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The endocannabinoid system: a new molecular target for the treatment of tobacco addiction

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Abstract

Tobacco addiction is one of the leading preventable causes of mortality in the world and nicotine appears to be the main critical psychoactive component in establishing and maintaining tobacco dependence. Several lines of evidence suggest that the rewarding effects of nicotine, which underlie its abuse potential, can be modulated by manipulating the endocannabinoid system. For example, pharmacological blockade or genetic deletion of cannabinoid CB₁ receptors reduces or eliminates many behavioral and neurochemical effects of nicotine that are related to its addictive potential.

This review will focus on the recently published literature about the role of the endocannabinoid system in nicotine addiction and on the endocannabinoid system as a novel molecular target for the discovery of medications for tobacco dependence.

Keywords

Addiction; tobacco dependence; nicotine; endocannabinoid system; CB₁ receptors

Introduction

Tobacco use through cigarette smoking constitutes a major public health problem in our society with devastating consequences, such as lung cancer and chronic obstructive pulmonary and coronary heart diseases [1]. Indeed, more than 5 million deaths a year are attributable to smoking worldwide and, in addition, exposure to tobacco smoke has been associated with about 38,000 deaths of non-smokers annually from second-hand exposure [2]. Although there has been a substantial reduction in the prevalence of smoking in most

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NOTICE TO THE READERS

After the present review was accepted for publication we were notified that, based on assessment of available information about psychiatric side effects, the European Medicines Agency (EMA) has recommended the suspension of marketing authorization for rimonabant, Acomplia, in the European Union [253].

developed countries over the last three decades, the use of tobacco is on the rise in developing countries, with rates exceeding 40% in many countries, and tobacco smoking is one of the few causes of mortality that is increasing, with deaths projected to reach 10 million annually in 30 to 40 years [3]. This morbidity and mortality can be mostly avoided if people stop smoking [4]. Over the past 20 years, several medications have been developed and utilized for smoking cessation such as nicotine replacement therapies, bupropion and varenicline [5–7], but their effectiveness, although significant and clinically useful, is limited, and the majority of people utilizing them still relapse to smoking tobacco [8–10]. The mechanisms underlying tobacco addiction are, therefore, of wide interest and there is clearly a need for new and more effective medications to assist in smoking cessation and relapse prevention.

Over the last few years there have been numerous discoveries in the field of cannabinoid research, which have led to the discovery and development of a variety of pharmacological tools able to modulate cannabinoid system neurotransmission. The development of this new class of compounds has boosted a world-wide research interest in potential therapeutic activities related to modulation of endocannabinoid transmission, due to the involvement of the endocannabinoid system in a wide variety of physiological and pathological processes, among them, motor behavior, learning, appetite, drug reward and addiction. This review focuses on pre-clinical and clinical studies supporting a role for the endocannabinoid system in the reinforcing/rewarding effects of nicotine, which ultimately lead to nicotine abuse and addiction, and on the development of therapeutic strategies based on drugs that modulate endocannabinoid system signalling in ways that might prove useful for smoking cessation.

1. Tobacco addiction

Tobacco addiction is described as a chronic, relapsing disorder in which compulsive drug-seeking behavior and drug-taking behavior persist despite negative consequences and the motivation to quit. To develop successful pharmacotherapies for tobacco addiction, it is important to understand the elements that play fundamental roles in the pathway that goes from acquisition and sampling of tobacco with exposure to its reinforcing effects, to its subsequent abuse and addiction. Indeed, many different intrinsic and environmental factors are implicated in and sustain tobacco addiction. Also, as noted below, genetic factors might play an important role in nicotine dependence.

Tobacco smoke contains thousands of chemical constituents, some natural and some formed during its combustion. It is largely accepted that nicotine is the main psychoactive ingredient in tobacco. Indeed, nicotine is thought to be primarily responsible for tobacco's addictive effects and for difficulties encountered in attempts to cease smoking [11, see ref. 12–15 for reviews]. Apart from nicotine, other factors contribute to whether tobacco use is started or sustained. These factors include environmental stimuli (cues) associated with tobacco smoking, tobacco constituents that provide reinforcing sensory stimulation, tobacco components that minimize excessive irritation from inhaled nicotine, and other pharmacologically active compounds in tobacco [16]. These factors provide rewarding effects, often surpassing the direct effects of nicotine. For example, exposure to smoking cues increases craving for cigarettes and can precipitate relapse [17] and smokers have been shown to have reduced levels of brain MAO, leading to speculation that MAO inhibition by tobacco smoke may underlie some of the behavioral and epidemiological features of smoking [18]. Furthermore, smoke constituents such as acetaldehyde and ammonia may also play a role in human smoking behavior [16, 19].

The primary pharmacological targets for nicotine are nicotinic acetylcholine receptors (nAChR), which are ligand-gated ion channels that are activated by the endogenous

neurotransmitter acetylcholine. These receptors are expressed in most tissues and organs, including the brain [see ref. 20, 21 for reviews]. It has been demonstrated that nAChR identified in mammalian brain are pentameric, heteromeric or homomeric ion-channels composed of different transmembrane subunits [see ref. 22 for review]. To date, two major nAChR subunits termed α () and β () have been characterized in the central nervous system (CNS), with nine different (2-10) and three different (2-4) subunits [see ref. 23, 24 for reviews]. All of the possible receptors derived from these subunit combinations are potential pharmacological targets for nicotine. However, the nAChR receptor containing 4/ 2 subunits is the predominant nicotinic receptor subtype in the brain, and nicotine shows a high affinity for this receptor [see ref. 21, 24 for reviews]. Several findings suggest that the 4/ 2 nAChR plays a pivotal role in nicotine's reinforcing effects [25–27] and physical dependence on nicotine [28]. For example, animals that lack expression of the 2 subunit do not readily self-administer nicotine and do not develop nicotine-induced conditioned place preference, two animal models used to assess nicotine-induced reinforcing/rewarding effects [29–32]. In addition, Tapper and colleagues [28] showed that the selective activation of the 4 subunit is sufficient to elicit nicotine-induced reward, tolerance and sensitization.

Although evidence from pre-clinical and clinical studies indicates that several neurotransmitter systems mediate the neurobiological effects underlying nicotine addiction [33, 34], the dopaminergic system is thought to be primarily involved in mediating the rewarding effects of this drug [13,15]. Using a change in [^{11}C] raclopride binding as an indirect measure of dopamine release in the ventral striatum/nucleus accumbens by positron emission tomography, Brody and colleagues [35] showed that smoking a regular cigarette resulted in a significantly greater mean reduction in [^{11}C] raclopride binding than smoking a denicotinized cigarette. Thus, nicotine delivered through cigarette smoking appears to be important for dopamine release in the ventral striatum/nucleus accumbens, providing some validation for the dopamine hypothesis of tobacco dependence [13,15]. Barrett and colleagues [36] also found that cigarette smoking is capable of displacing binding of [^{11}C] raclopride measured by positron emission tomography and that such displacement was correlated with verbal reports of feelings of “high” in human smokers.

The mesocorticolimbic dopamine system, which is comprised of cell bodies in the ventral tegmental area (VTA) and their projections to limbic brain regions, including the nucleus accumbens and other forebrain sites like the medial prefrontal cortex, is regarded as the major brain substrate mediating the rewarding/reinforcing effects of both natural reinforcers like food and addictive drugs like nicotine [see ref. 37, 38 for reviews, 39]. However, other brain substrates, such as the pedunculopontine tegmental nucleus, may be involved as well, and previous studies have found reductions in nicotine self-administration following manipulations of this brain nucleus [40, 41].

VTA neurons express a variety of nAChR subtypes, which, when activated by nicotine, promote a sustained increase in dopamine release in the nucleus accumbens that is considered pivotal to the development of addiction to nicotine [42–44; see ref. 45 for review], as well as addiction to other psychostimulants and to other classes of abused drugs [see ref. 46, 47 for reviews; 48, 49]. In the VTA, nAChR are located on cell bodies of dopaminergic neurons, on GABAergic cell bodies and terminals, and, as well, on presynaptic nerve endings of glutamatergic neurons which descend from the medial prefrontal cortex [see ref. 50 for review; 51, 52]. Nicotine can facilitate dopaminergic neurotransmission and dopamine release in the nucleus accumbens shell directly by stimulating nicotinic receptors on cell bodies of dopaminergic neurons. Stimulation of nAChR on dopaminergic neurons increases their firing rates, but these receptors desensitize rapidly [53–57]. Nicotine also increases glutamate and GABA transmission in the VTA [57,

58]. Moreover, whereas the nicotine-induced increase of GABA transmission rapidly desensitizes, the glutamate response does so to a much lesser extent [58]. Thus, following nicotine administration, there appears to be a net shift in the balance of excitatory and inhibitory inputs to dopaminergic neurons in the VTA such that inhibitory GABAergic transmission is decreased and excitatory glutamatergic transmission is increased [58].

In addition to the mesocorticolimbic dopamine system, various other neuronal systems have been shown to be involved in the psychoactive and addictive effects of nicotine. Indeed, nicotine is able to facilitate the presynaptic release of many additional neurotransmitters such as GABA, glutamate, serotonin, norepinephrine, and endogenous opioids [see ref. 33, 34, 59 for reviews]. Several recent lines of evidence suggest the endocannabinoid signalling system as an important component in the behavioral and motivational responses induced by nicotine, and cannabinoid CB₁ receptors seem to play a primary role in this interaction [see ref. 60, 61 for reviews; 62–65]. Cannabinoid CB₁ receptors are a natural target for endogenous cannabinoid ligands such as anandamide (AEA) and 2-arachidonoylglycerol (2-AG). Thus, it is possible that cannabinoid-nicotine interactions are related to the ability of nicotine to regulate endocannabinoid signalling by triggering the formation and release of endogenous cannabinoids. Consistent with this hypothesis, animals chronically treated with nicotine show an increase of AEA content in the limbic forebrain, a key brain region for reward [66]. Moreover, Solinas and colleagues [67] showed that nicotine potentiates the discriminative effects of low doses of THC, an effect reversed by the CB₁ antagonist rimonabant. Again, a mechanism that could account for this potentiation would be nicotine-induced release of endogenous cannabinoids, such as AEA. Additional support for this hypothesis comes from findings that blockade of fatty acid amide hydrolase (FAAH), the enzyme that metabolizes and inactivates endogenous AEA, by the selective FAAH inhibitor URB-597, results in nicotine producing significant THC-like discriminative stimulus effects. These THC-like discriminative effects of nicotine during FAAH inhibition are reversed by rimonabant, further suggesting that nicotine can release AEA [67]. Other indirect evidence of the ability of nicotine to stimulate endocannabinoid release comes from in-vivo Voltammetry studies. It has been reported that transient increases in dopamine signals in the nucleus accumbens, obtained with nicotine and other psychostimulants, are under a CB₁ receptor control. Thus, increased levels of endocannabinoids may also facilitate the stimulation of dopaminergic neuronal activity in the ventral tegmental area [68] elicited by nicotine and other drugs of abuse, an effect that could be reversed by blocking CB₁ receptors with rimonabant.

Finally, it is also important to remember that dependence on tobacco, like many other types of drug dependence, is a complex biological phenomenon with both genetic and environmental factors that contribute to the variance. Heritability estimates for smoking in twin studies have ranged from 46 to 84%, indicating a substantial genetic component in tobacco smoking [69]. Evidence that a genetic component is involved in the variance comes from studies of individuals who share genes (twin, family and adoption studies), from studies based on comparisons of unrelated affected and unaffected individuals from a population (association studies, case-control studies), and from inheritance patterns of phenotypes and genotypes in pedigrees (linkage studies) [69]. These studies indicate that there is an important and significant genetic component that participates in several aspects of smoking behavior, such as the initiation [70] and persistence of smoking behavior and the number of cigarettes smoked [71] in both men and women [72, see ref. 73 for review].

2. The endocannabinoid signalling system

The endocannabinoid signalling system is composed of cannabinoid receptors, their endogenous ligands, like AEA and 2-AG, the enzymes that produce and inactivate

endocannabinoids, and endocannabinoid transporters [see ref. 74–77 for reviews]. During the last decade, two subtypes of cannabinoid receptors, CB₁ and CB₂, have been characterized and cloned. They belong to the large family of G-protein-coupled receptors (GPCRs) that are composed of seven transmembrane domains, an extracellular N-terminal tail, three extracellular and three intracellular loops that link the transmembrane domains, and an intracellular C-terminal tail. [78; see ref. 79 for review]. CB₁ receptors are the most abundant GPCRs in the CNS, and they are found in high concentration in brain areas controlling motor coordination and cognitive, emotional, and sensory functions, i.e. the hippocampus, cerebellum, cortex and olfactory bulb [80, 81]. CB₁ receptors are also found in peripheral tissues [see ref. 69 for review]. CB₂ receptors are mainly located in the cells of the immune system [see ref. 82 for review], but they have also been recently identified in some areas of the brain [83–85]. Recent studies suggest the existence of another G-protein-coupled cannabinoid receptor (“CB₃”) but it has not yet been cloned and characterized [86–90; see ref. 91 for review]. Other recent studies suggest that GPR55, a G protein-coupled receptor, is a cannabinoid receptor. GPR55 is highly expressed in large dorsal root ganglion neurons and, upon activation by various cannabinoids, e.g. ⁹-tetrahydrocannabinol (THC) and methanandamide, increases intracellular calcium in these neurons. Examination of its signalling pathway in HEK293 cells transiently expressing GPR55 indicated that the calcium increase involves G(q), G(12), RhoA, actin, phospholipase C, and calcium release from IP(3)R-gated stores. GPR55 activation also inhibits M currents. These results have established GPR55 as a cannabinoid receptor with signaling distinct from CB₁ and CB₂ receptors [92, 93].

Both CB₁ and CB₂ receptors inhibit cAMP formation via G_i-proteins and activate mitogen-activated-protein kinases [94]. In addition, CB₁ receptors activate ion channels such as A-type, inwardly rectifying potassium channels, and inhibit voltage sensitive N-type and P/Q-type Ca²⁺ channels [95–97].

None of the several different endogenous ligands for CB₁ and CB₂ receptors that have been discovered so far, such as AEA, 2-AG and 2-arachidonoylglycerol ether [98–101], are stored in vesicles like other neuronal mediators. Instead, endocannabinoids are produced “on demand” by receptor-stimulated cleavage of lipid precursors from cell membranes and released from neurons immediately afterwards [see ref. 102–105 for reviews].

Endocannabinoid signaling is terminated by a two-step process consisting of: 1) transport inside the cell and 2) metabolic degradation by specific enzymes. Once taken up into the cell, AEA is primarily metabolized by the intracellular enzyme FAAH, which has been cloned and characterized [106–110]. FAAH is widely distributed in the CNS and its distribution mostly overlaps with that of the CB₁ receptor: high concentrations are localized in the hippocampus, cerebellum and cerebral cortex [see ref. 111, 112 for reviews; 113]. Pharmacological blockade of FAAH activity has been shown to prolong many behavioral and neurobiological effects of AEA [110, 114–166]. 2-AG, once taken up into the cell, is metabolized into arachidonic acid and glycerol mainly by the enzyme monoacylglycerol-lipase (MAGL), which has been cloned and characterized, and is also widely distributed in the CNS [117].

It is generally accepted that the endocannabinoid system plays a modulatory role in the control of motor behavior, nociception, memory and learning, emesis, appetite, temperature, and brain development [see ref. 118–120 for reviews; 121, 122]. Several lines of evidence also suggest that the endocannabinoid system is an important constituent of neuronal substrates involved in brain reinforcement/reward processes [see ref. 123, 124 for reviews]. As mentioned before, there is strong evidence that the mesocorticolimbic dopaminergic system, which projects from the VTA to the nucleus accumbens and to other forebrain sites, is the major substrate for reward and reinforcement processes [see ref. 125 for review].

Cannabinoid CB₁ receptors are present in the VTA and the nucleus accumbens and in several areas projecting to these two structures, including the prefrontal cortex, central amygdala and hippocampus, and CB₁ receptors appear to play an important role in brain reinforcement/reward processes [see ref. 126 for review]. These receptors are mainly located at the presynaptic level, and an important functional consequence of their activation is the inhibition of neurotransmitter release [see ref. 127 for review]. Acting as retrograde messengers, endocannabinoids can modulate excitatory and inhibitory inputs that control dopaminergic neurons of the mesocorticolimbic system [128–130]. Thus, endocannabinoids can be released following depolarization in the nucleus accumbens [131], and from dopaminergic neurons of the VTA [132, 133]. Activation of CB₁ receptors on axon terminals of GABAergic neurons in the VTA and on glutamatergic neurons in both the VTA and nucleus accumbens would inhibit both GABAergic and glutamatergic neurotransmission [132–135]. The final effect on modulation of VTA dopaminergic activity will depend upon the relative level of activation of these inputs under distinct behavioral circumstances [133, 136]. This retrograde signaling function of endocannabinoids appears to be widely distributed throughout the CNS [137], although it appears to be limited to only certain neuronal phenotypes within particular brain regions [138].

Thus, the endocannabinoid system plays an overall modulatory role for the brain reward circuitry. While it is well established that the endocannabinoid system is the primary site of action for the reinforcing/rewarding effects of cannabinoids [see ref. 123, 140 for reviews], it also participates in modulating the reinforcing/rewarding effects of other abused drugs, including nicotine [see ref. 141 for review].

As mentioned above, scientific evidence indicates a substantial genetic component in tobacco smoking [69]. The gene that codes the cannabinoid CB₁ receptor (CNR1) is an essential component of the endogenous cannabinoid system. It has been shown that the CNR1 gene is widely expressed in various regions of the brain [see ref. 142 for review] and direct association studies of this gene have been performed with substance abuse and dependence [143–149]; however, the results have not always been consistent. For example, Coming and colleagues [145] hypothesized that variants of the CNR1 gene could be associated with susceptibility to alcohol abuse or drug dependence. On the other hand, Li *et al.* [147] did not find evidence that polymorphism of the CNR1 gene confers susceptibility to heroin abuse. In relation to tobacco addiction, it is interesting to note that Chen *et al.* [150] showed that variants and haplotypes in the CNR1 gene may alter the risk for nicotine dependence, and the associations are likely sex specific. In addition to variants of the CNR1 gene, Sipe *et al.* [151] reported that a naturally occurring single nucleotide polymorphism in the human FAAH gene, the gene that codes the primary enzyme that inactivates neuromodulatory-signaling lipids including AEA, is strongly associated with drug/alcohol use.

Collectively, these results provide evidence that genetic mutations in CNR1 or FAAH genes may constitute important risk factors for problem drug use and provide an additional rationale for looking into endocannabinoid signaling as a novel molecular target for the discovery of medications for tobacco dependence and other types of drug dependence.

3. Endocannabinoid signaling modulation: new frontiers for nicotine addiction treatment?

Most available treatments for drug addiction substitute for the effects of the addictive substance (substitution therapy), reduce withdrawal symptoms, or reduce the reinforcing/rewarding effects of the addictive substance. Other pharmacological approaches are being developed that target the conditioning processes that strengthen the reinforcing/rewarding

effects of the addictive substance or that reduce delivery of the substances into the brain (antibodies). In this section we will review both pre-clinical and clinical evidence for modulation by the brain endocannabinoid system of the motivational effects of nicotine which are believed to underlie tobacco addiction.

There are several different available methods for modulating endocannabinoid system activity, each having beneficial therapeutic effects, but also some unwanted side effects. For instance, due to the presence and abundance of cannabinoid receptors in virtually all brain regions, pharmacological activation or blockade of CB₁ receptors by systemically administered CB₁ receptor agonists or antagonists leads to a widespread activation or blockade of endocannabinoid signalling throughout the brain. Modulation of endocannabinoid system activity by systemically administered CB₁ agonists or antagonists, although simple to obtain and therapeutically effective, can lead to unwanted psychotropic effects with CB₁ agonists, and unwanted psychiatric problems with CB₁ receptor antagonists, the latter only reported in obese populations to date [see ref. 152 for review]. The most widely used antagonists of cannabinoid CB₁ receptors, Rimonabant and AM251, have been shown to possess clear inverse agonist activities at cannabinoid receptors [153, 154]. These inverse agonist activities might be responsible for undesirable effects of these drugs. Among the new tools available for modulating the endocannabinoid system are recently developed neutral antagonists of cannabinoid CB₁ receptors, which might facilitate this therapeutic approach by avoiding undesirable side effects [153, 155]. Only a few preclinical studies using neutral antagonists of cannabinoid receptors are available to date. Results from some these experiments show experimental outcomes similar to those obtained with inverse agonists without unwanted side effects [156, 157].

Among the other new tools available for modulating the endocannabinoid system are endocannabinoid level enhancers, like inhibitors of endogenous cannabinoid uptake or metabolic degradation, and allosteric modulators of CB₁ receptors. One advantage of using endocannabinoid metabolic enzyme inhibitors instead of CB₁ receptor agonist/inverse agonist/neutral antagonist ligands arises from the fact that endocannabinoids are believed to be released on demand [see ref. 102, 105 for reviews]. Thus, blockade of FAAH or MAGL would increase levels of AEA or 2-AG only in those brain areas in which release of endocannabinoids has been triggered by a specific biological event. This feature is different from the widespread action of CB₁ agonists, and indeed a wide variety of therapeutic uses have been suggested for this metabolic inhibitor class of compounds [see ref. 158 for review].

On a more recent, yet to be systemically explored track, various allosteric modulators of CB₁ receptors are now available, which are as promising as endocannabinoid metabolic modulators and as potential medications, but with fewer side effects than CB₁ orthosteric ligands and ligands that enhance endogenous cannabinoid levels. Since endocannabinoids are released on-demand during physiological and/or pathological events [see ref. 102, 105 for reviews], region-selective increases in endocannabinoids concentrations could be obtained depending on the relative involvement of a specific brain area in a physiological or pathological state [see ref. 159 for review], and this could allow an area-specific activation of brain cannabinoid receptors. There is evidence that in the absence of a cannabinoid ligand, allosteric modulators of cannabinoid receptors show neither agonist nor inverse agonist activity in *in-vitro* assays [160]. On this basis, it has been hypothesized that allosteric modulators of CB₁ receptors could selectively modulate only those effects mediated by CB₁ receptors activated by on-demand released endocannabinoids [see ref. 161 for review]. Thus, allosteric modulators would not affect activity of unbound CB₁ receptors located in brain areas in which the on-demand release of endocannabinoids has not been stimulated by physiological/pathological events [see ref. 161 for review]. This implies a less

invasive systemic effect of this class of compounds compared to the widespread activation or inactivation of CB₁ receptors obtained by administration of CB₁ agonist or antagonist ligands.

Another interesting point in favour of using allosteric modulators reviewed by Ross [161] comes from the observation that these drugs might be effective in treating diseases due to alterations of endocannabinoid levels in brain areas in which FAAH and MGL enzymes are not the predominant metabolic pathways for endocannabinoid degradation. Moreover, some endocannabinoid release enhancers might block enzymes which are not selective for endocannabinoids, thus increasing levels of other non-cannabinoid endogenous substances in the brain, which themselves might have different pharmacological effects from those desired.

3.1 Neuropharmacological studies

3.1.1 Biochemical evidence—Within the rat brain, endocannabinoid transmission can be significantly altered during chronic exposure to psychoactive drugs, as described by several studies reporting marked changes in levels of AEA and 2-AG in cerebral regions related to reward and addiction. With some limitations (see below), biochemical studies have shown that chronic nicotine exposure affects endocannabinoid neurotransmission in numerous brain regions. Gonzalez and colleagues [66] found that animals treated chronically with nicotine (1mg/kg/day for 7 day, s.c.) have increased endocannabinoid levels, i.e., AEA, in the limbic forebrain, a brain structure that, among other nuclei, contains the nucleus accumbens and the amygdala. This finding is interesting because this region is a key area for the reinforcing/rewarding effects of addictive drugs [66]. These authors also found significant increases in AEA and 2-AG content in the brainstem, a region that contains the locus coeruleus, a brain area involved in processes related to the appearance of drug withdrawal symptoms. Furthermore, a significant decrease in endocannabinoid levels was found in the hippocampus, striatum (only AEA) and cerebral cortex. On the other hand, chronic nicotine exposure did not change mRNA levels or the binding capacity of CB₁ receptors. It should be noted that this study was performed in postmortem rat brain tissue, with endocannabinoid levels usually measured at a single time-point after chronic administration of the drugs. It is well known that endocannabinoids levels are affected by rapid deactivation of enzymes in post-mortem tissue, which could result in artificial increases in cannabinoid levels [162]. In addition, it has been repeatedly shown that differences in how the drugs are administered, from a motivational stand point (i.e. passive experimenter administered or passive yoked administered, versus active self-administered drug), can lead to differences in the related neurobiology [163]. For such reasons more work has to be done in order to provide evidence that active, motivated self-administration of nicotine would effectively alter brain endocannabinoid levels measured *in vivo*.

It is now well established that some addiction-related behaviors are strongly linked to molecular adaptation, such as gene regulation, in discrete areas of the brain [164, 165] and that c-Fos expression is a good index of neuronal activity produced by drug administration [166, 167]. As previously described [167,168], acute administration of cannabinoid agonists and nicotine induces c-Fos immunoreactivity preferentially in the terminal fields of neurons of the VTA (nucleus accumbens, central amygdala, lateral-septum, dorsal-lateral bed nucleus stria terminalis), which are involved in the rewarding effects of drugs of abuse [see ref. 169 for review]. Valjent and colleagues [170] investigated the consequences of the co-administration of nicotine and THC on c-Fos immunoreactivity in several brain structures where they found potentiation of c-Fos immunoreactivity, relative to either compound alone, in various limbic and cortical structures, such as the shell of the nucleus accumbens, the central and basolateral amygdala, the bed-nucleus of stria terminalis, the cingular and

piriform cortex and the paraventricular nucleus of the hypothalamus. Most of these areas are highly innervated by dopaminergic inputs [see ref. 169 for review], suggesting that the interaction between nicotine and cannabinoids could occur via the stimulation of mesolimbic and mesocortical dopaminergic systems.

3.1.2 *In vivo* microdialysis—As described above, drugs of abuse appear to produce their addictive effects primarily by acting on the dopaminergic mesolimbic system which projects from the VTA to the nucleus accumbens [see ref. 125 for review]. Nicotine shares the ability, with other abused drugs, to elevate extracellular levels of dopamine in the shell of the nucleus accumbens, as measured by *in vivo* microdialysis, and elevated dopamine levels in the accumbens shell are believed to play an important role in nicotine's reinforcing/rewarding effects [43]. Systemic pre-treatment with the CB₁ receptor antagonist Rimonabant has been shown to block the elevations in accumbal dopamine produced by nicotine in rats [62]. In addition, pre-treatment with Rimonabant also blocks nicotine-induced dopamine release in the bed nucleus of the stria terminalis, a brain structure interconnected with the nucleus accumbens and VTA that is critically involved in the acquisition and maintenance of drug addiction [62, 171, 172]. On the other hand, URB597, the selective inhibitor of FAAH that magnifies and prolongs many effects of the endogenous cannabinoid ligand AEA, also inhibits the development of nicotine self-administration behaviour and relapse to nicotine seeking in rats and, as well, significantly reduces the elevations in dopamine levels in the nucleus accumbens shell that are produced by nicotine [173]. Importantly, URB597 did not potentiate the effects of a low threshold dose of nicotine that by itself had no significant effect on dopamine levels [173]. This suggests that modulation of the endocannabinoid system in two fundamentally different ways (systemically-administered CB₁-receptor antagonists or pharmacological blockade of endogenous AEA degradation) can have the common effect of attenuating effects of nicotine that are mediated by the dopaminergic system, effects that are regarded as fundamental for nicotine addiction.

Cannabinoid CB₁ receptors are present in high concentrations on axon terminals of GABAergic neurons. Activation of these receptors inhibits GABA release [174–176], thereby increasing activation of dopaminergic neurons, which ultimately leads to stimulation of dopamine release in terminal areas. On the one hand, it is possible that Rimonabant, by blocking disinhibitory effects of activation of CB₁ receptors in different brain areas, elicits stimulation of GABA release, resulting in reduced firing activity of dopaminergic neurons and reduced release of dopamine in terminal areas. On the other hand, activation of nicotinic receptors on dopaminergic neurons in the VTA by nicotine results in depolarization, increased extracellular calcium, and release of AEA [132]. AEA, protected from rapid degradation by URB597, could then activate CB₁ receptors present on presynaptic nerve endings of glutamatergic neurons, reducing glutamate release from these neurons [132], thereby reducing activation of dopaminergic neurons and release of dopamine from their terminals in the nucleus accumbens shell. This hypothesis is supported by the finding that nicotine can increase dopaminergic neuron excitation facilitating glutamate release [39].

It has been recently shown that URB597 may block the endocannabinoid reuptake mechanism in addition to inhibiting the FAAH enzyme [177]. To our knowledge there are no published reports about a possible involvement of endocannabinoid uptake, or compounds able to block endocannabinoid uptake, in nicotine's behavioral/neurobiological effects. For these reasons it will be of interest to test the potential therapeutic efficacy of the well known endocannabinoid uptake blockers, like AM404, VDM11 and UCM707 on nicotine dependence.

3.1.3 Electrophysiology and Voltammetry—Like many other drugs abused by humans, nicotine stimulates the firing rate of dopamine neurons in the VTA, which

ultimately results in increased dopamine transmission in the nucleus accumbens shell [178, 179]. While it is well established that nicotine produces relatively long-lasting elevations in extracellular levels of dopamine in dialysates from the nucleus accumbens [62], the moment-to-moment effects of nicotine on dopamine neurotransmission are less well known. Using a voltammetric sensor with high temporal and spatial resolution, Cheer and colleagues [68] showed that the increased frequency of dopamine concentration transients caused by an acute dose of nicotine were inhibited by Rimonabant administration, suggesting that an increase in endocannabinoid tone facilitates the effects of nicotine on sub-second dopamine release. It is interesting to note that, due to the reduced nicotine-induced desensitization process occurring at 7 nAChR compared to $4/2 \text{ nAChR}$, the authors explained the immediate changes induced by nicotine on dopamine release as effects attributable to $4/2 \text{ nAChR}$. Since dopamine transients have been associated with associative learning and drug seeking [180,181], this suggests that $4/2 \text{ nAChR}$ might be more involved in these behaviors. On the other hand, independent of which nAChR is responsible for the rapid surge of dopamine transients, cannabinoid CB_1 antagonists attenuate these dopamine transients, supporting the hypothesis that the endocannabinoid system plays a facilitating role in the development of both the rewarding/reinforcing effects of nicotine, and of the conditioned effects of nicotine-associated environmental cues, which are thought to be a key feature in nicotine addiction.

Using a two-electrode voltage-clamp technique, Oz and colleagues [182] showed that AEA, at nanomolar concentrations, inhibited the function of 7 nAChR expressed in *Xenopus oocytes* in a cannabinoid receptor-independent and non-competitive manner. Finally, using an 86Rb^+ efflux assay in thalamic synaptosomes, Butt and colleagues [183] showed that AEA inhibits the function of $4/2 \text{ nAChR}$ via a cannabinoid-independent mechanism.

3.1.4 *In situ* hybridization—Morales and colleagues [184] established that the vast majority of hippocampal interneurons containing 7 nAChR mRNA co-expressed CB_1 mRNA suggesting a specific interaction between the nicotinic cholinergic and endocannabinoid systems. These hippocampal neurons relay inputs from different cortical and subcortical regions relating emotional, motivational and physiological states [184].

3.2 Behavioral studies in animals

3.2.1 Discriminative stimulus effects of nicotine—The drug-discrimination paradigm is a behavioral procedure based on the ability of a drug to induce a specific set of interoceptive stimulus conditions perceived by animals which might be predictive of the subjective reports of perceptions/feelings induced by the same drug in humans [185]. Although there may be no direct link between the discriminative stimulus effects of a drug and drug addiction, it is likely that subjective effects of abused drugs play an important role in establishing their reinforcing effects and in the likelihood that a drug will be abused by humans. Indeed, in human subjects, abused drugs produce euphoric or pleasurable sensations that are believed to be primary factors contributing to drug abuse [185], and, ultimately, to drug addiction.

Animal drug-discrimination procedures consist of an initial period of training, which may take several months. Subjects learn to press one of two levers during sessions preceded by injection of the training drug and to press the other lever during sessions preceded by a vehicle injection. Lever pressing is intermittently reinforced by food pellets or by postponement or escape from mild electric shocks. Once subjects reach a criterion level of correct lever-press performance, test sessions are occasionally scheduled, during which they receive either the training drug at a different dose or a different drug, to test whether or not such a drug/dose will produce a discriminative stimulus effect similar to the training drug.

Generally, if the training drug is an agonist, only agonist drugs belonging to the same pharmacological class will produce full generalization to the training stimulus and only antagonists belonging to the same pharmacological class will selectively block the training stimulus.

It is well known that in rats trained to discriminate nicotine from saline, nicotine produces discriminative stimulus effects which are mainly mediated by nAChRs containing the $\alpha 4 \beta 2$ subunit [186–188]. Blockade of CB₁ receptors by Rimonabant administration does not produce nicotine-like effects in rats trained to discriminate nicotine from saline and is ineffective in modifying the discriminative stimulus effects of nicotine [62, 63, 189]. However, Rimonabant appears to dose-dependently antagonize nicotine generalization to a d-amphetamine training stimulus [62]. It is interesting to note that the discriminative stimulus effects of nicotine do not appear to depend on dopamine, unlike the reinforcing/rewarding effects of nicotine [190]. Also, the nucleus accumbens has been found to be less involved than other brain areas (hippocampus for example) in mediating the discriminative stimulus effects of nicotine. [188]. Thus CB₁ cannabinoid antagonists may not modify the discriminative stimulus of effects of nicotine because nicotine discrimination does not depend on the release of dopamine in reward related areas. However, when the dopaminergic effects of nicotine play a role in discrimination, for example in rats trained to discriminate d-amphetamine from saline, cannabinoid CB₁ receptor antagonists can block those dopamine-mediated effects [62].

3.2.2 Nicotine-induced conditioned place preference—The conditioned place preference procedure (CPP) is an animal model for studying rewarding or reinforcing effects of psychoactive drugs that is based on an animal's behavioral choice after a number of pairings of a drug with one environment and placebo with another. The place conditioning procedure, though relatively simple, allows the study of the development and subsequent expression of preference or aversion for a distinctive environment associated with a drug [see ref. 191, 192 for reviews], with the final test for preference or aversion being performed when the animal is in a drug-free state. The ability of drugs to impair the development or expression of conditioned place preference could be related to a potential therapeutic value, in terms of ability to reduce or block the rewarding/reinforcing effects of the conditioning drug, or to reduce the strength of the association between the conditioning drug and associated environmental stimuli.

The involvement of the endocannabinoid system in the rewarding effects of nicotine obtained in the CPP procedure has been evaluated in several different studies. These studies provide clear evidence that blockade of CB₁ receptors by Rimonabant can impair both the acquisition and the expression of nicotine-induced CPP in rats [63, 64]. It is unlikely that the blockade of nicotine-induced CPP reported was due to nonspecific effects of Rimonabant, since, at the dose studied, the CB₁ antagonist did not decrease locomotor activity and did not disrupt food-maintained behavior [63]. However, inhibition of the expression of nicotine-induced CPP was effective when Rimonabant was administered 24 hours after the last conditioning session, but not when it was given 3 or 12 weeks after the acquisition phase [64]. These results suggest a different involvement of the endocannabinoid system in short- and long-term expression of incentive learning supported by nicotine.

The involvement of the endocannabinoid system in nicotine-induced CPP was also studied in mice. Co-administration of sub-threshold doses of the CB₁-receptor agonist THC and nicotine that were ineffective when administered alone induced significant CPP when administered together [170]. Furthermore, Castané and colleagues [194] showed that nicotine was not able to induce CPP in CB₁ receptor knockout mice as compared with their wild-type littermates. Also in mice, there is evidence that pharmacological inhibition of

FAAH by URB597, or genetic ablation of FAAH, can facilitate the rewarding effects of a subthreshold dose of nicotine using a CPP procedure [195]. On the other hand, Scherma and colleagues [173] found that pharmacological inhibition of FAAH by URB597 prevented the development of nicotine-induced CPP in rats without facilitating the rewarding effects of a subthreshold dose of nicotine. In addition, the same study found that URB597 reduced nicotine-induced reinstatement of CPP, after conditioning had been extinguished by substituting saline for nicotine. The contrasting findings in these studies might be due to species-related differences (mice vs. rats) or to procedural differences.

3.2.3 Nicotine self-administration behavior—Intravenous drug self-administration behavior has been one of the most direct and productive approaches for modeling and studying the reinforcing effects of drugs abused by humans. With this procedure, animals are given the opportunity to self-administer a drug by making intravenous drug delivery contingent upon an operant response such as a lever press. The administration of the drug constitutes the event that positively reinforces the lever pressing behavior, and reward is inferred if the frequency of responding subsequently increases (thus, defining reinforcement). A variety of schedules of reinforcement have been employed to study self administration behavior. For example, the number of lever presses required to obtain each injection of drug can be fixed (a fixed-ratio schedule, FR) or can increase during the session (a progressive-ratio schedule; PR), thus allowing assessment of the direct reinforcing effects of the drug and the motivation of the animal to self-administer it. With these behavioral procedures, some stimuli (visual and/or auditory) are repeatedly associated with drug administration and these drug-associated stimuli can progressively gain motivational value that allows them to induce and maintain drug-seeking behavior in the absence of actual drug administration [196, 197]. Using this methodology, it has been possible to study neuropharmacological mechanisms involved in drug-taking behaviors and to pre-clinically evaluate therapeutic strategies for treatment of drug abuse [see ref. 198 for review].

Numerous experiments have shown that nicotine can serve as a positive reinforcer and this has been extensively documented in diverse species, including monkeys, dogs, rodents [199–202], and humans [203]. It should be noticed, however, that the conditions under which nicotine maintains nicotine self administration behavior appear to be more limited than those with other drugs of abuse [204]. As is commonly observed with the intravenous drug self-administration paradigm, a crucial experimental parameter that governs development of self-administration behavior is the unit dose of nicotine. The optimal dose for acquisition is thought to be around 0.01–0.03 mg/kg/injection in many animal species [201, 202, 205, 206]. The maintenance of nicotine-seeking behavior once it develops is also dose dependent, and an inverted “U” shaped function is usually found when dose is varied. In addition, it is well established that environmental stimuli associated with nicotine administration may be crucial for maintaining nicotine-seeking behavior. In a study with squirrel monkeys, Goldberg and colleagues [207] showed that when a brief-light stimulus was paired intermittently with nicotine delivery under a second-order schedule of intravenous nicotine self-administration, rates of responding were about twice as high compared with rates of responding when the brief light was not presented. The crucial role environmental stimuli associated with nicotine administration play in developing and sustaining nicotine self-administration behavior has also been demonstrated in rodents [65]. However, squirrel monkeys self-administering nicotine will orient their behavior toward a lever where responses produce both the presentation of nicotine-associated stimuli and nicotine injection rather than to a lever where responses produce only nicotine-associated stimuli, suggesting that the delivery of nicotine is the primary reinforcing event for this self-administration behavior [208].

Preclinical studies have shown that pharmacological alteration of the endocannabinoid system activity can interfere with the ability of nicotine to maintain self administration behavior. In Sprague-Dawley rats that had learned to press an active, but not an inactive, lever for intravenous injections of nicotine, administration of Rimonabant reduced responding on the active lever and, consequently, number of nicotine injections self-administered [62]. These effects of Rimonabant did not seem to be related to motor impairment, since Rimonabant did not reduce responses on the inactive lever. Further, Rimonabant also blocked nicotine-induced dopamine release in the shell of the nucleus accumbens, in separate *in vivo* microdialysis experiments, suggesting that this reduced dopamine release was responsible for the decrease in nicotine self-administration behavior [62]. In agreement with these findings with Rimonabant, AM251, another selective CB₁ receptor antagonist, was found to reduce nicotine self administration behavior. Shoaib [209] showed that AM251 produced robust dose-related reductions in nicotine self-administration in rats during three successive days of AM251 pre-treatment. The effect was prominent following a low 1 mg/kg dose of AM251, which reduced nicotine self-administration behavior without significantly modifying responding for food in other rats. However, this suppression of nicotine self-administration behavior did not persist when AM251 pre-treatment was stopped. In contrast with these findings with Rimonabant and AM251, Cossu and colleagues [210] reported that the absence of CB₁ receptors in CB₁-knockout mice did not result in a significant attenuation of nicotine-maintained self-administration behavior. Compensatory or adaptive neurobiological mechanisms as a result of genetic ablation of CB₁ receptors might be an explanation for the negative results obtained with CB₁ receptor knockout mice.

Finally, in agreement with our findings described earlier where the FAAH enzyme inhibitor URB597 blocked the development of nicotine-induced CPP [173], we also found that pretreatments with URB597 significantly decreased the likelihood of rats developing nicotine self-administration behavior, with 90% of vehicle-treated rats, but only 50% of URB597-treated rats, meeting the acquisition criterion for nicotine self-administration. Among rats that met the acquisition criterion, URB597-treated rats took about only half of the daily amount of nicotine as compared to vehicle-treated rats [173].

3.2.4 Extinction and reinstatement of nicotine-seeking behavior—The reinstatement model is currently used in many laboratories to investigate mechanisms underlying relapse to drug-seeking behavior in abstinent animals [see ref 211 for review]. Recent advances using this animal model of relapse have shown that reinstatement of drug seeking can be triggered by drug-associated (conditioned) cues, by stressors (e.g. mild footshock, food restriction and yohimbine) and by re-exposure to the previously experienced drug [see ref 212, 213 for reviews; 214], which are all events known to trigger drug craving and relapse in human addicts [215–217]. Although there are methodological limitations, drug-induced or cue-induced reinstatement of extinguished drug self-administration behavior in laboratory animals is used as an animal model of relapse behavior in humans [for review, see 211, with related commentaries]. It has been repeatedly demonstrated that after extinction of nicotine self-administration behavior, reintroduction of stimuli previously paired with nicotine injections (nicotine cues) results in increased responding on a previously active lever [65, see ref. 218 for review]. Similarly, a priming dose of nicotine given before the session can also reinstate nicotine-seeking behavior in abstinent rats [219–221].

Recent preclinical studies have shown that endocannabinoid signaling is crucially implicated in reinstatement of nicotine-seeking and nicotine-taking behaviors following even prolonged periods of abstinence [see ref. 222, 223 for reviews]. For example, Rimonabant can attenuate reinstatement of nicotine-seeking behavior evoked by contextual cues [224].

Consistent with this report, De Vries and colleagues [225] showed that blockade of CB₁ receptors by Rimonabant attenuated responding for nicotine-associated stimuli in a long-term extinction reinstatement model. In another study, responding maintained by nicotine-associated stimuli was also attenuated by Rimonabant [65]. Furthermore, Shoaib [209] showed that the CB₁ antagonist AM251 dose-dependently attenuated the reinstatement effects produced by the combination of nicotine-associated cues and a priming dose of nicotine.

The neuronal mechanisms through which CB₁ antagonists attenuate relapse remain largely unknown. However, Kudas and colleagues [226] recently identified key cortico-limbic structures mediating the effects of Rimonabant on cue-induced nicotine seeking in rats. They found that Rimonabant injected into the shell of the nucleus accumbens dose-dependently reduced cue-induced nicotine-seeking behavior without affecting locomotor activity. Similar results were obtained when Rimonabant was injected into the basolateral amygdala and in the pre-limbic cortex. These three regions express CB₁ receptors [227, 228] and are involved in drug-seeking behavior in animals [229–231] and humans [35, 232]. On the other hand, in agreement with the CPP and self-administration findings described above, we recently found that FAAH inhibition by URB597 can also block reinstatement effects produced by nicotine-associated cues and by priming injections of nicotine [Forget *et al.*, submitted; 173].

3.2.5 Nicotine-induced physical dependence—Chronic administration of nicotine in rodents can result in a state of “physical dependence” that can be unmasked and characterized by abruptly interrupting the administration of nicotine, or by administering a nicotine receptor antagonist like mecamylamine, resulting in the occurrence of a nicotine withdrawal syndrome [see ref. 233 for review]. Nicotine withdrawal in animals consists of a syndrome of spontaneous or precipitated signs including teeth-chattering/chewing, yawning, abdominal writhing/gasping, ptosis, shakes/tremors, rearing and jumping [234]. Castané and colleagues [165] showed that the nicotine antagonist mecamylamine precipitated a withdrawal syndrome with a similar severity index in wild-type mice and CB₁ receptor knockout mice chronically treated with nicotine. In agreement, the CB₁ receptor antagonist Rimonabant was not able to precipitate a withdrawal syndrome in nicotine-dependent animals [235]. Together these findings suggest that the endocannabinoid system, through CB₁ receptors, might not participate in the development and expression of nicotine physical dependence. On the other hand, Balerio and colleagues [235] showed that acute THC pre-treatment significantly decreased the severity of somatic manifestations of nicotine withdrawal precipitated by mecamylamine and naloxone. Therefore, an involvement of the endocannabinoid system in nicotine physical dependence cannot be excluded.

3.3 Clinical studies with potential therapeutic cannabinoid drugs

Consistent with preclinical data showing that blockade of CB₁ receptors by Rimonabant can decrease nicotine self-administration [62], prevent development and expression of nicotine-induced CPP [63, 64], prevent relapse to nicotine-seeking behavior [65], and prevent nicotine-induced dopamine elevations in the nucleus accumbens shell [62] in rats, clinical studies have also shown Rimonabant to be effective as an aid for smoking cessation. Rimonabant has been evaluated in multicenter, randomized, placebo-controlled Phase III clinical trials for smoking cessation and obesity reduction and results (yet unpublished) have been presented at various scientific meetings. Studies with Rimonabant and Tobacco Use (STRATUS) have enrolled over 6500 patients combined (*Sanofi-Aventis*. <http://en.sanofi-aventis.com/press/ppc-1960.asp#3>).

In a trial conducted in the United States (STRATUS-US), 787 subjects, each smoking at last 10 cigarettes a day, and wishing to quit, were randomised to receive either placebo, or 5 mg/

day or 20 mg/day Rimonabant. There were an equal number of men and women who smoked an average of 23 cigarettes per day. The study included a 2-week screening period, 10 weeks of treatment period, and 42 weeks of follow-up. The subjects' self-reports of having quit smoking were verified by measurement of carbon monoxide in expired air (10 ppm indicating cessation) and plasma cotinine concentration (8ug/L indicating cessation). In this study, the quit-rates at 10 weeks were 27.6% for the 20 mg Rimonabant treatment group, and 16% for both the 5 mg Rimonabant and the placebo treatment groups. The difference in results between subjects in the 20 mg Rimonabant and the placebo groups was significant ($P=0.004$). In addition, subjects assigned to the 20 mg Rimonabant treatment group lost an average of 0.3 kg, body weight whereas subjects that received placebo gained an average weight of 1.1 kg ($P<0.001$). Subjects who were overweight or obese at study onset lost weight when treated with 20 mg Rimonabant, but non-obese patients did not lose weight (Anthenelli RM, Dale L, in: *Final Program and Abstracts of 6th Annual Conference on the SRNT Europe; Tübingen, Germany; 2004. p.35*; Dale L, Anthenelli R, Despress JP, in *53rd Annual Scientific Session of the American College of Cardiology: New Orleans, LA, USA. 2004*).

A further study conducted in Europe enrolled 789 subjects who smoked 10 or more cigarettes/day for at least two months, and were motivated to quit smoking. The study used the same design as the STRATUS-US trial. Subjects were given 10 weeks of active treatment with Rimonabant or placebo with a 42 week follow-up. Subjects were randomly assigned to one of three treatment conditions (placebo; Rimonabant 5mg/day; Rimonabant 20mg/day). In this STRATUS Europe study (www.sanofi-aventis.com; *Sanofi –Aventis website*), the quit-rates for smoking were 25, 24, and 20% for the 20 mg Rimonabant, the 5 mg Rimonabant, and the placebo treatment groups, respectively, with no significant statistical difference between the three treatment groups.

Another STRATUS-META trial was conducted in the United States and involved only a double-blind Rimonabant versus placebo 10-week treatment course, with no extended follow-up. In a pooled analysis of this STRATUS-META trial with the short-term STRATUS-US and STRATUS-EU trials, which was presented at a scientific meeting (Cinciripini PM, Aubin HJ, Dale LC, Anthenelli R in: *8th Annual Conference of the SRNT Europe, Kusadasi, Turkey. 2006*), it appeared that the 5 mg/day dose of Rimonabant was not able to increase abstinence rates in smokers. However, the largest 20 mg/day dose of Rimonabant, which was evaluated in around 800 subjects, significantly increased abstinence rates compared to placebo and produced significant effects on weight gain compared to placebo.

A STRATUS-WORLDWIDE trial with a different study design was carried out in Australia, Canada and the United States. This study included a total of 5055 cigarette smokers who were randomized into two treatment groups: 5 mg/day or 20 mg/day Rimonabant. Then, the successful quitters (1672) were re-randomized to either placebo, 5 mg Rimonabant, or 20 mg Rimonabant. This experimental design allowed evaluation of Rimonabant effects on relapse rates. Interestingly, equally high proportions of subjects treated with 5 mg/day Rimonabant and with 20 mg/day Rimonabant were abstinent at 32 weeks and the percent of subjects abstinent in both Rimonabant groups were significantly higher than those receiving placebo (51.9%; $p<.001$ and 50.9%; $p<.05$ vs. placebo, respectively), suggesting that a lower dose of 5 mg/day Rimonabant, that itself cannot increase rates of abstinence, may be able to prevent relapse to tobacco smoking (Niaura RD, Dale L, Anthenelli R, in: *101st International Conference of the Thoracic Society, San Diego, Ca, USA, 2005*). Interestingly, only the higher dose of Rimonabant produced effects on post-cessation weight gain, in agreement with the previous literature on effects of Rimonabant on weight gain [236–238].

Finally, a recent STRATUS-CIRRUS trial was designed to test the potential effectiveness of adding a nicotine patch to Rimonabant oral treatment for smoking cessation. This trial enrolled 755 smokers who were given Rimonabant (20 mg/day) open-label for nine weeks. Target quit date was set at 1 week. At that time participants were randomly assigned to receive a nicotine patch (21mg for 8 week, 14mg for 1 week and 7 mg for 1 week) or placebo (for 10 weeks) in addition to Rimonabant oral treatment. Participants had brief cessation counselling at each visit and were followed-up until 26 weeks post treatment. The primary endpoint was sustained abstinence from week 6 to week 9, verified by carbon monoxide concentration. Interestingly, the combination of Rimonabant and nicotine replacement therapy was more effective in elevating smoking abstinence rates than Rimonabant plus placebo (*Cinciripini PM, Aubin HJ, Dale LC, Niaura R, Anthenelli RM 8th Annual Conference of the SRNT Europe, Kusadasi, Turkey, 2006*).

4. Limitations of existing medications

The drugs currently approved by the US Food and Drug Administration (FDA) for smoking cessation include nicotine-replacement therapy (NTR), which can be found in the form of transdermal patches, gum, nasal sprays, oral inhalers, and lozenges [239]; the antidepressant, bupropion [240]; and, most recently, the 4- 2 selective nAChR partial agonist, varenicline [241]. As mentioned in the introduction, although available pharmacotherapy can improve success rates, the rate of smoking cessation by subjects entering into clinical trials that combine effective medication and behavioral and cognitive therapy is around 30% at the end of the first year; the vast majority of subjects relapse [7, 8].

The use of medications approved for nicotine dependence is associated with some side effects [7]. For instance, the most common side effects reported for bupropion were insomnia, occurring in 30–40% of patients, dry mouth (10%) and nausea [33]. The main reported adverse effects of varenicline were nausea and abnormal dreams. It is important also to consider that the main contraindication for the use of bupropion is the increased risk of seizures in those patients with a history of alcohol withdrawal, anorexia or head trauma. Moreover, in early 2008, the Food Drug Administration added serious neuropsychiatric symptoms to the warnings section of the prescribing information for varenicline. This measure was taken because symptoms such as changes in behavior, agitation, depressed mood, suicidal ideation, and attempted and completed suicide were recorded in patients taking varenicline. Although these symptoms are rare, they should be carefully monitored [242, 243].

Although the initial studies of the cannabinoid CB₁ inverse agonist/antagonist Rimonabant for smoking cessation that were performed in selected populations were reassuring, an increased risk of anxiety and depression conferred by exposure to Rimonabant was identified in two meta-analysis studies in obese populations [152, 244] and by a report from the US Food and Drug Administration (*Food and Drug Administration Endocrinologic and Metabolic Advisory, June 13, 2007*). Due to such psychiatric side effects, Rimonabant has not yet been approved for smoking cessation. It is also clear that since the prevalence of mental health disorders is very high in subjects addicted to marijuana or other illicit drugs, it is possible that these secondary effects will occur at even higher rates in those populations addicted to both tobacco and marijuana or other illicit drugs and tolerability studies should be performed.

Thus, there is a need for better therapeutic approaches for the clinical treatment of smokers. Clinicians typically use a monotherapy first, and if not effective, there is the possibility of combining treatments [7, 8]. Although there is no strong evidence yet to support treatment combinations over monotherapy, preliminary studies indicate that a combination of nicotine

replacement and Rimonabant is well tolerated and may produce increased efficacy (Cinciripini PM, Aubin HJ, Dale LC, Niaura R, Anthenelli RM 8th Annual Conference of the SRNT Europe, Kusadasi, Turkey, 2006). This effect should be validated and might allow future development of novel and alternative therapeutic strategies. It should also be mentioned that Rimonabant may be particularly useful for individuals who wish to avoid weight gain following smoking cessation. Post-cessation weight gain concerns may be an important deterrent to stop smoking. The probability of attempting to stop smoking appears to be lower in subjects reporting smoking-related weight concerns [245–249].

Since Rimonabant has been shown to be efficacious in reducing the effects of a wide range of addictive substances [250], its efficacy for reducing polydrug use could be further explored, for example in those subjects addicted to nicotine and alcohol. In this respect, it will be of particular clinical interest to evaluate the therapeutic effects of Rimonabant or other CB₁ antagonists in subjects addicted to both tobacco and marijuana, since CB₁ receptors clearly mediate the reinforcing effects of marijuana [251].

5. Conclusions

Nicotine addiction is still one of the main health concerns world-wide, and is likely the most preventable cause of death in developed countries. This is in spite of the social and medical efforts expended over the last 30 years in the search for behavioral or pharmacological therapeutic strategies that could help people effectively quit smoking and resist relapsing to nicotine addiction.

There is a growing body of scientific evidence pointing to the endocannabinoid system as a new pharmacological target for discovery and development of medications in the fields of drug abuse and obesity, diseases in which motivation likely plays a major role. Consequently, modulation of endocannabinoid system activity has been the subject of many exciting discoveries in the addiction field over the past few years and the endocannabinoid system is emerging as a novel molecular target for the discovery of medications for tobacco dependence. Initial studies suggested cannabinoid CB₁ receptor antagonists as a promising approach to development of medications for tobacco dependence while the most recent studies have focused on modulation of endocannabinoid signaling by blockade of endocannabinoid deactivation.

Although Rimonabant, the first discovered antagonist of cannabinoid CB₁ receptors, has been shown to attenuate several of the neurobiological consequences of nicotine administration, the demonstration of its clinical utility has been delayed. A recent review of non-nicotinic therapies reported that rimonabant produced inconsistent results in Phase 3 smoking cessation trials [see ref 252 for review]. It appears that its global efficacy is limited and that there is a potential for psychiatric side effects (*Food and Drug Administration Endocrinologic and Metabolic Advisory, June 13, 2007*). Such limitations have, so far, prevented the introduction of Rimonabant as an approved medication for smoking cessation. Nonetheless, further clinical trials may allow the introduction of Rimonabant as a nicotine addiction medication in the future. On the other hand, research in the cannabinoid field in the past ten years has made huge progress. We have available today different antagonists of CB₁ receptors, with some of them showing neutral competitive antagonism, as compared to the inverse-agonist activity of rimonabant and AM251 [155, 157]. These neutral antagonists together with newly developed allosteric agonists [160] might be promising pharmacological tools in developing efficacious medications for nicotine addiction with a lower incidence of unwanted side effects. Another new target for medication development for nicotine addiction might come from the possibility of modulating endocannabinoid neurotransmission through blockade of the enzymatic metabolic pathways involved in

endocannabinoid degradation. In this respect, in a recent series of experiments it has been suggested that blockade of FAAH by URB597 attenuates both neurochemical and behavioral effects of nicotine in rats [173].

In conclusion, the endocannabinoid system plays a fundamental role in different neurobiological processes related to nicotine dependence. There has been a rapid evolution of our knowledge about the brain endocannabinoid system which has provided new molecular targets for the development of medications for nicotine addiction. Some of these medications might be available to the public in the near future, if future clinical trials for rimonabant-like inverse agonists/antagonists provide favorable risk/benefit outcomes. The FAAH inhibitor URB597 is also entering clinical investigation for analgesia, so clinical testing of FAAH inhibitors for nicotine addiction might soon be possible. Finally, neutral competitive CB₁-receptor antagonists and allosteric inhibitors are now at the preclinical testing stage, and they hold promise as future compounds for clinical trials as nicotine addiction medications.

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