Non-nicotinic neuropharmacological strategies for nicotine dependence: beyond bupropion

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Smoking is a major health problem and is propelled, at least in part, by the addictive properties of nicotine. Two types of pharmacological therapies have been approved for smoking cessation by the US Food and Drug Administration. The first therapy consists of nicotine replacement, substituting the nicotine from cigarettes with safer nicotine formulations. The second therapy is bupropion (Zyban®), an atypical antidepressant, whose use has raised much debate as to how a non-nicotine-based agent can aid in smoking cessation. This review focuses on recent advances that could lead to the development of improved novel pharmacological treatments. These strategies focus on altering reward processes in the brain by modulating various neurotransmitter systems: the most promising include dopamine D_3 receptor antagonists, noradrenaline reuptake inhibitors, $GABA_B$ receptor agonists, metabotropic glutamate 5 (mGluR5) receptor antagonists, cannabinoid CB1 receptor antagonists, and corticotropin releasing factor (CRF) 1 receptor antagonists.

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▼ Smoking-related illness is a major public health problem in today's society, with smoking contributing to the prevalence of a variety of diseases. Smoking is implicated in 80% of deaths from lung cancer, 80% of deaths from chronic obstructive pulmonary disease and 17% of deaths from coronary heart disease ([1]; http://www.ash.org.uk). There are an estimated 1.1 billion smokers in the world, 500 million of whom will die prematurely from tobacco use if no attempts are made to reduce smoking. Within the USA and Europe, 70% of all smokers have considered quitting smoking at least once, and 35% try to quit at least once a year, yet only ~6% of these succeed in maintaining abstinence [2]. On average, smoking leads to an average loss of 12 healthy years and reduces the lifespan by 8 years [2]. Therefore, there is great impetus to develop effective therapies that will aid in facilitating smoking cessation and assist in maintaining abstinence.

Nicotine: the smoking gun of cigarette smoking

There are five different aspects of smoking that can be altered by effective treatments, and could thus contribute to smoking cessation and sustained abstinence:

- Stop smoking intake
- Reduce reinforcing value of nicotine
- Attenuate affective and somatic withdrawal symptoms
- Minimize craving
- Reduce relapse risk

It is largely accepted that nicotine is one of the – if not the main – active ingredients in tobacco smoke that leads to and maintains tobacco addiction [3]. Therefore, most preclinical research efforts are directed at developing interventions that prevent self-administration of nicotine, attenuate the nicotine withdrawal syndrome and prevent relapse to nicotine-seeking behavior. Some of the experimental procedures used to model these drug dependence-related processes in laboratory animals are described in Box 1 and Figure 1.

Two types of pharmacological therapies have been approved for smoking cessation by the US Food and Drug Administration (http://www.fda.gov) [4]. The first is nicotine replacement therapy, which enables the smoker to substitute the nicotine from cigarettes with other nicotine formulations that are safer than tobacco, such as chewing gum, transdermal patches or inhalers [4]. The second therapy is non-nicotine based and is the

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Box 1. Experimental procedures for evaluating potential therapeutics for smoking cessation

There are several aspects of the tobacco smoking habit that contribute to the perpetuation of dependence. Rodent experimental procedures have been developed that enable the assessment of the effects of compounds on the various aspects of dependence. Although a single procedure can not assess all aspects of dependence, the use of multiple procedures provides converging evidence about the potential therapeutic efficacy of test compounds. Because nicotine is the major ingredient in tobacco that contributes to dependence and the need to isolate aspects of the disease for systematic experimental investigations, most currently available procedures for the evaluation of potential therapeutics for smoking cessation involve the use of nicotine. Rat and mouse intravenous nicotine self-administration (Figure 1a) and place preference procedures (Figure 1b) assess the reinforcing effects of nicotine and enable the assessment of the effects of test compounds on these reinforcing effects. Acquisition of nicotine self-administration into the tail vein of the mouse (Figure 1c) involves the use of a yoked-control mouse, and is another procedure that permits the assessment of the effects of test compounds on the reinforcing effects of nicotine. Because this mouse acquisition procedure is short (30–60 min session), it has the potential for rapid screening of test compounds, as well as assessing genetically engineered mice. Elevations in intracranial self-stimulation (ICSS) thresholds (Figure 1d) are a measure of the negative affective aspects of nicotine withdrawal in rats and reflect a depression-like state (see Figure 2a). Somatic signs of nicotine withdrawal are also assessed through observational measures in rats (see Figure 2b). Finally, cue-induced reinstatement after a period of extinction of intravenous nicotine self-administration is a model of relapse in rats (Figure 1e). This reinstatement procedure assesses the effects of compounds on the motivational properties of stimuli that had previously been associated with the reinforcing effects of nicotine. The presentation of these stimuli leads to craving and relapse. Thus, blockade of the motivational effects of these stimuli is hypothesized to prevent relapse. 'Relapse' can also be induced by noncontingent administration of nicotine to the subjects before the test.

atypical antidepressant bupropion (Zyban®) [5]. Although the rationale behind the use of nicotine replacement therapies is intuitive, it remains unclear why bupropion is effective in this indication. Simple replacement of nicotine has some efficacy in the initial stages of withdrawal; however, first year relapse rates are as high as 80% [6]. Therefore, much research has been directed at developing non-nicotinic strategies for the facilitation of smoking cessation. This review will focus on several neuropharmacological targets that are generating interest both in experimental preclinical research and in the clinic, and will specifically concentrate on the nicotine-dependence aspects of smoking behavior.

What have we learned from bupropion?

The use of bupropion in smoking cessation was first realized serendipitously by clinical observations that depressed patients receiving this antidepressant drug decreased their tobacco smoking [7]. A possible rationale for the effectiveness of bupropion is provided from the multitude of clinical studies showing a strong link between negative affect and the propensity to smoke and difficulty in quitting [8]. Indeed, direct correlations have been made recently on the ability of bupropion to modify negative affective aspects of withdrawal and its potential as a cessation agent [9]. Also, nicotine intake and difficulty in quitting correlated with the magnitude of depressive symptomatology [10]. However, bupropion is also equally effective for smoking cessation independent of a past history of major depression [5]. Furthermore, the initial trials assessing the effectiveness of bupropion in smoking cessation excluded depressed patients [11]. Nevertheless, smoking cessation is associated with depressive symptomatology that is more pronounced in individuals who exhibited depressive symptoms while maintaining the smoking habit [4,10]. Thus, it is possible that the effectiveness of bupropion in smoking cessation could be attributed to reversal of the depressive symptoms of nicotine withdrawal, thus facilitating abstinence [12].

In support of this hypothesis, it was shown recently that bupropion reversed both the affective (depression-like) and somatic aspects of nicotine withdrawal in rats ([13]; Figure 2]. However, other antidepressants – including the selective serotonin reuptake inhibitors, with the possible exception of the tricyclic nortryptiline – have been shown to be less effective in reducing quit rates in non-depressed smokers although they could have some benefit in individuals with concomitant psychiatric disorders [14]. Bupropion, unlike other antidepressants, facilitated brain reward function as measured using the intracranial self-stimulation (ICSS) procedure [13]. Thus, the dual effects of bupropion in enhancing brain reward function and in reversing nicotine withdrawal, including the depression-like aspects of withdrawal, could explain the superiority of bupropion over other antidepressant agents in facilitating smoking cessation.

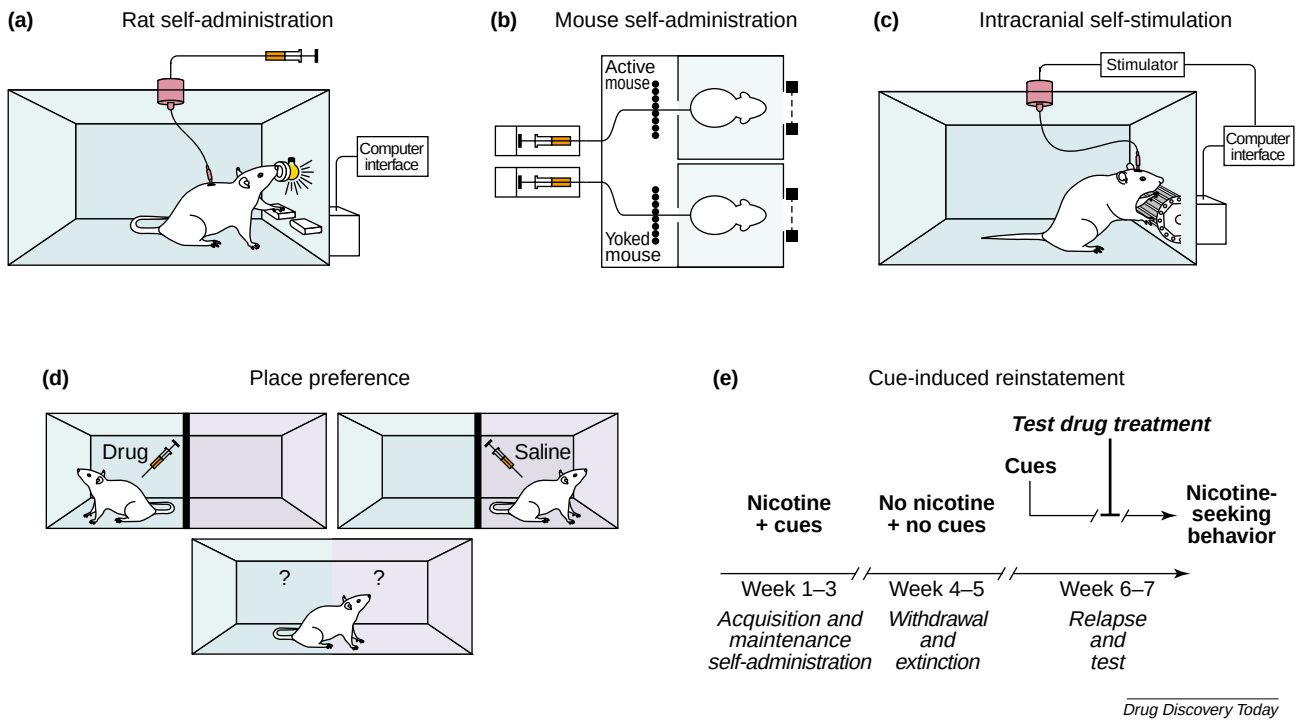

Figure 1. Experimental procedures for evaluating potential therapeutics for smoking cessation (see Box 1). **(a)** Rat intravenous nicotine self-administration. After a fixed number of lever presses, rats are administered an injection of nicotine. The reinforcing aspects of the drug are reflected in the number of injections that the animal self-administers. **(b)** Mouse intravenous nicotine self-administration (rapid test). A mouse nose-pokes to receive nicotine into the tail vein. This procedure involves the use of a yoked-control mouse, which receives vehicle and no drug. This is a rapid test assessing the acquisition of nicotine self-administration, and thus the reinforcing effects of the drug. **(c)** Intracranial self-stimulation (ICSS). Animals (rats and mice) find electrical stimulation of various brain sites rewarding. Thus, subjects will actively perform an operant (in this case, turn a wheel) to receive the stimulation. Stimulation currents are titrated to yield a threshold current or frequency, which is the minimal level of stimulation for which animals will work. Drugs of abuse, such as nicotine, lower ICSS thresholds that the animal finds rewarding. By contrast, withdrawal from nicotine results in marked elevations in thresholds indicating an anhedonic state. **(d)** Conditioned place preference. A rat or mouse is placed in one part of an arena, defined by contextual stimuli, where the subject receives a rewarding drug, such as nicotine. When the animal is placed back into the same arena and given the choice of moving to the drug- or non-drug-paired section, the subject chooses the drug-associated compartment indicating conditioned reinforcing effects of the drug. **(e)** Cue-induced reinstatement. Intravenous nicotine self-administration in animals is paired with specific environmental cues. After extinction of nicotine intake, exposure to the cues alone can prime the animal to engage in nicotine-seeking behavior, modeling relapse to drug-seeking in humans. Relapse can also be induced by non-contingent administration of nicotine or footshock stress to the subjects before the test.

Despite bupropion having been in clinical use for 25 years, the neurochemical mechanisms underlying its actions are still not well-elucidated [15]. Recent data indicate that antidepressant-like effects of bupropion could be due to its effects on the noradrenergic system [15–16]. Nonetheless, unlike many antidepressants, it also acts as a dopamine transporter (DAT) inhibitor (albeit in the micromolar range) [15], and microdialysis studies have shown that acute bupropion administration increased extracellular dopamine [17]. A recent imaging study in humans demonstrated that at therapeutically effective doses, bupropion has only 22% occupancy at the DAT [18], indicating that the efficacy of bupropion is probably not solely dopamine-mediated. Moreover, recent evidence suggests that bupropion might act as a functional antagonist at neuronal nicotinic acetylcholine receptors [13,19]. Thus, it is possible that bupropion could act to acutely attenuate the rewarding effects of nicotine, thus increasing the likelihood of cessation. Studies in rats demonstrated that bupropion, similarly to the nicotinic receptor antagonists mecamylamine and dihydro-β-erythroidine (Box 1), decreased intravenous nicotine self-administration in some [20,21] but not all studies [22,23]. Indeed, mecamylamine has been shown to be effective as a smoking cessation aid when used in combination with a nicotine patch [24] and is currently in Phase III clinical trials. Interestingly, bupropion generalizes to nicotine in drug discrimination paradigms. However, unlike the discriminatory properties of nicotine, those of bupropion are insensitive to blockade by the nicotinic antagonist mecamylamine indicating a

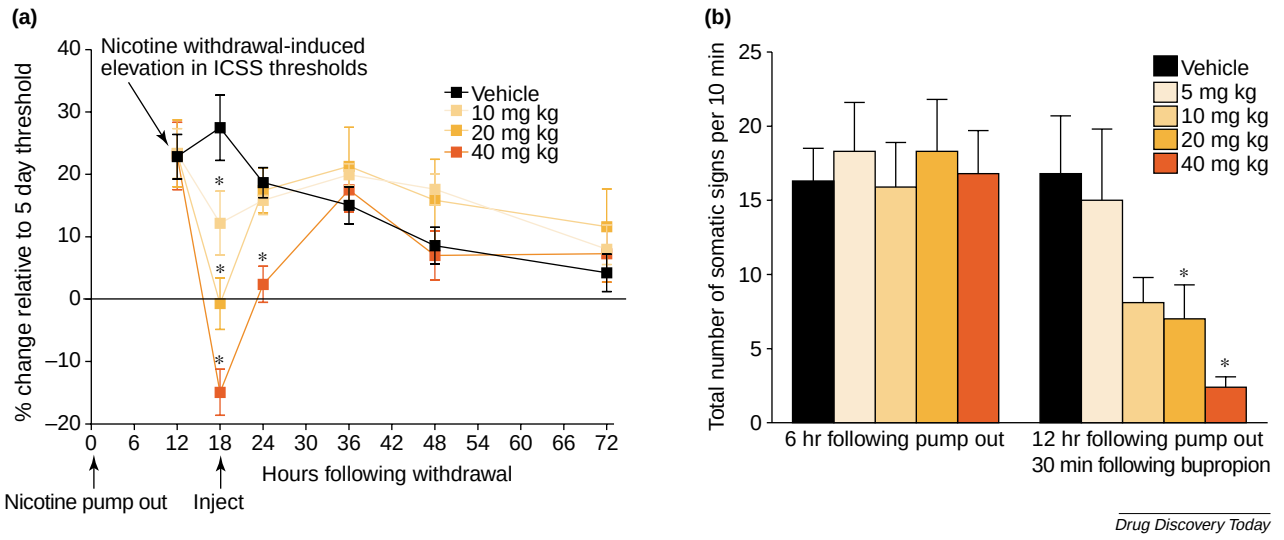

Figure 2. Bupropion reverses the affective and somatic aspects of nicotine withdrawal in the rat. **(a)** Bupropion dose dependently reversed the elevations in ICSS thresholds observed after cessation of nicotine administration via subcutaneous osmotic minipumps (3.16 mg $kg^{-1}day^{-1}$ for 7 days; free base). * = P <0.05 versus vehicle-treated controls. **(b)** Bupropion dose-dependently reversed the increases in somatic signs observed (these included gasps and writhes; cheek tremors, chews, teeth chattering, shakes, escape attempts, licks, scratches and yawns) after cessation of nicotine administration. * = P <0.05 versus vehicle-treated controls. Adapted with permission from reference [14].

differential mechanism-of-action mediating the discriminatory cues of bupropion and nicotine [25]. Taken together, it is reasonable to suggest that the actions of bupropion could be mediated, at least in part, via the nicotinic receptor system. Thus, the multifaceted effects of bupropion on behavior, coupled with its non-selectivity *in vitro*, make it a challenge to design more effective small-molecule therapies, based solely on the knowledge of bupropion's pharmacology. Nevertheless, the use of bupropion has propelled research into other neuropharmacological mechanisms outside of the nicotine cholinergic system that might lead to effective smoking cessation therapies. In the following sections we describe the evidence for some of the more promising strategies.

Targeting dopamine mechanisms

Dopaminergic mechanisms have also been implicated in both the rewarding aspects of nicotine and the manifestation of the nicotine withdrawal syndrome [26,27]. Thus, directly targeting the dopamine system is an attractive approach to the development of therapeutics for nicotine dependence and tobacco smoking. However, it is relevant to note that despite extensive clinical investigations, dopaminergic drugs have thus far not been effective in reducing other forms of psychostimulant abuse [28]. Furthermore, although there has been some evidence that targeting D_1 and D_2 dopamine receptors could be therapeutically useful for nicotine addiction, the most promising dopaminergic avenues for developing novel treatments are drugs that are specific for the DAT and the D_3 receptor.

DAT inhibitors

Selective inhibitors of DAT increase dopamine levels throughout the brain, which increases the probability that these drugs would be abused and hence limits their use. Nevertheless, it is possible that a DAT inhibitor with low potency and suitable pharmacokinetics might have reduced abuse liability [29]. In this regard, bupropion and nomifensine, both non-selective DAT inhibitors, have been used successfully as effective antidepressants without being abused in humans. Nomifensine was eventually withdrawn from the market because it induced a rare blood disorder in some patients [30]. Vanoxerine (GBR-12909), a selective DAT inhibitor, is undergoing clinical development by the National Institute on Drug Abuse (http://www.nida.nih.gov) for cocaine dependence [31] and might have potential therapeutic value in smoking cessation.

The dopamine D_3 receptor

One promising approach to treat various drug dependencies is the targeting of dopamine D_3 receptors [32]. In marked contrast with D_1 and D_2 receptors, this receptor has a restricted expression pattern in the brain, being selectively expressed in the shell subdivision of the nucleus accumbens [32], which neurochemical studies have identified as a crucial anatomical substrate for the effects of drugs

of abuse [32]. Indeed, repeated nicotine administration is associated with a marked increase in D_3 receptor binding and mRNA in this region [33]. In contrast to D_2 receptor antagonists, selective D_3 antagonists have no cataleptogenic or akinetic properties in animals [32]. The highly selective D_3 receptor partial agonist BP897 has the unprecedented property of reducing cocaine-seeking behavior maintained by cocaine-associated cues, without modifying cocaine self-administration and without being self-administered in animals [34]. These data suggest that it is possible to reduce the motivation induced by a drug-related cue without interfering with the primary drug-related reward. Because contextual stimuli previously associated with drug consumption elicit craving and relapse in abstinent human addicts, it is possible that compounds like BP897 could be useful in reducing relapse vulnerability, with minimal liability for abuse. Recent data demonstrated similar effects against cocaine with the full D_3 receptor antagonist SB-277011-A [35,36], and these results were also further extended to nicotine [37,38]. Such dopamine D_3 receptor partial agonists/antagonists now await clinical validation for prevention of relapse to smoking in humans.

Role of noradrenergic mechanisms in nicotine dependence

Nicotine increases cortical noradrenaline in rats [39]. In addition, increases in hypothalamic noradrenaline levels correlated with self-administration of nicotine in rats [40], thus modifying the animal's endocrine and behavioural response to stress. Furthermore, locus coeruleus α2 noradrenergic autoreceptors are markedly down-regulated in smokers [41], suggesting that the nicotine-induced noradrenaline release might have resulted in adaptive processes in feedback mechanisms that regulate noradrenaline function. As stated previously, there is accumulating evidence that bupropion mediates some of its effects through noradrenergic mechanisms [15–16]. Recent studies showed that the selective noradrenaline reuptake inhibitor reboxetine blocked self administration of nicotine in rats [42]. The effects of reboxetine could be facilitated by its ability to indirectly modulate the firing pattern of dopamine cells in the ventral tegmental area (VTA) and selectively increase dopamine availability in the prefrontal cortex [43]. Clinical studies in smoking cessation with the tricyclic antidepressant and noradrenaline reuptake inhibitor nortriptyline have also provided promising results [44].

Serotoninergic mechanisms for cessation

Nicotine has been shown to interact intricately with the serotonergic system [45,46]. Nonetheless, selective serotonin reuptake inhibitors (SSRIs) have limited efficacy in smoking cessation in non-depressed smokers [14]. Interestingly, acute administration of such drugs did not significantly alter the negative affective aspects of the nicotine withdrawal syndrome as assessed by ICSS in rats [47]. However, such deficits in reward function were counteracted when an SSRI was given in combination with a 5-HT_{1A} autoreceptor antagonist [47]. These findings are consistent with data demonstrating a role for the serotonergic dorsal raphe nucleus in mediating some of the affective aspects of nicotine withdrawal [46]. Furthermore, 5-HT_{1A} receptor antagonists alone reversed the increases in the amplitude of the auditory startle reflex observed during nicotine withdrawal in rats [48]. Such antagonists are currently in development by several pharmaceutical companies for smoking cessation. It is not clear whether any of the other 13 5-HT receptor subtypes might be useful targets for developing anti-smoking agents, although some evidence indicates that the 5-HT_{2C} receptor could be a useful target [49].

Targeting glutamate

The role of the glutamatergic system in drug dependence is only partially elucidated, although a large body of evidence suggests a close interaction between the dopaminergic and glutamatergic systems in the control of reward processes [50]. The recent availability of new pharmacological tools enabled the investigation of the role of different glutamate receptors in drug dependence. Glutamate receptors are broadly divided, on the basis of their molecular properties and effector systems, into two categories: ionotropic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate and *N*-methyl-D-aspartate (NMDA) receptors, and metabotropic receptors [51]. In terms of the ionotropic glutamate receptors, injections of relatively selective receptor antagonists in the nucleus accumbens suggest that inhibition of the AMPA receptor subtype has a greater effect on the facilitation of dopamine neurotransmission in the nucleus accumbens than blockade of NMDA receptors [52]. By contrast, blockade of the NMDA subtype in the VTA blocks nicotine-induced dopamine release in the nucleus accumbens, whereas blockade of the AMPA subtype had no effect [53]. Consistent with these findings, blockade of the NMDA subtype reduced the locomotor sensitizing effects of nicotine [54], whereas the AMPA/kainate receptor antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX) precipitated withdrawal in nicotine-dependent animals [55]. Taken together, these data suggest that NMDA-, rather than AMPA-, selective mechanisms could be useful targets for smoking cessation. Given the side-effects

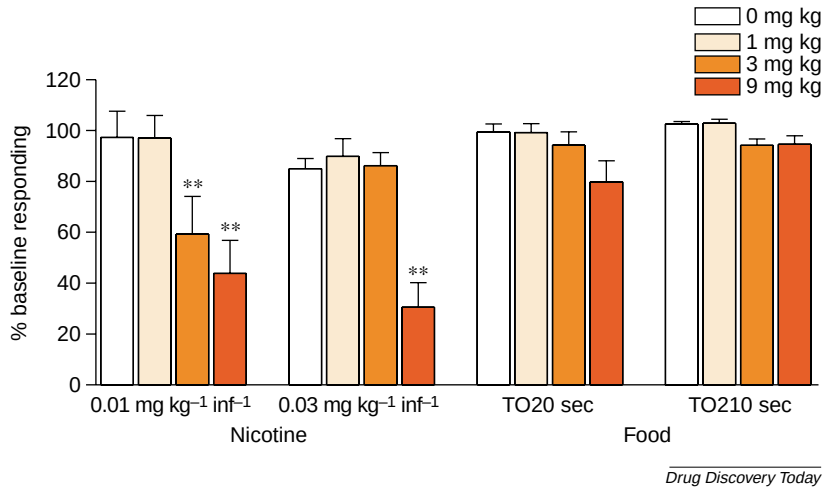

Figure 3. The mGluR5 receptor antagonist 6-methyl-2-(phenylethynyl)-pyridine (MPEP) blocks nicotine self-administration in rats. The effects of MPEP administration on nicotine- and food-maintained responding in the rat. The data are expressed as percent of baseline responding (mean + SEM). Asterisks indicate significant differences from control conditions for each reinforcer (** = P<0.01). Adapted with permission from reference [58].

associated with full NMDA antagonists, it is worthwhile to investigate whether subtype-selective NMDA subunit antagonists provide an advantage as a pharmacological approach to smoking cessation.

In terms of the metabotropic glutamate receptors, activation of the presynaptic mGluR2/3 receptors with LY354740 attenuated nicotine withdrawal-induced increases in the acoustic startle response [56]. By contrast, activation of mGluR2/3 precipitated ICSS threshold elevations in nicotine-dependent rats, while the blockade of mGluR2/3 attenuated the ICSS threshold elevations observed during spontaneous nicotine withdrawal [55]. A key role for mGluR5 in cocaine dependence was elucidated by the fact that mGluR5 knockout mice did not self-administer cocaine, and the selective mGluR5 antagonist 6-methyl-2-(phenylethynyl)-pyridine (MPEP) decreased cocaine self-administration in wildtype mice [57]. Building on these studies, MPEP was recently shown to decrease nicotine self-administration in mice and rats ([58]; Figure 3). Taken together, these data suggest that blockade of mGluR5 decreases the reinforcing effects of nicotine while blockade of mGluR2/3 receptors reverses the affective signs of nicotine withdrawal. Thus, both of these pharmacological approaches could be fruitful for smoking cessation.

Targeting γ-aminobutyric acid (GABA)

GABAergic mechanisms have been implicated in drug dependence largely because of known direct interactions of the GABA and the dopamine transmitter systems. Neurons projecting from the VTA to the nucleus accumbens receive descending GABAergic input from the ventral pallidum and the nucleus accumbens that have an inhibitory effect on dopaminergic tone at the level of both the VTA and the nucleus accumbens [59]. There are GABA inhibitory afferents to dopaminergic VTA neurons, inhibitory GABA interneurons within the VTA, and medium spiny GABA neurons in the nucleus accumbens that also inhibit mesolimbic dopamine release [59]. Given this intricate interaction, it is predicted that GABAergic manipulations will modulate nicotine reinforcement. GABA is the most important inhibitory neurotransmitter in the mammalian CNS. Like glutamate receptors, GABA receptors are divided into ionotropic $GABA_A$ and $GABA_c$ receptors and metabotropic $GABA_B$ receptors [60]. The $GABA_A$ receptors are the direct targets for drugs such as the benzodiazepine anxiolytics, barbiturates and anaesthetics and have limited therapeutic potential for smoking cessation [61]. $GABA_B$ receptors have a crucial role in the fine-tuning of CNS synaptic transmission [60] and are largely thought to be the most promising GABAergic targets for the treatment of drug dependence.

GABA transaminase inhibitors

Enhancement of GABAergic transmission using γ–vinyl GABA (GVG or vigabatrin), an irreversible inhibitor of GABA transaminase that is the primary enzyme involved in GABA metabolism, decreased nicotine self-administration in rats [62]. Furthermore, dose- and time-dependent GVG administration lowered the nicotine-induced increases in nucleus accumbens dopamine in both naïve and chronically nicotine-treated rats, as measured by *in vivo* microdialysis, and abolished nicotine-induced increases in dopamine in the striatum of primates, as measured by positron emission tomography [63]. Ashby and colleagues [64] also showed that 1R, 4S-4-amino-cyclopent-2-ene-carboxylic acid (ACC), a reversible inhibitor of GABA transaminase, blocked expression of conditioned place preference to nicotine in rats. Taken together, these data suggest that activation of the GABAergic system is a potential therapeutic strategy for nicotine dependence. Unfortunately, GVG-induced GABA-related visual problems could hamper the widespread use of GVG [65]. Given that the effects of GVG are anatomically non-specific,

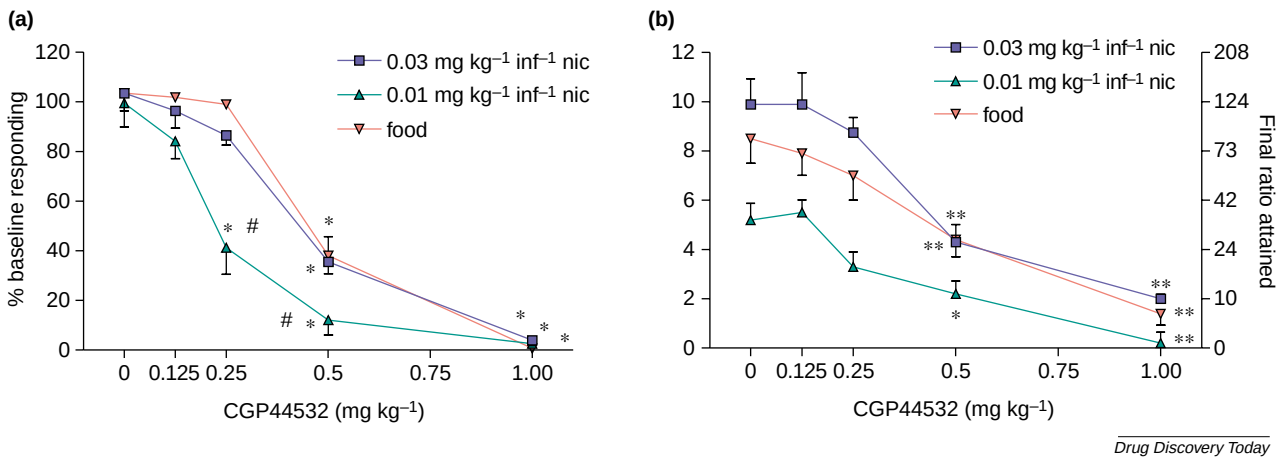

Figure 4. $GABA_B$ receptor agonist CGP 44532 blocks nicotine self-administration assessed under fixed and progressive ratio schedules of reinforcement. **(a)** Effects of CGP44532 administration on nicotine- and food-maintained responding under a Fixed Ratio (FR) 5 TO 20 schedule of reinforcement. Data are expressed as a percent of baseline. Asterisks indicate statistically significant differences from vehicle pretreatment for each CGP44532 dose (* = P <0.05; ** = P <0.01). # indicates statistically significant differences between nicotine- (0.01 mg kg^{-1}infusion^{-1}) and food-maintained responding (P <0.05). **(b)** The mean (± SEM) number of infusions earned under a progressive-ratio schedule of reinforcement for two available nicotine doses (0.01 and 0.03 mg kg^{-1}infusion^{-1}). The right ordinal axis shows the corresponding final ratios (i.e. break points) reached. Asterisks indicate statistically significant differences from vehicle pretreatment for each nicotine dose group (* = P <0.05; ** = P <0.01). Adapted with permission from reference [66].

GABA receptor-specific ligands could offer a more promising alternative for drug discovery.

$GABA_B$ receptor agonists and positive modulators

Accumulating evidence suggests that increased GABAergic transmission through the $GABA_B$ receptors might be a suitable approach for aiding cessation of smoking. Systemic [66,67] or intracerebral administration of baclofen or CGP44532, both $GABA_B$ receptor agonists, directly into the nucleus accumbens shell, the VTA or the peduncular pontine nucleus [68,69] (Figure 4), decreased the reinforcing effects of nicotine. Baclofen also attenuated nicotine-induced increases in accumbal dopamine [70]. Preliminary clinical studies demonstrated that baclofen is an effective treatment in cocaine-abstinent humans [71], and prevented cue-induced craving and concurrent activation of relevant brain areas in these subjects [72]. Furthermore, baclofen treatment reduced the rewarding effects of alcohol consumption and craving for alcohol in a double-blind randomized study [73]. Taken together, these results suggest that enhancement of GABA transmission through activation of $GABA_B$ receptors blocks the reinforcing effects of various drugs of abuse, including nicotine. One of the drawbacks of baclofen therapy is the marked muscle relaxation and sedative properties of the drug, which hamper its widespread use in indications outside of that as an antispastic medication. Recently, allosteric positive modulators for the $GABA_B$ receptor (e.g. CGP7930, CGP13501; GS39783) have become available [74]. These modulators potentiate the actions of endogenous agonists at the receptor, thus enhancing GABA transmission in an impulse-dependent manner. Thus, such modulators might be more efficacious and less disruptive to the normal functioning of the system than direct agonists, causing fewer side effects and lowering the chance for receptor desensitization and, thus, tolerance to the therapeutic effects [75]. Whether such ligands will be as effective as full agonists in animal models of nicotine dependence remains to be tested.

Cannabinoid (CB) mechanisms

CB_1 and CB_2 receptors are the known receptor subtypes for endogenous cannabinoids and for Δ9-THC (tetrahydrocannabinol), the active ingredient in marijuana smoke in addition to other CB agonists. In locomotor, anxiety, hypothermia and nociception studies a significant interaction was demonstrated between cannabinoid agonists and nicotine [76], while the rewarding effects of acute nicotine, as assessed by place conditioning, were blunted in CB_1 receptor knockout mice. However, the nicotinic antagonist mecamylamine was equally efficacious in precipitating withdrawal in nicotine-dependent animals from both genotypes [77]. Furthermore, the CB_1 knockout mice self-administered nicotine similar to wildtypes [78]. Pharmacological studies showed that the CB_1 receptor antagonist SR141716A (Rimonabant) reduced nicotine self-administration and nicotine-induced dopamine release in

the nucleus accumbens [79]. Furthermore, SR141716A increased dopamine, noradrenaline and 5-HT levels in the cortex and the nucleus accumbens [80], which could contribute to the ability of SR141716A to reverse nicotine-induced effects. In conclusion, although data from CB_1 receptor knockout animals are inconsistent, the pharmacolological data suggest that CB_1 receptor antagonists, such as Rimonabant, could have anti-smoking activity; accordingly, Rimonabant is currently being tested in placebo-controlled Phase III clinical trials.

Corticotropin-releasing factor (CRF)

CRF is a key neuropeptide in the orchestration of the normal stress response [81]. Increasing evidence points to a role for CRF in mediating the actions of drugs of abuse, including nicotine. First, acute administration of drugs of abuse activates the hypothalamic-pituitary-adrenocortical (HPA) stress axis [81]. Second, drug withdrawal is associated with physiological and behavioral changes associated with responses to stressors, which are linked to brain CRF activation [81]. Third, exposure to stressors is associated with increased drug-taking behavior and relapse to drugs in both humans and laboratory animals [81,82]. The influence of nicotine on CRF-containing neurons in the hypothalamus, which results in an increase in adrenocorticotropin hormone, is probably indirect through noradrenergic mechanisms [83]. Of interest, intermittent footshock stress effectively reinstates extinguished nicotine-seeking behaviors in rats [84], a phenomenon hypothesized to be related to relapse (Box 1). Similar studies with cocaine or heroin demonstrated that CRF_1 receptor antagonists effectively attenuated 'relapse' using such animal procedures [85]. CRF_1 receptor antagonists are currently under development by many large pharmaceutical companies for the treatment of depression and anxiety. It will be of interest to assess whether these compounds also have an effect on smoking cessation rates.

Other issues relating to smoking cessation

In addition to blocking cravings for tobacco smoking and withdrawal effects, promising smoking cessation aids should be devoid of major side effects to be successful in the market. As weight gain is often associated with smoking cessation, an ideal drug would prevent this; the concern of weight gain often is a contributing factor to the poor success rates for smoking cessation. It is possible that many of the pharmacological approaches described above might also have effects on natural rewards and, as such, could reduce food intake. However, such effects on reward processes might also lead to considerable side-effects, such as anhedonia, a core symptom of depression.

Although we have focused on studies investigating the effects of various non-nicotinic pharmacological strategies on animal models of nicotine self-administration and dependence, it is important to emphasize two points. First, considerable efforts are also directed towards nicotinic strategies for smoking cessation, focusing largely on ligands for specific nicotinic receptors or the development of nicotine vaccines [86,87]. Second, although pure nicotine can serve as a positive reinforcer, the reinforcing effects of cigarette smoke can not fully be accounted for by nicotine alone [88]. The sensory effects of tobacco can contribute to the primary and conditioned reinforcing properties of cigarette smoking. In addition, other chemicals (such as acetaldehyde and nornicotine) that are present in cigarette smoke can interact with nicotine to enhance the reinforcing effects of nicotine by either having their own primary pharmacological effects, or by potentially influencing the nicotine dose delivered and absorbed, and the speed of nicotine delivery, or both. Also worth noting is the important issue of genetic-based differences in individual treatment response, which could provide clues for future medication development [89].

Conclusion

Although the use of bupropion has shown that neuropharmacological strategies are effective in the treatment of nicotine dependence, with over 70% of bupropion-treated individuals relapsing after one year, it is clear that future treatments need to be more efficacious than bupropion. Given the promising preclinical data for many of the strategies outlined here, it is likely that these strategies will result in therapies that will improve long-term smoking quit rates, and become important tools in the struggle to extinguish smoking behavior and maintain smoking abstinence.

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