Review

Mechanism-based medication development for the treatment of nicotine dependence

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Tobacco use is a global problem with serious health consequences. Though some treatment options exist, there remains a great need for new effective pharmacotherapies to aid smokers in maintaining long-term abstinence. In the present article, we first discuss the neural mechanisms underlying nicotine reward, and then review various mechanism-based pharmacological agents for the treatment of nicotine dependence. An oversimplified hypothesis of addiction to tobacco is that nicotine is the major addictive component of tobacco. Nicotine binds to α4β2 and α7 nicotinic acetylcholine receptors (nAChRs) located on dopaminergic, glutamatergic and GABAergic neurons in the mesolimbic dopamine (DA) system, which causes an increase in extracellular DA in the nucleus accumbens (NAc). That increase in DA reinforces tobacco use, particularly during the acquisition phase. Enhanced glutamate transmission to DA neurons in the ventral tegmental area appears to play an important role in this process. In addition, chronic nicotine treatment increases endocannabinoid levels in the mesolimbic DA system, which indirectly modulates NAc DA release and nicotine reward. Accordingly, pharmacological agents that target brain acetylcholine, DA, glutamate, GABA, or endocannabonoid signaling systems have been proposed to interrupt nicotine action. Furthermore, pharmacokinetic strategies that alter plasma nicotine availability, metabolism and clearance also significantly alter nicotine's action in the brain. Progress using these pharmacodynamic and pharmacokinetic agents is reviewed. For drugs in each category, we discuss the mechanistic rationale for their potential anti-nicotine efficacy, major findings in preclinical and clinical studies, and future research directions.

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Introduction

Nicotine use and dependence is a world-wide health problem. In the United States alone, approximately 45 million people smoke cigarettes and the adverse health effects from cigarette smoking account for an estimated half million deaths each year[1]. Though many cigarette smokers report a desire to quit smoking, few are successful. In fact, according to the US Department of Health and Human Services, approximately 80% of smokers who attempt to quit relapse before achieving 6 months of abstinence. Of the remainder, relapses may occur years after a smoker initially quits[2]. Consequently, there is a great need for pharmacotherapies to aid smokers who wish to quit.

Although the mechanisms underlying tobacco addiction are not completely understood, accumulating evidence indicates that nicotine is the major addictive component[3, 4]. In preclinical experimental conditions, nicotine produces many hallmark behaviors observed with other addictive drugs. Non-contingent administration of nicotine stimulates locomotor activity[5, 6] and enhances electrical brain-stimulation reward[7]. It can also reinforce intravenous self-administration[3, 8], produce conditioned place preference[9], and serve as a discriminative stimulus in animals[10]. In addition, nicotine cessation produces withdrawal syndromes with both somatic and affective symptoms[4, 11], and those symptoms can be alleviated by nicotine replacement.

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Neural mechanisms underlying nicotine dependence

Nicotinic acetylcholine receptors (nAChRs) Nicotine is an alkaloid that binds to central and peripheral nicotinic acetylcholine receptors (nAChRs). Acetylcholine (ACh) is

an endogenous neurotransmitter that binds to and activates nAChRs. Neuronal nAChRs are ligand-gated ion channels with high permeability to Ca^{++}, and are formed from combinations of five subunits[12, 13]. To date, twelve different neuronal nAChR subunits have been cloned, including nine α-subunits (α2-α10) and three β-subunits (β2-β4). Non-neuronal subunits, α1, β1, γ, δ, and ε, form peripheral nicotinic receptors at the neuromuscular junction[14]. The neuronal subunits combine with a stoichiometry of two α- and three β-, or five α7-subunits to form nAChRs[15]. Both the α4β2 and α7 subtypes of nAChRs are the most abundant subtypes in the brain and are localized on presynaptic terminals, axons, somatodendrites or on postsynaptic cells[16, 17]. Overall, activation of presynaptic nAChRs by ACh or nicotine potentiates neurotransmitter release, while activation of postsynaptic nAChRs increases excitability of postsynaptic cells by increasing Ca^{++} influx via nAChR channels.

ACh-glutamate-GABA-DA mechanisms underlying nicotine dependence Although many areas of the brain are involved in reward, the mesocorticolimbic dopamine (DA) system serves a vital and fundamental role in mediating the rewarding and psychostimulant effects of addictive drugs, including nicotine[9, 18]. This system originates from DA neurons in the ventral tegmental area (VTA) in the midbrain and projects to the nucleus accumbens (NAc), the amygdala and the prefrontal cortex[18]. The activity of VTA DA neurons is regulated by excitatory glutamatergic inputs predominantly from the prefrontal cortex, cholinergic inputs from brainstem nuclei and inhibitory GABAergic inputs within the VTA or from the NAc (Figure 1). This DA model is supported by a number of findings[9, 19]. For example, nicotine self-administration elevates NAc DA, and that elevation reinforces nicotine self-administration, particularly during the acquisition phase[20]. In contrast, chemical lesion of DA terminals or pharmacological blockade of DA receptors in the NAc attenuates the rewarding effects of nicotine, as indicated by reduced self-administration[8, 21].

Several studies suggest that the α4β2 nAChR subtype plays a major role in nicotine reward. First, pretreatment with the selective α4β2 receptor antagonist dihydro-β-erythroidine (DHβE) or the partial agonist SSR591813 significantly inhibits nicotine self-administration in rats[22, 23]. Similarly, genetic deletion of β2 subunits abolishes nicotine self-administration[24]. Second, *in vivo* microdialysis studies indicate that the selective α4β2 receptor partial agonist SSR591813 or genetic deletion of α4 or β2 subunits prevents nicotine-induced increase in NAc DA[23–25]. Third, nicotine-mediated currents from VTA neurons are inhibited by DHβE[24, 26–28] or dramatically decreased on midbrain neurons in β2-null mice[24, 28]. Fourth, brain slices from α4- or β2-subunit knockout mice lack high-affinity nicotine binding, confirming that most (if not all) binding sites for nicotine in adult brains contain α4β2-containing receptors[24, 29]. These data suggest that both α4 and β2 subunits in the VTA are crucial in mediating nicotine's DA-releasing effects. Thus, the effects of nicotine on DA function could be mediated by activation of α4β2 nAChRs located on DA neurons in the VTA and on DA terminals in the NAc[30].

In addition, several studies suggest the involvement of other nAChR subunits, including α3, α6, α7, and β3, in the control of DA release induced by nicotine. It was reported α7 nAChRs are also expressed on VTA DA neurons [31, 32], and nicotine still activates midbrain neurons in β2-subunit knockout mice by a α7-nAChR mediated mechanism[28], suggesting the involvement of α7 nAChRs in nicotine's action. Differential distribution of nAChR subtypes has been found on glutamatergic (α7) and GABAergic (α4β2) terminals in the VTA[27, 28, 33]. Different nAChR subtypes appear to show different levels of desensitization: the α4β2 subtype desensitizes more rapidly than the α7 subtype[32]. Therefore, it has been proposed that nicotine first activates then desensitizes α4β2 nAChRs on VTA DA neurons, producing an initial fast increase in extracellular DA in the NAc[12, 24, 26]. At the same time, nicotine also excites α7 nAChRs located on presynaptic glutamatergic terminals and increases excitatory glutamatergic inputs to VTA DA neurons. Since the α7 nAChRs have much lower affinity for nicotine than α4β2 nAChRs, and are therefore much less susceptible to desensitization by low concentrations of nicotine obtained from tobacco smoking, the enhanced glutamate release causes prolonged DA neuron activation[34]. In addition, nicotine may also excite α4β2 receptors located on VTA GABAergic neurons to increase GABA release[35]. Thus, the DA-releasing effects of nicotine may result from a modification of the balance between excitatory and inhibitory inputs to DA neurons[12, 26, 36]. This hypothesis may in part explain the finding with *in vivo* microdialysis that a single injection of nicotine produces long-term (2 h) increases in extracellular NAc DA[9].

It should be pointed out that not all evidence supports this hypothesis. For example, conflicting findings were found in mutant mice lacking the α7 receptors or in rats injected with the relatively selective α7 nAChR antagonist methyllycaconitine[37, 38]. Although much attention has focused on the VTA-NAc pathway, many other brain sites that are not yet extensively studied are also likely to contribute to nicotine reward and addiction.

Chronic nicotine-induced neuroadaptations Most smokers report that the first cigarette of the day produces the

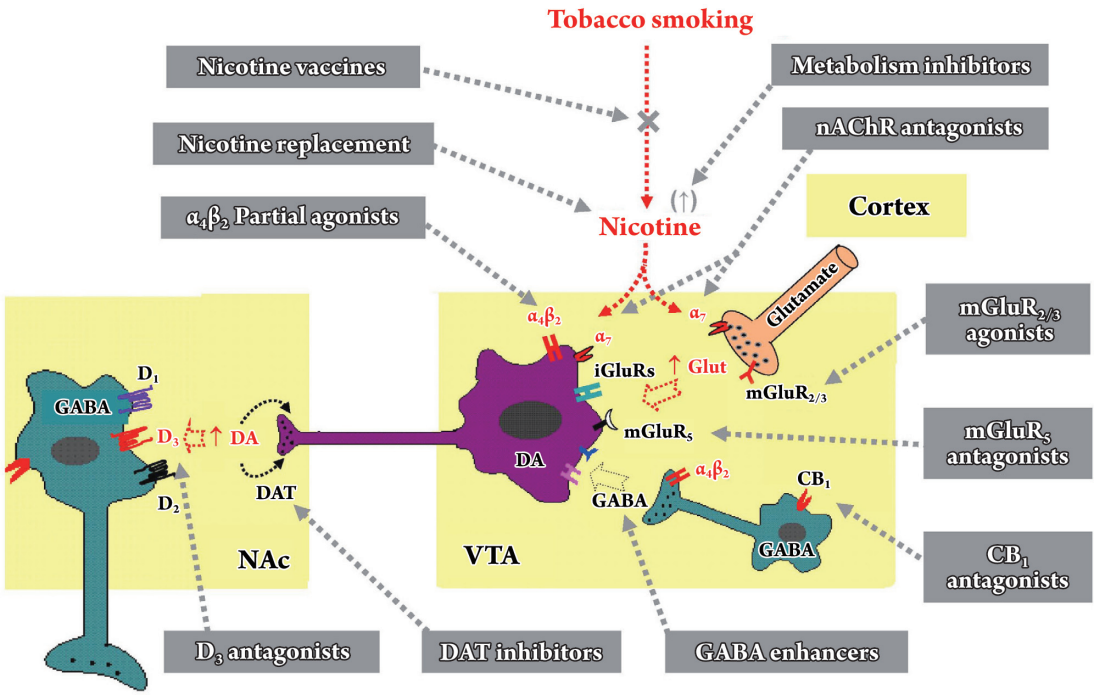

Figure 1. Schematic diagram of the mesolimbic dopamine (DA) projection pathway, illustrating the actions of nicotine on extracellular DA, glutamate and GABA in the ventral tegmental area (VTA), and the sites of action of various mechanism-based pharmacological agents in medication development for the treatment of tobacco dependence. The mesolimbic DA system originates in the VTA and projects to the nucleus accumbens (NAc). In the VTA, DA neurons (purple) are under tonic excitatory glutamatergic afferent influence from the medial prefrontal cortex (orange), and tonic inhibitory GABAergic afferent influence from GABAergic interneurons (teal) and also from long-loop GABAergic projections from the NAc (not shown). Nicotine activates mesolimbic DA neurons either via $\alpha_4\beta_2$ nAChRs located on VTA DA and GABAergic neurons or via α_7 nAChRs on DA neurons and glutamatergic terminals. Chronic nicotine exposure may also increase endocannabinoid contents in the VTA and NAc, which may remove the tonic inhibitory GABAergic control on VTA DA neurons via CB_1 receptors localized on VTA GABAergic neurons or their terminals. Based on this hypothesis, various pharmacological agents that target ACh, DA, glutamate, GABA, and endocannabinoid transmission have been proposed and studied for their potential use in the treatment of tobacco dependence. More details for each class of pharmacological agents are discussed in the text of this review.

most powerful effects[39], suggesting that fast tolerance and desensitization develop after repeated exposure to nicotine. This could be related to findings that chronic exposure to nicotine results in fast desensitization of α4β2 nAChRs, leading to upregulation of α4β2 nAChRs on the cell surface[12, 28, 40–42]. On the other hand, repeated administration of nicotine is also associated with sensitization, an effect that appears to be mediated (at least with respect to locomotor sensitization) by α4β2 subunits[23, 43]. Sensitization of the motivational effects of nicotine is also seen in the self-administration and conditioned place preference paradigms[44, 45]. These neuroadaptations may contribute to the development of nicotine addiction[36].

Animal models of nicotine dependence

Animal models of addiction are, by definition, approximations of human drug abuse. A major obstacle to the development of medication for nicotine dependence is the lack of animal models with sufficient predictive clinical validity, and therefore, multiple animal models have to be used to emulate different aspects of nicotine dependence in humans. Six behavioral animal models or measures have been widely used in research on nicotine dependence.

Self-Administration Drug self-administration reliably models drug reinforcement[46]. In this model, laboratory animals are allowed to operantly self-administer addictive drugs, such as nicotine. Two commonly used self-administration paradigms are fixed-ratio (FR) and progressive-ratio (PR) schedules of drug reinforcement. In the FR paradigm, a drug infusion follows after a fixed number of responses by the animal, *eg*, after every one (FR1) or two (FR2) lever presses. In the PR reinforcement paradigm, a progressively increasing work-load (*eg*, lever pressing) is imposed upon the animal in

order to receive one drug administration. Eventually, a point is reached at which the animal stops responding. This is termed the PR "break-point" and is considered a measure of rewarding efficacy[47].

Reinstatement of drug-seeking behavior Nicotine dependence is characterized by high rates of relapse to tobacco use. The reinstatement animal model is widely used to model relapse to tobacco use in humans[48]. In this model, rats are implanted with intravenous catheters and are allowed to self-administer nicotine until stable nicotine-taking is achieved. Then, vehicle is substituted for nicotine. Since the animals are no longer rewarded, they stop ("extinguish") the nicotine-seeking behavior. Next, the experimenter administers a stimulus to "trigger" the animal to relapse — to go back to the drug-seeking behavior that previously resulted in intravenous infusions of nicotine. Three triggers cause relapse in this model: 1) re-exposure to nicotine, 2) re-exposure to environmental cues that were previously associated with nicotine self-administration, or 3) exposure to mild stress. The face validity of the reinstatement models rest upon the fact that these are the triggers that provoke relapse to tobacco use in humans[46].

Conditioned Place Preference (CPP) The CPP model is an experimental procedure to study the rewarding effects of nicotine and/or reward-related learning and memory. In this model, a distinctive environment (in wall color, light, floor texture) in one compartment of a two- or three-compartment apparatus is paired repeatedly with nicotine or vehicle injections. CPP occurs when repeated nicotine administration in one particular environment results in the ability of previously neutral environmental stimuli to elicit approach behavior and increased time spent in that environment even in the absence of nicotine administration. It has been argued that CPP, like self-administration and several other behavioral measures, is an example of DA-mediated incentive learning and memory, and that the approach behavior and increased time spent by animals in nicotine-paired environment can be considered a measure of nicotine-seeking behavior[49].

Drug discrimination Drug discrimination procedures are often used as animal models for the subjective effects of an addictive drug[46]. The animal is trained to make one response when nicotine is given and a different response when vehicle is given. Well-trained animals typically make close to 100% appropriate responses to discriminate nicotine from vehicle. The degree to which a novel drug is perceived by the animal as "nicotine-like" versus "not-nicotine-like" is reflected in the percentage of nicotine-associated responses versus vehicle-associated responses. By combining a novel drug (*eg*, a putative anti-nicotine therapeutic agent) with nicotine in this paradigm, one can determine the degree to which the novel drug increases or decreases the subjective "nicotine-like" feeling experienced by the animal.

Brain stimulation reward Virtually all addictive drugs not only have rewarding actions of their own, but also potentiate the rewarding actions of other substances or events[50]. The brain stimulation reward (BSR) paradigm models this property of addictive drugs by directly assessing the degree of drug-induced enhancement of BSR in animals trained to respond for electrical stimulation of specific brain-reward loci such as the VTA, medial forebrain bundle, or NAc. To assess drug-induced enhancement of BSR, the "rate-frequency curve-shift" paradigm is commonly used to measure changes in BSR thresholds after drug administration. Addictive drugs (such as nicotine) produce highly characteristic leftward shifts (*eg*, decreased BSR threshold) in these functions, indicating summation between the reward provided by the electrical stimulation and the drug-induced reward. This paradigm is therefore useful in the search for compounds with potential anti-addictive therapeutic properties and, conversely, to screen compounds for reward-enhancing properties, which might be predictive of intrinsic addictive potential[46].

Withdrawal Many tobacco smokers report that they experience unpleasant withdrawal symptoms when they quickly quit smoking. In experimental animals, abrupt cessation of chronic nicotine or administration of nAChR antagonists causes somatic withdrawal symptoms, such as shakes/tremors, gasps/writhes, teeth chattering and ptosis[51, 52], which may in part mimic withdrawal symptoms experienced by abstinent smokers[53]. In addition, nicotine withdrawal also results in reduced DA overflow in the NAc[54] and elicits changes in behavior that are characteristic of anhedonia[11]. BSR, described above, can also be used to measure nicotine withdrawal-induced anhedonia[36, 46]. These changes are thought to model the dysphoria experienced by many smokers when they first quit[55]. Strikingly, all these symptoms can be reversed by nicotine replacement therapy[11]. Thus, relieving nicotine withdrawal symptoms, thought to be an important reason for relapse to tobacco use, may be another strategy to aid cessation of tobacco smoking.

Mechanism-based medication discovery

Although several types of pharmacological therapies have been approved for smoking cessation in both North America and Europe, long-term abstinence rates are less optimal. These approved pharmacological therapies include

nicotine replacement, the antidepressant bupropion and the α4β2 receptor partial agonist varenicline. The efficacy of bupropion and varenicline for smoking cessation has raised questions about how a non-nicotine drug can aid in smoking cessation. Here we review recent progress on "mechanism"-based medication strategies for the treatment of nicotine dependence at both preclinical and clinical levels. These strategies include various pharmacological agents that target brain ACh, DA, glutamate, GABA and endocannabinoid transmission, and pharmacokinetic approaches that alter blood nicotine concentrations, metabolism and clearance.

ACh-based medication development

Nicotine replacement Nicotine replacement therapy (NRT) is an early pharmacotherapy approved in the early 1980s for smoking cessation[56]. The rationale for NRT is similar to that for methadone or buprenorphine for the treatment of opiate dependence. That is, NRT uses safe delivery forms of nicotine to replace the nicotine obtained from cigarettes, thereby eliminating tobacco smoking and tobacco-related illnesses. Various NRTs are currently available and include gums, transdermal patches, lozenges, tablets, and inhalers. NRTs have been shown to be effective in aiding abstinence from cigarette smoking behavior, reducing the rewarding effects produced by nicotine from cigarettes, attenuating affective and somatic withdrawal symptoms, relieving craving and reducing relapse risk. However, efficacy is low and only lasts for a short period of time. At best only about 20% of smokers are able to maintain long-term abstinence with any of these approaches, and first year relapse rates are as high as 80%. The reasons underlying such low efficacy of NRTs are unclear, but likely to be related to their relatively poor pharmacokinetic properties compared to nicotine delivered via smoking. Thus, much research has been directed to develop other non-nicotine strategies for the facilitation of smoking cessation.

Nonselective nAChR antagonists In theory, a nonselective nAChR antagonist would block the physiological and reinforcing effects of cigarette smoking, and thereby lead to extinction of cigarette smoking behavior. A possible side-effect is that an antagonist may precipitate withdrawal symptoms, and thus increase the risk of relapse to cigarette smoking.

Mecamylamine Mecamylamine is a non-competitive nicotinic antagonist, originally used as an antihypertensive agent[57]. Widely used in the 1950s, this orally effective antihypertensive agent is now rarely used because of its widespread ganglionic side-effects at antihypertensive doses. However, recent studies suggest that mecamylamine, at relatively low doses, significantly attenuates the physiological and rewarding effects of nicotine, and improves abstinence rates in smoking cessation studies, particularly for women[58]. In particular, mecamylamine, when combined with NRT, significantly reduces craving for cigarettes and produces prolonged abstinence rates (37.5% versus 4.2% for 12 months) when compared with NRT alone[59]. Preclinical studies demonstrate that mecamylamine attenuates intravenous self-administration of nicotine[3, 60, 61], reduces nicotine-enhanced brain-stimulation reward[62], blocks nicotine-conditioned place preference[63], and inhibits the stimulant effect of nicotine on locomotor activity[64]. In contrast, it has also been reported that mecamylamine causes an increase rather than a decrease in smoking behavior, likely a compensatory response to partially reduced nicotine reward[65]. Further, a recent study did not find an added benefit of combining mecamylamine with use of a transdermal nicotine patch[66]. Thus, further studies are required to confirm mecamylamine's efficacy for smoking cessation. Mecamylamine is currently in Phase III clinical trials in the United States, and is not yet approved by the US Food and Drug Administration (FDA) for smoking cessation.

nAChR partial agonists Given the central role of $\alpha_4\beta_2$ nAChRs in nicotine reward as noted above, modulating the activity of these receptors is expected to have therapeutic benefits. Partial agonists, by definition, have lower intrinsic functional activity, and therefore, produce a smaller maximal effect at full receptor occupancy than do full agonists. By mimicking some of the agonist rewarding effects of nicotine, partial $\alpha_4\beta_2$ nAChR agonists should, theoretically, relieve craving and withdrawal symptoms during abstinence. In addition, high affinity α4β2 nAChR partial agonists may also prevent nicotine binding to α4β2 nAChRs, therefore producing an "antagonistic" anti-nicotine effect. These considerations prompted the search for ligands that act as partial agonists at the $\alpha_4\beta_2$ nAChR subtype of as novel treatments for smoking cessation.

Varenicline Varenicline is a partial agonist at α4β2 and a full agonist at α7 nicotinic receptors[67, 68]. Both chemically and pharmacologically, varenicline is similar to cytisine, a plant alkaloid with high affinity for several subtypes of nAChRs[69]. Cytisine has been used in Eastern Europe for over 40 years as a treatment for tobacco dependence in the form of an extract from the herb Cytisus Laborinum L (Golden Rain acacia)[70]. In 1994, it was reported that cytisine is a weak partial agonist at nAChRs with limited absorption into the brain[71], providing an additional rationale for the use of partial agonists for smoking cessation. Direct chemical modifications of cytisine have lead to two novel highly potent and selective α4β2 nAChR partial ago-

nists-varenicline and dianicline. Varenicline, developed by Pfizer Inc, has been approved by the US FDA as a therapeutic aid to quit smoking, while dianicline, developed by Sanofi-Aventis, is currently under Phase III clinical trials[69, 72, 73].

Preclinical studies demonstrate that varenicline elevates extracellular DA in the shell of the NAc, an effect that is weaker than that evoked by nicotine. Pretreatment with varenicline significantly inhibits nicotine-enhanced NAc DA and nicotine self-administration[69, 72, 73]. Varenicline itself partially substitutes for nicotine in animal self-administration paradigms and partially generalizes to nicotine as a discriminative stimulus[69, 74]. Consistent with these findings, we have recently reported that varenicline also significantly inhibits nicotine-enhanced electrical brain-stimulation reward, an effect that is mediated by activation of α4β2, but not α7, nAChRs[75]. It has also been reported that varenicline significantly reduces ethanol, but not sucrose, self-administration, and decreases voluntary ethanol, but not water, consumption in rats[76]. Clinical trials indicate superior efficacy of varenicline over placebo and bupropion for achieving abstinence from smoking, and varenicline has also been shown to significantly delay smoking relapse[73, 77, 78]. The safety profile of varenicline is generally good, with the most commonly occurring adverse event being nausea[79]. However, new safety warnings were added to the varenicline label in early 2008 because of post-marketing reports of neuropsychiatric symptoms including agitation, depression and suicidality[79]. A causal relationship between varenicline use and these symptoms has not been established.

Nicotine metabolism inhibitors In addition to targeting nAChRs, another approach is to elevate blood nicotine concentrations by reducing nicotine metabolism, thereby decreasing the number of cigarettes smoked[80]. In humans, approximately 80% of absorbed nicotine is metabolized to cotinine by the hepatic enzyme CYP2A6[81]. Nicotine is also excreted unchanged and metabolized to other minor metabolites, but these pathways account for only a small portion of nicotine. Based on this, it has been proposed that CYP2A6 inhibitors may have therapeutic potential for the treatment of tobacco dependence[82]. In support of this hypothesis, it was reported that the strong CYP2A6 inhibitors methoxsalen and tranylcypromine significantly elevate plasma nicotine levels during smoking or NRT treatment[83, 84] and significantly decrease the desire to smoke[85]. Similarly, human subjects with genetically low CYP2A6 activity have an increased likelihood (1.75 fold) of quitting smoking[86], suggesting that CYP2A6 inhibitors may hold some promise for smoking cessation.

Selegiline Compared to other CYP2A6 inhibitors, selegiline is not only a competitive CYP2A6 inhibitor, but also a selective and irreversible monoamine oxidase B (MAO-B) inhibitor. In the brain, MAO-B is the major enzyme that, together with MAO-A, metabolizes brain DA[87]. Since MAO-B activity is 40% lower in the brain of smokers compared to nonsmokers[88], and this decrease in MAO-B is reversed during long-term smoking abstinence[89], it has been suggested that a tobacco smoke component with MAO-B inhibition activity may contribute to the rewarding effects of cigarette smoking[90, 91]. Based on this, selegiline has been investigated as a potential therapy for smoking cessation. Several clinical studies suggest that selegiline is effective in reducing withdrawal symptoms and increasing abstinence compared with placebo. For instance, selegiline has been shown to significantly reduce smoking satisfaction during smoking and decrease craving during abstinence[92]. In addition, it has also been reported that oral selegiline increases smoking cessation trial endpoint (8-week) abstinence compared with placebo by 3-fold[93]. When combined with nicotine patch, selegiline doubled the 52-week continuous abstinence rate compared with nicotine patch alone[94]. In addition, there is no evidence indicating that selegiline is addictive[95]. Taken together, selegiline may have therapeutic potential for smoking cessation by inhibiting both nicotine and DA metabolism.

Nicotine vaccines The nicotine vaccine is a newer strategy being investigated for smoking cessation. The principle of this strategy is to prevent nicotine from entering the brain. In immunized individuals, nicotine obtained from smoking is bound by nicotine-specific antibodies and cannot cross the blood-brain barrier, thus preventing its central effects. Since nicotine itself is not immunogenic, it must be conjugated to larger carrier proteins that can act as immunogenic molecules. Currently, there are at least five companies developing nicotine vaccines using different antigenic molecular approaches. An advantage of nicotine vaccines is that daily administration of the drug is not required, and only occasional booster shots are needed to maintain an adequate antibody titer. A major concern with nicotine vaccines is that the titer of antibodies after immunization may not be sufficient to sequester all of the nicotine in blood, limiting vaccine utility for preventing nicotine entry into the brain during smoking[80].

Preclinical studies indicate that passive immunization in rats with nicotine antibodies prevents nicotine-conditioned place preference and attenuates withdrawal symptoms[96]. Active immunization with nicotine vaccines significantly reduces (~65%) nicotine distribution into the brain[97], and inhibits nicotine self-administration, although it failed to

prevent the acquisition of nicotine self-administration[98]. In addition, active immunization also significantly prevents nicotine-triggered reinstatement of nicotine-seeking behavior[99]. Small-size clinical trials indicate that high doses of nicotine vaccine significantly increase continuous abstinence rates compared with placebo (38% *vs* 10% for 30 days), and do not cause compensatory smoking behaviors or precipitate withdrawal[100]. There are two nicotine vaccines, developed by Cytos Biotechnology (http://www.cytos.com) and Nabi Biopharmaceuticals (NicVAXTM) (http://www.nabi.com), which are currently under Phase II clinical trials for smoking cessation. High abstinence rates were achieved with nicotine vaccines compared with the placebo (57% versus 31% at 6 months; 42% versus 21% at 12 months). So far, there have been no serious adverse events associated with such vaccines[80, 100]. These data suggest that nicotine vaccines may be useful for smoking cessation treatment.

DA-based medication development As noted above, the mesolimbic DA system is critically involved in drug reward and addiction, including addiction to nicotine[101, 102]. Based on this, much work on the development of new medications for treatment of tobacco addiction has focused on manipulation of DA transmission in the reward circuitry of the brain. Two major pharmacological strategies of manipulating brain DA transmission have emerged as the basis for anti-nicotine medication development: one being to target brain DA receptors with either partial agonists or antagonists, and another being to target brain DA transporters. Although both DA D_1 and D_2 receptors have been shown to be critically involved in drug reward and addiction[103, 104], clinical trials with D_1- or D_2-like receptor antagonists have failed, due to lack of therapeutic effect with D_1-like antagonists or severe side-effects with D_2-like antagonists – such as dysphoria, suppression of natural reward or abnormal movements[105]. In marked contrast to DA D_1 and D_2 receptors, the D_3 receptor subtype has a restricted distribution in the brain; that is, D_3 receptors are selectively expressed in the mesolimbic DA system with the highest receptor densities in the NAc, islands of Calleja and olfactory tubercle[106, 107]. This restricted neuroanatomic localization suggests that D_3 receptors may play an important role in drug reward and addiction[108]. In addition, D_3 receptors have the highest affinity for endogenous DA of all known receptors[109, 110], suggesting a crucial role for D_3 receptors in the normal functioning of the mesolimbic DA system. Moreover, chronic exposure to nicotine significantly increases the expression of D_3 receptor binding and mRNA levels in the mesolimbic DA system[111]. Based on this, it has been hypothesized that selective D_3 receptor partial agonists or antagonists would be effective in the treatment of nicotine dependence[108, 109, 112].

DA D_3 receptor partial agonists or antagonists The rationale for D_3 partial agonists as novel treatments for tobacco dependence is that: 1) D_3 partial agonists are expected to modestly activate D_3 receptors, and therefore blunt cigarette craving and withdrawal during abstinence; and 2) D3 partial agonists would have additional therapeutic anti-nicotine benefit by blocking nicotine-enhanced DA binding to D_3 receptors. In other words, such a compound can act either as an agonist or antagonist depending on the prevailing DA tone.

BP-897 BP-897 is the first developed D_3-selective partial agonist[113]. It has modest (60–70 fold) selectivity for human D_3 versus D_2 receptors, and similar (60–70 fold) selectivity over other receptors including α_1-, α_2-adrenergic, and 5-HT_{1A} receptors[113]. In experimental animals, BP-897 produces a significant dose-dependent reduction in the expression of nicotine-induced CPP[111], nicotine-enhanced brain stimulation reward (Xi and Gardner, unpublished data), nicotine-conditioned locomotor responses, and nicotine-induced increases in D_3 receptor expression in the NAc[111, 114]. In contrast, BP-897 failed to alter the dose–response curve for nicotine drug discrimination. When substituted for the training dose of nicotine, BP-897 did not produce nicotine-like discriminative-stimulus effects[111]. In addition, BP-897 fails to alter locomotor activity and food-maintained behavior[115]. These findings suggest that BP-897 may selectively reduce the motivational effects of nicotine without significant unwanted side-effects by itself. However, enthusiasm for BP-897 has waned due to recent findings that BP-897 also displays full antagonist properties at both DA D_2 and D_3 receptors[116–118], suggesting that its therapeutic anti-nicotine effects could be mediated by blockade of D_2 and/or D_3 receptors. Since D_2 receptor antagonism usually produces severe unwanted side-effects, such as dysphoria, inhibition of natural reward, and abnormal extra-pyramidal movements[103–105], it is suggested that BP-897, at high doses, may also produce such side-effects at the human level. BP-897 has recently entered Phase II clinical studies, but detailed pharmacokinetic and toxicological data have not yet been reported.

SB-277011A SB-277011A is the most well characterized full D_3 receptor antagonist to date. SB-277011A has high affinity for the human cloned DA D_3 receptor, and the ratio of *in vitro* D_3/D_2 affinity of SB-277011A for human and rat is 120 and 80, respectively[119]. SB-277011A has a 100-fold or better selectivity over 180 other receptors, enzymes and ion channels[119]. A series of studies has assessed the efficacy of SB-277011A in animal models of nicotine dependence[120, 121]. SB-277011A significantly inhibits

nicotine self-administration under progressive-ratio (PR), but not low FR reinforcement schedules[122, 123], nicotine-induced CPP[7], nicotine-enhanced brain reward, and nicotine-paired environmental cue functions[7]. In addition, SB-277011A also inhibits nicotine-induced reinstatement of drug-seeking behaviors[122] and nicotine cue-induced conditioned locomotor activity[7, 114]. However, further development of SB-277011A has been halted by Glaxo-SmithKline Pharmaceuticals, due to unexpectedly poor bioavailability (~2%) and a very short half-life (<20 min) in primates[124]. Therefore, development of other D_3-selective antagonists with higher bioavailability and more promising pharmacotherapeutic profiles is required[125]. There are two novel DA D3 receptor-selective antagonists, GSK598809, and GSK-618334, which are currently under clinical phase I for the treatment of drug addiction, including nicotine dependence (http://clinicaltrials.gov/ct2/results?term=GSK-618334).

DA transporter (DAT) inhibitors Although there is no evidence that nicotine can act directly on the DAT protein, several studies suggest a potential relationship[126]. First, a single dose of nicotine enhances DA clearance in rat NAc, suggesting that nicotine regulates extracellular DA concentration via the DAT[127]; and second, nAChR activation by acute and chronic nicotine augments amphetamine-induced reverse transport of DA by the DAT[128, 129]. This DA enhancement by nicotine was fully reversed by the nicotinic receptor antagonists DHβE and mecamylamine, suggesting that nAChRs modulate DAT function[128].

Bupropion Bupropion is an efficacious antidepressant and smoking cessation agent which inhibits the DAT and the norepinephrine transporter in addition to acting as a nicotinic antagonist at α3β2 and α3β4 nAChRs in rat striatum and hippocampus[130]. Bupropion is endorsed by the US Clinical Practice Guideline as a first-line pharmacotherapy for treatment of tobacco abstinence[56]. Since tobacco smokers undergoing cessation often experience symptoms of depression[130, 131], it is speculated that bupropion's pharmacotherapeutic efficacy may be mediated by its antidepressant effects combined with blockade of the DAT and some nAChRs. In animal studies, acute administration of bupropion attenuated both nicotine-enhanced brain-stimulation reward and the brain-stimulation inhibition associated with nicotine withdrawal[132]. Also, bupropion blocks the acquisition of nicotine-induced CPP and mecamylamine-precipitated withdrawal[133]. In the self-administration paradigm, bupropion produces a biphasic effect: low dose bupropion increases, whereas high dose bupropion decreases, nicotine self-administration in rats[134]. The increase in nicotine self-administration could be a compensatory response to a reduction in nicotine reward after low doses of bupropion. In humans, the majority of studies have demonstrated that bupropion is more effective at improving smoking cessation than placebo[135, 136]. Bupropion has also been reported to reduce nicotine abstinence-associated depression, difficulty concentrating, and irritability, relative to placebo[137]. A recent meta-analysis of several trials shows that bupropion nearly doubles smoking cessation rates with a similar efficacy to NRT[138, 139].

Glutamate-based medication development Glutamate is the major excitatory neurotransmitter in the brain and plays a critical role in the acute and long-term effects of nicotine. The actions of glutamate are mediated by both ionotropic (iGluR) and metabotropic (mGluR) glutamate receptors. The iGluRs include N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate receptors, which are located primarily on postsynaptic cells and regulate cellular excitability by opening glutamate-gated ion channels. The mGluRs are classified into three groups based on sequence homology, signal transduction pathways and pharmacological actions. Group I (mGluR1 and mGluR5) receptors are predominately located postsynaptically where they couple to G_q-proteins to activate phospholipase C. Group II (mGluR2 and mGluR3) and Group III (mGluR4, mGluR6, mGluR7 and mGluR8) receptors are primarily found presynaptically and on glial cells, and couple to $G_{i/o}$ proteins to negatively regulate adenylyl cyclase activity. Activation of group II or III mGluRs negatively modulates glutamate release.

Nicotine binds with high affinity to nAChRs located on presynaptic glutamatergic terminals in various brain sites, including the VTA, NAc, prefrontal cortex and hippocampus, producing an increase in glutamate release[32, 140, 141]. In the VTA, the α7 nAChR subtype has been shown to be localized on VTA DA neurons[31, 32] and presynaptic glutamatergic afferents[142]. Activation of the α7 receptors by nicotine increases glutamate release in the VTA and activates iGluRs located postsynaptically on VTA DA neurons (Figure 1), with the end result being an increase in the activity of the mesolimbic reward circuit[139, 141]. Behaviorally, repeated administration of nicotine causes a long-lasting motor sensitization[143, 144] that has been suggested to play a role in nicotine's addictive properties[145]. Glutamatergic and dopaminergic mechanisms within the VTA and NAc have been implicated in this nicotine sensitization[145]. Pharmacological studies on nicotine reinforcement, relapse, and withdrawal have provided important information regarding possible glutamate-based interventions for the treatment of nicotine addiction[141]. The effects of glutamate compounds on nico-

tine dependence are likely to be mediated by attenuation of nicotine-stimulated glutamate transmission in the mesolimbic system via blockade of either presynaptic mGluR2/3 receptors or postsynaptic mGluR5 or NMDA receptors.

mGluR2/3 receptor agonists Considering that mGluR2/3 are located presynaptically and negatively modulate glutamate release, it has been proposed that activation of mGluR2/3 receptors by agonists would decrease presynaptic glutamate release, and therefore block the rewarding effects of nicotine and cigarette smoking.

LY379268 LY379268 is a potent, systemically active mGluR2/3 agonist. Systemic or local administration of LY379268 into the posterior VTA or the NAc shell dose dependently inhibits nicotine self-administration at doses that have no effect on food-taking behavior. LY379268 also reverses cue-induced reinstatement of both nicotine- and food-seeking behaviors[146]. In addition, LY379268 attenuates reward deficits associated with spontaneous nicotine withdrawal in rats[147]. However, when LY379268 is given alone, it inhibits brain-stimulation reward in rats[148]. These data suggest that LY379268 or other mGluR2/3 agonists may have some utility for the treatment of nicotine withdrawal and dependence. However, LY379268, at doses that inhibited cue-induced reinstatement of nicotine seeking, also inhibited food seeking, suggesting that stimulatory actions at presynaptic inhibitory mGluR2/3 have general effects on the motivational impact of conditioned reinforcers. In addition, rapid tolerance occurred to the LY379268-induced decreases in nicotine self-administration[146], which may also limit the potential use of this compound for the treatment of nicotine dependence.

mGluR5 receptor antagonists The mGluR5 receptor has become an important target in medication discovery for treatment of addiction, largely because of its relatively selective regional distribution in the brain and predominantly postsynaptic location[149]. mGluR5 blockade has been proposed to attenuate nicotine-enhanced glutamate transmission in the mesolimbic DA system, and therefore attenuating the rewarding effects of nicotine. A large body of literature indicates that mGluR5s play an important role in behavioral responses to nicotine.

MPEP 2-methyl-6-(phenylethynyl)-pyridine (MPEP) is a selective mGluR5 antagonist. It has been shown to decrease nicotine self-administration in mice and rats[150–152]. MPEP also decreases progressive-ratio reinforcement breaking points for nicotine self-administration more than break points for food-taking behavior[153]. MPEP also reduces reinstatement of nicotine-seeking behavior induced by a nicotine priming injection[152] or by re-exposure to environmental cues previously associated with nicotine self-administration, but not by cues associated with food-taking behavior[154]. However, MPEP does not block the ability of nicotine to enhance brain-reward[140], nor does it block the development of nicotine-induced CPP[155]. Thus, although MPEP may be of some clinical benefit in reducing cigarette smoking or relapse during attempts to quit, it may not significantly attenuate the effects of nicotine on brain reward functions[141]. In addition to MPEP, it was recently reported that MTEP (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine), a novel highly selective mGluR5 antagonist, also significantly inhibits nicotine seeking, but does not affect the reinforcement enhancing effects of nicotine[156].

NMDA receptor antagonists NMDA receptor blockade either globally via systemic administration of an NMDA receptor antagonist or locally via injections of an NMDA receptor antagonist directly into the VTA or the central nucleus of the amygdala-decreases intravenous nicotine self-administration in rats[157]. The effects of NMDA receptor antagonists on intravenous nicotine self-administration occur at doses that do not effect responding for food reinforcement under similar schedules of reinforcement.

Memantine Memantine is a non-competitive, selective NMDA receptor antagonist[158], and is clinically used for the treatment of dementia. The efficacy of memantine for the treatment of nicotine addiction has been investigated as well. In a preclinical study, memantine was found to block the acquisition of nicotine self-administration[159]. However, in humans, memantine does not influence cigarette consumption, craving, or estimation of nicotine's hedonic effects under conditions of instructed smoking reduction, nor does it significantly disturb sensory components of learning mechanisms relevant for the acquisition and maintenance of nicotine dependence[160].

GABA-based medication development GABA is the most important inhibitory neurotransmitter in the mammalian CNS and it has been shown to play an important role in mediating the reinforcing effects of nicotine. GABAergic afferents to the VTA originate from the pedunculopontine tegmental nucleus, ventral pallidum and NAc. Also, GABAergic interneurons within the VTA exert inhibitory control over VTA DA neurons[161]. The central effects of GABA are mediated by both ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors. $GABA_A$ receptors are located predominantly on postsynaptic cells and functionally lower their excitability. In contrast, $GABA_B$ receptors are predominantly located on presynaptic terminals, and inhibit presynaptic neurotransmitter release. Consequently, any pharmacological strategy that increases GABAergic trans-

mission within brain reward circuits by either elevating extracellular GABA levels or directly activating GABA receptors would inhibit nicotine-induced increases in NAc DA and subsequent nicotine reinforcement[132].

Gamma-vinyl GABA (GVG, vigabatrin) GVG is an irreversible inhibitor of GABA transaminase, the primary enzyme involved in GABA metabolism[162]. GABA transaminase is essential for GABA's metabolic breakdown, and therefore its inhibition elevates brain GABA levels. GVG has been shown to dose-dependently attenuate nicotine-induced increases in extracellular DA in the NAc[163]. GVG also decreases nicotine self-administration[164] and abolishes both the acquisition and the expression of nicotine-conditioned place preference[165]. In addition, GVG dose-dependently lowers nicotine-induced increases in NAc DA in both naive and chronically nicotine-treated rats, and blocks nicotine-induced increases in striatal DA in non-human primates as measured by positron emission tomography[166]. These results suggest that GVG may have potential utility as an anti-nicotine therapeutic medication. GVG is currently in Phase II clinical trials for cocaine dependence, but not for nicotine dependence.

Baclofen Baclofen is a systemically active $GABA_B$ receptor agonist. It has been reported that baclofen dose-dependently inhibits nicotine-induced increases in NAc DA release[167]. Systemic injections or microinjections of baclofen into the VTA, NAc shell, or pedunculopontine tegmental nucleus (that sends cholinergic, GABAergic and glutamatergic projections to the VTA) inhibits nicotine self-administration in rats and mice[35, 168–171]. In addition, baclofen, at high doses, completely inhibits nicotine-induced CPP and food-reinforced responding, but fails to reduce nicotine's drug discriminative effects[49]. A small-scale clinical study (16 patients) indicated that a single dose of baclofen (20 mg/kg) significantly altered the sensory properties of smoked cigarettes (*eg*, increasing ratings of 'harsh' and decreasing ratings of 'like cigarette's effects), produced mild sedative-like effects, but failed to reduce cigarette craving or the number of cigarettes smoked[172]. Large-scale clinical trials with multiple drug treatment regimens are required for fully evaluating baclofen's efficacy in the treatment of nicotine dependence. On a cautionary note, baclofen may have undesired side-effects, as indicated by preclinical findings that high dose baclofen significantly inhibited locomotor activity and rotarod locomotor performance[49, 173], and decreased responding for non-drug rewards, such as food and electrical brain stimulation reward[164, 171, 174].

GS39783 and BHF177 GS39783 and BHF177 are novel $GABA_B$ receptor-positive allosteric modulators[175, 176]. Since positive allosteric modulators bind to a site distinct from the agonist binding pocket, they do not alter or perturb receptor signaling on their own, but potentiate the effect of GABA when endogenous GABA is released. Recent studies demonstrate that such positive allosteric compounds significantly inhibits nicotine self-administration under both FR and PR reinforcement schedules and attenuates nicotine-induced CPP and nicotine-enhanced brain-stimulation reward[177, 178]. Strikingly, these effects were seen at a range of doses that neither altered food-taking behavior nor impaired rotarod locomotor performance in rats[173, 178]. These findings suggest that $GABA_B$ receptor positive allosteric modulators may have similar pharmacotherapeutic effects for smoking cessation as the full $GABA_B$ receptor agonist baclofen, but with fewer side-effects.

Endocannabinoid-based medication development Recent studies suggest that the endocannabinoid system also plays an important role in nicotine's addictive properties[179, 180]. This is supported by evidence that: 1) co-administration of sub-threshold doses of a cannabinoid agonist and nicotine produces an enhanced rewarding effect[181]; 2) chronic administration of nicotine in rats produces increases in endocannabinoid (anandamide) levels in the limbic forebrain and in both anandamide and 2-arachidonolyglycerol in the brainstem, although CB_1 receptor binding and CB1 mRNA levels were not affected[182]; 3) the rewarding effects of nicotine, assessed in the CPP paradigm, are absent in CB1 receptor knockout mice[183], though the absence of CB1 receptors does not modify the acquisition of nicotine self-administration[184]; and 4) endocannabinoid agonists have been shown to facilitate DA neuron activity in the VTA and increase DA release in the NAc[185], whereas cannabinoid CB1 receptor antagonists inhibit nicotine self-administration and nicotine-seeking behavior[181, 186]. It is generally believed that such cannabinoid effects are mediated by activation of CB1 receptors located on presynaptic GABAergic neurons in the VTA and/or the NAc, causing a decrease in GABA release and an increase in NAc DA release[181]. Based on this, CB1 receptor antagonists may have utility for smoking cessation.

SR141716A (*Rimonabant*) SR141716A is the first developed CB1 receptor antagonist[187], and has become an important tool for research on cannabinoid involvement in nicotine's addictive properties. In preclinical studies, rimonabant dose-dependently blocks the nicotine-induced elevations in NAc DA and attenuates nicotine self-administration[188]. Rimonabant also attenuates the expression and development of nicotine-induced CPP[189, 190] and blocks environmental cue-induced reinstatement of nicotine-seeking[180, 181]. In

humans, one trial gave both rimonabant and a nicotine patch or rimonabant and a placebo patch to smokers who were motivated to quit. The rimonabant and nicotine patch produced abstinence rates of 39% during weeks 6–9 of treatment, compared with 21.3% of patients treated with rimonabant and placebo[191]. However, it has been recently reported that rimonabant increases anxiety and depressive symptoms[192, 193]. Because of these potential adverse effects, the US FDA has not currently approved its use in humans.

AM251 AM251 is a novel CB1 antagonist, structurally similar to rimonabant[194, 195]. AM251 reverses locomotor sensitization to a nicotine challenge and nicotine-induced elevations in extracellular serotonin in the rat hippocampus[196]. AM251 also dose-dependently suppresses intravenous nicotine self-administration in rats. The self-administration behavior was reinstated by suspending AM251 treatment. Also, pretreatment with AM251 dose-dependently attenuates nicotine-induced and nicotine-associated cue-induced relapse to nicotine-seeking behavior[197]. AM251 has not yet been evaluated clinically.

Conclusion

Given the prevalence and serious consequences of smoking and nicotine dependence, the development of effective therapies to aid smoking cessation is imperative. In this article, we have reviewed the neurochemical bases underlying the pharmacological actions of nicotine and nicotine reinforcement, and evaluated the pharmacological actions of a number of promising agents that target brain substrates on which nicotine acts in both preclinical and clinical models. Though these compounds are different pharmacologically and mechanistically, they all work by interfering with nicotine's actions in the mesolimbic DA reward and relapse system. Recent success with some of these compounds, such as varenicline and bupropion, highlights the importance of preclinical medication development with animal models of drug dependence. These mechanism-based pharmacological strategies may lead to more novel compounds for evaluation in human trials for smoking cessation. If any one demonstrates significant anti-nicotine reward, anti-nicotine craving and anti-relapse efficacy in humans, the beneficial impact on addiction medicine will be considerable.

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