Title

Advances in smoking cessation pharmacotherapy: Non-nicotinic approaches in animal models

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Introduction

Tobacco-related illness remains the leading cause of preventable death worldwide, with over 7 million deaths annually (WHO 2017). A worldwide gender gap is seen in tobacco use, in which the prevalence is 4- to 10-times higher in males than in females. This gap is much higher in developing countries than in developed countries because of the stigma against tobacco smoking. However, with increasing tobacco use in adolescents, especially in developing countries, the gender gap is closing (Warren 2008, Higgins, Kurti et al. 2015). Some evidence suggests that socioeconomic status may affect the onset of tobacco use and cessation success (Daniel, Hickman et al. 2009, van Wijk, Landais et al. 2019). Further complicating the treatment of tobacco dependence is the occurrence of comorbidity with other disorders. High comorbidity is seen between nicotine dependence and mental health disorders, such as depression, anxiety, and schizophrenia (Pettey and Aubry 2018, Tidey, Davis et al. 2018). The prevalence of nicotine use is high among individuals who engage in poly-substance abuse. Poly-drug use is widespread among adolescents, people with lower socioeconomic status, and people with a high comorbidity of mental health disorders (Bello, Khoddam et al. 2018). Despite the higher risk of cardiovascular and inflammatory disease (Campagna, Alamo et al. 2019), the rate of smoking in people who are diagnosed with diabetes is comparable to the general population (Ford, Mokdad et al. 2004).

Electronic cigarettes

The rates of tobacco smoking have been declining in recent decades, but the use of electronic cigarettes has rapidly increased since their introduction to the market (WHO 2017). Electronic cigarettes are currently under scrutiny in the United States after several vaping-related

lung injuries and deaths were reported (Schier, Meiman et al. 2019). Countries in the European Union are recommending electronic cigarettes as smoking cessation aids (Erku, Gartner et al. 2019, Filippidis, Laverty et al. 2019). Researchers have struggled to stay apprised of the rapid progression of vaping technology. Nearly a decade after the advent of electronic cigarettes, however, we are beginning to understand their effects on nicotine dependence. Nicotine-containing electronic cigarette liquid produces a similar lowering of intracranial self-stimulation (ICSS) reward thresholds as nicotine alone in rats (Harris, Muelken et al. 2018). The effects of precipitated withdrawal from chronic nicotine-containing electronic cigarette liquid and nicotine alone on ICSS thresholds were similar in rats (Harris, Muelken et al. 2017). Passive nicotine vapor studies have characterized nicotine vapor-induced dependence in rats and mice (George, Grieder et al. 2010, Kallupi and George 2017, Shao, Lopez et al. 2019), but no studies have investigated the possible attenuation of intravenous nicotine self-administration by passive vapor exposure (Cohen and George 2013). The lack of a nicotine vapor self-administration model in rats has limited further explorations of the neuropharmacological mechanisms of electronic cigarette use. Our laboratory developed the first such model, which was shown to produce nicotine dependence and cardiopulmonary abnormalities in rats (Smith, Kallupi et al. 2019). Further investigations of this animal model may shed light on the neurobiological mechanisms of electronic cigarette use and the development of novel treatments.

Nicotine

Nicotine is absorbed from tobacco smoke or electronic cigarettes into the lungs (Benowitz 1990). From the lungs, nicotine diffuses into the central nervous system within seconds where it activates nicotinic acetylcholine receptors (Langley 1905). The rapid absorption

and rise in blood nicotine levels after the inhalation of nicotine-containing smoke or vapor contribute to the reinforcing properties and abuse liability of these products (Benowitz 1990, Henningfield and Keenan 1993). Neuronal nAChRs are pentameric ligand-gated ion channels that consist of 12 receptor subunits (α2-10, β2-4). The most widely expressed nAChRs in the central nervous system contain the α4β2, α3β4, and α7 subunits (Deneris, Connolly et al. 1988, Connolly, Boulter et al. 1992, Gotti, Moretti et al. 2008). The reinforcing properties of nicotine are mainly attributed to binding at β2-containing nAChRs (Picciotto, Zoli et al. 1998, Maskos, Molles et al. 2005), particularly nAChRs that contain the α4β2 subunits for which nicotine has the highest affinity (Watkins, Epping-Jordan et al. 1999, Tapper, McKinney et al. 2004). The nicotine-induced activation of α4β2 nAChRs increases dopamine release in the prefrontal cortex, ventral striatum, and nucleus accumbens (Koob 1992, Leshner and Koob 1999, Di Chiara 2000). The mesolimbic dopamine system is associated with positive reinforcement for most drugs of abuse, including nicotine (Koob 1992, Leshner and Koob 1999). Recent genome-wide association studies have also identified a role for the α5, α3, and β4 subunits in nicotine dependence (Improgo, Scofield et al. 2010). Chronic nicotine use leads to receptor changes in the central nervous system and muscular-skeletal system, which contribute to tolerance, negative reinforcing effects, and withdrawal upon cessation (Marks, Burch et al. 1983, Schwartz and Kellar 1983, Benwell, Balfour et al. 1988, Wonnacott 1990, Grieder, Besson et al. 2019). Environmental cues that are associated with substance use contribute to the habituation of drug intake and contribute to relapse (Niaura, Rohsenow et al. 1988). In nicotine-dependent individuals, cues that are associated with a specific method of nicotine use may trigger craving, drug seeking, and withdrawal symptoms (Carter and Tiffany 1999). Upon cessation, nicotine-

dependent individuals exhibit negative affective states, characterized by depressed mood, anxiety, irritability, and insomnia (Cohen, Pickworth et al. 1991, Parrott 2003).

Food and Drug Administration-approved pharmacotherapies

Seven smoking cessation therapies are currently approved by the United States Food and Drug Administration (FDA): varenicline, bupropion, and nicotine replacement therapy in the form of nicotine patches, gum, lozenges, sprays, and inhalers (Prochaska and Benowitz 2016). Nicotine replacement therapy products are designed to deliver nicotine in a controlled manner, with a gradual rise in blood nicotine levels. The slow pharmacokinetics of these products lowers their abuse liability compared with inhalation and oral tobacco products (West, Hajek et al. 2000). Varenicline is an $\alpha4\beta2$ and $\alpha7$ nAChR partial agonist (Coe, Brooks et al. 2005) that was shown to significantly increase abstinence rates compared with placebo in clinical studies (Gonzales et al. 2006). Bupropion is an atypical antidepressant and the only FDA-approved nicotine cessation therapy that does not contain or mimic nicotine. Bupropion blocks the reuptake of dopamine via the dopamine transporter, the norepinephrine transporter, and the nicotinic acetylcholine receptors. All of these interactions may contribute to bupropion's mechanism of action during smoking cessation.

Modulation of the Nicotinic Acetylcholine Receptor

Modulation of nAChRs remains a central strategy for the development of smoking cessation pharmacotherapies. Non-specific nAChR antagonists act as a general blockade of nicotine's activity, however the lack of specificity may result in unwanted side effects. Mecamylamine is a non-selective, non-competitive antagonist of nAChRs (Papke, Sanberg et al.

2001). Mecamylamine was originally FDA approved for the treatment of hypertension, but it is most often used as a repurposed pharmacologic in experimental models of neuropsychiatric disorders (Shytle, Penny et al. 2002, Bacher, Wu et al. 2009). In animal models, mecamylamine blocks the discriminative stimulus effects of nicotine (Morrison and Stephenson 1969, Stolerman, Naylor et al. 1999, Moerke, Zhu et al. 2017), and is widely used to precipitate withdrawal after establishment of nicotine dependence (Malin, Lake et al. 1994, Damaj, Kao et al. 2003, Paterson and Markou 2004). Mecamylamine and a mecamylamine enantiomer have been assessed in clinical trials as a treatment for smoking cessation and as an antidepressant, both without much success (Rose, Sampson et al. 1989, Rose, Behm et al. 1994, Rose, Behm et al. 1998, Moller, Demyttenaere et al. 2015). The hypertension drug, pempidine, a non-selective, non-competitive antagonist of nAChRs, has shown antagonism of the physiological effects of nicotine in animal studies (Haikala and Ahtee 1988, Martin, Suchocki et al. 1990). Subtype specific antagonists would allow for modulation of specific nicotine-mediated behaviors with limited side-effects. The β2 specific nAChR competitive antagonist dihydro-β-erythroidine shows some promise in blocking the discriminative stimulus effects of nicotine in animal models (Williams and Robinson 1984, Stolerman, Chandler et al. 1997, Gommans, Stolerman et al. 2000, Moerke, Zhu et al. 2017). One strategy for the development of nAChR antagonists with high subtype specificity is the utilization of natural toxins (Hone, Scadden et al. 2012, Kini 2019, You, Li et al. 2019). The diterpenoid alkaloid methyllycacotinine, a competitive agonist for the α7 nAChR subtype (Alkondon, Pereira et al. 1992), reduced nicotine self-administration in rats (Markou and Paterson 2001). The peptide α-conotoxin AuIB blocks α3β4 nAChRs in several brain regions involved in nicotine dependence (Luo, Kulak et al. 1998, Fu, Matta et al. 1999, Grady, Meinerz et al. 2001, Kulak, McIntosh et al. 2001). Investigations with α-conotoxin AuIB show modulation of nicotine

reward and physical withdrawal signs via α3β4 containing nAChRs, but not the acute behaviors or affective withdrawal signs of nicotine (Jackson, Sanjakdar et al. 2013). In mice, α-conotoxin TxIB, an antagonist of α6β2 containing nAChRs, was found to inhibit the expression and reinstatement of conditioned place preference induced by nicotine (You, Li et al. 2019). Additionally, α-conotoxin TxIB inhibited the increase in concentrations of dopamine, GABA, and norepinephrine in the nucleus accumbens, hippocampus, and prefrontal cortex induced by nicotine. Mecamylamine and pempidine have similar pharmacodynamic effects, with mecamylamine more commonly used now as a pharmacological tool in animal models of nicotine dependence. Dihydro-*β*-erythroidine, methyllycacotinine, and α-conotoxin need further validation in animal models of nicotine dependence. While they are unlikely to be useful in a clinical setting, their nAChR subtype specificity could provide insight into the mechanisms involved in nicotine dependence and lead to the development of smoking cessation therapies. For more comprehensive reviews on nAchR ligands and modulators in the scope of nicotine dependence, see (Papke and Lindstrom 2020, Wilkerson, Deba et al. 2020).

Acetylcholinesterase inhibitors

Acetylcholinesterase is a catabolic enzyme that is responsible for metabolizing acetylcholine in the synapse (Thapa, Lv et al. 2017). Acetylcholinesterase inhibitors, such as galantamine and donepezil, increase extracellular acetylcholine levels in the brain, augmenting cholinergic transmission (Shaikh, Verma et al. 2014). Nicotine acetylcholine receptors are endogenously activated via binding of acetylcholine, making the cholinergic system an attractive therapeutic target for nicotine dependence (Turner, Gold et al. 2013). Acetylcholinesterase inhibitors are FDA-approved for the treatment of cognitive impairments that are associated with

mild to moderate Alzheimer's disease (Lazarevic-Pasti, Leskovac et al. 2017). The acetylcholinesterase inhibitor donepezil has been shown to reverse deficits in contextual fear conditioning induced by nicotine withdrawal in mice (Poole, Connor et al. 2014). In rats, donepezil has been found to attenuate nicotine self-administration and reinstatement of nicotine seeking (Kimmey, Rupprecht et al. 2014, Ashare, Kimmey et al. 2016). Chronic donepezil treatment was found to induce the up-regulation of α7 nAChRs and nicotine induced calcium ion induction in rat cortical neurons (Takada-Takatori, Kume et al. 2008). The acetylcholinesterase inhibitor galantamine was shown to reverse the nicotine withdrawal-induced disruption of contextual fear conditioning in mice (Wilkinson and Gould 2011) and attenuate nicotine taking and seeking in rats (Hopkins, Rupprecht et al. 2012, Liu 2013). In rhesus monkeys, galantamine and donepezil were shown to exert nicotine-like discriminative stimulus effects under a fixed-ratio 5 schedule of stimulus-shock termination (Moerke and McMahon 2019). Some evidence indicates that galantamine acts as a positive allosteric modulator of nAChRs, perhaps potentiating the effects of nicotine (Maelicke, Samochocki et al. 2001, Farlow 2003, Inden, Takata et al. 2016, Hahn, Shrieves et al. 2019). Galantamine has seen some success in reducing nicotine intake in human subjects (Sofuoglu, Herman et al. 2012, Ashare, Kimmey et al. 2016, MacLean, Waters et al. 2018), but larger clinical trials are necessary to determine the efficacy of galantamine for smoking cessation.

Pharmacotherapeutic Approaches for the Treatment of Nicotine Dependence: Beyond Nicotinic Acetylcholine Receptors

The present review focuses on the newest pharmacotherapeutic strategies that do not target nAChRs. Two biotherapeutic strategies (i.e., nicotine vaccines and nicotine-degrading enzymes)

target nicotine peripherally to reduce its effective concentration in the central nervous system. An opposing pharmacokinetic strategy is the inhibition of nicotine metabolism to shift its effective dose and prolong its duration of action. Other possible therapeutic approaches target cannabinoid receptors 1 and 2, peroxisome proliferator-activated receptor alpha and gamma, or repurpose metformin (i.e., which was approved by the FDA for the treatment of type 2 diabetes) for the treatment of nicotine dependence.

Vaccines

The development of nicotine vaccines began two decades ago, centered around the hypothesis that targeting nicotine systemically rather than targeting nAChRs in the brain would more successfully treat nicotine dependence (Fig. 1A) (Hieda, Keyler et al. 1997). High-affinity antibody production relies on hapten design. Research on optimizing nicotine haptens is summarized in Table 1. The evolution of hapten development is presented in Fig. 2. Active vaccination was the initial strategy for preventing and attenuating nicotine-taking behavior in animal models (Table 2). Active vaccination is defined as administration of a vaccine to the subject in order to stimulate an immune response to the hapten (i.e. nicotine-immunogen conjugate). Active vaccination against nicotine suppresses nicotine-induced dopamine release in the nucleus accumbens shell and prevents the reinstatement of nicotine-seeking behavior in rats (de Villiers, Lindblom et al. 2002, Lindblom, de Villiers et al. 2002). One major concern about nicotine vaccines, however, was their efficacy in active smokers. Vaccination against nicotine in rats during continued nicotine administration produced anti-nicotine antibodies that reduced brain distribution by 40-60%, suggesting that vaccination could be a feasible strategy in current smokers (Hieda, Keyler et al. 2000). However, both human and rat studies suggested that a 40-

60% reduction of brain distribution may not be sufficient to prevent smoking because a > 95% reduction of nicotine was shown to be required to reduce nicotine seeking and smoking (Smith, Cassidy et al. 2017, Kallupi, Xue et al. 2018).

Because of the inconsistent or lack of robust titer production following active vaccination, the next strategy to emerge was passive vaccination using an anti-nicotine antibody. Passive vaccination is defined as administration of monoclonal antibodies raised against the hapten (i.e. nicotine-immunogen conjugate) to the subject. The experimental production of anti-nicotine antibodies is detailed in Table 3. Studies of passive vaccination with anti-nicotine antibodies in animal models are summarized in Table 4. Pretreatment with anti-nicotine immunoglobulin G (IgG) reduced the brain distribution of nicotine by 65%, attenuated the nicotine-induced elevation of systolic blood pressure, and prevented the nicotine-induced stimulation of locomotor activity in rats (Pentel, Malin et al. 2000). Improvements in hapten design led to development of the anti-nicotine IgG NIC9D9, which reduced the brain distribution of nicotine by 89% and prevented the nicotine-induced stimulation of locomotor activity in rats (Meijler, Matsushita et al. 2003, Carrera, Ashley et al. 2004). Combining active and passive vaccination strategies was shown to increase the efficacy of immunotherapy against the brain distribution of nicotine and nicotine-induced locomotor activity in rats by increasing total plasma antibody concentrations (Roiko, Harris et al. 2008). Several iterations of immunogenic carrier protein-conjugate vaccines have undergone clinical trials, but none have passed Phase II because of the inconsistent production of high-affinity antibodies and interindividual variations in the antibody response (Heekin, Shorter et al. 2017).

The latest vaccination strategy involves alternative hapten-carrier encapsulation methods using nanoparticle and lipid technology, the progress of which is summarized in Table 5. Recent

efforts to improve nicotine vaccines include nanoparticle encapsulation (Zhao, Hu et al. 2017) and liposome complex formation (Lockner, Ho et al. 2013, Hu, Zheng et al. 2014). The encapsulation of nicotine-KLH conjugates by poly(lactic-co-glycolic acid (PLGA) nanoparticles improved titers by 400% compared with vaccination with free nicotine-KLH conjugates in mice (Hu, Smith et al. 2016). Although the majority of preclinical studies of nicotine vaccines and monoclonal antibodies have been conducted in rodent models, some studies have been performed in nonhuman primates (Table 6). The majority of preclinical work on anti-nicotine vaccines has been conducted only in males, which is a major issue. Further compounding this issue is the lack of genetic diversity in mouse and rat strains that are used in these studies, which may contribute to the lack of success in clinical trials of anti-nicotine vaccines. Small sample sizes in many behavioral studies may also lead to reproducibility and translation issues across species. Future studies should incorporate both males and females and focus on obtaining reproducible, robust titers across animal species before progressing to clinical trials. Another limitation of the vaccination strategy is the ability of the subjects to compensate for the vaccine by increasing self-administration. Future studies could potentially address this issue using combination therapy with known pharmacotherapies for smoking cessation.

Nicotine-degrading enzymes

In the continued effort to target systemic nicotine (Fig. 1B), researchers turned to nature after the discovery of an enzyme that was isolated from *Pseudomonas putida* S16, a non-pathogenic member of the *Pseudomonas* family, which metabolizes nicotine to fumaric acid (Tang, Wang et al. 2013). From this strain, the nicotine-degrading enzyme NicA2 was isolated and shown to have a promising binding profile for therapeutic use (K_m = 91.9 ± 10.4 nM, k_{cat} =

[1.32 ± 0.04] × 10^{-2} s^{-1}, k_{cat}/K_m = 1.44 × 10^5 s^{-1}·M^{-1} at 37°C; (Xue, Schlosburg et al. 2015). NicA2 has a half-life of 1 day in rat sera and an optimum temperature of 70°C. Crystallography and kinetic analyses of the flavoenzyme showed that NicA2 is a monoamine oxidase with high specificity for S-nicotine (Tararina, Xue et al. 2018). The oxidoreductase features a hydrophobic binding site with a solvent-exclusive cavity that promotes a hydride transfer mechanism. The enzyme's half-life was further improved to 3 days by N-terminal albumin fusion with an albumin binding domain (Xue, Kallupi et al. 2018). With this mutation, the enzyme completely blocked the brain distribution of nicotine and prevented somatic signs, hyperalgesia, and irritability-like behavior that are associated with nicotine withdrawal in rats. Chronic administration of the modified NicA2 decreased compulsive-like nicotine intake and prevented nicotine- and stress-induced relapse in rats with a history of the escalation of nicotine self-administration (Kallupi, Xue et al. 2018). The wildtype enzyme (20 mg/kg) reduced nicotine discrimination and reinforcement in rats (Pentel, Raleigh et al. 2018). Compensation for the enzyme with an increase in nicotine self-administration was attenuated with a higher dose of the enzyme (70 mg/kg). Improvements in potency, the pharmacokinetic profile, and immunogenicity in a human HLA DR4 transgenic mouse model showed promise for human therapeutic use (Thisted, Biesova et al. 2019). Table 7 summarizes experimental progress that has been made with nicotine-degrading enzymes. However, important issues (e.g., compensation with greater nicotine intake by the user) need to be addressed before NicA2 can proceed to clinical trials. Similar to all biotherapeutics, the route of administration and frequency of administration are also important issues because injectables are not desirable by consumers.

In contrast to biotherapeutics targeting nicotine in the periphery to reduce nicotine concentration in the central nervous system, an alternative strategy involves slowing nicotine metabolism (Fig. 1C). The initial metabolism of nicotine and its co-metabolites primarily involves hepatic cytochrome P-450 2A6 (CYP2A6) (Murphy, Raulinaitis et al. 2005). One proposed strategy for smoking cessation has been slowing nicotine metabolism by inhibition of CYP2A6. For this strategy, the mouse model is more translational than the rat since the mouse CYP2A5 and human CYP2A6 orthologs share close structural and functional similarity (Murphy, Raulinaitis et al. 2005, Siu, Wildenauer et al. 2006). Whereas in rats, nicotine is primarily metabolized by enzymes in the CYP2B family (Nakayama, Okuda et al. 1993). A summary of the studies of CYP2A6 inhibition in mice is shown in Table 8. Inhibition of CYP2A5 with methoxsalen in mice increases nicotine's Cmax, prolongs the plasma half-life of nicotine, and decreases its clearance (Damaj, Siu et al. 2007, Alsharari, Siu et al. 2014, Bagdas, Muldoon et al. 2014). This enhancement of nicotine's bioavailability by methoxsalen is accompanied by altered behavioral effects of nicotine, such as analgesia, hyperthermia, and conditioned place preference (Damaj, Siu et al. 2007, Alsharari, Siu et al. 2014, Bagdas, Muldoon et al. 2014). Similar results have been found with coumarins which inhibit CYP2A5 in mice, resulting in prolonged nicotine-induced behaviors, including enhanced memory and learning processes (Budzynska, Skalicka-Wozniak et al. 2016). Recently, a new CYP2A6 inhibitor, DLCI-1, has been validated in a mouse model of intravenous self-administration, where CYP2A5 inhibition significantly reduced nicotine self-administration (Chen, Fowler et al. 2020). In this study, lower rates of self-administration were accompanied with similar behavior that of mice showing higher nicotine self-administration. These studies suggest that low-dose nicotine replacement therapy could be effectively combined with a CYP2A6 inhibitor for

smoking cessation. Table 8 summarizes the findings in mice with CYP2A5 inhibitors. Although this strategy shows translational promise, and potential to reduce nicotine intake, there is no evidence that this strategy prevents cue- or nicotine-induced reinstatement. Therefore, in terms of smoking cessation, this potential therapeutic could be useful for reducing intake, however it is unclear whether it would be useful in complete cessation.

Endocannabinoid System

Substances that activate the cannabinoid receptor system have shown promise in attenuating the effects of drugs of abuse (Sloan, Gowin et al. 2017). Table 9 summarizes studies of cannabinoid receptor agents in animal models of nicotine dependence. Cannabinoid-1 (CB_1) receptor antagonism (or inverse agonism) affects several nicotine-mediated behaviors (Fig. 2A). CB_1 antagonism by rimonabant decreased nicotine self-administration and attenuated nicotine-induced dopamine release in the nucleus accumbens (Cohen, Perrault et al. 2002). Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal, and CB_1 receptor antagonism by rimonabant reversed this nicotine-conditioned behavior in rats (Cohen, Perrault et al. 2005). Pretreatment with the CB_1 receptor antagonist AM251 suppressed nicotine self-administration, but continued pretreatment was necessary to maintain such a beneficial effect (Shoaib 2008). The CB_1 receptor inverse agonist rimonabant also reduced the nicotine- and cue-induced reinstatement of nicotine seeking. However, rimonabant, which was approved as an anti-obesity drug in Europe, was withdrawn from market because of serious psychiatric side effects, including mood disorders and suicidal ideation. Alternative strategies of modulating the cannabinoid system, such as indirectly by modulating fatty acid amide hydrolase and directly using cannabidiol, have been proposed. Fatty acid amide

hydrolase is the enzyme that is responsible for hydrolyzing endocannabinoids and has been evaluated as a potential therapeutic target for several indications (Tripathi 2019). Investigations of URB597, a fatty acid amide hydrolase inhibitor, suggest a role for the endocannabinoid system in regulating the rewarding properties of nicotine and nicotine dependence liability (Fig. 2A-B) (Merritt, Martin et al. 2008). The mechanism of action of URB597 in mediating nicotine behaviors is likely mediated by CB_1 receptors and peroxisome proliferator-activated receptor α (Luchicchi, Lecca et al. 2010, Justinova, Panlilio et al. 2015, Forget, Guranda et al. 2016). Another promising strategy for smoking cessation is agonism of peroxisome proliferator-activated receptors (PPARs, Fig. 2A-B). Peroxisome proliferator-activated receptors are a family of nuclear ligand-activated transcription factors that upregulates the transcription of genes regulating peripheral physiological responses (i.e. inflammation) when activated by endocannabinoids, such as oleoylethanolamide and palmitoylethanolamide (Zhu, Kan et al. 2000). Proliferator-activated receptor α (PPARα) has been found to play a role in nicotine reward and withdrawal, mediated by interactions with the α7 nAChR (Melis, Scheggi et al. 2013, Jackson, Bagdas et al. 2017). The fibrate medication clofibrate, a PPARα agonist, prevented intravenous nicotine acquisition in rats and decreased ongoing intravenous nicotine self-administration in rats and non-human primates and prevented relapse-like behavior in non-human primates (Panlilio, Justinova et al. 2012). Clofibrates effects on nicotine-mediated behavior were found to be specific to PPARα by co-administration of PPARα antagonist MK886 during behavior experiments. Exogenous administration of an endogenous lipid with structural similarity to endocannabinoids, *N*-oleoyl glycine, was found to prevent withdrawal behaviors in nicotine dependent mice and nicotine induced conditioned place preference (Donvito, Piscitelli et al. 2019). The mechanism of action for the effect of *N*-oleoyl glycine on nicotine-dependent

behaviors is presumed to involve PPARα activation leading to downstream negative modulation of β2 containing nAchR expression on dopaminergic neurons in the ventral tegmental area (Melis, Scheggi et al. 2013). The ɣ isoform of proliferator-activated receptor (PPARɣ) has the highest level of expression in the central nervous system, with localization in brain areas involved in drug addiction (i.e. the amygdala and hippocampus) (Searcy, Phelps et al. 2012, Ferguson, Most et al. 2014). Agonism of PPARɣ by pioglitazone in rats showed reduced somatic signs of withdrawal and anxiety-like behavior induced by nicotine withdrawal (Domi, Caputi et al. 2019). The behavioral effect of PPARɣ manipulation on nicotine withdrawal in mice was investigated via neuron specific PPARɣ deletion and pharmacologic blockade of PPARɣ by antagonist GW9662. PPARɣ expression increased in GABAergic and glutamatergic cells of the amygdala and hippocampus, respectively. PPARɣ agonism with pioglitazone showed some effect on craving in a small clinical trial (Jones, Comer et al. 2017), however further pre-clinical and clinical evaluation is needed to assess the potential for agonism of PPARs as a smoking cessation pharmacotherapeutic in humans. The recent legalization of cannabidiol in the United States, coupled with the FDA approval of a cannabidiol formulation for the treatment of epilepsy (2018), makes it a possible candidate for pharmacological development. In animal models of alcohol and cocaine dependence, cannabidiol prevented relapse (Gonzalez-Cuevas, Martin-Fardon et al. 2018). However, the effect of cannabidiol on nicotine dependence has yet to be examined in animal models. There is still much to be explored on the endocannabinoid receptor system front in terms of smoking cessation pharmacotherapy.

Metformin

Smokers have a higher chance of developing diabetes, and nicotine is known to increase blood glucose levels (Maddatu, Anderson-Baucum et al. 2017). A recently discovered link between nicotine use and diabetes involves the diabetes-associated gene *Tcf7l2*, which is densely expressed in the medial habenula region of the rodent brain (Duncan, Heyer et al. 2019). TCF7L2 protein was found to regulate nicotine intake in rats, with a polysynaptic connection from the medial habenula to the pancreas. Metformin is a synthetic biguanide that is most commonly prescribed for the treatment of type 2 diabetes. It is also prescribed for polycystic ovarian syndrome, metabolic syndrome, and diabetes prevention (Markowicz-Piasecka, Huttunen et al. 2017). Metformin works by activating adenosine monophosphate-activated protein kinase (AMPK). AMPK can be activated by an increase in the intracellular AMP/adenosine triphosphate ratio, which can result from hypoxia, oxidant stress, and glucose deprivation (Fig. 3A). High nicotine levels in chronic smokers was shown to be associated with AMPK activation that resulted from stress signaling and high reactive oxygen species levels in adipose tissue (An, Wang et al. 2007). The chronic activation of hypothalamic AMPK by nicotine results in negative energy balance, which is responsible for nicotine-induced weight loss (Fig. 3B). Upon nicotine withdrawal, AMPK is no longer activated, and this energy imbalance is reversed, contributing to smoking cessation-induced weight gain (Fig. 3C) (Martinez de Morentin, Whittle et al. 2012). In models of type 2 diabetes, metformin-induced AMPK activation lowers glucose levels. In models of nicotine dependence, metformin may alleviate the effects of nicotine withdrawal that are induced by a lack of AMPK activation (Fig. 3D). Metformin-induced AMPK activation was shown to improve signs of nicotine withdrawal in mice (Brynildsen, Lee et al. 2018). Metformin reduced the oxidative and inflammatory risk for stroke and post-ischemic brain injury that was promoted by smoking and electronic cigarette use

(Kaisar, Villalba et al. 2017). Metformin activates counteractive mechanisms that are associated with the nuclear factor erythroid 2-related factor (Nrf2) pathway, which reduces the toxic effects of tobacco smoke (Prasad, Sajja et al. 2017). Table 10 summaries the findings to date with metformin. The effects of metformin on nicotine self-administration and its efficacy in preventing cue-induced relapse have not yet been evaluated. Future studies are also needed to investigate the efficacy of metformin in co-morbid disease models of diabetes and nicotine dependence.

Conclusion

Anti-nicotine vaccine and antibody strategies need to be reevaluated in preclinical studies before conducting clinical trials. The nicotine-degrading enzyme NicA2 could be an alternative to vaccination as a biotherapeutic strategy. In contrast to a biotherapeutic approach to target nicotine in the periphery, CYP2A6 inhibitors could slow the metabolism of nicotine leading to a decrease in smoking behavior. Metformin and cannabidiol are the only two pharmacotherapeutic strategies that are already approved by the FDA and thus may be more amenable to bringing to market for the treatment of nicotine dependence. Further preclinical studies are needed to demonstrate the efficacy of metformin and cannabidiol in alleviating the symptoms of nicotine withdrawal and preventing relapse. The majority of preclinical behavioral work on nicotine cessation pharmacotherapies has been conducted only in male subjects. Future studies must incorporate both sexes to produce translatable results. The changing landscape of tobacco use calls for adaptation by the research field. With the rise of nicotine dependence in adolescents, future preclinical studies must also evaluate effects of pharmacological strategies for nicotine cessation in adolescent animals. New nicotine cessation therapies must also consider the

pharmacological effects of nicotine vapor delivery methods. Finally, new models of poly-substance use including nicotine are necessary to evaluate the effectiveness of pharmacotherapies in poly-substance use disorder.

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References

(2018). "Cannabidiol (Epidiolex) for epilepsy." Med Lett Drugs Ther **60**(1559): 182-184.

Alkondon, M., E. F. Pereira, S. Wonnacott and E. X. Albuquerque (1992). "Blockade of nicotinic currents in hippocampal neurons defines methyllycaconitine as a potent and specific receptor antagonist." Mol Pharmacol **41**(4): 802-808.

Alsharari, S. D., E. C. Siu, R. F. Tyndale and M. I. Damaj (2014). "Pharmacokinetic and pharmacodynamics studies of nicotine after oral administration in mice: effects of methoxsalen, a CYP2A5/6 inhibitor." Nicotine Tob Res **16**(1): 18-25.

An, Z., H. Wang, P. Song, M. Zhang, X. Geng and M. H. Zou (2007). "Nicotine-induced activation of AMP-activated protein kinase inhibits fatty acid synthase in 3T3L1 adipocytes: a role for oxidant stress." J Biol Chem **282**(37): 26793-26801.

Ashare, R. L., B. A. Kimmey, L. E. Rupprecht, M. E. Bowers, M. R. Hayes and H. D. Schmidt (2016). "Repeated administration of an acetylcholinesterase inhibitor attenuates nicotine taking in rats and smoking behavior in human smokers." Transl Psychiatry **6**: e713.

Bacher, I., B. Wu, D. R. Shytle and T. P. George (2009). "Mecamylamine - a nicotinic acetylcholine receptor antagonist with potential for the treatment of neuropsychiatric disorders." Expert Opin Pharmacother **10**(16): 2709-2721.

Bagdas, D., P. P. Muldoon, A. Z. Zhu, R. F. Tyndale and M. I. Damaj (2014). "Effects of methoxsalen, a CYP2A5/6 inhibitor, on nicotine dependence behaviors in mice." Neuropharmacology **85**: 67-72.

Bello, M. S., R. Khoddam, M. D. Stone, J. Cho, Y. Yoon, J. O. Lee and A. M. Leventhal (2018). "Poly-product drug use disparities in adolescents of lower socioeconomic status: Emerging trends in nicotine products, marijuana products, and prescription drugs." Behav Res Ther.

Benowitz, N. L. (1990). "Clinical pharmacology of inhaled drugs of abuse: implications in understanding nicotine dependence." NIDA Res Monogr **99**: 12-29.

Benowitz, N. L. (1990). "Pharmacokinetic considerations in understanding nicotine dependence." Ciba Found Symp **152**: 186-200; discussion 200-189.

Benwell, M. E., D. J. Balfour and J. M. Anderson (1988). "Evidence that tobacco smoking increases the density of (-)-[3H]nicotine binding sites in human brain." J Neurochem **50**(4): 1243-1247.

Brynildsen, J. K., B. G. Lee, I. J. Perron, S. Jin, S. F. Kim and J. A. Blendy (2018). "Activation of AMPK by metformin improves withdrawal signs precipitated by nicotine withdrawal." Proc Natl Acad Sci U S A **115**(16): 4282-4287.

Budzynska, B., K. Skalicka-Wozniak, M. Kruk-Slomka, M. Wydrzynska-Kuzma and G. Biala (2016). "In vivo modulation of the behavioral effects of nicotine by the coumarins xanthotoxin, bergapten, and umbelliferone." Psychopharmacology (Berl) **233**(12): 2289-2300.

Campagna, D., A. Alamo, A. Di Pino, C. Russo, A. E. Calogero, F. Purrello and R. Polosa (2019). "Smoking and diabetes: dangerous liaisons and confusing relationships." Diabetol Metab Syndr **11**: 85.

Carrera, M. R., J. A. Ashley, T. Z. Hoffman, S. Isomura, P. Wirsching, G. F. Koob and K. D. Janda (2004). "Investigations using immunization to attenuate the psychoactive effects of nicotine." Bioorg Med Chem **12**(3): 563-570.

Carter, B. L. and S. T. Tiffany (1999). "Cue-reactivity and the future of addiction research." Addiction **94**(3): 349-351.

Cerny, E. H., R. Levy, J. Mauel, M. Mpandi, M. Mutter, C. Henzelin-Nkubana, L. Patiny, G. Tuchscherer and T. Cerny (2002). "Preclinical development of a vaccine 'against smoking'." Onkologie **25**(5): 406-411.

Chen, X., M. Pravetoni, B. Bhayana, P. R. Pentel and M. X. Wu (2012). "High immunogenicity of nicotine vaccines obtained by intradermal delivery with safe adjuvants." Vaccine **31**(1): 159-164.

Chen, X. Z., R. Y. Zhang, X. F. Wang, X. G. Yin, J. Wang, Y. C. Wang, X. Liu, J. J. Du, Z. Liu and J. Guo (2019). "Peptide-free Synthetic Nicotine Vaccine Candidates with alpha-Galactosylceramide as Adjuvant." Mol Pharm **16**(4): 1467-1476.

Chen, Y. C., J. P. Fowler, J. Wang, C. J. W. Watson, Y. Sherafat, A. Staben, P. Lazarus, T. T. Denton and C. D. Fowler (2020). "The Novel CYP2A6 Inhibitor, DLCI-1, Decreases Nicotine Self-Administration in Mice." J Pharmacol Exp Ther **372**(1): 21-29.

Cippitelli, A., G. Astarita, A. Duranti, G. Caprioli, M. Ubaldi, S. Stopponi, M. Kallupi, G. Sagratini, F. Rodriguez de Fonseca, D. Piomelli and R. Ciccocioppo (2011). "Endocannabinoid regulation of acute and protracted nicotine withdrawal: effect of FAAH inhibition." PLoS One **6**(11): e28142.

Coe, J. W., P. R. Brooks, M. G. Vetelino, M. C. Wirtz, E. P. Arnold, J. Huang, S. B. Sands, T. I. Davis, L. A. Lebel, C. B. Fox, A. Shrikhande, J. H. Heym, E. Schaeffer, H. Rollema, Y. Lu, R. S. Mansbach, L. K. Chambers, C. C. Rovetti, D. W. Schulz, F. D. Tingley, 3rd and B. T. O'Neill (2005). "Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation." J Med Chem **48**(10): 3474-3477.

Cohen, A. and O. George (2013). "Animal models of nicotine exposure: relevance to second-hand smoking, electronic cigarette use, and compulsive smoking." Front Psychiatry **4**: 41.

Cohen, C., G. Perrault, G. Griebel and P. Soubrie (2005). "Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB1) receptor antagonist, rimonabant (SR141716)." Neuropsychopharmacology **30**(1): 145-155.

Cohen, C., G. Perrault, C. Voltz, R. Steinberg and P. Soubrie (2002). "SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats." Behav Pharmacol **13**(5-6): 451-463.

Cohen, C., W. B. Pickworth and J. E. Henningfield (1991). "Cigarette smoking and addiction." Clin Chest Med **12**(4): 701-710.

Collins, K. C. and K. D. Janda (2014). "Investigating hapten clustering as a strategy to enhance vaccines against drugs of abuse." Bioconjug Chem **25**(3): 593-600.

Connolly, J., J. Boulter and S. F. Heinemann (1992). "Alpha 4-2 beta 2 and other nicotinic acetylcholine receptor subtypes as targets of psychoactive and addictive drugs." Br J Pharmacol **105**(3): 657-666.

Cornish, K. E., S. H. de Villiers, M. Pravetoni and P. R. Pentel (2013). "Immunogenicity of individual vaccine components in a bivalent nicotine vaccine differ according to vaccine formulation and administration conditions." PLoS One **8**(12): e82557.

Cornish, K. E., A. C. Harris, M. G. LeSage, D. E. Keyler, D. Burroughs, C. Earley and P. R. Pentel (2011). "Combined active and passive immunization against nicotine: minimizing monoclonal antibody requirements using a target antibody concentration strategy." Int Immunopharmacol **11**(11): 1809-1815.

Damaj, M. I., W. Kao and B. R. Martin (2003). "Characterization of spontaneous and precipitated nicotine withdrawal in the mouse." J Pharmacol Exp Ther **307**(2): 526-534.

Damaj, M. I., E. C. Siu, E. M. Sellers, R. F. Tyndale and B. R. Martin (2007). "Inhibition of nicotine metabolism by methoxysalen: Pharmacokinetic and pharmacological studies in mice." J Pharmacol Exp Ther **320**(1): 250-257.

Daniel, J. Z., M. Hickman, J. Macleod, N. Wiles, A. Lingford-Hughes, M. Farrell, R. Araya, P. Skapinakis, J. Haynes and G. Lewis (2009). "Is socioeconomic status in early life associated with drug use? A systematic review of the evidence." Drug Alcohol Rev **28**(2): 142-153.

De, B. P., O. E. Pagovich, M. J. Hicks, J. B. Rosenberg, A. Y. Moreno, K. D. Janda, G. F. Koob, S. Worgall, S. M. Kaminsky, D. Sondhi and R. G. Crystal (2013). "Disrupted adenovirus-based vaccines against small addictive molecules circumvent anti-adenovirus immunity." Hum Gene Ther **24**(1): 58-66.

de Villiers, S. H., K. E. Cornish, A. J. Troska, M. Pravetoni and P. R. Pentel (2013). "Increased efficacy of a trivalent nicotine vaccine compared to a dose-matched monovalent vaccine when formulated with alum." Vaccine **31**(52): 6185-6193.

de Villiers, S. H., N. Lindblom, G. Kalayanov, S. Gordon, A. Malmerfelt, A. M. Johansson and T. H. Svensson (2002). "Active immunization against nicotine suppresses nicotine-induced dopamine release in the rat nucleus accumbens shell." Respiration **69**(3): 247-253.

de Villiers, S. H. L., N. Lindblom, G. Kalayanov, S. Gordon, I. Baraznenok, A. Malmerfelt, M. M. Marcus, A. M. Johansson and T. H. Svensson (2010). "Nicotine hapten structure, antibody selectivity and effect relationships: results from a nicotine vaccine screening procedure." Vaccine **28**(10): 2161-2168.

Deneris, E. S., J. Connolly, J. Boulter, E. Wada, K. Wada, L. W. Swanson, J. Patrick and S. Heinemann (1988). "Primary structure and expression of beta 2: a novel subunit of neuronal nicotinic acetylcholine receptors." Neuron **1**(1): 45-54.

Desai, R. I. and J. Bergman (2015). "Effects of the Nanoparticle-Based Vaccine, SEL-068, on Nicotine Discrimination in Squirrel Monkeys." Neuropsychopharmacology **40**(9): 2207-2216.

Di Chiara, G. (2000). "Role of dopamine in the behavioural actions of nicotine related to addiction." Eur J Pharmacol **393**(1-3): 295-314.

Dickerson, T. J., N. Yamamoto and K. D. Janda (2004). "Antibody-catalyzed oxidative degradation of nicotine using riboflavin." Bioorg Med Chem **12**(18): 4981-4987.

Domi, E., F. F. Caputi, P. Romualdi, A. Domi, G. Scuppa, S. Candeletti, A. Atkins, M. Heilig, G. Demopulos, G. Gaitanaris, R. Ciccocioppo and M. Ubaldi (2019). "Activation of PPARgamma Attenuates the Expression of Physical and Affective Nicotine Withdrawal Symptoms through Mechanisms Involving Amygdala and Hippocampus Neurotransmission." J Neurosci **39**(49): 9864-9875.

Donvito, G., F. Piscitelli, P. Muldoon, A. Jackson, R. M. Vitale, E. D'Aniello, C. Giordano, B. M. Ignatowska-Jankowska, M. A. Mustafa, F. Guida, G. N. Petrie, L. Parker, R. Smoum, L. Sim-Selley, S. Maione, A. H. Lichtman, M. I. Damaj, V. Di Marzo and R. Mechoulam (2019). "N-Oleoyl-glycine reduces nicotine reward and withdrawal in mice." Neuropharmacology **148**: 320-331.

Duncan, A., M. P. Heyer, M. Ishikawa, S. P. B. Caligiuri, X. A. Liu, Z. Chen, M. V. Micioni Di Bonaventura, K. S. Elayouby, J. L. Ables, W. M. Howe, P. Bali, C. Fillinger, M. Williams, R. M. O'Connor, Z. Wang, Q. Lu, T. M. Kamenecka, A. Ma'ayan, H. C. O'Neill, I. Ibanez-Tallon, A. M. Geurts and P. J. Kenny (2019). "Habenular TCF7L2 links nicotine addiction to diabetes." Nature **574**(7778): 372-377.

Erku, D. A., C. E. Gartner, J. T. Do, K. Morphett and K. J. Steadman (2019). "Electronic nicotine delivery systems (e-cigarettes) as a smoking cessation aid: A survey among pharmacy staff in Queensland, Australia." Addict Behav **91**: 227-233.

Farlow, M. R. (2003). "Clinical pharmacokinetics of galantamine." Clin Pharmacokinet **42**(15): 1383-1392.

Ferguson, L. B., D. Most, Y. A. Blednov and R. A. Harris (2014). "PPAR agonists regulate brain gene expression: relationship to their effects on ethanol consumption." Neuropharmacology **86**: 397-407.

Filippidis, F. T., A. A. Laverty, U. Mons, C. Jimenez-Ruiz and C. I. Vardavas (2019). "Changes in smoking cessation assistance in the European Union between 2012 and 2017: pharmacotherapy versus counselling versus e-cigarettes." Tob Control **28**(1): 95-100.

Ford, E. S., A. H. Mokdad and E. W. Gregg (2004). "Trends in cigarette smoking among US adults with diabetes: findings from the Behavioral Risk Factor Surveillance System." Prev Med **39**(6): 1238-1242.

Forget, B., K. M. Coen and B. Le Foll (2009). "Inhibition of fatty acid amide hydrolase reduces reinstatement of nicotine seeking but not break point for nicotine self-administration--comparison with CB(1) receptor blockade." Psychopharmacology (Berl) **205**(4): 613-624.

Forget, B., M. Guranda, I. Gamaleddin, S. R. Goldberg and B. Le Foll (2016). "Attenuation of cue-induced reinstatement of nicotine seeking by URB597 through cannabinoid CB1 receptor in rats." Psychopharmacology (Berl) **233**(10): 1823-1828.

Fu, Y., S. G. Matta, J. M. McIntosh and B. M. Sharp (1999). "Inhibition of nicotine-induced hippocampal norepinephrine release in rats by alpha-conotoxins MII and AuIB microinjected into the locus coeruleus." Neurosci Lett **266**(2): 113-116.

Gamaleddin, I., A. Zvonok, A. Makriyannis, S. R. Goldberg and B. Le Foll (2012). "Effects of a selective cannabinoid CB2 agonist and antagonist on intravenous nicotine self administration and reinstatement of nicotine seeking." PLoS One **7**(1): e29900.

George, O., T. E. Grieder, M. Cole and G. F. Koob (2010). "Exposure to chronic intermittent nicotine vapor induces nicotine dependence." Pharmacol Biochem Behav **96**(1): 104-107.

Gommans, J., I. P. Stolerman and M. Shoaib (2000). "Antagonism of the discriminative and aversive stimulus properties of nicotine in C57BL/6J mice." Neuropharmacology **39**(13): 2840-2847.

Gonzalez-Cuevas, G., R. Martin-Fardon, T. M. Kerr, D. G. Stouffer, L. H. Parsons, D. C. Hammell, S. L. Banks, A. L. Stinchcomb and F. Weiss (2018). "Unique treatment potential of cannabidiol for the prevention of relapse to drug use: preclinical proof of principle." Neuropsychopharmacology **43**(10): 2036-2045.

Gotti, C., M. Moretti, N. M. Meinerz, F. Clementi, A. Gaimarri, A. C. Collins and M. J. Marks (2008). "Partial deletion of the nicotinic cholinergic receptor alpha 4 or beta 2 subunit genes changes the acetylcholine sensitivity of receptor-mediated 86Rb+ efflux in cortex and thalamus and alters relative expression of alpha 4 and beta 2 subunits." Mol Pharmacol **73**(6): 1796-1807.

Grady, S. R., N. M. Meinerz, J. Cao, A. M. Reynolds, M. R. Picciotto, J. P. Changeux, J. M. McIntosh, M. J. Marks and A. C. Collins (2001). "Nicotinic agonists stimulate acetylcholine release from mouse interpeduncular nucleus: a function mediated by a different nAChR than dopamine release from striatum." J Neurochem **76**(1): 258-268.

Grieder, T. E., M. Besson, G. Maal-Bared, S. Pons, U. Maskos and D. van der Kooy (2019). "beta2* nAChRs on VTA dopamine and GABA neurons separately mediate nicotine aversion and reward." Proc Natl Acad Sci U S A **116**(51): 25968-25973.

Hahn, B., M. E. Shrieves, C. K. Olmstead, M. B. Yuille, J. J. Chiappelli, E. F. R. Pereira, E. X. Albuquerque and W. P. Fawcett (2019). "Evidence for positive allosteric modulation of cognitive-enhancing effects of nicotine in healthy human subjects." Psychopharmacology (Berl).

Haikala, H. and L. Ahtee (1988). "Antagonism of the nicotine-induced changes of the striatal dopamine metabolism in mice by mecamylamine and pempidine." Naunyn Schmiedebergs Arch Pharmacol **338**(2): 169-173.

Harris, A. C., P. Muelken, J. R. Smethells, M. Krueger and M. G. LeSage (2017). "Similar precipitated withdrawal effects on intracranial self-stimulation during chronic infusion of an e-cigarette liquid or nicotine alone." Pharmacol Biochem Behav **161**: 1-5.

Harris, A. C., P. Muelken, J. R. Smethells, K. Yershova, I. Stepanov, T. T. Olson, K. J. Kellar and M. G. LeSage (2018). "Effects of nicotine-containing and "nicotine-free" e-cigarette refill liquids on intracranial self-stimulation in rats." Drug Alcohol Depend **185**: 1-9.

Heekin, R. D., D. Shorter and T. R. Kosten (2017). "Current status and future prospects for the development of substance abuse vaccines." Expert Rev Vaccines **16**(11): 1067-1077.

Henningfield, J. E. and R. M. Keenan (1993). "Nicotine delivery kinetics and abuse liability." J Consult Clin Psychol **61**(5): 743-750.

Hieda, Y., D. E. Keyler, S. Ennifar, A. Fattom and P. R. Pentel (2000). "Vaccination against nicotine during continued nicotine administration in rats: immunogenicity of the vaccine and effects on nicotine distribution to brain." Int J Immunopharmacol **22**(10): 809-819.

Hieda, Y., D. E. Keyler, J. T. Vandevoort, J. K. Kane, C. A. Ross, D. E. Raphael, R. S. Niedbalas and P. R. Pentel (1997). "Active Immunization Alters the Plasma Nicotine Concentration in Rats." Journal of Pharmacology and Experimental Therapeutics **283**(3): 1076-1081.

Higgins, S. T., A. N. Kurti, R. Redner, T. J. White, D. E. Gaalema, M. E. Roberts, N. J. Doogan, J. W. Tidey, M. E. Miller, C. A. Stanton, J. E. Henningfield and G. S. Atwood (2015). "A literature review on prevalence of gender differences and intersections with other vulnerabilities to tobacco use in the United States, 2004-2014." Prev Med **80**: 89-100.

Hone, A. J., M. Scadden, J. Gajewiak, S. Christensen, J. Lindstrom and J. M. McIntosh (2012). "alpha-Conotoxin PeIA[S9H,V10A,E14N] potently and selectively blocks alpha6beta2beta3 versus alpha6beta4 nicotinic acetylcholine receptors." Mol Pharmacol **82**(5): 972-982.

Hopkins, T. J., L. E. Rupprecht, M. R. Hayes, J. A. Blendy and H. D. Schmidt (2012). "Galantamine, an acetylcholinesterase inhibitor and positive allosteric modulator of nicotinic acetylcholine receptors, attenuates nicotine taking and seeking in rats." Neuropsychopharmacology **37**(10): 2310-2321.

Hu, Y., D. Smith, E. Frazier, R. Hoerle, M. Ehrich and C. Zhang (2016). "The next-generation nicotine vaccine: a novel and potent hybrid nanoparticle-based nicotine vaccine." Biomaterials **106**: 228-239.

Hu, Y., H. Zheng, W. Huang and C. Zhang (2014). "A novel and efficient nicotine vaccine using nano-lipoplex as a delivery vehicle." Hum Vaccin Immunother **10**(1): 64-72.

Improgo, M. R., M. D. Scofield, A. R. Tapper and P. D. Gardner (2010). "The nicotinic acetylcholine receptor CHRNA5/A3/B4 gene cluster: dual role in nicotine addiction and lung cancer." Prog Neurobiol **92**(2): 212-226.

Inden, M., K. Takata, D. Yanagisawa, E. Ashihara, I. Tooyama, S. Shimohama and Y. Kitamura (2016). "alpha4 nicotinic acetylcholine receptor modulated by galantamine on nigrostriatal terminals regulates dopamine receptor-mediated rotational behavior." Neurochem Int **94**: 74-81.

Isomura, S., P. Wirsching and K. D. Janda (2001). "An immunotherapeutic program for the treatment of nicotine addiction: hapten design and synthesis." J Org Chem **66**(12): 4115-4121.

Izzotti, A., R. Balansky, F. D'Agostini, M. Longobardi, C. Cartiglia, R. T. Micale, S. La Maestra, A. Camoirano, G. Ganchev, M. Iltcheva, V. E. Steele and S. De Flora (2014). "Modulation by metformin of molecular and histopathological alterations in the lung of cigarette smoke-exposed mice." Cancer Med **3**(3): 719-730.

Jackson, A., D. Bagdas, P. P. Muldoon, A. H. Lichtman, F. I. Carroll, M. Greenwald, M. F. Miles and M. I. Damaj (2017). "In vivo interactions between alpha7 nicotinic acetylcholine receptor and nuclear peroxisome proliferator-activated receptor-alpha: Implication for nicotine dependence." Neuropharmacology **118**: 38-45.

Jackson, K. J., S. S. Sanjakdar, P. P. Muldoon, J. M. McIntosh and M. I. Damaj (2013). "The alpha3beta4* nicotinic acetylcholine receptor subtype mediates nicotine reward and physical nicotine withdrawal signs independently of the alpha5 subunit in the mouse." Neuropharmacology **70**: 228-235.

Jones, J. D., S. D. Comer, V. E. Metz, J. M. Manubay, S. Mogali, R. Ciccocioppo, S. Martinez, M. Mumtaz and A. Bisaga (2017). "Pioglitazone, a PPARgamma agonist, reduces nicotine craving in humans, with marginal effects on abuse potential." Pharmacol Biochem Behav **163**: 90-100.

Justinova, Z., L. V. Panlilio, G. Moreno-Sanz, G. H. Redhi, A. Auber, M. E. Secci, P. Mascia, T. Bandiera, A. Armirotti, R. Bertorelli, S. I. Chefer, C. Barnes, S. Yasar, D. Piomelli and S. R. Goldberg (2015). "Effects of Fatty Acid Amide Hydrolase (FAAH) Inhibitors in Non-Human Primate Models of Nicotine Reward and Relapse." Neuropsychopharmacology **40**(9): 2185-2197.

Kaisar, M. A., H. Villalba, S. Prasad, T. Liles, A. E. Sifat, R. K. Sajja, T. J. Abbruscato and L. Cucullo (2017). "Offsetting the impact of smoking and e-cigarette vaping on the cerebrovascular system and stroke injury: Is Metformin a viable countermeasure?" Redox Biol **13**: 353-362.

Kallupi, M. and O. George (2017). "Nicotine Vapor Method to Induce Nicotine Dependence in Rodents." Curr Protoc Neurosci **80**: 8 41 41-48 41 10.

Kallupi, M., S. Xue, B. Zhou, K. D. Janda and O. George (2018). "An enzymatic approach reverses nicotine dependence, decreases compulsive-like intake, and prevents relapse." Sci Adv **4**(10): eaat4751.

Keyler, D. E., M. B. Dufek, A. D. Calvin, T. J. Bramwell, M. G. LeSage, D. E. Raphael, C. A. Ross, C. T. Le and P. R. Pentel (2005). "Reduced nicotine distribution from mother to fetal brain in rats vaccinated against nicotine: time course and influence of nicotine dosing regimen." Biochem Pharmacol **69**(9): 1385-1395.

Keyler, D. E., S. A. Roiko, C. A. Earley, M. P. Murtaugh and P. R. Pentel (2008). "Enhanced immunogenicity of a bivalent nicotine vaccine." Int Immunopharmacol **8**(11): 1589-1594.

Kimmey, B. A., L. E. Rupprecht, M. R. Hayes and H. D. Schmidt (2014). "Donepezil, an acetylcholinesterase inhibitor, attenuates nicotine self-administration and reinstatement of nicotine seeking in rats." Addict Biol **19**(4): 539-551.

Kini, R. M. (2019). "Toxins for decoding interface selectivity in nicotinic acetylcholine receptors." Biochem J **476**(10): 1515-1520.

Koob, G. F. (1992). "Drugs of abuse: anatomy, pharmacology and function of reward pathways." Trends Pharmacol Sci **13**(5): 177-184.

Koob, G. F. (1992). "Neural mechanisms of drug reinforcement." Ann N Y Acad Sci **654**: 171-191.

Kulak, J. M., J. M. McIntosh, D. Yoshikami and B. M. Olivera (2001). "Nicotine-evoked transmitter release from synaptosomes: functional association of specific presynaptic acetylcholine receptors and voltage-gated calcium channels." J Neurochem **77**(6): 1581-1589.

Langley, J. N. (1905). "On the reaction of cells and of nerve-endings to certain poisons, chiefly as regards the reaction of striated muscle to nicotine and to curari." J Physiol **33**(4-5): 374-413.

Lazarevic-Pasti, T., A. Leskovac, T. Momic, S. Petrovic and V. Vasic (2017). "Modulators of Acetylcholinesterase Activity: From Alzheimer's Disease to Anti-Cancer Drugs." Curr Med Chem **24**(30): 3283-3309.

Le Foll, B. and S. R. Goldberg (2004). "Rimonabant, a CB1 antagonist, blocks nicotine-conditioned place preferences." Neuroreport **15**(13): 2139-2143.

LeSage, M. G., D. E. Keyler, Y. Hieda, G. Collins, D. Burroughs, C. Le and P. R. Pentel (2006). "Effects of a nicotine conjugate vaccine on the acquisition and maintenance of nicotine self-administration in rats." Psychopharmacology (Berl) **184**(3-4): 409-416.

Leshner, A. I. and G. F. Koob (1999). "Drugs of abuse and the brain." Proc Assoc Am Physicians **111**(2): 99-108.

Lindblom, N., S. H. de Villiers, G. Kalayanov, S. Gordon, A. M. Johansson and T. H. Svensson (2002). "Active immunization against nicotine prevents reinstatement of nicotine-seeking behavior in rats." Respiration **69**(3): 254-260.

Liu, X. (2013). "Positive allosteric modulation of alpha4beta2 nicotinic acetylcholine receptors as a new approach to smoking reduction: evidence from a rat model of nicotine self-administration." Psychopharmacology (Berl) **230**(2): 203-213.

Lockner, J. W., S. O. Ho, K. C. McCague, S. M. Chiang, T. Q. Do, G. Fujii and K. D. Janda (2013). "Enhancing nicotine vaccine immunogenicity with liposomes." Bioorg Med Chem Lett **23**(4): 975-978.

Lockner, J. W., J. M. Lively, K. C. Collins, J. C. Vendruscolo, M. R. Azar and K. D. Janda (2015). "A conjugate vaccine using enantiopure hapten imparts superior nicotine-binding capacity." J Med Chem **58**(2): 1005-1011.

Luchicchi, A., S. Lecca, S. Carta, G. Pillolla, A. L. Muntoni, S. Yasar, S. R. Goldberg and M. Pistis (2010). "Effects of fatty acid amide hydrolase inhibition on neuronal responses to nicotine, cocaine and morphine in the nucleus accumbens shell and ventral tegmental area: involvement of PPAR-alpha nuclear receptors." Addict Biol **15**(3): 277-288.

Luo, S., J. M. Kulak, G. E. Cartier, R. B. Jacobsen, D. Yoshikami, B. M. Olivera and J. M. McIntosh (1998). "alpha-conotoxin AuIB selectively blocks alpha3 beta4 nicotinic acetylcholine receptors and nicotine-evoked norepinephrine release." J Neurosci **18**(21): 8571-8579.

MacLean, R. R., A. J. Waters, E. Brede and M. Sofuoglu (2018). "Effects of galantamine on smoking behavior and cognitive performance in treatment-seeking smokers prior to a quit attempt." Hum Psychopharmacol **33**(4): e2665.

Maddatu, J., E. Anderson-Baucum and C. Evans-Molina (2017). "Smoking and the risk of type 2 diabetes." Transl Res **184**: 101-107.

Maelicke, A., M. Samochocki, R. Jostock, A. Fehrenbacher, J. Ludwig, E. X. Albuquerque and M. Zerlin (2001). "Allosteric sensitization of nicotinic receptors by galantamine, a new treatment strategy for Alzheimer's disease." Biol Psychiatry **49**(3): 279-288.

Malin, D. H., J. R. Lake, V. A. Carter, J. S. Cunningham, K. M. Hebert, D. L. Conrad and O. B. Wilson (1994). "The nicotinic antagonist mecamylamine precipitates nicotine abstinence syndrome in the rat." Psychopharmacology (Berl) **115**(1-2): 180-184.

Markou, A. and N. E. Paterson (2001). "The nicotinic antagonist methyllycaconitine has differential effects on nicotine self-administration and nicotine withdrawal in the rat." Nicotine Tob Res **3**(4): 361-373.

Markowicz-Piasecka, M., K. M. Huttunen, L. Mateusiak, E. Mikiciuk-Olasik and J. Sikora (2017). "Is Metformin a Perfect Drug? Updates in Pharmacokinetics and Pharmacodynamics." Curr Pharm Des **23**(17): 2532-2550.

Marks, M. J., J. B. Burch and A. C. Collins (1983). "Effects of chronic nicotine infusion on tolerance development and nicotinic receptors." J Pharmacol Exp Ther **226**(3): 817-825.

Martin, T. J., J. Suchocki, E. L. May and B. R. Martin (1990). "Pharmacological evaluation of the antagonism of nicotine's central effects by mecamylamine and pempidine." J Pharmacol Exp Ther **254**(1): 45-51.

Martinez de Morentin, P. B., A. J. Whittle, J. Ferno, R. Nogueiras, C. Dieguez, A. Vidal-Puig and M. Lopez (2012). "Nicotine induces negative energy balance through hypothalamic AMP-activated protein kinase." Diabetes **61**(4): 807-817.

Mascia, P., M. Pistis, Z. Justinova, L. V. Panlilio, A. Luchicchi, S. Lecca, M. Scherma, W. Fratta, P. Fadda, C. Barnes, G. H. Redhi, S. Yasar, B. Le Foll, G. Tanda, D. Piomelli and S. R. Goldberg (2011). "Blockade of nicotine reward and reinstatement by activation of alpha-type peroxisome proliferator-activated receptors." Biol Psychiatry **69**(7): 633-641.

Maskos, U., B. E. Molles, S. Pons, M. Besson, B. P. Guiard, J. P. Guilloux, A. Evrard, P. Cazala, A. Cormier, M. Mameli-Engvall, N. Dufour, I. Cloez-Tayarani, A. P. Bemelmans, J. Mallet, A. M. Gardier, V. David, P. Faure, S. Granon and J. P. Changeux (2005). "Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors." Nature **436**(7047): 103-107.

McCluskie, M. J., D. C. Pryde, D. P. Gervais, D. R. Stead, N. Zhang, M. Benoit, K. Robertson, I. J. Kim, T. Tharmanathan, J. R. Merson and H. L. Davis (2013). "Enhancing immunogenicity of a 3'aminomethylnicotine-DT-conjugate anti-nicotine vaccine with CpG adjuvant in mice and non-human primates." Int Immunopharmacol **16**(1): 50-56.

McCluskie, M. J., J. Thorn, D. P. Gervais, D. R. Stead, N. Zhang, M. Benoit, J. Cartier, I. J. Kim, K. Bhattacharya, J. I. Finneman, J. R. Merson and H. L. Davis (2015). "Anti-nicotine vaccines: Comparison of adjuvanted CRM197 and Qb-VLP conjugate formulations for immunogenicity and function in non-human primates." Int Immunopharmacol **29**(2): 663-671.

McCluskie, M. J., J. Thorn, P. R. Mehelic, P. Kolhe, K. Bhattacharya, J. I. Finneman, D. R. Stead, M. B. Piatchek, N. Zhang, G. Chikh, J. Cartier, D. M. Evans, J. R. Merson and H. L. Davis (2015). "Molecular attributes of conjugate antigen influence function of antibodies induced by anti-nicotine vaccine in mice and non-human primates." Int Immunopharmacol **25**(2): 518-527.

Meijler, M. M., M. Matsushita, L. J. Altobell, 3rd, P. Wirsching and K. D. Janda (2003). "A new strategy for improved nicotine vaccines using conformationally constrained haptens." J Am Chem Soc **125**(24): 7164-7165.

Melis, M., G. Pillolla, A. Luchicchi, A. L. Muntoni, S. Yasar, S. R. Goldberg and M. Pistis (2008). "Endogenous fatty acid ethanolamides suppress nicotine-induced activation of mesolimbic dopamine neurons through nuclear receptors." J Neurosci **28**(51): 13985-13994.

Melis, M., S. Scheggi, G. Carta, C. Madeddu, S. Lecca, A. Luchicchi, F. Cadeddu, R. Frau, L. Fattore, P. Fadda, M. G. Ennas, M. P. Castelli, W. Fratta, B. Schilstrom, S. Banni, M. G. De Montis and M. Pistis (2013). "PPARalpha regulates cholinergic-driven activity of midbrain dopamine neurons via a novel mechanism involving alpha7 nicotinic acetylcholine receptors." J Neurosci **33**(14): 6203-6211.

Merritt, L. L., B. R. Martin, C. Walters, A. H. Lichtman and M. I. Damaj (2008). "The endogenous cannabinoid system modulates nicotine reward and dependence." J Pharmacol Exp Ther **326**(2): 483-492.

Miller, K. D., R. Roque and C. H. Clegg (2014). "Novel Anti-Nicotine Vaccine Using a Trimeric Coiled-Coil Hapten Carrier." PLoS One **9**(12): e114366.

Moerke, M. J. and L. R. McMahon (2019). "Nicotine-like discriminative stimulus effects of acetylcholinesterase inhibitors and a muscarinic receptor agonist in Rhesus monkeys." Drug Dev Ind Pharm **45**(5): 861-867.

Moerke, M. J., A. Z. Zhu, R. F. Tyndale, M. A. Javors and L. R. McMahon (2017). "The discriminative stimulus effects of i.v. nicotine in rhesus monkeys: Pharmacokinetics and apparent pA2 analysis with dihydro-beta-erythroidine." Neuropharmacology **116**: 9-17.

Moller, H. J., K. Demyttenaere, B. Olausson, J. Szamosi, E. Wilson, D. Hosford, G. Dunbar, R. Tummala and H. Eriksson (2015). "Two Phase III randomised double-blind studies of fixed-dose TC-5214 (dexmecamylamine) adjunct to ongoing antidepressant therapy in patients with major depressive disorder and an inadequate response to prior antidepressant therapy." World J Biol Psychiatry **16**(7): 483-501.

Moreno, A. Y., M. R. Azar, G. F. Koob and K. D. Janda (2012). "Probing the protective effects of a conformationally constrained nicotine vaccine." Vaccine **30**(47): 6665-6670.

Moreno, A. Y., M. R. Azar, N. A. Warren, T. J. Dickerson, G. F. Koob and K. D. Janda (2010). "A critical evaluation of a nicotine vaccine within a self-administration behavioral model." Mol Pharm **7**(2): 431-441.

Morrison, C. F. and J. A. Stephenson (1969). "Nicotine injections as the conditioned stimulus in discrimination learning." Psychopharmacologia **15**(5): 351-360.

Murphy, S. E., V. Raulinaitis and K. M. Brown (2005). "Nicotine 5'-oxidation and methyl oxidation by P450 2A enzymes." Drug Metab Dispos **33**(8): 1166-1173.

Nakayama, H., H. Okuda, T. Nakashima, S. Imaoka and Y. Funae (1993). "Nicotine metabolism by rat hepatic cytochrome P450s." Biochem Pharmacol **45**(12): 2554-2556.

Niaura, R. S., D. J. Rohsenow, J. A. Binkoff, P. M. Monti, M. Pedraza and D. B. Abrams (1988). "Relevance of cue reactivity to understanding alcohol and smoking relapse." J Abnorm Psychol **97**(2): 133-152.

Panlilio, L. V., Z. Justinova, P. Mascia, M. Pistis, A. Luchicchi, S. Lecca, C. Barnes, G. H. Redhi, J. Adair, S. J. Heishman, S. Yasar, M. Aliczki, J. Haller and S. R. Goldberg (2012). "Novel use of a lipid-lowering fibrate medication to prevent nicotine reward and relapse: preclinical findings." Neuropsychopharmacology **37**(8): 1838-1847.

Papke, R. L. and J. M. Lindstrom (2020). "Nicotinic acetylcholine receptors: Conventional and unconventional ligands and signaling." Neuropharmacology **168**: 108021.

Papke, R. L., P. R. Sanberg and R. D. Shytle (2001). "Analysis of mecamylamine stereoisomers on human nicotinic receptor subtypes." J Pharmacol Exp Ther **297**(2): 646-656.

Parrott, A. C. (2003). "Cigarette-derived nicotine is not a medicine." World J Biol Psychiatry **4**(2): 49-55.

Paterson, N. E. and A. Markou (2004). "Prolonged nicotine dependence associated with extended access to nicotine self-administration in rats." Psychopharmacology (Berl) **173**(1-2): 64-72.

Pentel, P. R., D. H. Malin, S. Ennifar, Y. Hieda, D. E. Keyler, J. R. Lake, J. R. Milstein, L. E. Basham, R. T. Coy, J. W. Moon, R. Naso and A. Fattom (2000). "A nicotine conjugate vaccine reduces nicotine distribution to brain and attenuates its behavioral and cardiovascular effects in rats." Pharmacol Biochem Behav **65**(1): 191-198.

Pentel, P. R., M. D. Raleigh, M. G. LeSage, T. Thisted, S. Horrigan, Z. Biesova and M. W. Kalnik (2018). "The nicotine-degrading enzyme NicA2 reduces nicotine levels in blood, nicotine distribution to brain, and nicotine discrimination and reinforcement in rats." BMC Biotechnol **18**(1): 46.

Pettey, D. and T. Aubry (2018). "Tobacco use and smoking behaviors of individuals with a serious mental illness." Psychiatr Rehabil J **41**(4): 356-360.

Picciotto, M. R., M. Zoli, R. Rimondini, C. Lena, L. M. Marubio, E. M. Pich, K. Fuxe and J. P. Changeux (1998). "Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine." Nature **391**(6663): 173-177.

Poole, R. L., D. A. Connor and T. J. Gould (2014). "Donepezil reverses nicotine withdrawal-induced deficits in contextual fear conditioning in C57BL/6J mice." Behav Neurosci **128**(5): 588-593.

Prasad, S., R. K. Sajja, M. A. Kaisar, J. H. Park, H. Villalba, T. Liles, T. Abbruscato and L. Cucullo (2017). "Role of Nrf2 and protective effects of Metformin against tobacco smoke-induced cerebrovascular toxicity." Redox Biol **12**: 58-69.

Pravetoni, M., D. E. Keyler, R. R. Pidaparthi, F. I. Carroll, S. P. Runyon, M. P. Murtaugh, C. A. Earley and P. R. Pentel (2012). "Structurally distinct nicotine immunogens elicit antibodies with non-overlapping specificities." Biochem Pharmacol **83**(4): 543-550.

Pravetoni, M., D. E. Keyler, M. D. Raleigh, A. C. Harris, M. G. Lesage, C. K. Mattson, S. Pettersson and P. R. Pentel (2011). "Vaccination against nicotine alters the distribution of nicotine delivered via cigarette smoke inhalation to rats." Biochem Pharmacol **81**(9): 1164-1170.

Prochaska, J. J. and N. L. Benowitz (2016). "The Past, Present, and Future of Nicotine Addiction Therapy." Annu Rev Med **67**: 467-486.

Pryde, D. C., L. H. Jones, D. P. Gervais, D. R. Stead, D. C. Blakemore, M. D. Selby, A. D. Brown, J. W. Coe, M. Badland, D. M. Beal, R. Glen, Y. Wharton, G. J. Miller, P. White, N. Zhang, M. Benoit, K. Robertson, J. R. Merson, H. L. Davis and M. J. McCluskie (2013). "Selection of a novel anti-nicotine vaccine: influence of antigen design on antibody function in mice." PLoS One **8**(10): e76557.

Roiko, S. A., A. C. Harris, D. E. Keyler, M. G. Lesage, Y. Zhang and P. R. Pentel (2008). "Combined active and passive immunization enhances the efficacy of immunotherapy against nicotine in rats." J Pharmacol Exp Ther **325**(3): 985-993.

Rose, J. E., F. M. Behm and E. C. Westman (1998). "Nicotine-mecamylamine treatment for smoking cessation: the role of pre-cessation therapy." Exp Clin Psychopharmacol **6**(3): 331-343.

Rose, J. E., F. M. Behm, E. C. Westman, E. D. Levin, R. M. Stein and G. V. Ripka (1994). "Mecamylamine combined with nicotine skin patch facilitates smoking cessation beyond nicotine patch treatment alone." Clin Pharmacol Ther **56**(1): 86-99.

Rose, J. E., A. Sampson, E. D. Levin and J. E. Henningfield (1989). "Mecamylamine increases nicotine preference and attenuates nicotine discrimination." Pharmacol Biochem Behav **32**(4): 933-938.

Rosenberg, J. B., B. P. De, M. J. Hicks, K. D. Janda, S. M. Kaminsky, S. Worgall and R. G. Crystal (2013). "Suppression of nicotine-induced pathophysiology by an adenovirus hexon-based antinicotine vaccine." Hum Gene Ther **24**(6): 595-603.

Sanderson, S. D., S. R. Cheruku, M. P. Padmanilayam, J. L. Vennerstrom, G. M. Thiele, M. I. Palmatier and R. A. Bevins (2003). "Immunization to nicotine with a peptide-based vaccine composed of a conformationally biased agonist of C5a as a molecular adjuvant." Int Immunopharmacol **3**(1): 137-146.

Satoskar, S. D., D. E. Keyler, M. G. LeSage, D. E. Raphael, C. A. Ross and P. R. Pentel (2003). "Tissue-dependent effects of immunization with a nicotine conjugate vaccine on the distribution of nicotine in rats." Int Immunopharmacol **3**(7): 957-970.

Scherma, M., Z. Justinova, C. Zanettini, L. V. Panlilio, P. Mascia, P. Fadda, W. Fratta, A. Makriyannis, S. K. Vadivel, I. Gamaleddin, B. Le Foll and S. R. Goldberg (2012). "The anandamide transport inhibitor AM404 reduces the rewarding effects of nicotine and nicotine-induced dopamine elevations in the nucleus accumbens shell in rats." Br J Pharmacol **165**(8): 2539-2548.

Schier, J. G., J. G. Meiman, J. Layden, C. A. Mikosz, B. VanFrank, B. A. King, P. P. Salvatore, D. N. Weissman, J. Thomas, P. C. Melstrom, G. T. Baldwin, E. M. Parker, E. A. Courtney-Long, V. P. Krishnasamy, C. M. Pickens, M. E. Evans, S. V. Tsay, K. M. Powell, E. A. Kiernan, K. L. Marynak, J. Adjemian, K. Holton, B. S. Armour, L. J. England, P. A. Briss, D. Houry, K. A. Hacker, S. Reagan-Steiner, S. Zaki, D. Meaney-Delman and C. D. C. L. I. R. Group (2019). "Severe Pulmonary Disease Associated with Electronic-Cigarette-Product Use - Interim Guidance." MMWR Morb Mortal Wkly Rep **68**(36): 787-790.

Schwartz, R. D. and K. J. Kellar (1983). "Nicotinic cholinergic receptor binding sites in the brain: regulation in vivo." Science **220**(4593): 214-216.

Searcy, J. L., J. T. Phelps, T. Pancani, I. Kadish, J. Popovic, K. L. Anderson, T. L. Beckett, M. P. Murphy, K. C. Chen, E. M. Blalock, P. W. Landfield, N. M. Porter and O. Thibault (2012). "Long-term pioglitazone treatment improves learning and attenuates pathological markers in a mouse model of Alzheimer's disease." J Alzheimers Dis **30**(4): 943-961.

Shaikh, S., A. Verma, S. Siddiqui, S. S. Ahmad, S. M. Rizvi, S. Shakil, D. Biswas, D. Singh, M. H. Siddiqui, S. Shakil, S. Tabrez and M. A. Kamal (2014). "Current acetylcholinesterase-inhibitors: a neuroinformatics perspective." CNS Neurol Disord Drug Targets **13**(3): 391-401.

Shao, X. M., B. Lopez, D. Nathan, J. Wilson, E. Bankole, H. Tumoyan, A. Munoz, J. Espinoza-Derout, K. M. Hasan, S. Chang, C. Du, A. P. Sinha-Hikim, K. Lutfy and T. C. Friedman (2019). "A mouse model for chronic intermittent electronic cigarette exposure exhibits nicotine pharmacokinetics resembling human vapers." J Neurosci Methods **326**: 108376.

Shoaib, M. (2008). "The cannabinoid antagonist AM251 attenuates nicotine self-administration and nicotine-seeking behaviour in rats." Neuropharmacology **54**(2): 438-444.

Shytle, R. D., E. Penny, A. A. Silver, J. Goldman and P. R. Sanberg (2002). "Mecamylamine (Inversine): an old antihypertensive with new research directions." J Hum Hypertens **16**(7): 453-457.

Siu, E. C., D. B. Wildenauer and R. F. Tyndale (2006). "Nicotine self-administration in mice is associated with rates of nicotine inactivation by CYP2A5." Psychopharmacology (Berl) **184**(3-4): 401-408.

Sloan, M. E., J. L. Gowin, V. A. Ramchandani, Y. L. Hurd and B. Le Foll (2017). "The endocannabinoid system as a target for addiction treatment: Trials and tribulations." Neuropharmacology **124**: 73-83.

Smith, L. C., M. Kallupi, L. Tieu, A. Jaquish, J. Barr, Y. Su, N. Velarde, S. Sedighim, M. Klodnicki, L. L. G. Carrette, X. Sun, G. de Guglielmo and O. George (2019). "Vaping nicotine-containing electronic cigarettes produces addiction-like behaviors and cardiopulmonary abnormalities in rats." bioRxiv: 797084.

Smith, T. T., R. N. Cassidy, J. W. Tidey, X. Luo, C. T. Le, D. K. Hatsukami and E. C. Donny (2017). "Impact of smoking reduced nicotine content cigarettes on sensitivity to cigarette price: further results from a multi-site clinical trial." Addiction **112**(2): 349-359.

Sofuoglu, M., A. I. Herman, Y. Li and A. J. Waters (2012). "Galantamine attenuates some of the subjective effects of intravenous nicotine and improves performance on a Go No-Go task in abstinent cigarette smokers: a preliminary report." Psychopharmacology (Berl) **224**(3): 413-420.

Stolerman, I. P., C. J. Chandler, H. S. Garcha and J. M. Newton (1997). "Selective antagonism of behavioural effects of nicotine by dihydro-beta-erythroidine in rats." Psychopharmacology (Berl) **129**(4): 390-397.

Stolerman, I. P., C. Naylor, G. I. Elmer and S. R. Goldberg (1999). "Discrimination and self-administration of nicotine by inbred strains of mice." Psychopharmacology (Berl) **141**(3): 297-306.

Takada-Takatori, Y., T. Kume, Y. Ohgi, T. Fujii, T. Niidome, H. Sugimoto and A. Akaike (2008). "Mechanisms of alpha7-nicotinic receptor up-regulation and sensitization to donepezil induced by chronic donepezil treatment." Eur J Pharmacol **590**(1-3): 150-156.

Tang, H., L. Wang, W. Wang, H. Yu, K. Zhang, Y. Yao and P. Xu (2013). "Systematic unraveling of the unsolved pathway of nicotine degradation in Pseudomonas." PLoS Genet **9**(10): e1003923.

Tapper, A. R., S. L. McKinney, R. Nashmi, J. Schwarz, P. Deshpande, C. Labarca, P. Whiteaker, M. J. Marks, A. C. Collins and H. A. Lester (2004). "Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization." Science **306**(5698): 1029-1032.

Tararina, M. A., S. Xue, L. C. Smith, S. N. Muellers, P. O. Miranda, K. D. Janda and K. N. Allen (2018). "Crystallography Coupled with Kinetic Analysis Provides Mechanistic Underpinnings of a Nicotine-Degrading Enzyme." Biochemistry **57**(26): 3741-3751.

Thapa, S., M. Lv and H. Xu (2017). "Acetylcholinesterase: A Primary Target for Drugs and Insecticides." Mini Rev Med Chem **17**(17): 1665-1676.

Thisted, T., Z. Biesova, C. Walmacq, E. Stone, M. Rodnick-Smith, S. S. Ahmed, S. K. Horrigan, B. Van Engelen, C. Reed and M. W. Kalnik (2019). "Optimization of a nicotine degrading enzyme for potential use in treatment of nicotine addiction." BMC Biotechnol **19**(1): 56.

Tidey, J. W., D. R. Davis, M. E. Miller, I. Pericot-Valverde, R. L. Denlinger-Apte and D. E. Gaalema (2018). "Modeling nicotine regulation: A review of studies in smokers with mental health conditions." Prev Med **117**: 30-37.

Tripathi, R. K. P. (2019). "A perspective review on fatty acid amide hydrolase (FAAH) inhibitors as potential therapeutic agents." Eur J Med Chem **188**: 111953.

Tuncok, Y., Y. Hieda, D. E. Keyler, S. Brown, S. Ennifar, A. Fattom and P. R. Pentel (2001). "Inhibition of nicotine-induced seizures in rats by combining vaccination against nicotine with chronic nicotine infusion." Exp Clin Psychopharmacol **9**(2): 228-234.

Turner, J. R., A. Gold, R. Schnoll and J. A. Blendy (2013). "Translational research in nicotine dependence." Cold Spring Harb Perspect Med **3**(3): a012153.

van Wijk, E. C., L. L. Landais and J. Harting (2019). "Understanding the multitude of barriers that prevent smokers in lower socioeconomic groups from accessing smoking cessation support: A literature review." Prev Med **123**: 143-151.

Warren, C. W. (2008). "The Global Youth Tobacco Survey (GYTS): linking data to the implementation of the WHO Framework Convention on Tobacco Control." BMC Public Health **8 Suppl 1**: S1.

Watkins, S. S., M. P. Epping-Jordan, G. F. Koob and A. Markou (1999). "Blockade of nicotine self-administration with nicotinic antagonists in rats." Pharmacol Biochem Behav **62**(4): 743-751.

West, R., P. Hajek, J. Foulds, F. Nilsson, S. May and A. Meadows (2000). "A comparison of the abuse liability and dependence potential of nicotine patch, gum, spray and inhaler." Psychopharmacology (Berl) **149**(3): 198-202.

WHO (2017). WHO report on the global tobacco epidemic, 2017: monitoring tobacco use and prevention policies. W. H. Organization.

Wilkerson, J. L., F. Deba, M. L. Crowley, A. K. Hamouda and L. R. McMahon (2020). "Advances in the In vitro and In vivo pharmacology of Alpha4beta2 nicotinic receptor positive allosteric modulators." Neuropharmacology **168**: 108008.

Wilkinson, D. S. and T. J. Gould (2011). "The effects of galantamine on nicotine withdrawal-induced deficits in contextual fear conditioning in C57BL/6 mice." Behav Brain Res **223**(1): 53-57.

Williams, M. and J. L. Robinson (1984). "Binding of the nicotinic cholinergic antagonist, dihydro-beta-erythroidine, to rat brain tissue." J Neurosci **4**(12): 2906-2911.

Wonnacott, S. (1990). "The paradox of nicotinic acetylcholine receptor upregulation by nicotine." Trends Pharmacol Sci **11**(6): 216-219.

Xue, S., M. Kallupi, B. Zhou, L. C. Smith, P. O. Miranda, O. George and K. D. Janda (2018). "An enzymatic advance in nicotine cessation therapy." Chem Commun (Camb) **54**(14): 1686-1689.

Xue, S., J. E. Schlosburg and K. D. Janda (2015). "A New Strategy for Smoking Cessation: Characterization of a Bacterial Enzyme for the Degradation of Nicotine." J Am Chem Soc **137**(32): 10136-10139.

You, S., X. Li, J. Xiong, X. Zhu, D. Zhangsun, X. Zhu and S. Luo (2019). "alpha-Conotoxin TxIB: A Uniquely Selective Ligand for alpha6/alpha3beta2beta3 Nicotinic Acetylcholine Receptor Attenuates Nicotine-Induced Conditioned Place Preference in Mice." Mar Drugs **17**(9).

Zhao, Z., Y. Hu, R. Hoerle, M. Devine, M. Raleigh, P. Pentel and C. Zhang (2017). "A nanoparticle-based nicotine vaccine and the influence of particle size on its immunogenicity and efficacy." Nanomedicine **13**(2): 443-454.

Zheng, H., Y. Hu, W. Huang, S. de Villiers, P. Pentel, J. Zhang, H. Dorn, M. Ehrich and C. Zhang (2015). "Negatively Charged Carbon Nanohorn Supported Cationic Liposome Nanoparticles: A Novel Delivery Vehicle for Anti-Nicotine Vaccine." J Biomed Nanotechnol **11**(12): 2197-2210.

Zhu, Y., L. Kan, C. Qi, Y. S. Kanwar, A. V. Yeldandi, M. S. Rao and J. K. Reddy (2000). "Isolation and characterization of peroxisome proliferator-activated receptor (PPAR) interacting protein (PRIP) as a coactivator for PPAR." J Biol Chem **275**(18): 13510-13516.

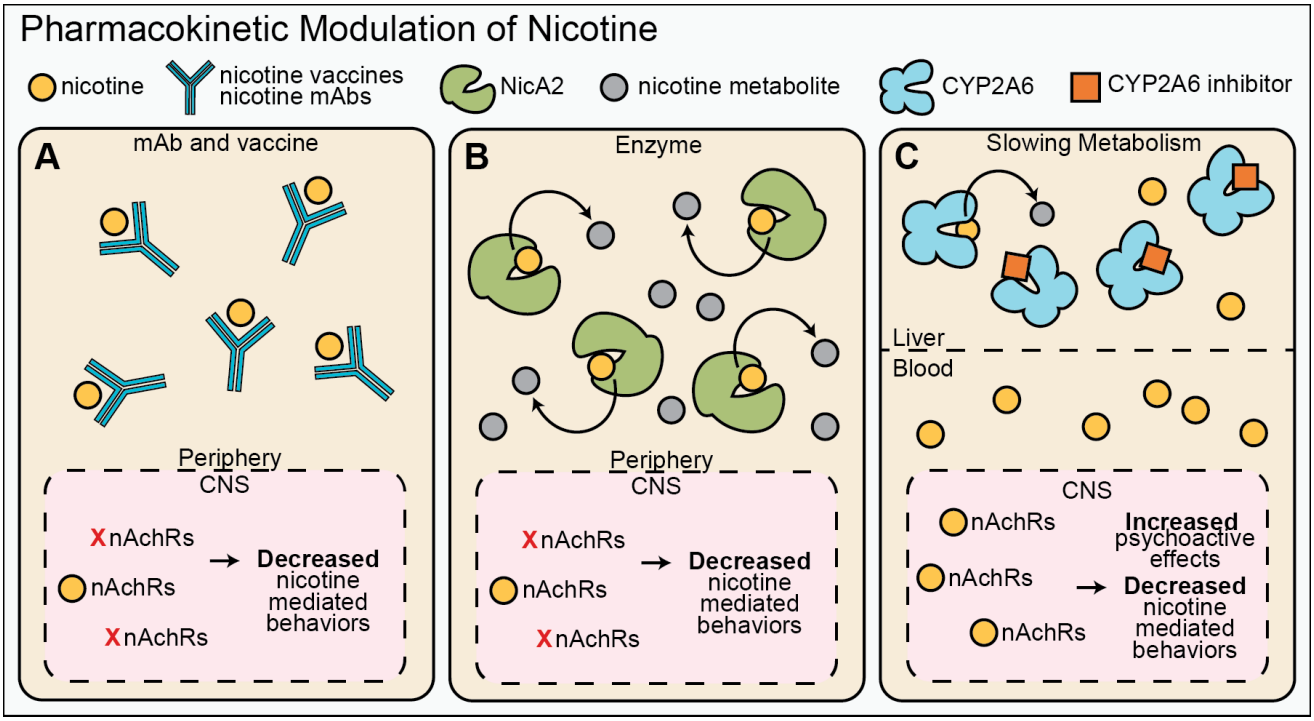

Figure 1. Pharmacotherapy strategies targeting nicotine pharmacokinetics. A) Monoclonal antibodies (mAbs) and vaccines against nicotine increase nicotine half-life, concentration in the periphery, and decrease nicotine concentration in the central nervous system (CNS) via high affinity nicotine-mAb binding. The nicotine-mAb construct is too large to pass the blood-brain-barrier, thereby decreasing the concentration of nicotine bound nAchRs, leading to a decrease or prevention in nicotine mediated behaviors; B) Exogenous delivery of a nicotine degrading enzyme, such as NicA2, decreases nicotine concentration in the periphery with an increase in concentration of an inactive metabolite. Decrease in active nicotine bound nAchRs in the CNS leads to a decrease in nicotine mediated behaviors; C) Inhibitors of hepatic CYP2A6 lead to an increase the half-life and of peripheral concentration of nicotine. Increased nicotine bound nAchRs in the CNS results in a lower effective dose of nicotine.

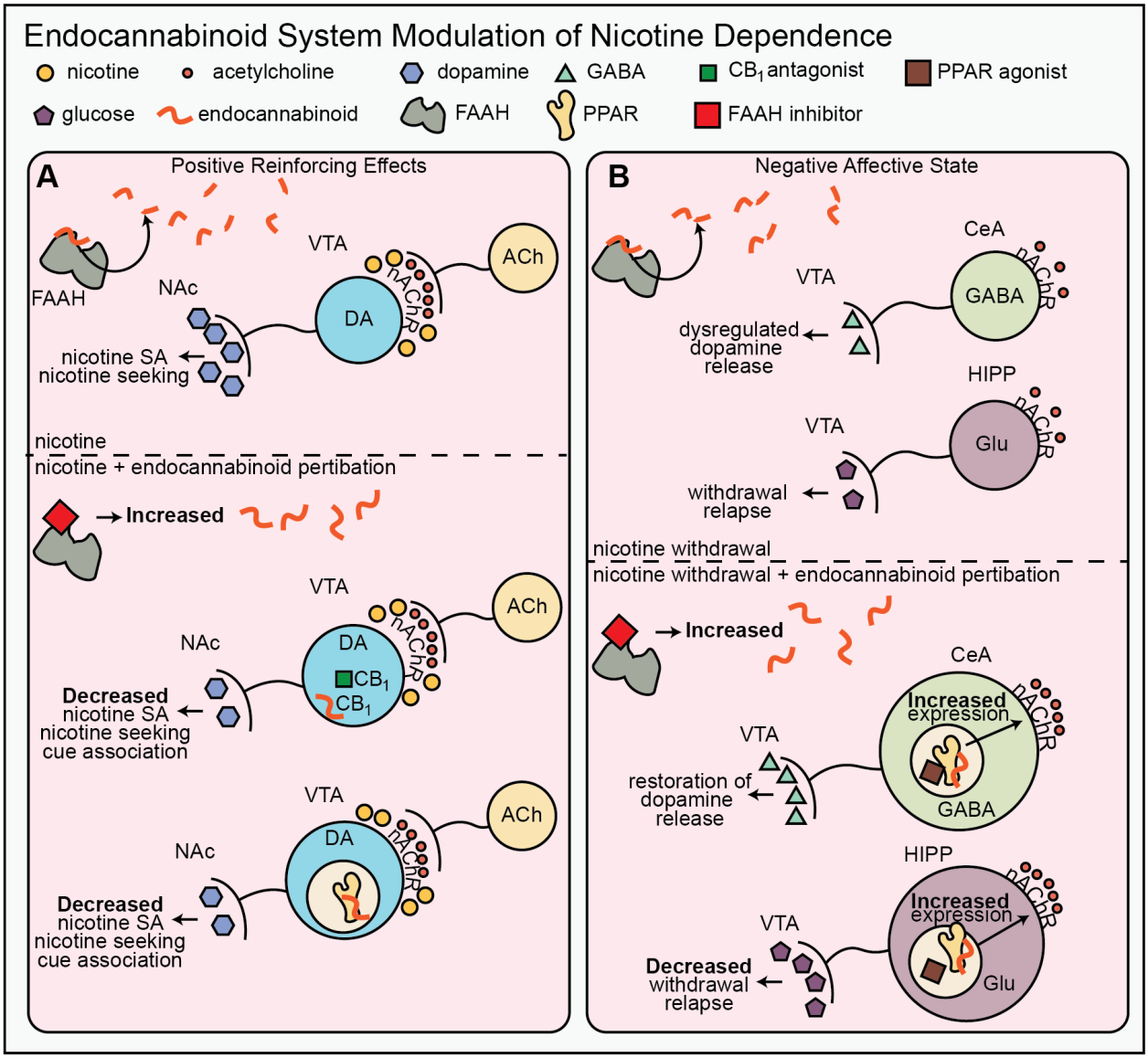

Figure 2. Pharmacodynamic modulation of nicotine dependence by the endocannabinoid system. A) Nicotine activates nicotinic acetylcholine receptors (nAChR) on dopaminergic (DA) neurons in the ventral tegmental area (VTA) projecting to the nucleus accumbens (NAc), this pathway is associated with increased nicotine seeking and self-administration (SA) in animal models. The enzyme, fatty acid amide hydrolase (FAAH) metabolizes endocannabinoids, such as anandamide. FAAH inhibitors increase the concentration of endocannabinoids, which act as inhibitors of the cannabinoid 1 (CB_1) receptor in the VTA, decreasing dopamine release. This results in decreased nicotine self-administration, nicotine seeking, and cue-association behaviors in animal models. Endocannabinoids also act on the transcription regulating peroxisome proliferator-activated receptors (PPARs) in the VTA, leading to downstream regulation of dopamine in the NAc; B). nAChR expression is dysregulated during nicotine withdrawal, decreasing dopamine release mediated by GABAergic and glutamatergic neurons projecting to the VTA, which results in a negative affective state that contributes to relapse and withdrawal behaviors in animal models. Endocannabinoids act on PPARs in GABAergic and glutamatergic

neurons in the CeA and HIPP, respectively, resulting in the upregulation of dopamine in the VTA via a pathway involving increased nAChR expression. This is accompanied with decreased withdrawal and relapse behaviors in animal models.

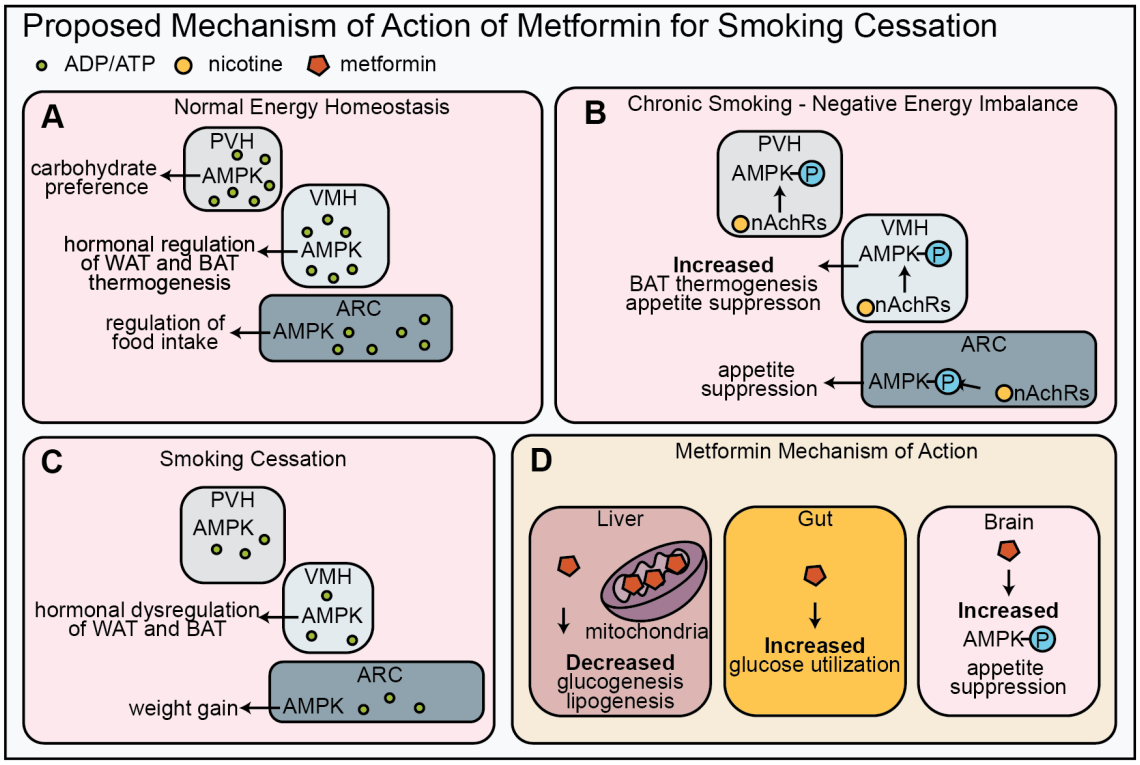

Figure 3. Proposed mechanism of action of metformin for smoking cessation. A) Adenosine monophosphate (AMP) regulates AMP-activated protein kinase (AMPK) via the rate of its phosphorylation to adenosine diphosphate or adenosine triphosphate (ADP/ATP). AMPK activates cellular glucose and fatty acid uptake and oxidation throughout the body to maintain energy homeostasis. In the hypothalamus, carbohydrate preference is regulated by the paraventricular (PVH), white adipose tissue (WAT) and brown adipose tissue (BAT) thermogenesis is regulated by the ventromedial (VMH), and regulation of food intake is regulated by the arcuate (ARC). Nicotine use causes an increase in phosphorylated AMPK (AMPK-P), likely mediated by nicotinic acetylcholine receptors (nAChRs), causing a state of negative energy imbalance. Increased AMPK-P in the VMH is associated with increased thermogenesis in BAT cell cultures and appetite suppression in animal models. Increased AMPK-P in the ARC is associated with appetite suppression in animal models. These effects likely contribute to nicotine-induced weight loss; C) During smoking cessation, there is dysregulation of AMPK resulting in a positive energy imbalance and weight gain; D) The mechanism of action of metformin is not entirely understood, however it is thought to act by indirectly increasing AMPK phosphorylation to regulate blood glucose levels. Metformin is readily absorbed into hepatic mitochondria, decreasing glucogenesis and lipogenesis. In the gut, metformin X to increase glucose utilization. In the brain, metformin was found to have neuroprotective effects in animal models of smoking. Metformin has been found to increase hypothalamic AMPK phosphorylation, perhaps contributing to appetite suppression and weight loss. Metformin may help return the body to homeostasis during the cessation and withdrawal period due to its similar effects on AMPK as nicotine.

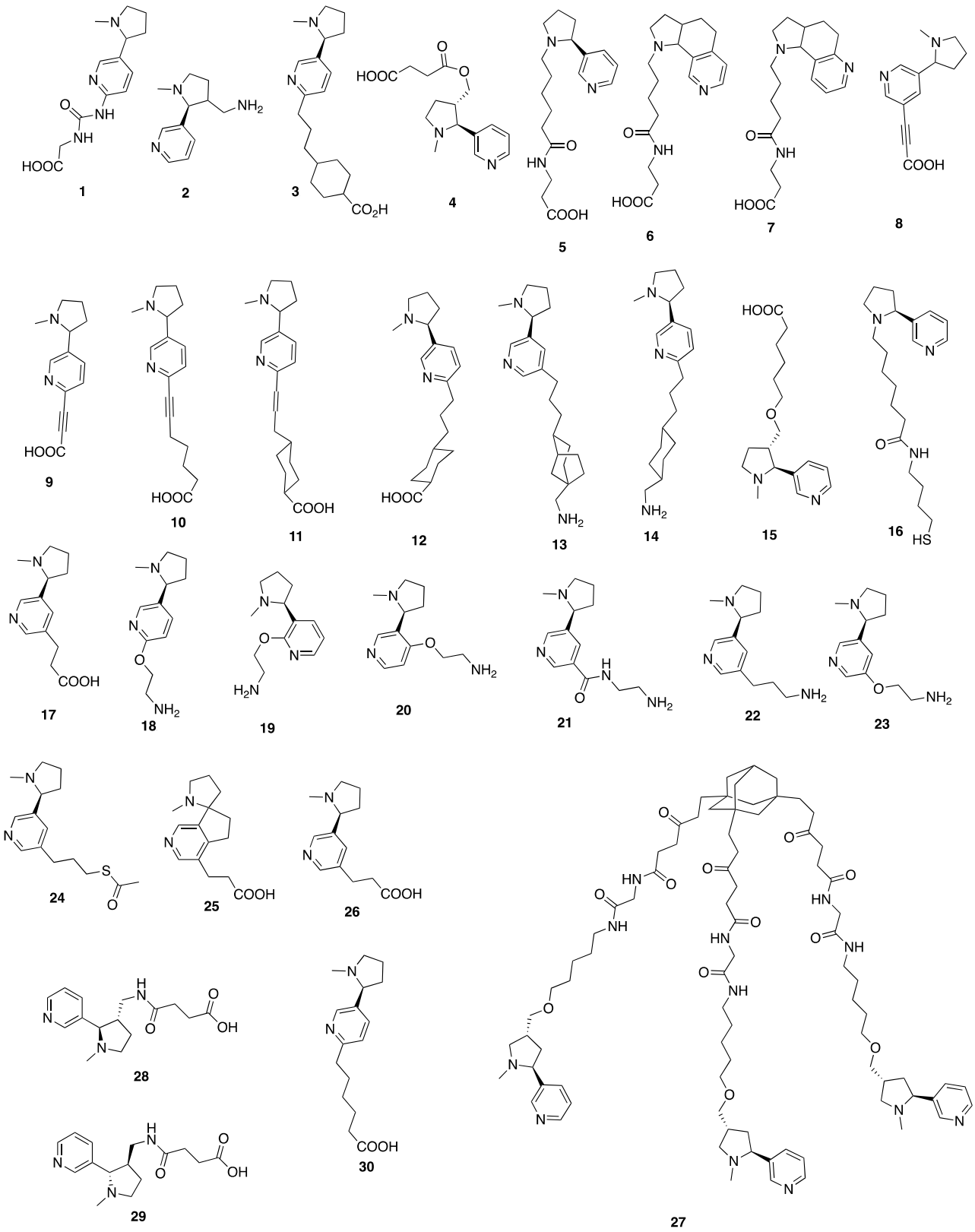

Figure 2. Nicotine hapten design. See Table 1 for corresponding studies.

Table 1. Nicotine hapten design.

Hapten	**Structure # (presented in Fig. 1)**	***Carrier***	***Adjuvant***	***Dose***	***Strain/Species***	***Sex, n***	***Nicotine* affinity (K_d)**	**Reference**
6-(carboxymethylureido)-(±)-nicotine	**1**	KLH	Complete Freund's adjuvant	25 μg (s.c.)	Holtzmann rats	Male, *n* = 8	1.5×10^{-6} M	(Hieda, Keyler et al. 1997)
trans-3'-aminomethylnicotine	**2**	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Sprague-Dawley rats	Male, *n* = 5	$2.6 \pm 0.6 \times 10^{-7}$ M	(Pentel, Malin et al. 2000)
IP18	**3**	KLH	Incomplete Freund's adjuvant	100 μg (s.c.)	Wistar rats	Male, *n* = 5	8.4×10^{-6} M*	(Lindblom, de Villiers et al. 2002)
trans-3'-succinylmethylnicotine	**4**	rCTB	Alum	100 μg (s.c.)	Balb/c mice	Female, *n* = 5	1.8×10^{-7} M	(Cerny, Levy et al. 2002)
NIC	**5**	KLH	Sigma adjuvant system	100 μg (s.c.)	129GIX+ mice	n/a	$1.7 \pm 0.2 \times 10^{-6}$ M	(Meijler, Matsushita et al. 2003)
CNA	**6**	KLH	Sigma adjuvant system	100 μg (s.c.)	129GIX+ mice	n/a	$1.0 \pm 0.1 \times 10^{-6}$ M	(Meijler, Matsushita et al. 2003)
CNI	**7**	KLH	Sigma adjuvant system	100 μg (s.c.)	129GIX+ mice	n/a	$0.6 \pm 0.1 \times 10^{-6}$ M	(Meijler, Matsushita et al. 2003)
GK56	**8**	KLH	Complete Freund's adjuvant	100 μg (i.p.)	Wistar rats	Male, *n* = 6	$0.850 \pm 0.382 \times 10^{-3}$ M	(de Villiers, Lindblom et al. 2010)
GK60	**9**	KLH	Complete Freund's adjuvant	100 μg (i.p.)	Wistar rats	Male, *n* = 6	$0.615 \pm 0.173 \times 10^{-3}$ M	(de Villiers, Lindblom et al. 2010)
GK81	**10**	KLH	Complete Freund's adjuvant	90 μg (i.p.)	Wistar rats	Male, *n* = 6	$0.165 \pm 0.076 \times 10^{-3}$ M	(de Villiers, Lindblom et al. 2010)
GK83	**11**	KLH	Complete Freund's adjuvant	100 μg (i.p.)	Wistar rats	Male, *n* = 12	$0.273 \pm 0.092 \times 10^{-3}$ M	(de Villiers, Lindblom et al. 2010)
IP31	**12**	KLH	Complete Freund's adjuvant	100 μg (i.p.)	Wistar rats	Male, *n* = 12	$0.014 \pm 0.007 \times 10^{-3}$ M	(de Villiers, Lindblom et al. 2010)
SG62	**13**	KLH	Complete Freund's adjuvant	100 μg (i.p.)	Wistar rats	Male, *n* = 12	$0.144 \pm 0.081 \times 10^{-3}$ M	(de Villiers, Lindblom et al. 2010)
IB87	**14**	KLH	Complete Freund's adjuvant	100 μg (i.p.)	Wistar rats	Male, *n* = 12	$0.020 \pm 0.009 \times 10^{-3}$ M	(de Villiers, Lindblom et al. 2010)
AM1	**15**	TT	AS-03 adjuvant	100 μg (s.c.)	129GIX+ mice	nr, *n* = 4	$14.63 \pm 2.19 \times 10^{-6}$ M	(Moreno, Azar et al. 2010)
1'-SNic	**16**	KLH	Complete Freund's adjuvant	25 μg (i.p.)	Rats	nr, *n* = 3	12×10^{-9} M	(Pravetoni, Keyler et al. 2012)
(S)-3-(5-(1-methylpyrrolidin-2-yl)pyridin-3-yl)propanoic acid	**17**	DT	Alum	25 μg (i.m.)	BALB/c mice	Female, *n* = 10	$19.2 \pm 3.2 \times 10^{-6}$ M**	(Pryde, Jones et al. 2013)

(S)-2-(5-(1-methylpyrrolidin-2-yl)pyridin-2-yloxy)ethanamine	**18**	DT	Alum	25 μg (i.m.)	BALB/c mice	Female, *n* = 10	$8.7 \pm 0.7 \times 10^{-6}$ M**	(Pryde, Jones et al. 2013)
(S)-2-(3-(1-methylpyrrolidin-2-yl)pyridin-2-yloxy)ethanamine	**19**	DT	Alum	25 μg (i.m.)	BALB/c mice	Female, *n* = 10	$15.4 \pm 1.6 \times 10^{-6}$ M**	(Pryde, Jones et al. 2013)
(S)-2-(3-(1-methylpyrrolidin-2-yl)pyridin-4-yloxy)ethanamine	**20**	DT	Alum	25 μg (i.m.)	BALB/c mice	Female, *n* = 10	Antibody titers were too low to determine affinity	(Pryde, Jones et al. 2013)
(S)-N-(2-aminoethyl)-5-(1-methylpyrrolidin-2-yl)nicotinamide	**21**	DT	Alum	25 μg (i.m.)	BALB/c mice	Female, *n* = 10	$15.2 \pm 3.6 \times 10^{-6}$ M**	(Pryde, Jones et al. 2013)
(S)-3-(5-(1-methylpyrrolidin-2-yl)pyridin-3-yl)propan-1-amine	**22**	DT	Alum	25 μg (i.m.)	BALB/c mice	Female, *n* = 10	$18.7 \pm 1.8 \times 10^{-6}$ M**	(Pryde, Jones et al. 2013)
(S)-2-(5-(1-methylpyrrolidin-2-yl)pyridin-3-yloxy)ethanamine	**23**	DT	Alum	25 μg (i.m.)	BALB/c mice	Female, *n* = 10	$20.9 \pm 8.0 \times 10^{-6}$ M**	(Pryde, Jones et al. 2013)
(S)-S-3-(5-(1-methylpyrrolidin-2-yl)pyridin-3-yl)propyl ethanethioate	**24**	DT	Alum	25 μg (i.m.)	BALB/c mice	Female, *n* = 10	$17.4 \pm 5.9 \times 10^{-6}$ M**	(Pryde, Jones et al. 2013)
3-(1'-methyl-5,6-dihydrospiro[cyclopenta[c]pyridine-7,2'-pyrrolidine]-4-yl)propanoic acid	**25**	DT	Alum	25 μg (i.m.)	BALB/c mice	Female, *n* = 10	Antibody titers were too low to determine affinity	(Pryde, Jones et al. 2013)
(S)-3-(5-(1-methylpiperidin-2-yl)pyridin-3-yl)propanoic acid	**26**	DT	Alum	25 μg (i.m.)	BALB/c mice	Female, *n* = 10	$163.4 \pm 11.6 \times 10^{-6}$ M**	(Pryde, Jones et al. 2013)
triAM1	**27**	OVA	Sigma adjuvant system	100 μg (i.p.)	BALB/c mice	Female, *n* = 6	$155 \pm 23.0 \times 10^{-9}$ M	(Collins and Janda 2014)
(+)-3'-succinylmethylnicotine	**28**	TT	CpG	100 μg (i.m.)	Wistar rats	Male, *n* = 10	$23.9 \pm 3.2 \times 10^{-9}$ M	(Lockner, Lively et al. 2015)
(-)-3'-succinylmethylnicotine	**29**	TT	CpG	100 μg (i.m.)	Wistar rats	Male, *n* = 10	$23.8 \pm 3.2 \times 10^{-9}$ M	(Lockner, Lively et al. 2015)
nicotine-6-hexanoic acid	**30**	TCC	—	2.5 μg (i.m.)	C57BL/6 mice	Female, *n* = 5	4.2×10^{-9} M	(Miller, Roque et al. 2014)
5-aminoethoxy-nicotine	**31**	CRM	CpG	10 μg (i.m.)	BALB/c mice	Female, *n* = 10	$3.87 \pm 0.27 \times 10^{-6}$ M - $19.8 \pm 11.64 \times 10^{-6}$ M**	(McCluskie, Thorn et al. 2015)

*Value reported is IC_{20}. **Value reported is IC_{50}. nr, not reported; KLH, keyhole limpet hymocyanin; rEPA, *Pseudomonas aeruginosa* exoprotein A; rCTB, recombinant cholera toxin B subunit; NIC, *N*-[1-oxo-6-[(2*S*)-2-(3-pyridinyl)-1-pyrrolidinyl]hexyl]-β-alanine; Alum, aluminium hydroxide gel; CNA, *N*-[6-(2,3,3*a*,4,5,9*b*-hexahydro-1*H*-pyrrolo[3,2-*h*]isoquinolin-1-yl)hexanoyl]-β-alanine; CNI, *N*-[6-(2,3,3*a*,4,5,9*b*-hexahydro-1*H*-pyrrolo[2,3-f]quinolin-1-yl)hexanoyl]-β-alanine; OVA, ovalbumin; TT, tetanus toxin; DT, diphtheria toxin; i.m., intramuscular; s.c., subcutaneous; i.p., intraperitoneal; TCC, trimeric coiled-coil peptide; CRM, cross-reactive material 197; CpG, type B oligodeoxynucleotide (5'-TCG TCG TTT TTC GGT GCT TTT-3').

Table 2. Studies of active vaccination against nicotine.

Hapten	Carrier	Adjuvant	Dose	Strain/Species	Sex, *n*	Brain nicotine C reduction	Measurements	Reference
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Sprague-Dawley rats	Male, *n* = 4	75%	Reduction of brain nicotine concentration measured after daily saline injection	(Hieda, Keyler et al. 2000)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Sprague-Dawley rats	Male, *n* = 5	60%	Reduction of brain nicotine concentration measured after daily nicotine injection	(Hieda, Keyler et al. 2000)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Sprague-Dawley rats	Male, *n* = 8	42%	Reduction of brain nicotine concentration measured after chronic saline infusion	(Hieda, Keyler et al. 2000)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Sprague-Dawley rats	Male, *n* = 8	29%	Reduction of brain nicotine concentration measured after chronic nicotine infusion	(Hieda, Keyler et al. 2000)
—	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Sprague-Dawley rats	Male, *n* = 10	—	90% incidence of seizures induced by nicotine after chronic saline infusion	(Tuncok, Hieda et al. 2001)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Sprague-Dawley rats	Male, *n* = 10	38%	70% incidence of seizures induced by nicotine after chronic nicotine infusion	(Tuncok, Hieda et al. 2001)
—	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Sprague-Dawley rats	Male, *n* = 10	—	80% incidence of seizures induced by nicotine after chronic saline infusion	(Tuncok, Hieda et al. 2001)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Sprague-Dawley rats	Male, *n* = 10	38%	40% incidence of seizures induced by nicotine after chronic nicotine infusion	(Tuncok, Hieda et al. 2001)
—	—	Incomplete Freund's adjuvant	100 μg (s.c.)	Wistar rats	Male, *n* = 13	n/a	~25% increase in dopamine release following nicotine injection	(de Villiers, Lindblom et al. 2002)
IP18	KLH	Incomplete Freund's adjuvant	100 μg (s.c.)	Wistar rats	Male, *n* = 5	n/a	~0% increase in dopamine release following nicotine injection	(de Villiers, Lindblom et al. 2002)
—	—	Incomplete Freund's adjuvant	100 μg (s.c.)	Wistar rats	Male, *n* = 5	n/a	17.8 ± 5 rewards/1 h in reinstatement session after nicotine priming	(Lindblom, de Villiers et al. 2002)
IP18	KLH	Incomplete Freund's adjuvant	100 μg (s.c.)	Wistar rats	Male, *n* = 5	n/a	5.6 ± 0.8 rewards/1 h in reinstatement session after nicotine priming	(Lindblom, de Villiers et al. 2002)
trans-3′-succinylmethylnicotine	rCTB	Alum	100 μg (s.c.)	Balb/c mice	Female, *n* = 5	90%	Titers were unaffected by chronic nicotine infusion	(Cerny, Levy et al. 2002)
trans-3′-succinylmethylnicotine	rCTB	—	30 μg (intranasal)	Balb/c mice	Female, *n* = 5	90%	No adjuvant required for titer response similar to s.c. vaccination	(Cerny, Levy et al. 2002)
trans-3′-*N*-succinylaminomethylnicotine	B cell epitope	Molecular adjuvant	200 μg (s.c./i.p.)	Sprague-Dawley rats	Male, *n* = 8	n/a	Most psychomotor effects of nicotine were prevented No effect on nicotine/saline discrimination	(Sanderson, Cheruku et al. 2003)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, *n* = 30	64%	Nicotine concentrations significantly reduced in muscle, testis, spleen, kidney, and heart but to a lesser extent than in brain	(Satoskar, Keyler et al. 2003)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, *n* = 6	n/a	Tissue distribution of antibody production: 200 μg/ml in serum, 0.4 μg/g in brain, 4.5 μg/g in muscle, 25.4 μg/g in fat, 13.7 μg/g in lung, 8.6 μg/g in heart	(Satoskar, Keyler et al. 2003)
—	KLH	Sigma Adjuvant System	250 μg (s.c.)	Wistar rats	Male, *n* = nr	n/a	16% decrease in ambulatory measure following 6th challenge with nicotine in locomotor assay	(Carrera, Ashley et al. 2004)
NIC	KLH	Sigma Adjuvant System	250 μg (s.c.)	Wistar rats	Male, *n* = 15	n/a	45% decrease in ambulatory measure following 6th challenge with nicotine in locomotor assay	(Carrera, Ashley et al. 2004)
—	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Sprague-Dawley rats	Female, *n* = 7	—	Breeding began 1 week after final booster. Nicotine (0.03 mg/kg) was delivered i.v. on gestational day 16-22	(Keyler, Dufek et al. 2005)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Sprague-Dawley rats	Female, *n* = 6	42%	Fetal brain nicotine c reduction was 42% after nicotine (0.03 mg/kg, i.v.) was delivered on gestational day 16-22	(Keyler, Dufek et al. 2005)
—	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, *n* = 10	—	Self-administration began 2 weeks after final booster	(LeSage, Keyler et al. 2006)

							79% reduction of nicotine self-administration during extinction	
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, $n = 14$	n.s.	38% reduction of mean number of nicotine infusions during fixed-ratio 3 self-administration 73% reduction of nicotine self-administration during extinction	(LeSage, Keyler et al. 2006)
—	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, $n = 9$	—	Nicotine sensitization began 10 days after final booster	(Roiko, Harris et al. 2008)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, $n = 15$	n.s.	No effect in locomotor assay after nicotine (0.3 mg/kg, s.c.) administration	(Roiko, Harris et al. 2008)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, $n = 15$	~40%	Concurrent with passive vaccination (Nic311, 30 mg/kg) Attenuation of nicotine (0.3 mg/kg, s.c.)-induced increase in locomotor activity	(Roiko, Harris et al. 2008)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, $n = 12$	~60%	94% serum nicotine bound after 0.03 mg/kg nicotine, i.v.	(Keyler, Roiko et al. 2008)
6-carboxymethlureido nicotine	KLH	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, $n = 12$	~40%	85% serum nicotine bound after 0.03 mg/kg nicotine, i.v.	(Keyler, Roiko et al. 2008)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, $n = 12$	~80	Bivalent vaccination approach 98% serum nicotine bound after 0.03 mg/kg nicotine, i.v.	(Keyler, Roiko et al. 2008)
6-carboxymethlureido nicotine	KLH	Complete Freund's adjuvant	25 μg (i.p.)					
—	TT	AS-03 adjuvant	100 μg (i.p.)	Wistar rats	Male, $n = 5$	n/a	50 mean lever presses during progressive ratio	(Moreno, Azar et al. 2010)
AM1	TT	AS-03 adjuvant	100 μg (i.p.)	Wistar rats	Male, $n = 5$	n/a	43% increase in nicotine intake during 1 h FR1 session 171 mean lever presses during progressive ratio	(Moreno, Azar et al. 2010)
—	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, $n = 12$	—	Brain nicotine c measured after 10 min and 2 h cigarette smoke exposure	(Pravetoni, Keyler et al. 2011)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, $n = 12$	90%, 35%	Brain nicotine c measured after 10 min and 2 h cigarette smoke exposure	(Pravetoni, Keyler et al. 2011)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, $n = 12$	55%	No change in nicotine-induced locomotor activity compared with non-immunized control	(Cornish, Harris et al. 2011)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, $n = 12$	78%	In combination with passive vaccination (Nic311) Significant decrease in nicotine-induced locomotor activity compared with non-immunized control	(Cornish, Harris et al. 2011)
—	KLH	Alum	100 μg (s.c.)	Wistar rats	Male, $n = 8$	n/a	Breakpoint of 14.6 ± 2.4 during progressive-ratio self-administration	(Moreno, Azar et al. 2012)
NIC	KLH	Alum	100 μg (s.c.)	Wistar rats	Male, $n = 8$	n/a	Nicotine self-administration and breakpoint during progressive-ratio self-administration was not significantly different than KLH control Nicotine-induced analgesia was not blocked by NIC-KLH	(Moreno, Azar et al. 2012)
CNI	KLH	Alum	100 μg (s.c.)	Wistar rats	Male, $n = 8$	n/a	Nicotine self-administration was not significantly different from KLH control Breakpoint of 39.1 ± 10 during progressive-ratio self-administration Nicotine-induced analgesia was blocked by CNI-KLH	(Moreno, Azar et al. 2012)
6-(carboxymethylureido)-(±)-nicotine	KLH	—	2.8 μg (i.m.)	BALB/c mice	Male, $n = 5$	37%	Brain nicotine c reduction measured 5 min after 0.01 mg/kg nicotine, i.v.	(Chen, Pravetoni et al. 2012)
6-(carboxymethylureido)-	KLH	—	2.8 μg (intradermal)	BALB/c mice	Male, $n = 5$	40%	Brain nicotine c reduction measured 5 min after 0.01 mg/kg nicotine, i.v.	(Chen, Pravetoni et al. 2012)

(±)-nicotine								
6-(carboxymethylureido)-(±)-nicotine	KLH	Alum	2.8 μg (i.m.)	BALB/c mice	Male, *n* = 5	58%	Brain nicotine c reduction measured 5 min after 0.01 mg/kg nicotine, i.v.	(Chen, Pravetoni et al. 2012)
6-(carboxymethylureido)-(±)-nicotine	KLH	Laser vaccine adjuvant	2.8 μg (intradermal)	BALB/c mice	Male, *n* = 5	34%	Brain nicotine c reduction measured 5 min after 0.01 mg/kg nicotine, i.v.	(Chen, Pravetoni et al. 2012)
6-(carboxymethylureido)-(±)-nicotine	KLH	Monophosphoryl lipid A	2.8 μg (intradermal)	BALB/c mice	Male, *n* = 5	48%	Brain nicotine c reduction measured 5 min after 0.01 mg/kg nicotine, i.v.	(Chen, Pravetoni et al. 2012)
6-(carboxymethylureido)-(±)-nicotine	KLH	Monophosphoryl lipid A, CpG	2.8 μg (intradermal)	BALB/c mice	Male, *n* = 5	62%	Brain nicotine c reduction measured 5 min after 0.01 mg/kg nicotine, i.v.	(Chen, Pravetoni et al. 2012)
AM1	dAd5	Adjuplex (Advanced BioAdjuvants, LLC)	4 μg (i.m.)	BALB/c mice	Female, *n* = 4	~33%	Pre Ad5 immunity did not alter vaccine performance	(De, Pagovich et al. 2013)
trans-3′-aminomethylnicotine	DT	Alum	10 μg (i.m.)	BALB/c mice	Female, *n* = 10	3%	Brain nicotine c reduction measured 5 min after 0.05 mg/kg nicotine, i.v.	(McCluskie, Pryde et al. 2013)
trans-3′-aminomethylnicotine	DT	CpG, Alum	10 μg (i.m.)	BALB/c mice	Female, *n* = 10	30%	Brain nicotine c reduction measured 5 min after 0.05 mg/kg nicotine, i.v.	(McCluskie, Pryde et al. 2013)
AM1	Hexon	Adjuplex (Advanced BioAdjuvants, LLC)	4 μg (i.m.)	C57BL/6 mice	Male, *n* = 8	53%	70-80% reduction of nicotine-induced locomotor activity	(Rosenberg, De et al. 2013)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, *n* = 12	~60%	50 μg KLH added to match carrier protein content in trivalent vaccine	(de Villiers, Cornish et al. 2013)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	25 μg (i.p.)	Holtzman rats	Male, *n* = 12	~80%	Trivalent vaccination strategy	(de Villiers, Cornish et al. 2013)
6-(carboxymethylureido)-(±)-nicotine	KLH	Complete Freund's adjuvant	25 μg (i.p.)					
1′-SNic	KLH	Complete Freund's adjuvant	25 μg (i.p.)					
trans-3′-aminomethylnicotine	rEPA	Alum	75 μg (s.c.)	Holtzman rats	Male, *n* = 12	20%	Monovalent vaccine dose matched to trivalent vaccine	(de Villiers, Cornish et al. 2013)
trans-3′-aminomethylnicotine	rEPA	Alum	25 μg (s.c.)	Holtzman rats	Male, *n* = 12	40%	Trivalent vaccination strategy	(de Villiers, Cornish et al. 2013)
6-(carboxymethylureido)-(±)-nicotine	KLH	Alum	25 μg (s.c.)					
1′-SNic	KLH	Alum	25 μg (s.c.)					
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	100 μg (i.p.)	Holtzman rats	Male, *n* = 12	n/a	Complete Freund's adjuvant did not significantly alter antibody titers or serum concentration compared with alum	(Cornish, de Villiers et al. 2013)
6-(carboxymethylureido)-(±)-nicotine	KLH	Complete Freund's adjuvant	100 μg (i.p.)	Holtzman rats	Male, *n* = 12	n/a	Produced higher antibody titers and serum antibody concentrations than 3'-AmNic-rEPA	(Cornish, de Villiers et al. 2013)
trans-3′-aminomethylnicotine	rEPA	Complete Freund's adjuvant	50 μg (i.p.)	Holtzman rats	Male, *n* = 12	n/a	Bivalent vaccination strategy Produced higher antibody titers and serum antibody concentrations than 3'-AmNic-rEPA but not 6-CMUNic-KLH	(Cornish, de Villiers et al. 2013)
6-(carboxymethylureido)-(±)-nicotine	KLH	Complete Freund's adjuvant	50 μg (i.p.)					
trans-3′-aminomethylnicotine	rEPA	Alum	100 μg (s.c.)	Holtzman rats	Male, *n* = 12	n/a	Alum did not significantly alter antibody titers or serum concentrations compared with complete Freund's adjuvant	(Cornish, de Villiers et al. 2013)
6-(carboxymethylureido)-(±)-nicotine	KLH	Alum	100 μg (s.c.)	Holtzman rats	Male, *n* = 12	n/a	Produced higher antibody titers and serum antibody concentrations than 3'-AmNic-rEPA	(Cornish, de Villiers et al. 2013)
trans-3′-aminomethylnicotine	rEPA	Alum	50 μg (s.c.)	Holtzman rats	Male, *n* = 12	n/a	Bivalent vaccination strategy	(Cornish, de Villiers et al. 2013)

6-(carboxymethylureido)-(±)-nicotine	KLH	Alum	50 μg (s.c.)	Produced higher antibody titers and serum antibody concentrations than 3'-AmNic-rEPA but not 6-CMUNic-KLH

n/a, not applicable; KLH, keyhole limpet hymocyanin; rEPA, *Pseudomonas aeruginosa* exoprotein A; rCTB, recombinant cholera toxin B subunit; NIC, *N*-[1-oxo-6-[(2*S*)-2-(3-pyridinyl)-1-pyrrolidinyl]hexyl]-β-alanine; CNA, *N*-[6-(2,3,3*a*,4,5,9*b*-hexahydro-1*H*-pyrrolo[3,2-h]isoquinolin-1-yl)hexanoyl]-β-alanine; CNI, *N*-[6-(2,3,3*a*,4,5,9*b*-hexahydro-1*H*-pyrrolo[2,3-f]quinolin-1-yl)hexanoyl]-β-alanine; Alum, aluminium hydroxide gel; i.v., intravenous; n.s., not significant; CpG, type B oligodeoxynucleotide (5'-TCG TCG TTT TTC GGT GCT TTT-3'); dAd5, disrupted adenovirus 5; DT, diptheria toxoid; Hexon, adenovirus capsid hexon; 1'-SNic, (2*S*)-*N*,*N'*-(disulfanediyldiethane-2,1-diyl)bis[4-(2-pyridin-3-ylpyrrolidin-1-yl)butanamide].

Table 3. Anti-nicotine antibody production

Antibody	Hapten	Carrier	Adjuvant	Dose	Strain/Species	Sex, n	Nicotine affinity	Measurement	Reference
Nic-IgG	*trans*-3'-aminomethylnicotine	rEPA	Complete Freund's adjuvant	100 μg	New Zealand white rabbits	nr	$1.0 \pm 0.2 \times 10^{8}$ M^{-1}	2.7% cotinine crossreactivity	(Pentel, Malin et al. 2000)
NIC9D9	NIC	KLH	nr	nr	Mice	nr	3×10^{-7} M	n/a	(Isomura, Wirsching et al. 2001)
TD1-10E8	TD1	KLH	nr	nr	Mice	nr	6.3×10^{-3} M	50% nicotine catalysis	(Dickerson, Yamamoto et al. 2004)

nr, not reported; n/a, not available; rEPA, regulatory protein A (*Escherichia coli*); NIC, *N*-[1-oxo-6-[(2*S*)-2-(3-pyridinyl)-1-pyrrolidinyl]hexyl]-β-alanine; KLH, keyhole limpet hymocyanin; TD1, 5-oxo-5-(2-(pyridin-3-yl)pyrrolidin-1-yl)pentanoic acid.

Table 4. Passive vaccination studies

Antibody	*Dose*	*Strain/Species*	*Sex, n*	*Brain* nicotine *C* reduction	*Measures*/behavior	Reference
control-IgG	50 mg, i.v.	Sprague-Dawley rats	Male, *n* = 6	—	n/a	(Pentel, Malin et al. 2000)
Nic-IgG	12.5 mg, i.v.	Sprague-Dawley rats	Male, *n* = 6	33%	n/a	(Pentel, Malin et al. 2000)
Nic-IgG	25 mg, i.v.	Sprague-Dawley rats	Male, *n* = 6	52%	n/a	(Pentel, Malin et al. 2000)
Nic-IgG	50 mg, i.v.	Sprague-Dawley rats	Male, *n* = 6	65%	n/a	(Pentel, Malin et al. 2000)
—	—	Sprague-Dawley rats	Male, *n* = 4	n/a	~30% increase in systolic blood pressure induced by nicotine	(Pentel, Malin et al. 2000)
Nic-IgG	50 mg, i.v.	Sprague-Dawley rats	Male, *n* = 4	n/a	~20% increase in systolic blood pressure induced by nicotine	(Pentel, Malin et al. 2000)
Nic-IgG	100 mg, i.v.	Sprague-Dawley rats	Male, *n* = 4	n/a	~15% increase in systolic blood pressure induced by nicotine	(Pentel, Malin et al. 2000)
Nic-IgG	150 mg, i.v.	Sprague-Dawley rats	Male, *n* = 4	n/a	~4% increase in systolic blood pressure induced by nicotine	(Pentel, Malin et al. 2000)
—	—	Sprague-Dawley rats	Male, *n* = 6	n/a	Increase from baseline of 70.7 ± 33.9 activity counts/5 min in locomotor assay after nicotine	(Pentel, Malin et al. 2000)
control-IgG	50 mg, i.v.	Sprague-Dawley rats	Male, *n* = 6	n/a	Increase from baseline of 71.0 ± 32.7 counts/ 5 min in locomotor assay after nicotine	(Pentel, Malin et al. 2000)
Nic-IgG	50 mg, i.v.	Sprague-Dawley rats	Male, *n* = 8	n/a	Decrease from baseline of 15.0 ± 24.1 counts/5 min in locomotor assay after nicotine	(Pentel, Malin et al. 2000)
NIC9D9	30 mg/kg, i.v.	Wistar rats	Male, *n* = 7	89.9%	Reduction of brain concentration after 0.28 mg/kg nicotine, s.c.	(Carrera, Ashley et al. 2004)
NIC9D9	30 mg/kg, i.v.	Wistar rats	Male, *n* = 7	57.8%	Reduction of brain concentration after 0.56 mg/kg nicotine, s.c.	(Carrera, Ashley et al. 2004)
saline	1.5 ml/kg, i.v.	Wistar rats	Male, *n* = nr	n/a	3.4% decrease in ambulatory measure following 6th challenge with nicotine in locomotor assay	(Carrera, Ashley et al. 2004)
NIC9D9	50 mg/kg, i.v.	Wistar rats	Male, *n* = 15	n/a	66.9% decrease in ambulatory measure following 6th challenge with nicotine in locomotor assay	(Carrera, Ashley et al. 2004)
control-IgG	21 mg, i.v.	Sprague-Dawley rats	Female, *n* = 7	—	IgG was delivered i.v. on gestational day 16-22, followed by nicotine (0.03 mg/kg, i.v.) 30 min later	(Keyler, Dufek et al. 2005)
Nic-IgG	7 mg, i.v.	Sprague-Dawley rats	Female, *n* = 7	66%	63% reduction of fetal brain nicotine c after 0.03 mg/kg nicotine, i.v.	(Keyler, Dufek et al. 2005)
Nic-IgG	21 mg, i.v.	Sprague-Dawley rats	Female, *n* = 7	82%	Fetal brain nicotine c was not significantly reduced after 0.03 mg/kg nicotine, i.v.	(Keyler, Dufek et al. 2005)
Nic311	30 mg/kg, i.v.	Holtzman rats	Male, *n* = 15	n.s.	No effect in locomotor assay after nicotine (0.3 mg/kg, s.c.) administration	(Roiko, Harris et al. 2008)
Nic311	30 mg/kg, i.v.	Holtzman rats	Male, *n* = 15	~40%	Concurrent with active vaccination (3'-AmNic-rEPA, 25 μg) Attenuation of nicotine (0.3 mg/kg, s.c.)-induced increase in locomotor activity	(Roiko, Harris et al. 2008)
IgG	36 mg/kg, i.v.	Holtzman rats	Male, *n* = 12	—	Brain nicotine c measured after 2 h cigarette smoke exposure	(Pravetoni, Keyler et al. 2011)
Nic311	36 mg/kg, i.v.	Holtzman rats	Male, *n* = 12	29%	Brain nicotine c measured after 2 h cigarette smoke exposure	(Pravetoni, Keyler et al. 2011)
Nic311	36 mg/kg, i.v.	Holtzman rats	Male, *n* = 12	15%	No change in nicotine-induced locomotor activity compared with non-immunized control	(Cornish, Harris et al. 2011)
Nic311	36 mg/kg, i.v.	Holtzman rats	Male, *n* = 12	78%	In combination with active vaccination (3'-AmNic-rEPA) Significant decrease in nicotine-induced locomotor activity compared with non-immunized control	(Cornish, Harris et al. 2011)

n/a, not available; nr, not reported; s.c., subcutaneous; n.s., non significant; 3'-AmNic-rEPA, *trans*-3'-aminomethylnicotine conjugated to regulatory protein A (*Escherichia coli*)

Table 5. Liposome vaccine studies.

Hapten	*Carrier*	*Adjuvant*	*Liposome* content	*Dose*	*Strain/ Species*	*Sex, n*	*Titers*	Reference
AM1 nicotine	KLH	Sigma Adjuvant System	DMPC, DMPG, cholesterol, lipid-maleimide	200 µl	BALB/c mice	nr, *n* = 5	20000-25000	(Lockner, Ho et al. 2013)
AM1 nicotine	KLH	n/a	DMPC, DMPG, cholesterol, lipid-maleimide	200 µl	BALB/c mice	nr, *n* = 5	10000-15000	(Lockner, Ho et al. 2013)
AM1 nicotine	KLH	Pam_3CAG	DMPC, DMPG, cholesterol, lipid-maleimide	200 µl	BALB/c mice	nr, *n* = 5	10000-15000	(Lockner, Ho et al. 2013)
AM1 nicotine	KLH	MPLA	DMPC, DMPG, cholesterol, lipid-maleimide	200 µl	BALB/c mice	nr, *n* = 5	25000-30000	(Lockner, Ho et al. 2013)
AM1 nicotine	KLH	MPLA, Pam_3CAG	DMPC, DMPG, cholesterol, lipid-maleimide	200 µl	BALB/c mice	nr, *n* = 5	< 30000	(Lockner, Ho et al. 2013)
rac-trans-3′-hydroxymethylnicotine hemisuccinate	BSA	Alum	DOTAP, DSPE- PEG(2000)-maleimide	50 µg	BALB/c mice	Female, *n* = 8	5000-10000	(Hu, Zheng et al. 2014)
rac-trans-3′-hydroxymethylnicotine hemisuccinate	BSA	n/a	DOTAP, DSPE- PEG(2000)-maleimide	50 µg	BALB/c mice	Female, *n* = 8	~10000	(Hu, Zheng et al. 2014)
rac-trans-3′-hydroxymethylnicotine hemisuccinate	BSA	Alum	n/a	50 µg	BALB/c mice	Female, *n* = 8	< 10000	(Hu, Zheng et al. 2014)
rac-trans-3′-hydroxymethylnicotine hemisuccinate	BSA	Alum	n/a	50 µg	BALB/c mice	Female, *n* = 8	< 10000	(Zheng, Hu et al. 2015)
rac-trans-3′-hydroxymethylnicotine hemisuccinate	BSA	n/a	DOTAP, DSPE- PEG(2000)-maleimide, SWNH	50 µg	BALB/c mice	Female, *n* = 8	45000-50000	(Zheng, Hu et al. 2015)
rac-trans-3′-hydroxymethylnicotine hemisuccinate	BSA	Alum	DOTAP, DSPE- PEG(2000)-maleimide, SWNH	50 µg	BALB/c mice	Female, *n* = 8	55000-60000	(Zheng, Hu et al. 2015)
rac-trans-3′-hydroxymethylnicotine hemisuccinate	BSA	n/a	DOTAP, DSPE- PEG(2000)-maleimide	50 µg	BALB/c mice	Female, *n* = 8	10000-15000	(Zheng, Hu et al. 2015)
rac-trans-3′-hydroxymethylnicotine hemisuccinate	BSA	Alum	DOTAP, DSPE- PEG(2000)-maleimide	50 µg	BALB/c mice	Female, *n* = 8	10000-15000	(Zheng, Hu et al. 2015)
Nic-βGalCer	n/a	αGalCer	cholesterol, DSPC	3 µg	BALB/c mice	Female, *n* = 5	60000-70000	(Chen, Zhang et al. 2019)
Nic-βGalCer	n/a	Pam_3CSK_4	Cholesterol, DSPC	3 µg	BALB/c mice	Female, *n* = 5	30000-40000	(Chen, Zhang et al. 2019)
Nic-βGalCer	n/a	Freund's adjuvant	Cholesterol, DSPC	3 µg	BALB/c mice	Female, *n* = 5	< 10000	(Chen, Zhang et al. 2019)
Nic-βGalCer	n/a	n/a	Cholesterol, DSPC	3 µg	BALB/c mice	Female, *n* = 5	~10000	(Chen, Zhang et al. 2019)
Nic-βGalCer	n/a	αGalCer, Pam_3CSK_4	Cholesterol, DSPC	3 µg	BALB/c mice	Female, *n* = 5	20000-30000	(Chen, Zhang et al. 2019)
Nic-αGalCer	n/a	n/a	Cholesterol, DSPC	0.6 µg	BALB/c mice	Female, *n* = 5	10000-20000	(Chen, Zhang et al. 2019)
Nic-αGalCer	n/a	n/a	Cholesterol, DSPC	3 µg	BALB/c mice	Female, *n* = 5	10000-20000	(Chen, Zhang et al. 2019)
Nic-αGalCer	n/a	n/a	Cholesterol, DSPC	15 µg	BALB/c mice	Female, *n* = 5	10000-20000	(Chen, Zhang et al. 2019)

All vaccinations delivered subcutaneously. n/a, not applicable; nr, not reported; Alum, aluminium hydroxide gel; BSA, bovine serum albumin; KLH, keyhole limpet hymocyanin; Pam_3CAG, *S*-[2,3-bis(palmitoyloxy)-(2*RS*)-propyl]-*N*-palmitoyl-(*R*)-cysteinyl-alanyl-glycine (Toll-like receptor agonist); MPLA, monophosphoryl lipid A (Toll-like receptor agonist); DMPC, 1,2-dimyristoyl-*SN*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*SN*-glycero-3-phospho-(1′-*rac*-glycerol); DOTAP, *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methyl sulfate; DSPE-PEG(2000)-maleimide, 1,2-distearoyl-*SN*-glycero-3-phosphoethanolamine-*N*-[maleimide(polyethylene glycol)-2000]; SWNH, single-walled nanohorns (negatively charged); DSPC, 1,2-distearoyl-*SN*-glycero-3-phosphocholine; Pam_3CSK_4, *N*-palmitoyl-*S*-[2,3-bis(palmitoyloxy)-(2*RS*)- propyl]-[*R*]-cysteinyl-[*S*]-seryl-[*S*]-lysyl-[*S*]-lysyl-[*S*]-lysyl-[*S*]-lysine (Toll-like receptor agonist).

Table 6. Nicotine vaccination in nonhuman primates.

Vaccine	*Dose*	*Species*	*Sex, n*	*Measures*	Reference
5-aminoethoxy-nicotine-CRM, CpG	100 μg, i.m.	Cynomolgus monkeys	nr, $n = 7$	Up to ~34% reduction of nicotine in brain	(McCluskie, Thorn et al. 2015)
SEL-068 nanoparticle vaccine	2 mg, s.c.	Squirrel monkeys	Male, $n = 8$	Vaccination decreased discrimination for nicotine (i.v.) under fixed-ratio 10 schedule of stimulus termination	(Desai and Bergman 2015)
NIC7-CRM, Alum	150 μg, i.m.	Cynomolgus monkeys	Male and female, $n = 7$-14	Sera $IC_{50} = 0.63 \pm 0.15 \times 10^{-6}$ M 10% reduction of brain nicotine levels	(McCluskie, Thorn et al. 2015)
NIC7-CRM, CpG, Alum	150 μg, i.m.	Cynomolgus monkeys	Male and female, $n = 7$-14	Sera $IC_{50} = 0.10 \pm 0.02 \times 10^{-6}$ M ~80% reduction of brain nicotine levels	(McCluskie, Thorn et al. 2015)
NIC-VLP, Alum	1000 μg, i.m.	Cynomolgus monkeys	Male and female, $n = 7$-14	Sera $IC_{50} = 29.0 \pm 18.45 \times 10^{-6}$ M ~10% reduction of brain nicotine levels	(McCluskie, Thorn et al. 2015)

nr, not reported; CRM, cross-reactive material 197; CpG, type B oligodeoxynucleotide (5'-TCG TCG TTT TTC GGT GCT TTT-3'); i.m., intramuscular; s.c., subcutaneous; i.v., intravenous; Alum, aluminium hydroxide gel.

Table 7. Nicotine-degrading enzymes.

Enzyme	*Dose*	*Strain/ Species*	*Sex, n*	*Measurements*	Reference
NicA2-ABD	10 mg/kg, i.p.	Wistar rats	Male, $n = 8$	NicA2 reduced responding during footshock-, nicotine-, and stress(yohimbine)-induced reinstatement	(Kallupi, Xue et al. 2018)
NicA2 WT	20-70 mg/kg, i.v.	Sprague-Dawley rats	Female, $n = 8$	NicA2 reduced nicotine-induced discrimination and reinforcement	(Pentel, Raleigh et al. 2018)
NicA2-ABD	10 mg/kg, i.p.	Wistar rats	Male and Female, $n = 8$	NicA2 reduced brain nicotine concentration, somatic signs of nicotine withdrawal, nicotine withdrawal-induced hyperalgesia, and irritability during withdrawal	(Xue, Kallupi et al. 2018)
NicA2-PEG1, -PEG2, PEG3	5 mg/kg, s.c.	HLA DR4 mice	Male and female, $n = 6$	PEGylation of NicA2 decreased immunogenicity ≥ 10-fold, measured by NicA2-specific antibody titers	(Thisted, Biesova et al. 2019)

ABD, albumin binding domain; WT, wildtype; i.p., intraperitoneal; s.c., subcutaneous; i.v., intravenous.

Table 8. Slowing nicotine metabolism.

Inhibitor	*Target*	*Dose*	*Strain/ species*	*Sex, n*	*Measurements*	Reference
methoxsalen	CYP2A5	—	—	—	Methoxsalen inhibited nicotine metabolism *in vitro* with a K_i of $0.32 \pm 0.03 \times 10^{-6}$ M	(Damaj, Siu et al. 2007)
methoxsalen	CYP2A5	2.5 mg/kg, s.c.	ICR mice	Male, *n* = 4-6	Methoxsalen increased the AUC and half-life of plasma nicotine *in vivo*	(Damaj, Siu et al. 2007)
methoxsalen	CYP2A5	2.5 mg/kg, s.c.	ICR mice	Male, *n* = 8-12	Methoxsalen prolonged nicotine-induced antinociception and hypothermia	(Damaj, Siu et al. 2007)
methoxsalen	CYP2A5	15 mg/kg, i.p.	ICR mice	Male, *n* = 8-12	Methoxsalen increased the Cmax, AUC, and half-life of plasma nicotine *in vivo* (15 mg/kg, p.o.)	(Alsharari, Siu et al. 2014)
methoxsalen	CYP2A5	15 mg/kg, i.p.	ICR mice	Male, *n* = 8-12	Methoxsalen pretreatment potentiated nicotine-induced (15 mg/kg, p.o.) antinociception and hypothermia	(Alsharari, Siu et al. 2014)
—	—	—	ICR mice	Male, *n* = 8	a low dose of nicotine (0.1 mg/kg, s.c.) was unable to produce conditioned place preference or precipitated withdrawal, but did reduce open arms time and paw withdrawal latency	(Bagdas, Muldoon et al. 2014)
methoxsalen	CYP2A5	15, 30, and 45 mg/kg, i.p.	ICR mice	Male, *n* = 8	Conditioned place preference for a low dose of nicotine (0.1 mg/kg, s.c.) was induced after pretreatment with methoxsalen at 15 and 30 mg/kg, but not 45 mg/kg, this effect of methoxsalen corresponded with dose-dependent increase in plasma nicotine levels	(Bagdas, Muldoon et al. 2014)
methoxsalen	CYP2A5	15 mg/kg, i.p.	ICR mice	Male, *n* = 8	Pretreatment with methoxsalen resulted in increased somatic signs of withdrawal, further decrease in open arms time, and increased hyperalgesia precipitated by mecamylamine after repeated administration of low-dose nicotine	(Bagdas, Muldoon et al. 2014)
methoxsalen	CYP2A5	30 mg/kg, i.p.	ICR mice	Male, *n* = 8	Combination pretreatment with methoxsalen and nicotine (0.05 mg/kg, s.c.) completely reversed spontaneous somatic signs of withdrawal after chronic nicotine infusion (36 mg/kg/day, 7 days)	(Bagdas, Muldoon et al. 2014)
xanthotoxin	CYP2A5	15 mg/kg, i.p.	Swiss albino mice	Male, *n* = 8-10	Xanthotoxin prolonged nicotine's depressive effects and the duration of nicotine-induced acquisition and consolidation of memory	(Budzynska, Skalicka-Wozniak et al. 2016)
bergapten	CYP2A5	25 mg/kg, i.p.	Swiss albino mice	Male, *n* = 8-10	Bergapten prolonged nicotine's depressive effects and nicotine-induced acquisition of memory, but not consolidation	(Budzynska, Skalicka-Wozniak et al. 2016)
umbelliferone	CYP2A5	25 mg/kg, i.p.	Swiss albino mice	Male, *n* = 8-10	Umbelliferone prolonged nicotine's depressive effects and nicotine-induced acquisition of memory, but not consolidation	(Budzynska, Skalicka-Wozniak et al. 2016)
DLCI-1	CYP2A5	25, 50, and 75 mg/kg, p.o.	C57BL/6J mice	Male and female, *n* = 13	DLCI-1 (25 and 50 mg/kg) reduced intravenous nicotine self-administration, and was found to be more effective than bupropion (1 mg/kg)	(Chen, Fowler et al. 2020)
DLCI-1	CYP2A5	50 mg/kg, p.o.	C57BL/6J mice	Male, *n* = 5	Despite reduced intravenous nicotine self-administration after treatment with DLCI-1, mice had similar locomotor activity to saline treated mice after nicotine self-administration	(Chen, Fowler et al. 2020)

i.p., intraperitoneal; s.c., subcutaneous; p.o., per oral; CYP2A5, mouse ortholog of human cytochrome P450 CYP2A6; AUC, area under curve; Cmax, concentration max

Table 9. Cannabinoid studies.

Cannabinoid	*Mechanism of* action	*Dose*	*Strain*/species	*Sex, n*	*Measurements*	Reference
SR141716	Central CB_1 receptor antagonist	0.1 mg/kg, i.p.	Wistar rats	Male, $n = 6$	Reduced nicotine self-administration Prevented of substitution of nicotine for D-amphetamine	(Cohen, Perrault et al. 2002)
SR141716	Central CB_1 receptor antagonist	3 mg/kg, i.p.	Sprague-Dawley rats	Male $n = 6$	Prevented nicotine-induced increase in extracellular dopamine levels in nucleus accumbens shell in freely moving rats	(Cohen, Perrault et al. 2002)
rimonabant	CB_1 receptor inverse agonist	1, 3 mg/kg, i.p.	Sprague-Dawley rats	Male $n = 13$	Prevented nicotine-induced conditioned place preference	(Le Foll and Goldberg 2004)
—	—	—	Lister hooded rats	Male, $n = 12$	No reduction of nicotine self-administration after 3 days of vehicle pretreatment	(Shoaib 2008)
AM251	Selective CB_1 receptor antagonist	1 mg/kg, i.p.	Lister hooded rats	Male, $n = 12$	~60% reduction of nicotine self-administration after 3 days of AM251 pretreatment	(Shoaib 2008)
AM251	Selective CB_1 receptor antagonist	3 mg/kg, i.p.	Lister hooded rats	Male, $n = 12$	~90% reduction of nicotine self-administration after 3 days of AM251 pretreatment	(Shoaib 2008)
AM251	Selective CB_1 receptor antagonist	10 mg/kg, i.p.	Lister hooded rats	Male, $n = 12$	~98% reduction of nicotine self-administration after 3 days of AM251 pretreatment	(Shoaib 2008)
—	—	—	Lister hooded rats	Male, $n = 18$	No reduction of reinstatement responses after vehicle pretreatment	(Shoaib 2008)
AM251	Selective CB_1 receptor antagonist	1 mg/kg, i.p.	Lister hooded rats	Male, $n = 18$	~25% reduction of reinstatement responses after vehicle pretreatment	(Shoaib 2008)
AM251	Selective CB_1 receptor antagonist	3 mg/kg, i.p.	Lister hooded rats	Male, $n = 18$	~50% reduction of reinstatement responses after vehicle pretreatment	(Shoaib 2008)
AM251	Selective CB_1 receptor antagonist	10 mg/kg, i.p.	Lister hooded rats	Male, $n = 18$	~95% reduction of reinstatement responses after vehicle pretreatment	(Shoaib 2008)
rimonabant	CB_1 receptor inverse agonist	0.3, 1, and 3 mg/kg, i.p.	CB1 knockout mice	Male and female, $n =$ 6-8	Nicotine (0.5 or 0.7 mg/kg) did not produce conditioned place preference Rimonabant had no effect	(Merritt, Martin et al. 2008)
rimonabant	CB_1 receptor inverse agonist	0.3, 1, and 3 mg/kg, i.p.	C57BL/6J mice	Male and female, $n =$ 6-8	Nicotine (0.5 or 0.7 mg/kg) produced conditioned place preference, which was dose-dependently blocked by rimonabant	(Merritt, Martin et al. 2008)
rimonabant	CB_1 receptor inverse agonist	3 mg/kg, i.p.	FAAH knockout mice	Male and female, $n =$ 10-12	Nicotine reward at 0.1 mg/kg was increased during conditioned place preference, which was blocked by rimonabant	(Merritt, Martin et al. 2008)
rimonabant	CB_1 receptor inverse agonist	3 mg/kg, i.p.	C57BL/6J mice	Male and female, $n =$ 10-12	Nicotine reward at 0.1 mg/kg was ineffective during conditioned place preference	(Merritt, Martin et al. 2008)
URB597	FAAH inhibitor	0.3, 3, 5, and 10 mg/kg, i.p.	FAAH knockout mice	Male and female, $n =$ 10-12	The potency of nicotine reward at 0.1 mg/kg was increased by URB597 in U-shaped dose-dependent manner, with high doses (5 and 10 mg/kg) not having an effect	(Merritt, Martin et al. 2008)
URB597	FAAH inhibitor	0.3, 3, 5, and 10 mg/kg, i.p.	C57BL/6J mice	Male and female, $n =$ 10-12	No change in locomotor activity after URB597 administration	(Merritt, Martin et al. 2008)
rimonabant	CB_1 receptor inverse agonist	3 mg/kg, i.p.	C57BL/6J mice	Male and female, $n =$ 6-8	Mecamylamine (1 mg/kg, s.c.) but not rimonabant blocked acute nicotine-induced antinociception, measured in tail-flick and hot-plate tests	(Merritt, Martin et al. 2008)
—	—	—	CB1 knockout mice	Male and female, $n =$ 8-10	CB_1 receptor knockout mice exhibited similar extent of somatic signs of withdrawal to wildtype mice	(Merritt, Martin et al. 2008)
rimonabant	CB_1 receptor inverse agonist	3 mg/kg, i.p.	C57BL/6J mice	Male and female, $n =$ 8-10	Somatic signs of withdrawal were decreased by rimonabant	(Merritt, Martin et al. 2008)
—	—	—	FAAH knockout mice	Male and female, $n =$ 10-15	FAAH knockout mice exhibited two-fold increase in somatic signs of withdrawal compared with wildtype mice	(Merritt, Martin et al. 2008)

URB597	FAAH inhibitor	3, 5, and 10 mg/kg, i.p.	C57BL/6J mice	Male and female, n = 10-15	FAAH inhibitor dose-dependently increased number of somatic signs of withdrawal	(Merritt, Martin et al. 2008)
—	—	—	FAAH knockout mice	Male and female, n = 10-15	FAAH knockout mice exhibited an increase in conditioned place aversion compared with wildtype mice	(Merritt, Martin et al. 2008)
URB597	FAAH inhibitor	10 mg/kg, i.p.	C57BL/6J mice	Male and female, n = 10-15	FAAH inhibitor increased conditioned place aversion during nicotine withdrawal	(Merritt, Martin et al. 2008)
URB597	FAAH inhibitor	0.1 mg/kg, i.v.	Sprague-Dawley rats	Male, n = 14-48	URB597 prevented nicotine's effects on dopaminergic neurons in the ventral tegmental area	(Melis, Pillolla et al. 2008)
rimonabant	CB_1 receptor inverse agonist	0.5 mg/kg, i.v.	Sprague-Dawley rats	Male, n = 9	Blockade of CB_1 by rimonabant reduced the ability of URB597 to block nicotine-induced stimulation of dopaminergic neuron discharge rate	(Melis, Pillolla et al. 2008)
MK886	PPARα antagonist	3 mg/kg, i.p.	Sprague-Dawley rats	Male, n = 13	Antagonism of PPARα by MK886 prevented URB597 from altering nicotine-induced stimulation of bursting, but not firing rate of dopaminergic neurons	(Melis, Pillolla et al. 2008)
URB597	FAAH inhibitor	0.3 – 3 mg/kg, i.p.	Long Evans rats	Male, n = 7	FAAH inhibition by URB597 at varying doses had no effect on breakpoint for nicotine during progressive-ratio schedule of self-administration	(Forget, Coen et al. 2009)
URB597	FAAH inhibitor	0.3 and 1 mg/kg, i.p.	Long Evans rats	Male, n = 7	FAAH inhibition by URB597 partially prevented cue-induced and nicotine-induced reinstatement of nicotine seeking	(Forget, Coen et al. 2009)
rimonabant	CB_1 receptor inverse agonist	0.3 – 1 mg/kg, i.p.	Long Evans rats	Male, n = 8	CB_1 blockade by rimonabant dose-dependently reduced the breakpoint for nicotine during progressive-ratio schedule of self-administration	(Forget, Coen et al. 2009)
rimonabant	CB_1 receptor inverse agonist	1 mg/kg, i.p.	Long Evans rats	Male, n = 8	CB_1 blockade by rimonabant prevented cue-induced and nicotine induced reinstatement of nicotine seeking	(Forget, Coen et al. 2009)
URB597	FAAH inhibitor	0.1 mg/kg, i.v.	Sprague-Dawley rats	Male, n = 6	Pretreatment with URB597 blocked nicotine's depression of medium spiny neurons in the shell of the nucleus accumbens	(Luchicchi, Lecca et al. 2010)
rimonabant	CB_1 receptor inverse agonist	0.5 mg/kg, i.v.	Sprague-Dawley rats	Male, n = 6	Rimonabant fully reversed URB597's blockade of nicotine's effects in medium spiny neurons in the shell of the nucleus accumbens	(Luchicchi, Lecca et al. 2010)
MK886	PPARα antagonist	3 mg/kg, i.p.	Sprague-Dawley rats	Male, n = 6	MK886 fully reversed URB597's blockade of nicotine's effects in medium spiny neurons in the shell of the nucleus accumbens	(Luchicchi, Lecca et al. 2010)
URB597	FAAH inhibitor	0.1 and 0.3 mg/kg, i.p.	Wistar rats	Male, n = 8	URB597 did not alter somatic signs of withdrawal induced by nicotine abstinence, locomotor activity or weight.	(Cippitelli, Astarita et al. 2011)
URB597	FAAH inhibitor	0.1 and 0.3 mg/kg, i.p.	Wistar rats	Male, n = 7-11	URB597 reversed nicotine withdrawal-induced anxiety in the elevated plus maze test and in the shock-probe defensive burying test	(Cippitelli, Astarita et al. 2011)
WY14643	PPARα agonist	20 and 40 mg/kg, i.p.	Sprague-Dawley rats	Male, n = 6	WY14643 dose-dependently reduced intravenous nicotine self-administration (FR5) during three consecutive sessions, nicotine responding returned to baseline after three sessions of pre-treatment with WY14643 (40 mg/kg)	(Mascia, Pistis et al. 2011)
methyl oleoylethanolamide	PPARα agonist	10 mg/kg, i.m.	Sprague-Dawley rats	Male, n = 5	methyl oleoylethanolamide reduced intravenous nicotine self-administration (FR5) during three consecutive sessions	(Mascia, Pistis et al. 2011)
WY14643	PPARα agonist	10, 20, and 40 mg/kg, i.p.	Squirrel monkeys	Male, n = 10	WY14643 dose-dependently reduced intravenous nicotine self-administration (FR10) during five consecutive sessions, nicotine responding returned to baseline after five sessions of pre-treatment with WY14643 (40 mg/kg)	(Mascia, Pistis et al. 2011)
methyl oleoylethanolamide	PPARα agonist	10 mg/kg, i.m.	Squirrel monkeys	Male, n = 10	methyl oleoylethanolamide reduced intravenous nicotine self-administration (FR10) during five consecutive sessions	(Mascia, Pistis et al. 2011)
WY14643	PPARα agonist	20 and 40 mg/kg, i.p.	Sprague-Dawley rats	Male, n = 11-15	WY14643 dose-dependently reduced the nicotine-induced reinstatement of extinguished nicotine-seeking	(Mascia, Pistis et al. 2011)
WY14643	PPARα agonist	20, and 40 mg/kg,	Squirrel monkeys	Male, n = 3	WY14643 dose-dependently reduced the nicotine-induced	(Mascia, Pistis et al. 2011)

		i.p.			reinstatement of extinguished nicotine-seeking, this effect was prevented by MK886 (1 mg/kg, i.m.)	
WY14643	PPARα agonist	40 mg/kg, i.p.	Sprague-Dawley rats	Male, *n* = 5-7	WY14643 inhibited nicotine-induced activation of dopamine neurons in the ventral tegmental area, this effect was blocked by MK886 (3 mg/kg)	(Mascia, Pistis et al. 2011)
methyl oleoylethanolamide	PPARα agonist	5 and 10 mg/kg, i.v.	Sprague-Dawley rats	Male, *n* = 7	methyl oleoylethanolamide inhibited nicotine-induced activation of dopamine neurons in the ventral tegmental area	(Mascia, Pistis et al. 2011)
WY14643	PPARα agonist	20 and 40 mg/kg, i.p.	Sprague-Dawley rats	Male, *n* = 5-6	WY14643 inhibited nicotine-induced elevations in dopamine levels in the nucleus accumbens of freely moving rats, the effect of 40 mg/kg WY14643 was blocked by MK886 (3 mg/kg)	(Mascia, Pistis et al. 2011)
methyl oleoylethanolamide	PPARα agonist	10 mg/kg, i.p.	Sprague-Dawley rats	Male, *n* = 5	methyl oleoylethanolamide inhibited nicotine-induced elevations in dopamine levels in the nucleus accumbens of freely moving rats, this effect was blocked by MK886 (3 mg/kg)	(Mascia, Pistis et al. 2011)
—	—	—	Long Evans rats	Male, *n* = 22	No effect on nicotine self-administration under FR5 and PR schedules of reinforcement No effect on cue- and nicotine-induced reinstatement	(Gamaleddin, Zvonok et al. 2012)
AM630	CB_2 receptor antagonist	1.25, 2.5, and 5 mg/kg, i.p.	Long Evans rats	Male, *n* = 12	No effect on nicotine self-administration under FR5 and PR schedules of reinforcement No effect on cue- and nicotine-induced reinstatement	(Gamaleddin, Zvonok et al. 2012)
AM1241	CB_2 receptor agonist	1, 3, and 10 mg/kg, i.p.	Long Evans rats	Male, *n* = 10	No effect on nicotine self-administration under FR5 and PR schedules of reinforcement No effect on cue- and nicotine-induced reinstatement	(Gamaleddin, Zvonok et al. 2012)
AM404	anandamide transport inhibitor	5 mg/kg, i.p.	Sprague-Dawley rats	Male, *n* = 9-10	AM404 prevented nicotine-induced conditioned place preference	(Scherma, Justinova et al. 2012)
AM404	anandamide transport inhibitor	5 mg/kg, i.p.	Sprague-Dawley rats	Male, *n* = 9-10	AM404 prevented nicotine-induced reinstatement of abolished conditioned place preference	(Scherma, Justinova et al. 2012)
AM404	anandamide transport inhibitor	5 mg/kg, i.p.	Sprague-Dawley rats	Male, *n* = 9-10	AM404 did not affect the locomotor suppressant or anxiolytic effects of nicotine	(Scherma, Justinova et al. 2012)
AM404	anandamide transport inhibitor	5 mg/kg, i.p.	Sprague-Dawley rats	Male, *n* = 9-10	AM404 reduced nicotine-induced elevations in dopamine levels	(Scherma, Justinova et al. 2012)
Clofibrate	PPARα agonist	300 mg/kg, i.p.	Sprague-Dawley rats	Male, *n* = 9	Clofibrate prevented acquisition of intravenous nicotine self-administration in naïve rats	(Panlilio, Justinova et al. 2012)
Clofibrate	PPARα agonist	300 mg/kg, i.p.	Sprague-Dawley rats	Male, *n* = 13	Clofibrate decreased intravenous nicotine self-administration in experienced rats, this effect was blocked by MK886 (3 mg/kg, i.m., n=7); food self-administration was not affected by clofibrate	(Panlilio, Justinova et al. 2012)
Clofibrate	PPARα agonist	100 mg/kg, i.m.	Squirrel monkeys	Male, *n* =4	Clofibrate decreased intravenous nicotine self-administration in experienced monkeys, this effect was blocked by MK886 (1 mg/kg, i.m.); food self-administration was not affected by clofibrate	(Panlilio, Justinova et al. 2012)
Clofibrate	PPARα agonist	300 mg/kg, i.p.	Sprague-Dawley rats	Male, *n* = 9	Clofibrate blocked the effects of nicotine on firing of ventral tegmental area dopamine cells and nicotine0induced elevations of dopamine in the nucleus accumbens shell, these effects were blocked by MK886 (10 mg/kg, i.p., n=5)	(Panlilio, Justinova et al. 2012)
Clofibrate	PPARα agonist	100 mg/kg, i.m.	Squirrel monkeys	Male, *n* =4	Clofibrate decreased nicotine-induced and cue-induced reinstatement, this effect was dose-dependently blocked by MK886 (1 and 3 mg/kg, i.m.)	(Panlilio, Justinova et al. 2012)
URB597 and URB694	FAAH inhibitor	1 mg/kg, i.v.	Squirrel monkeys	Male, *n* =4	URB597 and URB694 both inhibited brain and liver FAAH activity, increasing brain and liver anandamide, 2-arachidonoylglycerol, and oleoylethanolamide	(Justinova, Panlilio et al. 2015)
URB597 and URB694	FAAH inhibitor	1 mg/kg, i.v.	Squirrel monkeys	Male, *n* =4	URB597 and URB694 both induced a rightward shift in nicotine's dose-response curve (3, 10, 30, and 100 μg/kg/inj, i.v., FR10)	(Justinova, Panlilio et al. 2015)
URB597	FAAH inhibitor	0.1, 0.3, and 1 mg/kg, i.v.	Squirrel monkeys	Male, *n* =4	URB597 dose dependently reduced nicotine (30 μg/kg/inj, i.v.) self-administration (FR10), this effect was reversed by MK886 (0.3 and 1 mg/kg, i.m.), a PPARα antagonist	(Justinova, Panlilio et al. 2015)

URB694	FAAH inhibitor	0.03, 0.1, and 1 mg/kg, i.v.	Squirrel monkeys	Male, *n* =4	URB694 dose dependently reduced nicotine (30 μg/kg/inj, i.v.) self-administration (FR10), this effect was reversed by MK886 (1 mg/kg, i.m.), a PPARα antagonist	(Justinova, Panlilio et al. 2015)
URB597	FAAH inhibitor	0.3 mg/kg, i.p.	Long Evans rats	Male, *n* = 8-13	URB597 attenuated cue-induced reinstatement of nicotine seeking, which was reversed by rimonabant (0.15 mg/kg), but not AM630 (5 mg/kg) or MK886 (1 mg/kg), antagonists of the CB_2 and PPARα receptors, respectively	(Forget, Guranda et al. 2016)
pioglitazone	PPARγ agonist	15 and 30 mg/kg, i.p.	Wistar rats	Male, *n* = 27	Pioglitazone (15 and 30 mg/kg) reduced somatic signs of withdrawal and anxiety-like behavior induced by nicotine withdrawal (nicotine patches)	(Domi, Caputi et al. 2019)
pioglitazone	PPARγ agonist	15 and 30 mg/kg, i.p.	$PPAR\gamma^{(+/+)}$ mice	Male, *n* = 6-8	Pioglitazone (15 and 30 mg/kg) reduced somatic signs of withdrawal and anxiety-like behavior induced by nicotine withdrawal (nicotine patches)	(Domi, Caputi et al. 2019)
pioglitazone	PPARγ agonist	15 and 30 mg/kg, i.p.	$PPAR\gamma^{(-/-)}$ mice	Male, *n* = 6-8	Pioglitazone (15 and 30 mg/kg) had no effect on somatic signs of withdrawal and anxiety-like behavior induced by nicotine withdrawal (nicotine patches)	(Domi, Caputi et al. 2019)
pioglitazone	PPARγ agonist	30 mg/kg, i.p.	$PPAR\gamma^{(+/+)}$ mice	Male, *n* = 6-8	GW9662 reversed the effect of pioglitazone on the symptoms of nicotine withdrawal	(Domi, Caputi et al. 2019)
N-oleoyl-glycine	PPARα agonist	10, 30, and 60 mg/kg, i.p.	ICR mice	Male, *n* = 7-8	N-oleoyl-glycine dose dependently reversed nicotine withdrawal-induced decreased time in open arms during elevated plus maze and reversed mecamylamine precipitated somatic signs of withdrawal in nicotine-dependent mice	(Donvito, Piscitelli et al. 2019)
N-oleoyl-glycine	PPARα agonist	1, 10, and 30 mg/kg, i.p.	ICR mice	Male, *n* = 7-8	N-oleoyl-glycine dose dependently prevented the development of nicotine-induced conditioned place preference, this effect was selective to nicotine compared to morphine	(Donvito, Piscitelli et al. 2019)

i.p., intraperitoneal; i.v., intravenous; FR, fixed ratio; PR, progressive ratio; FAAH, fatty acid amide hydrolase; PPARα, peroxisome proliferator-activated receptor alpha.

Table 10. Metformin studies.

Dose	*Strain/Species*	*Sex, n*	*Measurements*	Reference
800 mg/kg diet, 6 weeks	Strain H mice	Male and female, $n = 10$	Metformin decreased oxidative DNA damage and the incidence and multiplicity of microadenomas and normalized miRNA and RNA expression in electronic cigarette-exposed mice but did not affect the yield of electronic cigarette-induced lung adenomas or malignant tumors	(Izzotti, Balansky et al. 2014)
*200 mg/kg/*day, *i.p.,* 10 days	C57BL/6J mice	Male, $n = 6$	Animals that were treated with metformin exhibited better stroke outcome and reduction of inflammation after chronic tobacco smoke exposure	(Kaisar, Villalba et al. 2017)
*200 mg/kg/*day, *i.p.,* 10 days	C57BL/6J mice	Male, $n = 6$	Animals that were treated with metformin exhibited better stroke outcome and reduction of inflammation after chronic nicotine-containing electronic cigarette exposure	(Kaisar, Villalba et al. 2017)
*100-200 mg/kg/*day, *i.p.,* 4 weeks	C57BL/6J mice	Male, $n = 8$	Metformin prevented cigarette smoke-induced loss of blood-brain barrier integrity and function, mediated by Nrf2 activation	(Prasad, Sajja et al. 2017)
*250 mg/kg/*day, *i.p., 7* days	129SvEv; C57BL/6J mice	Male, $n = 7$-16	Systemically delivered metformin reduced nicotine withdrawal-induced anxiety-like behavior, measured by novelty-induced hypophagia and marble-burying behavior	(Brynildsen, Lee et al. 2018)
*50 μg/*day, *i.c.v., 7* days *(osmotic minipump)*	129SvEv; C57BL/6J mice	Male, $n = 7$-8	Centrally delivered metformin reduced nicotine withdrawal-induced anxiety-like behavior, measured by novelty-induced hypophagia and marble-burying behavior	(Brynildsen, Lee et al. 2018)

i.p., intraperitoneal; i.c.v., intracerebroventricular.