumption in depressed patients. The precise mechanism through which bupropion facilitates abstinence is unclear but nevertheless raises the intriguing possibility that other FDA-approved medications could likewise facilitate smoking cessation through novel mechanisms of action.

Repurposing of FDA-approved medications as novel smoking-cessation agents represents an opportunistic way to accelerate the process of drug discovery and has the potential to save considerable financial resources usually devoted to developing, testing, and obtaining regulatory approval for new drugs. Table 3 highlights examples of FDA-approved medications for other indications that are undergoing clinical assessment as potential smoking-cessation agents. As can be seen, repurposing strategies are now being widely adopted. For example, EMB-001 is in development by Embera Neuro Therapeutics Inc. for smoking cessation. EMB-001 is a combination of two FDA-approved medications: the benzodiazepine oxazepam and the cortisol-synthesis inhibitor metyrapone. This combination of drugs was recently shown to decrease intravenous nicotine self-administration in rats in a manner similar to varenicline (Goeders et al., 2012), with this drug combination hypothesized to act by attenuating the known stimulatory effects of stress on nicotine consumption (Goeders et al., 2012). Tolcapone (Tasmar), an inhibitor of the COMT enzyme involved in degradation of catecholamine neurotransmitters, is FDA-approved for the treatment of the symptoms of Parkinson's disease when used in conjunction with L-dopa. Tolcapone is also currently in clinical trials for smoking cessation, the outcome of which will be particularly intriguing considering that genetic variation in COMT is associated with increased risk of tobacco dependence (Nedic et al., 2010) (see above). Interestingly, an ongoing trial is assessing the effects of the antihistamine meclizine, used to treat motion sickness, on smoking cessation. Recently, blockade of H1 histamine receptors was shown to decrease intravenous nicotine self-administration in rats (Levin et al., 2011), supporting the notion that FDA-approved drugs that modify histaminergic transmission may indeed serve as novel smoking-cessation agents. Three putative mechanisms have been proposed to account the inhibitory effects of H1 receptor antagonists on nicotine consumption (Levin et al., 2011): First, H1 receptor antagonists have cocaine-like inhibitory effects on the reuptake of serotonin and catecholamine neurotransmitters (Lapa, Mathews, Harp, Budygin, & Jones, 2005; Tanda, Kopajtic, & Katz, 2008), suggesting that they may decrease nicotine intake through a mechanism similar to bupropion; second, nicotine is known to antagonize H1 receptors (Ercan & Turker, 1985), suggesting that H1 antagonists may at least partially substitute for this action of nicotine; third, hypocretin (orexin) neuropeptides (see below also) are known to regulate nicotine intake (Hollander, Lu, Cameron, Kamenecka, & Kenny, 2008) and have been shown to activate histaminergic neurons (Yamanaka et al., 2002). This suggests that histamine receptors may be downstream effectors of the hypocretin system. The acetylcholinesterase (AChE) inhibitor galantamine, approved for the treatment of cognitive symptoms of Alzheimer's disease, is also being tested as a smoking-cessation agent. Previously, it was shown that galantamine reduced the number of cigarettes smoked in alcohol-dependent patients (Diehl et al., 2006). Furthermore, low doses of galantamine reduced intravenous nicotine self-administration behavior in rats (Liu & Stewart, 2009). This suggests that boosting endogenous cholinergic transmission may be an effective strategy to facilitate smoking cessation and abstinence. Interestingly, it was recently shown that galantamine is also a PAM at α4β2α5 nAChRs (Kuryatov, Onksen, & Lindstrom, 2008). This is an intriguing finding because, as described above,

Table 3. Repurposing of Existing Therapeutics as Smoking-Cessation Aids

Compound	FDA-approved indication	Phase	Sponsor	Status
Cycloserine	Partial agonist at NMDA receptors used for tuberculosis	I	Baylor College of Medicine	Recruiting
Galantamine	Acetylcholinesterase inhibitor used for Alzheimer's disease	I	Yale University	Completed
Labetalol	Adrenergic antagonist used for hypertension	II	University of Minnesota	Completed
Meclizine	Antihistamine used to treat motion sickness	II	Duke University	Recruiting
Methylphenidate	Stimulant used for treatment of ADHD	III	University of Cincinnati	Completed
Nortriptyline (combined with nicotine patch)	Tricyclic antidepressant	II	Department of Veterans Affairs	Completed
Pioglitazone	PPARγ agonist used for dyslipidemia	I	New York State Psychiatric Institute	Recruiting
Progesterone	Postmenopause hormone-replacement therapy	I	Yale University	Completed
Tolcapone	COMT inhibitor used for Parkinson's disease	II	University of Pennsylvania	Completed
Topiramate	Anticonvulsant with unclear mechanism of action	II	Department of Veterans Affairs	Ongoing

human genetics evidence demonstrate that genetic deficits in α5* nAChR signaling increases vulnerability to tobacco dependence (Bierut et al., 2008; Wang et al., 2009), suggesting that α5* nAChR PAMs may be an effective strategy to promote smoking cessation. Hence, it is an interesting prospect that galantamine and other putative α5* nAChR PAMs may have clinical utility for smoking cessation, particularly in those individuals carrying *CHRNA5* risk alleles that result in decreased function of α5* nAChRs (Bierut et al., 2008).

An example of an FDA-approved medication that could serve as an effective smoking therapeutic, but has yet to be formally tested in clinical trials, is the peroxisome proliferator-activated receptors (PPARα) agonist clofibrate. PPARα agonists are lipid-lowering drugs used to reduce circulating cholesterol levels, although there is debate over their effectiveness and safety (Oliver, 2012). Recently, Goldberg and colleagues demonstrated that clofibrate reduced intravenous nicotine self-administration in rats and squirrel monkeys and attenuated reinstatement of extinguished nicotine seeking triggered by exposure to nicotine or nicotine-associated cues (i.e., reduced relapse-like behavior) (Panlilio et al., 2012). In addition, clofibrate attenuated the stimulatory effects of nicotine on midbrain dopamine systems (Panlilio et al., 2012). Hence, it will be interesting to test whether FDA-approved PPARα agonists can facilitate abstinence in human smokers. Interestingly, there is some evidence that other nuclear hormone receptors may also serve as targets for smoking cessation. In particular, the PPARγ agonist pioglitazone is currently being testing for treatment of alcohol dependence by Omeros Corporation. Pioglitazone (Actos) was originally developed as an antidiabetic drug that improves whole-body insulin sensitivity. Promisingly, Omeros recently completed a successful clinical trial for pioglitazone in alcohol drinking (Stopponi et al., 2011), and it has reported in the patent literature that pioglitazone decreases smoking behavior in a manner comparable with varenicline (Ciccocioppo, 2010).

A growing trend in clinical trials has been the testing of compounds that do not require FDA approval as they are classified as food supplements, such as S-adenosyl-L-methionine (SAMe), *N*-acetyl-cysteine (NAC) and dehydroepiandrosterone (DHEA). SAMe is a methyl donor involved in the synthesis of monoamine neurotransmitters and has been reported to have utility for the treatment of depression (Kagan, Sultzer, Rosenlicht, & Gerner, 1990). It has been reasoned that increasing monoamine neurotransmitter levels through consumption of SAMe, particularly during withdrawal from tobacco smoking when dopamine transmission is hypothesized to decline, may attenuate the aversive effects of tobacco withdrawal and thereby facilitate long-term abstinence. However, no reports on the effects of SAMe on smoking abstinence have appeared in the published literature. In preclinical models of drug dependence, NAC normalizes otherwise decreased levels of basal glutamate in the accumbens and attenuates cue-evoked drug-seeking behaviors (Baker et al., 2003). Moreover, NAC reduced the number of cigarettes consumed by smokers (Knackstedt et al., 2009). Hence, it is possible that NAC may be successfully used to facilitate smoking cessation. DHEA is a neuroactive steroid whose levels were shown to be inversely related to the magnitude of craving and negative affect in abstinent smokers (Marx et al., 2006) and may predict nicotine-dependence severity (Marx et al., 2006). Hence, boosting DHEA levels may decrease craving and negative affect during abstinence and thereby promote continued cessation of the tobacco habit. Based on these observations, it is possible that other compounds currently listed as food supplements may have beneficial effects on smoking cessation.

As repurposing efforts continue, an important challenge for medications development is to identify those molecules most likely to success in clinical trials. Key features in this regard will likely include (a) the molecule should have good compliance and tolerability profiles and present minimal toxicity or other safety concerns (particularly mood-related); (b) candidate molecules for repurposing should target circuits in the brain known to play an important role in tobacco dependence; (c) supporting evidence suggesting that the target modified by the molecule plays a role in tobacco dependence would greatly strengthen the rationale for its clinical assessment, for example, human genetics data suggesting a role for the target in tobacco dependence vulnerability (see above). Molecules that fulfill these three criteria should be considered as strong candidates for repurposing that should undergo testing as novel smoking-cessation aids. For this strategy to identify strong candidates for repurposing to succeed, it is important that knowledge of the neurobiology of tobacco dependence is leveraged to identify the most promising candidates suitable for clinical assessment. As summarized below, current evidence suggests that three key brain circuits in particular that regulate the reinforcing properties of nicotine are the mesoaccumbens dopamine circuit, the habenulo-interpeduncular circuit, and the insular cortex. It is important to note, however, that other brain circuits not considered here are also likely to play a role in tobacco dependence independent of nicotine reinforcement, such as the extended amygdala and its involvement in withdrawal-induced increases in nicotine consumption (George et al., 2007).

The Mesoaccumbens Dopamine Circuit

As described in detail above, the reinforcing actions of nicotine are related to its stimulatory effects on mesoaccumbens dopamine transmission. Indeed, the therapeutic effects of varenicline are hypothesized to be related to its ability to provide a low level of stimulation on midbrain dopamine systems in the absence of nicotine and to attenuate the stimulatory effects of nicotine when the drug is consumed in tobacco smoke. Hence, FDA-approved drugs that modulate midbrain dopamine transmission may be particularly promising candidate for repurposing as smoking-cessation agents.

Habenulo-Interpeduncular Circuit

In addition to the mesoaccumbens system, emerging evidence suggests that the habenulo-interpeduncular system also plays a key role in controlling nicotine intake. The habenula is a diencephalic structure located on the dorsomedial surface of the caudal thalamus that is segregated into medial (MHb) and lateral (LHb) domains (Hikosaka, 2010; Lecourtier & Kelly, 2007). The MHb and LHb are anatomically, chemically, and functionally distinct subnuclei, each with different complements of afferent and efferent connections. LHb receives afferent inputs from, and projects extensively to, midbrain and hindbrain sites. In particular, the LHb projects densely to the rostromedial tegmental nucleus (RMTg) (Jhou, Fields, Baxter, Saper, & Holland, 2009) and has a well-established inhibitory effect on the firing

of midbrain dopamine neurons (Bromberg-Martin & Hikosaka, 2011; Hikosaka, 2010; Lecourtier & Kelly, 2007; Matsumoto & Hikosaka, 2009). LHb neurons are excited by omission of anticipated rewards or exposure to aversive stimuli (Bromberg-Martin & Hikosaka, 2011; Hikosaka, 2010; Lecourtier & Kelly, 2007; Matsumoto & Hikosaka, 2009). This has prompted considerable interest in the role for LHb neurons in encoding negative motivational states. Unlike the LHb, the MHb projects almost exclusively to the interpeduncular nucleus (IPN) via the fasciculus retroflexus (Fr) (Hikosaka, 2010; Lecourtier & Kelly, 2007). MHb is comprised of neurons that produce the neurotransmitters acetylcholine or substance P (Cuello, Emson, Paxinos, & Jessell, 1978; Eckenrode, Barr, Battisti, & Murray, 1987) and a small population that produce the cytokine interleukin-18 (IL-18) (Sugama et al., 2002). However, it is believed that most MHb neurons also produce and co-release glutamate, with this excitatory neurotransmitter considered the major functional transmitter at the MHb-IPN synapse (Girod, Barazangi, McGehee, & Role, 2000; Mata, Schrier, & Moore, 1977; Ren et al., 2011; Vincent, Staines, McGeer, & Fibiger, 1980). The MHb contains some of the highest densities of nicotine binding sites in brain (Mugnaini et al., 2002). In particular, the highest densities of α5, α3 and β4 nAChR subunits expression in brain are detected in MHb and/or IPN (De Biasi & Salas, 2008).

Consistent with an important role for the habenulo-interpeduncular system in regulating nicotine intake, mice with null mutation in the α5 subunit gene were shown to intravenously self-administer far more nicotine than their wildtype littermates (Fowler et al., 2011). Moreover, by using Fos immunoreactivity as a measure of neuronal activation, it was shown that the MHb-IPN pathway of the KO mice was far less sensitive to nicotine-induced activation than wildtype mice (Fowler et al., 2011). Virus-mediated re-expression of the α5 nAChR subunit in the MHb-IPN pathway of KO mice abolished the increased nicotine intake typically seen at higher doses of nicotine (Fowler et al., 2011). Conversely, RNA interference-mediated knockdown of α5 nAChR subunits in the MHb-IPN pathway of rats resulted in increased nicotine intake at higher unit doses of the drug, very similar to the same behavioral profile detected in the KO mice (Fowler et al., 2011). Moreover, chemical inactivation of the MHb or the IPN using the local anesthetic lidocaine or disruption of NMDA receptor-mediated glutamatergic transmission in these sites using the competitive antagonist LY2358959 increased nicotine self-administration in rats in a manner similar to the α5 nAChR subunit KO mice (Fowler et al., 2011). Intriguingly, knockdown of α5nAChR subunits in the MHb-IPN pathway in rats decreased their sensitivity to the reward-inhibiting (i.e., aversive) actions of higher nicotine doses compared with control rats, as measured by nicotine-induced elevations of intracranial self-stimulation (ICSS) reward thresholds (Fowler et al., 2011). These findings suggest that the MHb-IPN system regulates aspects of the aversive properties of nicotine such that deficits in the responsiveness of this circuit may diminish nicotine aversion and increase vulnerability to tobacco dependence. As such, FDA-approved drugs that can amplify the stimulatory effects of nicotine on the MHb-IPN system may be suitable for repurposing as smoking-cessation agents.

Insular Cortex

It has been reported that human smokers with damage to the bilateral posterior (i.e., granular) and right anterior (i.e., agranular) insula cortex are more likely to experience a disruption in tobacco addiction than those with damage to tissues that did not include the insular cortex (Naqvi, Rudrauf, Damasio, & Bechara, 2007). Disruption of tobacco addiction in these individuals was characterized by increased likelihood to spontaneously quit smoking and concomitantly reduced conscious urges to smoke (Naqvi et al., 2007). The insula is comprised of a dorsally located granular region, a ventral agranular region, and the dysgranular region located between these areas. The dorsolateral striatum, a brain area heavily implicated in the expression of habitual responding for drugs of abuse (Chikama, McFarland, Amaral, & Haber, 1997), receives dense innervation from the granular and dysgranular portions of insula. Temporary inactivation of the granular insula reversibly disrupted nicotine and amphetamine seeking in rats (Contreras, Ceric, & Torrealba, 2007; Forget, Pushparaj, & Le Foll, 2010). The insular cortex is also densely innervated by hypocretin-1 (orexin-1) peptide-containing neurons (Hollander et al., 2008), and direct administration of a selective hypocretin-1 receptor antagonist (SB-334867) into the insula, but not into the adjacent somatosensory cortex, dose-dependently decreased nicotine self-administration but not responding for food rewards in rats (Hollander et al., 2008). Based on these data, and the fact that bilateral disruption to the posterior (i.e., granular) insula disrupted tobacco addiction in human smokers, it is likely that molecules that target proteins expressed in the insular cortex and that regulate the activity of this structure may influence the motivation to consume nicotine/tobacco and represent novel targets for therapeutic development.

Finally, the advent of new tools for assessing gene expression patterns in discrete brain sites, such as the Allen Brain Atlas, and novel mouse genetic approaches to identify gene expression patterns in discrete neuronal populations, such as Bac-TRAP mice, should facilitate efforts to determine which candidate drugs for repurposing as smoking cessation agents target brain circuits involved in tobacco dependence.

Development of Smoking-Cessation Therapeutics Based on Novel Targets in the Brain

There are many proteins that have been identified over recent years as key players in regulating the reinforcing properties of nicotine, many of which may be suitable for the development of new products for smoking cessation. For example, hypocretin-1 (orexin-1) receptors are well known to regulate intravenous nicotine self-administration behavior in rats and also play a key role in reinstatement of otherwise extinguished nicotine-seeking behaviors in mice (Hollander et al., 2008; Kenny, 2011b; Plaza-Zabala, Martin-Garcia, de Lecea, Maldonado, & Berrendero, 2010). These findings suggest that hypocretin-1 receptors may be viable targets for the development of smoking-cessation agents (Bartlett & Heilig, 2011; Kenny, 2011a; Plaza-Zabala, Maldonado, & Berrendero, 2012). Similar to hypocretin-1 receptors, metabotropic glutamate 5 (mGlu5) receptor antagonists have also been shown to reduce consumption of nicotine and other addictive in rats and mice (Bespalov et al., 2005; Chiamulera et al., 2001; Kenny et al., 2003; Liechti & Markou, 2007; Palmatier, Liu, Donny, Caggiula, & Sved, 2008; Paterson & Markou, 2005; Paterson, Semenova, Gasparini, & Markou,

2003). Promisingly, the safety of the novel mGlu5 receptor antagonist AFQ056 was recently tested in a phase I clinical trial for smoking cessation. In addition to hypocretin and mGlu5 receptor systems, other signaling systems implicated in nicotine reinforcement that may serve as targets for therapeutic development include neurotensin, NPY Y2 receptor, and κ-opioid receptor (Boules et al., 2011; Ismayilova & Shoaib, 2010; Liu & Jernigan, 2011), and transient receptor potential (TRP) channels (Feng et al., 2006); see Table 4 for a summary of other potential targets for the development of novel therapeutics.

A relatively unexplored opportunity for developing novel smoking-cessation agents is utilizing current knowledge in other fields of addiction biology, most notably cocaine addiction, by extending this knowledge to nicotine dependence. In particular, it is now well established that chromatin remodeling is a major genomic response to cocaine exposure that can alter the subsequent motivational properties of the drug (Borrelli, Nestler, Allis, & Sassone-Corsi, 2008; Renthal & Nestler, 2008). Indeed, proteins involved in epigenetic processes, including histone acetylation (HDACs) (Kumar et al., 2005; Renthal et al., 2007), deacetylation (SIRT1) (Renthal et al., 2009), methylation (G9a) (Maze et al., 2010), DNA methylation (DNMT1 and 3A, 3B) (LaPlant et al., 2010), methyl CpG binding proteins (MeCP2) (Im, Hollander, Bali, & Kenny, 2010), and microRNAs including miR-212 and miR-132 (Hollander et al., 2010; Im et al., 2010; Schaefer et al., 2010), all play essential roles in regulating cocaine reward and cocaine-taking behaviors. However, very little is known concerning the roles for this epigenetic machinery in the reinforcing properties of nicotine, or if targeting this machinery is a viable approach to developing new products for smoking cessation. Intriguingly, it was recently shown that the HDAC inhibitor phenylbutyrate greatly attenuated the rewarding properties of nicotine in rats, as measured in a place conditioning procedure (Pastor, Host, Zwiller, & Bernabeu, 2011). Furthermore, genetic variation in a genomic locus encoding a noncoding RNA (LOC100188947) was recently associated with tobacco dependence ("Genome-wide meta-analyses identify multiple loci associated with smoking behavior," 2010), suggesting that noncoding RNAs may regulate nicotine dependence. Hence, it will be important to assess whether already available pharmacological agents that can modulate chromatin remodeling in brain reward circuitries, particularly those already approved by the FDA such as the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA), may have therapeutic utility as smoking-cessation aids.

Conclusion

Pharmacotherapy is an effective strategy to facilitate smoking cessation, but even the most effective medications currently available have limited clinical efficacy. Moreover, Chantix and Zyban now carry "black box" warnings on their labeling because of reported serious mental-health events associated with their use. Hence, there is a pressing need to develop new effective medications. Much emphasis is currently being placed on repurposing FDA-approved compounds for other indications as potential smoking-cessation medications, reflected in the types of compounds currently in clinical trials. This is an appealing strategy as such compounds are likely to be safe for use in smokers and could be available for use rapidly. In this review, we have recommended some guidelines that may serve to facilitate the identification of already approved drugs for repurposing as smoking-cessation agents. Specifically, three key characteristics of already approved molecules are particularly attractive when considering which may be suitable for clinical assessment: Those molecules that are known to be safe in human with minimal toxicity issues and favorable safety and tolerability profiles; those that modulate the activity of targets expressed in brain circuits known to control the reinforcing properties of nicotine, such as the mesoaccumbens dopamine system, the habenulo-interpeduncular system, and the insular cortex; and finally, those molecules that modulate the activity of targets for

Table 4. Representative Studies in Which Compounds With Various Modes of Action Were Shown to Modulate Nicotine Intake or Withdrawal in Rats

Compound	Mechanism of action	Citation
AT-1001	α3β4* nAChR antagonist	(Toll et al., 2012)
CGP7930, GS39783, BHF177, CGP56433A, CGP44532	GABA-B receptor positive allosteric modulators	(Paterson et al., 2008; Vlachou et al., 2011)
Lorcaserin	5-HT2Creceptor agonist	(Higgins et al., 2012)
LY379268	Metabotropic glutamate 2/3 receptor agonist	(Liechti, Lhuillier, Kaupmann, & Markou, 2007)
PNU-282987	α7 nAChR agonist	(Brunzell & McIntosh, 2012)
R278995/CRA0450	CRF1 receptor antagonist	(Bruijnzeel et al., 2012)
Ro60-0175, M100907	5-HT2Creceptor agonists	(Fletcher et al., 2012)
Sazetidine	α4β3* nAChR desensitizing and partial agonist	(E. D. Levin et al., 2010)
SB-334867	Hypocretin-1 (orexin-1) receptor antagonist	(Hollander et al., 2008; Lesage, Perry, Kotz, Shelley, & Corrigall, 2010; Plaza-Zabala et al., 2010)
Various substituted heteroaromatic compounds	hCYP-2A6 inhibitors	(Cashman et al., 2012)
VDM11	Anandamide transport inhibitor	(Gamaleddin, Guranda, Goldberg, & Le Foll, 2011)

which there is evidence to support a role in tobacco dependence, for example, human genetics evidence or anecdotal reports of decreases in smoking behavior.

It is important to point that our understanding of the basic neurobiological mechanisms of tobacco dependence now far exceeds the established contribution of α4β2* nAChRs, which served as the major target for the development of Chantix. Existing knowledge on the contribution of targets suitable for medications development should be leveraged to develop new therapeutics with novel modes of action. As highlighted in this review article, we now know of a plethora receptors involved in regulating nicotine reinforcement, many of which may be suitable for medications development. A prime example in this regard is the hypocretin-1 receptors, which in preclinical studies has been shown to regulate nicotine reinforcement, yet this information has not translated into new therapeutics for smoking cessation. As our understanding of the basic mechanisms to nicotine addiction and tobacco-dependence increase, it is to be hoped that this information will indeed translate into new, highly effective medications.

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None declared.

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