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Rimonabant: Just an Anti-obesity Drug?

Current Evidence on Its Pleiotropic Effects

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Running title: Current evidence on rimonabant

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ABBREVIATIONS: 2-AG, 2-arachidonoyl glycerol; ACC1, acetyl coenzyme-A carboxylase; AEA, anandamide, *N*-arachidonoylethanolamine; BDV, Borna disease virus; BMI, body mass index; CART, cocaine-amphetamine-regulated trascript; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; CHD, coronary heart disease; CHO, chinese hamster ovary; CVD, cardiovascular disease; DA, dopamine; ERK, extracellular signal-regulated kinase; FAS, fatty acid synthase; GABA, gamma-aminobutyric acid; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GTPγS, guanosine 5'-*O*-(thiotriphosphate); HDL, high-density lipoproteins; HOMA-IR, homeostasis model assessment - insuline resistance; IGF1 insulin-like growth factor 1; Ki-Mol, K-ras-transformed FRTL-5 thyroid cells; LPS, lipopolysaccharide; MAP mitogen-activated protein; MAPK, MAP kinase; Met-F-AEA, 2-methylarachidonyl-2'-fluoro-ethylamide; METH, metamphetamine; msP rats, Marchigian Sardinian alcohol preferring rats; NCEP-ATPIII, national cholesterol education program's adult treatment panel III; NPY, neuropeptide Y; OX1R, orexin 1 receptor; (PDGF)-β, platelet-derived growth factor β; RIO, rimonabant in obesity; SR141716, *N*-(piperidino-1-yl)-5-(4-chlorophenyl)-1-(2,4dichlorophenyl)-4-methyl-pyrazole-3-carboxamide; SREBP, sterol regulatory element binding proteins; STRATUS, studies with rimonabant and tobacco use; TGF-β1, transforming growth factor β1; THC, Δ^9-tetrahydrocannabinol; TNFα, tumor necrosis factor α.

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ABSTRACT

The advent of the highly selective cannabinoid receptor (CB1) antagonist, rimonabant (SR141716, Acomplia^{TM}) can revolutionize the ability of the clinicians to manage obesity. Large-scale clinical trials have demonstrated that rimonabant therapy can reduce obesity. Although, the precise mechanisms of action of rimonabant have to be further dissected, it is emerging, from both pre-clinical and clinical research, that rimonabant is not only an anti-obesity drug but its pleiotropic functions affect a broad range of diseases, from obesity related co-morbidities to drug dependence and cancer. Here we review recent data from the literature and discuss the full pharmacological potential of this drug.

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Introduction

Studies on the effect of marijuana psychoactive principle Δ^9-tetrahydrocannabinol (THC) have evolved into the discovery and description of the endocannabinoid system. So far, this system is composed of two receptors, the widely expressed CB1 and the more restricted CB2, five endogenous lipid-like ligands including the well known endocannabinoids anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) and the enzymes involved in their biosynthesis and degradation (for a review see Mechoulam et al., 1998; De Petrocellis et al., 2004; Di Marzo et al., 2004). Starting from the discovery of the endocannabinoid system, a number of studies have pointed out that altered endocannabinoid signaling and CB1 receptor expression are involved in several pathophysiological situations ranging from neurological and psychiatric diseases to eating, cardiovascular and reproductive disorders. More recently it has been described that CB1 receptor stimulation by the endocannabinoid AEA can negatively modulate cancer cell proliferation in vitro (Bifulco et al., 2001, 2004) as well as tumour growth and metastatic spreading in vivo (Portella et al., 2003; Bifulco et al., 2006a, 2006b). CB1 or CB2 antagonistic or inverse agonistic compounds have been used to investigate the endocannabinoid network and its integration with other signalling transduction pathways (for a review see Lange and Kruse, 2005). The first highly selective CB1 receptor antagonist was discovered by Sanofi-Aventis and was the so-called rimonabant (SR141716, Acomplia™, Sanofi-Aventis) (Rinaldi-Carmona et al., 1994). It showed a number of biological effects in vitro and in vivo in several pathological situations. Because an update on the pleiotropic effects of rimonabant does not exist, while the knowledge concerning endocannabinoid system has been expanded considerably, we critically analyze the current literature on the pharmacological potential of rimonabant. We aim to describe both the clinical efficacy and the biological activity of rimonabant examining, as well as possible, also the molecular aspects at the basis of rimonabant-induced effects. Firstly, we will discuss the efficacy and

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safety of rimonabant in reducing body weight and cardiometabolic risk factors. Secondly, we will review the increasing literature on the other potential therapeutic properties of the CB1 receptor blocker rimonabant on behavior and in disorders related to the central nervous system. Thirdly, we will examine recent results about the anti-proliferative effects of rimonabant. Finally, we will discuss current data on rimonabant action as a modulator of reproductive system functions.

In Vitro And In Vivo Pharmacology of Rimonabant

The discovery of endocannabinoid system prompted the development of CB1- and CB2-selective antagonists, the first of which was the CB1-selective rimonabant, SR141716 (*N*-(piperidino-1-yl)-5-(4-chlorophenyl)-1-(2,4dichlorophenyl)-4-methyl-pyrazole-3-carboxamide) (Fig. 1). Binding studies have demonstrated that rimonabant is a potent (pK_i=8.4) and selective ligand for CB1 receptors, showing a high affinity (K_i=5.6 nM) for the CB1, and low affinity (K_i>1000nM) for the CB2 receptor (Rinaldi-Carmona et al., 1994). Moreover it displays a weak affinity to $Galanin_2$, MC_5, $opioid_\kappa$, and PCP receptors (Compton et al., 1996; Shire et al., 1996).

Functional studies confirmed its potent (pA_2 7.98–8.85) and selective CB1 receptor antagonistic activity. This compound readily displaced [^{3}H]CP 55,940 from specific binding sites (K_i=1.98 nM), and has been shown to prevent cannabinoids from producing several of their typical effects, both in vitro and in vivo. Rimonabant potency as an antagonist has been shown by comparing its ability to attenuate WIN 55,212-2-induced inhibition of electrically evoked contractions of the mouse isolated vas deferens (K_d=2.4 nM) with that of WIN 56,098, bromopravadoline and iodopravadoline (Pertwee et al., 1995). It was an effective antagonist also in vivo, by suppressing the hypothermia elicited by WIN 55,212-2 and psychomotor effects in mice and rats (Perio et al., 1996) (see also Table 1).

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Saturation binding experiments with membranes prepared from rat cerebellum have shown that radiolabeled rimonabant undergoes specific, rapid, saturable, high-affinity binding to a single class of sites. This specific binding is little affected by micromolar concentrations of a variety of noncannabinoid receptor ligands. However, it is readily attenuated by the cannabinoids CP 55,940, WIN 55,212-2, Δ^9-THC, ll-hydroxy- Δ^9-THC, and AEA. Interactions with rimonabant are competitive in nature for CP 55,940, WIN 55,212-2 and Δ^9-THC, but noncompetitive for AEA, since the latter compound decreases both the affinity constant and the B_{max} of radiolabeled rimonabant (Petitet et al., 1996; Rinaldi-Carmona et al., 1996). Shire et al. (1996) have carried out experiments to identify the domain(s) of the cannabinoid CB1 receptor responsible for the recognition and binding of rimonabant. Their approach was to transfect COS-3 cells with mutated CB1 receptors or with a range of different chimeric CBl/CB2 receptors. The results obtained suggest that the fourth and fifth transmembrane domains of the CB1 receptor are essential for high-affinity binding of rimonabant, whereas the extracellular loop between these two domains is unimportant.

In some experiments, rimonabant has been found to produce effects that are opposite in direction to those produced by cannabinoid receptor agonists. In particular, it can increase locomotor activity in mice (Compton et al., 1996), improve social short-term memory in rats and mice (Terranova et al., 1996), augment forskolin-induced stimulation of cyclic AMP production in cells transfected with CB1 (Felder et al., 1995), increase the amplitude of electrically evoked contractions of various isolated tissue preparations (Pertwee et al., 1996) and enhance electrically evoked release of acetylcholine both from rat hippocampal slices and the myenteric longitudinal muscle of guinea-pig small intestine (Gifford and Ashby, 1996).

Increasing evidence suggest that rimonabant behaves also as an inverse agonist in some membrane preparations. Indeed, Bouaboula et al. (1997) found that CHO cells, transfected with the CB1 receptor, displayed high constitutive activity of both MAPK and adenylate

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cyclase and this increase was inhibited by rimonabant. They also observed that [^{35}S]GTPγS enhanced the binding of rimonabant, a feature usually described for inverse agonists. The issue on inverse agonistic properties of rimonabant has been reviewed thoroughly by Pertwee (2005).

The pharmacokinetic/pharmacodynamic profile of rimonabant, as expected by both preclinical and clinical studies showed that rimonabant is distributed widely in brown fat, it could reduce total energy intake and body weight gain in obese rats and the most effective dose in reducing body weight in obese human subjects was 20 mg/day (Table 1).

Effect of Rimonabant on Weight Loss and Cardiovascular Risk Factors

The assessment of the clinical efficacy of rimonabant as an anti-obesity drug was carried out in multi-national, randomized and placebo-controlled trials on overweight (with a body mass index - BMI - higher than 27 kg/m^2) or obese (BMI ≥ 30 kg/m^2) patients (Van Gaal et al., 2005; Pi-Sunyer et al., 2006). A cumulative weight loss and a significant change of waist circumference from the baseline were observed in patients receiving 20 mg/day of rimonabant. Interestingly, rimonabant caused also an increase of high density lipoprotein (HDL) cholesterol levels, and a reduction of triglycerides, fasting insulin and insulin resistance derived from homeostasis model assessment (HOMA-IR) which was calculated by multiplying fasting insulin by fasting glucose and dividing by 22.5 (Pi-Sunyer et al., 2006).

After one year of rimonabant treatment (20 mg/day) the levels of HDL cholesterol, triglycerides and the other above mentioned parameters were twice those attributable to the concurrent weight loss alone as assessed by analysis of covariance (Pi-Sunyer et al., 2006). In addition, in patients who completed the study in the second year and received 20 mg of rimonabant, levels of triglycerides and fasting insulin declined rapidly from baseline

suggesting a direct pharmacological effect of rimonabant on glucose and lipid metabolism outside the weight loss achieved (Pi-Sunyer et al., 2006).

In another recent trial aimed to assess the effects of rimonabant in overweight patients with dyslipidemia (Déspres et al., 2005), a significant weight loss was reached in the majority of patients completing the 12 month study and receiving 20 mg/day of rimonabant. During the treatment, the levels of triglycerides significantly decreased, whereas HDL cholesterol increased compared with both placebo group and that treated with 5 mg/day rimonabant. Furthermore, the prevalence of metabolic syndrome, a collection of factors (abdominal adiposity, hypertriglyceridemia, low HDL cholesterol, hypertension and fasting hyperglycemia) increasing the risk of type 2 diabetes and cardiovascular disease (Nesto, 2005), was reduced in the same subset of patients (Pi-Sunyer et al., 2006). Indeed, the population of patients matching the National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATPIII) criteria for the metabolic syndrome (54% of the total), fell to the half of baseline after they received 20 mg rimonabant (Déspres et al., 2005).

The overall observations strongly suggest that rimonabant could modulate positively risk factors for a number of obesity related co-morbidities through weight loss dependent and independent pathways. In the sense of testing the clinical efficacy of the drug in new therapeutic strategies, we point out that future research should be focused on its potential pharmacological action in primary clinical outcome of the metabolic syndrome e.g. cardiovascular disease (CVD), coronary hearth disease (CHD), cerebrovascular disease and peripheral artery disease, that have been associated with the high prevalence of the metabolic syndrome (Nesto, 2005). Of note, the treatment with 20 mg rimonabant for 1 year increases significantly the levels of plasma adiponectin compared to the placebo group (Déspres et al., 2005). This finding is not unexpected since an enhanced adiponectin serum level has been associated with weight reduction (Yang et al., 2001). Indeed, serum adiponectin levels are

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inversely correlated to obesity, metabolic syndrome and type 2 diabetes (Gable et al., 2006), therefore the observation that rimonabant improves the levels of either adiponectin or fasting insulin and induces favorable changes in HOMA-IR, strongly suggests a possible pharmacological application of rimonabant in diabetes. Recently it has been demonstrated that 20 mg/day of rimonabant can improve a number of cardiovascular and metabolic risk factors in overweight or obese patients with type 2 diabetes (Scheen et al., 2006).

To date, safety evaluation and recording of adverse events from all RIO studies have reported nausea, diarrhea and upper respiratory tract infections in the first month of treatment. Serious adverse effects, such as psychiatric (depressed mood disorders), nervous system and gastrointestinal disorders occur more frequently in 20 mg/day treated patients compared to the placebo group during the first year (Van Gaal et al., 2005; Pi-Sunyer et al., 2006; Despres et al., 2005). Further studies are required to assess the long term effects of rimonabant, beneficial or adverse, aimed to prove the safety and efficacy of rimonabant beyond two years.

Although rimonabant has been shown to be a powerful agent for the treatment of obesity, as assessed by the mentioned clinical trials, its biological mechanism of action is not clear yet. There are key questions that still have to be tackled, *id est*, is the weight loss induced by the CB1 receptor blocker rimonabant a consequence of an action to central level or is it the result of a control at peripheral energy metabolism and which are the signal transducing events involved in weight loss?

In nongenetic-induced obese mice, body weight loss seems to consist of an early phase that depends on regulation of food intake and in a second phase, food intake regulation independent, in which weight loss is maintained probably through a sustained reduction of adiposity. Therefore, it is conceivable that rimonabant interferes with both the regulation of the expression and release of hypothalamic neuropeptides involved in the control of appetite and with peripheral metabolism. In agreement with this hypothesis, the treatment with

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rimonabant of mice lacking the cocaine-amphetamine-regulated transcript (CART), an anorexigenic peptide, does not affect feeding behavior (Ravinet Trillou et al., 2003), whereas this compound inhibits starvation-induced hyperphagia in neuropeptide Y (NPY) deficient mice and reduced body weight in leptin knockout mice (Di Marzo et al., 2001). It is likely that the reduction of body weight by rimonabant involves the modulation of more than one orexigenic pathway controlling food intake at central level. In this sense, recent data showing a functional co-localization of CB1 receptor and orexin 1 receptor (OX1R), have demonstrated that the treatment with rimonabant of CHO cells expressing both receptors, completely prevented orexin response, thus providing further insights that the weight loss achieved in vivo could be partly ascribed to the blockade of CB1/OX1R cross-talk (Hilairet et al., 2003). On the other hand the finding that the CB1 receptor-mediated lipogenetic activity in primary murine adipocytes can be blocked by rimonabant (Cota et al., 2003), intriguingly supports the idea that this compound may also function at peripheral level by decreasing lipoprotein lipase activity. In line with this hypothesis, in obese Zucker (fa/fa) rats rimonabant stimulates Acrp30 mRNA, an adipocytokine exclusively expressed by adipocytes and regulating fatty acid oxidation. This stimulation has been also found to occur within 30 min of rimonabant treatment of cultured mouse 3T3 F442A preadipocyte cells (Bensaid et al., 2003). The Acrp30 mRNA inhibition was accompanied by a reduced adipocyte proliferation and an enhanced cell maturation (Gary-Bobo et al., 2006). The effect is mediated by the inhibition of MAPK phosphorylation. Interestingly, rimonabant working as an inverse agonist, is able to switch off MAPK activation from the insulin receptor-tyrosine kinase and insulin-like growth factor (IGF1) receptors (Bouaboula et al., 1997). Moreover, endocannabinoid tonic activation of CB1 receptor in liver induces mRNA expression of the lipogenic transcription factor SREBP-1c, its target enzymes acetyl coenzyme-A carboxylase (ACC1) and fatty acid synthase (FAS), also increasing the rate of fatty acid synthesis (Osei-Hyiaman et al., 2005).

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Blockage of CB1 receptor stimulation by rimonabant significantly reduces de novo fatty acid synthesis in mice, thus providing the evidence for the involvement of fatty acid biosynthetic pathway in the sustained reduction of body weight. The reported outcomes explain how rimonabant induces its effects at central and peripheral level, even though it would be of such interest to study whether other G-protein and/or orexigenic pathways could be responsible for the rimonabant-induced anti-obesity effects and whether there are mechanisms of action independent from the interaction with the CB1 receptor.

Rimonabant Induced Effects on Drug Dependence

CB1 receptors are expressed at high levels in brain regions thought to play a key role in relapse-like behavior and conditioning processes. In these regions, CB1 receptors modulate the release of a variety of neurotransmitters (De Vries and Schoffelmeer, 2005a) implicated in drug seeking behavior, smoking cessation and alcohol addiction suggesting that rimonabant could offer a novel approach for the treatment of behavioral related disorders (for a review see Beardsley and Thomas 2005; De Vries and Schoffelmeer, 2005a).

Smoking cessation. Pharmacological studies aimed to investigate the effect of rimonabant on motivational effects of nicotine in rats, demonstrated that a single administration of rimonabant (0.3 mg/Kg and 1.0 mg/Kg) on two consecutive days reduced nicotine infusions and the presses of active lever from the first day of treatment (Cohen et al., 2002). Giving the findings that rimonabant reduced responding maintained by nicotine-associated cues, even after several months of nicotine abstinence, and antagonizes dopamine release caused by nicotine addiction, it is reasonable that the CB1 receptor antagonist can reduce both nicotine seeking behavior and nicotine reinforcing effects, probably by preventing nicotine induced dopamine (DA) release in limbic dopaminergic areas during self administration (Cohen et al., 2002; Cohen et al., 2005). The action of rimonabant on both

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reducing nicotine craving and weight gain may be, at least in part, the result of a common mechanism involving dopamine receptors mediated process (Duarte et al., 2004). With this respect, studies of rimonabant treatment on conditioned responding for nicotine and sucrose associated cues in a long term extinction-reinstatement animal model, demonstrated a strongly decrease cue-induced reinstatement of nicotine and sucrose seeking behavior (De Vries et al., 2005b). The blockade of CB1 receptors attenuated responding for stimuli previously associated with nicotine infusion and sucrose delivery in a similar fashion. Preclinical studies seem to support this evidence: *a)* rimonabant reduces nicotine self administration, DA turnover in nucleus accumbens and attenuates reinstatement of nicotine seeking behaviour (Fagerstrom and Balfour, 2006); *b)* The STudies with Rimonabant And Tobacco Use (STRATUS-US) enrolling 787 smokers at 11 clinical trial sites in the U.S. showed that 36% of patients treated with 20 mg/day rimonabant quitted smoking, whereas only 20.6 % of patients treated with placebo and 20.2% of patients treated with 5 mg/day of rimonabant were successful (Anthenelli and Despres, 2004). Previous findings suggest that rimonabant may represent a novel drug for the treatment of tobacco dependence and may be effective not only as an aid for smoking cessation, but also in the maintenance of abstinence.

Ethanol dependence. A large body of research lines suggests functional interactions between the cannabinoid receptor CB1 and ethanol dependence; CB1 receptor is also involved in the mechanism mediating alcohol relapse (Maldonado et al., 2006). Rimonabant treatment induces a decrease in voluntary ethanol intake in alcohol preferring rats (Colombo et al., 1998) and decreases ethanol consumption in mice (Arnone et al., 1997). Recently, Cippitelli and co-workers (2005) analyzed the effects of cannabinoid receptor blockade by rimonabant on alcohol self-administration and cue-induced relapse in wistar and genetically selected alcohol preferring (msP) rats, in which the acquisition of ethanol self-administration was quicker than in wild-type strain. Rimonabant was found to reduce either ethanol self

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administration or cue-induced relapse to ethanol self administration, being more efficacious in the msP rats than in wistar rats. Interestingly, strong differences in CB1 receptor mRNA levels between ethanol-naïve msP and wistar rats have been found, suggesting that the effects of rimonabant could be ascribable to the genetically determined different levels of CB1 receptor in brain region responsible for ethanol dependence. This assumption is also supported by the finding that $CB1^{-/-}$ mice and rimonabant treated mice consume significantly less alcohol than the corresponding untreated wild-type mice (Wang et al., 2003). However, a reduction of the reinforcing properties of ethanol in the self administration paradigm has also been observed in animal models of ethanol seeking elicited by environmental stimuli (Economidou et al., 2006). Therefore, considering the rimonabant-mediated reduction of reward-related responding to ethanol, it could be of relevant interest to test this compound in preclinical studies, aimed at proving its pharmacoterapeutic properties in the treatment of ethanol dependence.

Psycostimulants and opioid seeking. Although rimonabant administration does not interfere with cocaine self administration in monkeys (Tanda et al., 1997) and relapse induced by exposure to stress in rats, it reduced relapse to cocaine seeking behavior produced by re-exposure to cocaine associated cues (De Vries et al., 2001). Similarly, rimonabant blocked the relapse of methamphetamine (METH) seeking behaviour in rats also preventing the reinstatement of METH-seeking behavior, when administered prior to the cue phase of the test session (Anggadiredja et al., 2004).

Concerning opioids, several studies have revealed solid evidence for the existence of a functional interaction between cannabinoid and opioid system, suggesting that CB1 receptor may play an important role in the mechanism underlying relapse to heroin seeking (Tanda, et al., 1997, Navarro et al., 2001). Mainly, in opiate self administration and opiate induced place preference in rats and mice, using heroin and morphine, acute administration of rimonabant

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(3.0 mg/Kg) reduced heroin self administration in wistar rats and blocked heroin and morphine self administration in mice. Since rimonabant does not interact directly with opioid receptors, it could interact with dopaminergic system in the nucleus accumbens, attenuating extra-cellular dopamine release (Navarro et al., 2001). Furthermore, rimonabant attenuated (about of 50%) the reinstatement of heroin seeking behavior caused by both a priming injection of heroin and a re-exposure to heroin paired stimuli. In addiction, rimonabant dose dependently reduced responding for heroin in fixed and progressive ratio schedules of reinforcement (De Vries et al., 2003; Spano et al., 2004). These observations indicate that the selective CB1 receptor antagonist rimonabant might be used to attenuate both the reinforcing/motivational properties of heroin and the reinstatement of heroin seeking after prolonged withdrawal (Fattore et al., 2005).

Rimonabant Induced Effects on Neurodegenerative Disorders

Until endocannabinoid system was discovered, the role of CB1 receptor in the physiology and pathology of nervous system has received particular attention because of its selective and relatively high expression within the central nervous system. The CB1 receptor is localized mostly in the brain (Herkenham et al., 1990) and spinal cord (Herkenham et al., 1991a) being expressed in the output nuclei of basal ganglia, in the substantia nigra pars reticulata and globus pallidus. Intermediate receptor levels have also been found in the cortex, hippocampus, thalamic nuclei, hypothalamus and cerebellum (Herkenham et al., 1990; 1991a, b; Jansen et al., 1992; Thomas et al., 1992). The endogenous ligands of CB1 receptor are synthesized upon demand by neurons in response to depolarization (Freund et al., 2003) and, once released from postsynaptic neurons, they can function as retrograde synaptic messengers. They travel backward across synapses, activate CB1 on presynaptic axons and inhibit neurotransmitters release (Szabo et al., 1998; Wilson and Nicoll, 2002). Because of

this properties, the endocannabinoid system could offer new pharmacological targets to alleviate motor symptoms and supply neuroprotection in neurological disorders such as Parkinson's and Alzheimer's disease, Huntington's chorea and multiple sclerosis. Despite the projectile findings on this issue, recently well reviewed (Fernandez Ruiz and Gonzales, 2005; Robson, 2005; Valverde et al., 2005; Walker and Hohmann, 2005; Pertwee, 2006), inconclusive results were reported on early study carried out with rimonabant. Rimonabant increased the frequency and duration of seizures in a rat model of viral encephalopathy (Borna disease virus rats) (Solbrig et al., 2005), whereas the hyperkinetic state (vertical activity) induced by L-dopa was decreased by the subcutaneous injection of rimonabant in the reserpine-treated rat model of Parkinson's disease. On the other hand, discrepant results have been obtained about the effects of this compound on quinpirole-induced hyperactivity. Rimonabant administered alone does not show evident effects on motor activity (Giuffrida et al., 1999; Segovia et al., 2003) but when injected 1 h prior to quinpirole, it potentiates motor stimulation, thus suggesting a complex interaction between CB1 receptor, its agonists and dopamine receptors (Segovia et al., 2003). Moreover, rimonabant reverses the neuroprotective effect of CB1 agonists in primary neuronal cultures from spinal cords in vitro (Abood et al., 2001) and in animal models of both genetic and pharmacological-induced Huntington's disease in vivo (Lastres-Beker et al., 2003; Centonze et al., 2005), causing also epileptic activity during development (Bernard et al., 2005). Surprisingly, rimonabant was ineffective in producing hyperalgesia in rats and by itself is able to exert anti-nociceptive effect probably by blocking the action of CB1 receptor stimulation on presynaptic GABA release (Naderi et al., 2005).

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Rimonabant Anti-Proliferative Effects

First evidence on the anti-proliferative effects of rimonabant took place from the hypothesis that rimonabant highly anti-obesity effects in rodent models, could be the result of both a central and metabolic peripheral action on adipose tissue. Since hyperplasia of adipose tissue is a crucial event for the development of obesity, the effect of rimonabant has been investigated on mouse preadipocytes. A reduced proliferation and an induced late maturation of adipocytes, without lipid droplets accumulation, mediated by an inhibition of basal and serum-induced p42/44 MAPK pathway was observed (Gary-Bobo et al., 2006). The MAPK pathway, strongly activated by high-fat diet in white adipose tissue and required for the development of obesity condition (Bost et al., 2005), is inhibited by rimonabant through pertussin toxin-sensitive tyrosine kinase receptors such as insulin or insulin-like growth factor 1 receptors, therefore displaying a negative intrinsic activity ascribable to inverse agonism (Bouaboula et al., 1997; Landsman et al., 1997).

The endocannabinoid system is implicated in the pathogenesis of chronic liver diseases associated with hepatic fibrosis (Mallat and Lotersztajn, 2006) and particularly CB1 receptors are highly upregulated in human cirrhotic specimens and in liver fibrogenic cells. Recent findings have underlined that CB1 receptor antagonism by rimonabant administration in mice, counteracts the wound-healing response to acute liver injuries, by decreasing the accumulation of hepatic myofibroblasts and the levels of the profibrogenic cytokine TGF-β1. The anti-proliferative effect depends on CB1 receptor signaling, as revealed by the absence of anti-proliferative effects in $Cnr1^{-/-}$ hepatic myofibroblasts. As previously observed in several cell systems, the molecular pathways mediating CB1 antagonism effects involve decreased phosphorylation of ERK MAPK and Akt, both in $Cnr1^{-/-}$ cells and in wild-type cells treated with rimonabant (Teixeira-Clerc et al., 2006).

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Altogether these findings provide evidence for an anti-fibrotic effect of rimonabant and suggest that rimonabant might represent a therapeutic tool for the treatment of some liver pathological conditions in humans.

Rimonabant Anti-Tumour Effects

The studies conducted from the late 1990s on the endocannabinoid system have provided strong evidence for a key role of the endocannabinoids in the control of cancer cell growth, invasion and metastasis processes in a way dependent on CB receptor activation (for a review see Bifulco and Di Marzo, 2002; Bifulco et al., 2006a; 2006b).

The selective CB1 receptor antagonist rimonabant, attenuates the anti-tumour effects of anandamide related compounds or other cannabinoid agonists in thyroid, breast and prostate cancers (Grimaldi et al., 2006; Portella et al., 2003; Bifulco et al., 2001; Sarfaraz et al., 2005), the effects being dependent on CB1 receptor activation. In other tumour types, like glioma, rimonabant failed to revert the anti-proliferative action of cannabinoid agonists whereas the selective CB2 antagonist, SR144528 (Sanchez et al., 2001) or a combination of the CB1/CB2 antagonists can partially prevent this effect (Jacobsson et al., 2001). However, a 48 h preincubation with these antagonists seems to enhance the AEA-mediated cell death of glioma cells, suggesting a more complex mechanism of action (Maccarrone et al., 2000a).

Considering the anti-tumour properties of the cannabinoid receptor agonists, it could be expected that cannabinoid receptor antagonists, like rimonabant, if used alone, would enhance proliferation of normal and malignant cells leading to cancer. Some data excluded this possibility, rather reporting that not only agonists to cannabinoid receptors but also antagonists, used alone, are able to inhibit cancer growth (Bifulco et al., 2004) or induce apoptosis in cancer cells (Derocq et al., 1998; Powles et al., 2005).

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The first observation of a rimonabant potential anti-tumour action was provided by our group in rat thyroid cancer cells (KiMol) in vitro and in thyroid tumour xenografts induced by KiMol injection in athymic mice. In this model, rimonabant was able to partially prevent the anti-tumour effect of the inhibitors of endocannabinoid degradation, and of the anandamide metabolically stable analogue (Met-F-AEA). However, rimonabant, when used alone, in the same model and at the same dose previously shown to counteract the Met-F-AEA effect (0.7 mg/kg intratumoural, twice a week for two weeks), did not enhance tumour growth exerting a small, but significant, anti-tumour effect on thyroid tumours, both in vitro and in vivo (Bifulco et al., 2004).

Interestingly, micromolar concentrations of rimonabant decreased viability of primary mantle lymphoma cells isolated from tumor biopsies of two patients, after treatment with micromolar concentrations of rimonabant (Flygare et al., 2005). Moreover rimonabant showed an additive negative effect also on the viability of the mantle cell lymphoma cell line Rec-1 when combined with equipotent doses of AEA. Both Bifulco and Flygare supported the evidence of the anti-tumour action of rimonabant but they did not investigate or provide a molecular mechanism of action. They proposed that the observed effects could be ascribed to: *a)* a tonic anti-proliferative action mediated by the local endocannabinoids through mechanisms independent from CB1 receptor, particularly when CB1 receptors are blocked by the antagonist rimonabant; *b)* the inverse agonist properties of rimonabant on the receptor. These possibilities could explain the paradox whereby both CB1 agonists and antagonists display anti-tumour activity.

Recently, we reported that rimonabant exerts anti-tumour effects on breast cancer in vitro and in a mouse model in vivo, providing for the first time a new mechanism of action for this drug (Sarnataro et al., 2006). Rimonabant, at nanomolar concentrations, is able to inhibit human breast cancer cell proliferation being more effective in highly invasive metastatic

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MDA-MB-231 cells, than in less invasive T47D and MCF-7 cells, depending both on the presence and the different expression levels of the CB1 receptor. The anti-proliferative effect is characterized by a G1/S phase cell cycle arrest, without induction of apoptosis. The in vitro observed effect has also been confirmed in vivo: rimonabant reduces the volume of xenografts tumours induced by MDA-MB-231 injection in mice, after two weeks of treatment. The molecular mechanism at the basis of rimonabant function implicates an inhibition of p42/44 MAPK phosphorylation and needs lipid rafts/caveolae integrity to occur. This suggests that rimonabant effects on cell proliferation and signaling requires the presence of CB1 receptor in lipid rafts (Sarnataro et al., 2006).

Rimonabant Induced Effects on Fertility

During the last years accumulating evidence have indicated that the endocannabinoid system may play an important role in modulating reproductive system functions and fertility. Some reports have underlined the presence of both CB1 receptor subtype in human sperm (Rossato et al., 2005) and significant concentration of endocannabinoids in female and male genital tract fluids (Schuel et al., 2002). This finding suggests that the control of endogenous tone of endocannabinoids and its interaction with the CB receptors are checkpoints in reproduction (for a review see Maccarrone and Finazzi-Agrò, 2004). However, CB1 receptor activation by AEA is responsible for a reduced sperm motility and inhibition of capacitation-induced acrosome reaction in human sperm specimens. The CB1 selective antagonist rimonabant is capable to block the negative effects of AEA on motility of sperm without compromising sperm viability or motility *per se* (Rossato et al., 2005). Moreover, Melis and coworkers (2006) reported that rimonabant was able to induce penile erection in male rats, when injected into the paraventricular nucleus of male rat hypothalamus. This effect was associated with an increase of glutamic acid leading to the activation of neuronal and nitric

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oxide synthase in oxytocinergic neurons mediating penile erection (Succu et al., 2006). However, it is possible that rimonabant-induced penile erection implicates also an increase in dopaminergic neurotransmission (da Silva et al., 2003).

Few data on the effects of rimonabant on fertility are available, whereas a large body of the recent literature has been focused on the interaction and possible regulation of reproductive processes by endocannabinoid system. Endocannabinoids are involved in implantation (attachment and outgrowth of blastocystis), pregnancy (myometrial contractility) (Liu et al., 2002; Dennedy et al., 2004) and miscarriage (Maccarrone et al., 2000b). Therefore, it is possible to speculate that not only a decreasing AEA concentration in human reproductive tract secretions but also the administering of rimonabant may represent a therapeutic tool in pathological situations such as recurrent abortions characterized by increased levels of AEA.

Other Effects of Rimonabant

The promising results obtained in several experimental model systems, proposing rimonabant as a potential therapeutic tool for the treatment of several pathologies, have recently promoted investigations to ascertain the potential benefit effects of this compound, mainly as a CB1 receptor antagonist, in other disorders affecting the central nervous system, the immune system and the circulatory system. In this sense, the high concentration of cannabinoid CB1 receptors expressed in hippocampus suggests that cannabinoid neurochemical system may play a role in learning and memory processes (Takahashi et al., 2005). Some evidence support the idea that the natural and synthetic cannabinoids impair cognitive processes in human, non human primates and rodents (Braida and Sala, 2000) and appear to inhibit hippocampal extracellular acetylcoline release (Terranova et al., 1996). Rimonabant reverses many of the biochemical physiological and behavioral effects of

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cannabinoid receptor agonists, *e.g*. it attenuates the memory impairment produced by AEA and THC (Mallet and Beringer, 1998; Mishima et al., 2001). Rimonabant, which *per se* does not influence memory processes at the dose of 0.5 mg/kg, completely antagonizes the impairment produced by the synthetic cannabinoid CP 55,940 (Braida and Beringer, 2000).

Finally, blockade of CB1 receptor by rimonabant, improves amnesia induced by the β-amyloid fragment in mice, suggesting that endogenous cannabinoids may be involved in cognitive impairment induced by these fragments. The injection of rimonabant alone does not cause any significant change in the capacity of mice to retain passive avoidance responses and its effect may be observed only when CB1 are activated by their antagonists (Mazzola et al., 2003). From these few available data, it is not yet possible to support a potential therapeutic application of rimonabant in memory impairment, or to propose a mechanism of action, therefore further researches are needed in this direction. Since CB1 receptor mRNA has been detected outside the brain in many other tissues including immune system cells (Klein et al., 2003), recent papers on the role of endocannabinoids in the modulation of immune system have led to consider the therapeutic potential of rimonabant in the inflammation process.

Croci et al. (2003) proposed that rimonabant may interfere with immune-inflammatory pathogenic mechanisms like that underlying indomethacin's ulcerogenic action. They observed that oral administration of rimonabant is able to dose-dependently prevent the indomethacin-induced small intestinal ulcers in rats. This effect was associated with a higher inhibition of TNFα levels and myeloperoxidase activity compared to CB2 receptor antagonist SR144528. Rimonabant produced similar inhibitory effects also in CB1 receptor knock-out mice, suggesting that its anti-ulcerogenic action does not rely on CB1 antagonism. However, in the same CB1 knockout mice, rimonabant failed to counteract the increase of LPS-induced TNFα plasma levels that is CB1 receptor dependent. This observation suggests that rimonabant could probably act with distinct mechanism of actions modulating in a different

way the inflammatory process. The inhibition of TNFα levels by rimonabant is of particular interest, since an increase in plasma levels of this cytokine has been found in obese patients and it could be involved in the regulation of glucose transport and insulin sensitivity (Hube and Howner, 1999). In supporting the rimonabant anti-inflammatory action, its systemic administration could improve rat survival and endotoxin LPS-induced hypotension (Varga et al., 1998). Interestingly, the inhibition of LPS-induced hypotension by rimonabant does not depend on the presence of CB1 receptor, since rimonabant induces similar effects in CB1 deficient mice (Bátkai et al., 2004). Furthermore, other findings also pointed out that rimonabant was able to raise blood pressure maybe by counteracting the increased expression of CB1 receptor (Bátkai et al., 2001). On the bases of the previous findings it appears clear that the role of cannabinoid system and the complex action of rimonabant on the circulatory system and its pathologies needs further insights, in order to better clarify what are the molecular mechanisms and the signaling pathways evoked by rimonabant in determining such CB1 dependent and independent effects.

Conclusions and Perspectives

Collected results clearly show that rimonabant can have a plethora of pharmacological effects in a number of physiopathological conditions (Table 2). Due to its selectivity for the cannabinoid CB1 receptor, the effects are mainly ascribable to its antagonistic properties, even though some evidence for its inverse agonistic action has also been provided (Bouaboula et al., 1997; Landsman et al., 1997; Navarro et al, 2001). Rimonabant represents a promising therapeutic tool in the treatment of obesity, as witnessed by clinical trials and weight loss is achieved probably via central and peripheral mechanisms. Rimonabant is able to centrally target food intake regulation acting on neurotransmitter release. The blockage of the CB1 receptor could result in a reduction of dopamine release that has been found enhanced in

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cortico-limbic structures as a consequence of the rewarding effect of palatable food (Spanagel and Weiss, 1999). Furthermore, rimonabant reduced obesity in leptin (ob/ob) and leptin receptor (db/db) knockout mice (Di Marzo et al., 2001) and blocked CB1/OX1 receptor cross talk (Hilairet et al., 2003), suggesting that it could exert a negative effect on genetically obese animals with an altered leptin neuroendocrine pathway, and inhibit the CB1-mediated tonic orexigenic effect due to increased levels of endocannabinoids. The modulation of nervous system functions is also at the basis of its pharmacological action on ethanol and sucrose consumption, drug seeking behavior, and nicotine addiction. Mainly, rimonabant can operate by preventing drug-induced DA release and turnover in dopaminergic areas, leading to an attenuation of either ethanol reward-related response or to nicotine, psycostimulant and opioid-related relapse. However, by blocking CB1 receptor, rimonabant can act on GABAergic neurons stimulating GABA release (Naderi, 2005). This effect results in an induction of convulsive phenomena and epileptic activity in animal models of encephalopathy and Parkinson's disease (Bernard et al., 2005; Solbrig et al., 2005). These observations, together with the finding that rimonabant prevents the benefit effects of CB1 receptor agonists in genetic and pharmacological-induced Huntington's disease (Lastres-Beker et al., 2003; Centonze et al., 2005), suggest that the drug is not suitable in the treatment of neurodegenerative diseases and motor related disorders. We should point out that rimonabant, used alone, does not induce an alteration of motor activity in normal rats therefore we could hypothesize that its adverse effects on nervous system functions could be limited to an already established pathological condition. On the other hand, this issue needs further evaluations in order to better clarify the exact role and physiopathological consequences of rimonabant-regulated GABA and DA release.

The molecular aspects at the basis of rimonabant-induced effects are not fully understood but appear to be related to the inhibition of the key steps of the CB1 signaling pathway. The

molecular mechanism of action involves the inhibition of MAPK signaling. This pathway is activated by high-fat diet in white adipose tissue and is required for the development of obesity (Bost et al., 2005). Rimonabant, in agreement with its inverse agonistic properties, is able to inhibit CB1 receptor and switch off MAPK activation from the insulin receptor-tyrosine kinase and IGF1 receptors (Bouaboula et al., 1997). It is possible that this mechanism could be implicated also in the modulation of cardiovascular risk factors through a weight reduction independent pathway, but how this occurs and what are the other biological pathways regulated by rimonabant and involved in glucose metabolism remain to be established. MAPK inhibition usually correlate with anti-proliferative effects in both normal and cancer cells (Hou et al., 2004; Stepulak et al., 2005; Yoon and Seger, 2006) and the treatment of adipocytes and hepatic myofibroblasts with rimonabant, strongly reduces proliferation through this pathway. More recently we have provided evidence for an anti-proliferative effect of rimonabant in thyroid and breast cancer cells (Bifulco et al., 2004; Sarnataro et al., 2006). In the last cellular model, we found that rimonabant strongly reduces cell growth by perturbing rafts/caveolae integrity and excluding CB1R from lipid rafts. Interestingly, we found that rimonabant inhibitory effect on ERK 1/2 in the highly invasive and metastatic MDA-MB-231 breast cancer cells, requires lipid raft integrity to occur, thus suggesting that the role of lipid rafts in the receptor-dependent signaling would be to make favorable the CB1R-ligand encounter and the activation of CB1-dependent signaling (Sarnataro et al., 2006). Moreover, rimonabant causes a down-regulation of both the Acrp30 protein, SREBP-1c and FAS enzyme. At the same time it is able to induce an up-regulation of adiponectin and GAPDH, markers of adipose tissue functions, finally causing a reduction of adipocyte cell proliferation, a stimulation of fatty acid oxidation (Bensaid et al., 2003) or, alternatively, an inhibition of de novo fatty acid synthesis (Osey-Hyaman et al., 2005).

Additional targets for the pharmacological effects of rimonabant include reproduction system functions. Recently the role of endocannabinoid system in reproduction and fertility has been reported (Dennedy et al., 2004; Maccarrone et al., 2004) but still few available data demonstrated an increase in the number of penile erections in animal models probably due to an activation of dopaminergic and oxitocinergic neurotrasmission mediated by rimonabant (da Silva et al., 2003; Melis et al., 2006; Succu et al., 2006). Therefore, it could be of great interest to intensify this issue taking into account that the reproductive cascade of hormones and their regulation is tightly associated with energy metabolism and thus with the leptin pathway (Chehab, 2000). Finally, rimonabant seems to exhibit some beneficial effects in indomethacin-induced intestinal ulcer in rats, the effect being associated to a significant reduction of TNFα levels and myeloperoxidase activity (Croci et al., 2003), but these data on the potential application of rimonabant in inflammatory process, such as the results on a potential role of rimonabant in the treatment of memory impairment, are still quite scanty.

In light of the public health implications of the obesity pandemic, CB1 blockade strategy aimed to treat obesity and related disorders has encouraged several pharmaceutical companies to develop new and more selective CB1 antagonists, some of which are already in clinical trials (Table 3). At the moment, available data are not exhaustive to state the advantage of rimonabant competitor compounds, indeed, reported side effects are comparable and there are not data on long term safety and efficacy. Moreover in the pharmacotherapy of obesity it would be necessary to take into account clinical and genetic parameters. We recently demonstrated a strong association between a polymorphic variant of CB1 receptor and glycemia and triglycerides concentration in overweight and obese patients (Gazzerro et al., 2006) and polymorphic variants in the codifying or promoting regions of CB1 receptor have been also associated to mood disorders and predisposition to depression (Barrero et al., 2005). Therefore, a sub-selection of patients' eligibility based on polymorphic CB1 receptor variants

could influence the efficacy of the treatment and the incidence of side effects. Finally, rimonabant showing both anti-obesity, anti-inflammatory and anti-tumour properties, could be a preferential choice in breast cancer patients treated with chemotherapy since excess adiposity is linked to risk of postmenopausal breast cancer and the weight gain following chemotherapy is linked with higher frequency of recurrent breast cancer (Harvie et al., 2005). Several authors proposed that local production of adipokines and inflammatory cytokines by adipocytes within the stroma surrounding breast epithelial cells may be directly linked to the growth of breast cancer (Manabe et al., 2003). Taken together these evidence suggest that rimonabant could limit secretions by adipose tissue and improve the recurrence control in breast cancer obese and overweight patients.

In conclusion we prospect other potential applications of rimonabant in related and non related obesity pathologies. On the bases of the pleiotropic effects here described, it represents a promise beyond its anti-obesity action. Further studies will improve our understanding of the mechanisms of several diseases and will clarify the potential clinical impact of rimonabant “pleiotropic effects”.

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FOOTNOTES

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LEGENDS FOR FIGURES

Fig. 1: Chemical structure of rimonabant.

TABLE 1

Pharmacodynamics and pharmacokinetics of rimonabant

	Preclinical studies*	**Clinical studies***
Primary and secondary pharmacodynamics	Potent and selective CB1 antagonistic activity *in vitro* (pA_2 7.98-8.85) Reversion of WIN55212-2-induced hypothermia and psychomotor effects in mice and rats (either *i.p.* or *p.o.*; ID50 from 0.2 to 1.7 mg/kg) Long-lasting and selective inhibition in sucrose drinking (75%) in models for obesity (1 and 3 mg/kg, *p.o.*) Reduced intake of the preferred high-fat diet (37%), total energy intake in obese rats (by 30%) and body weight gain (93%) (3 mg/kg/day; for 12 days, *p.o.*). Reduction of known risk factors associated with obesity, serum leptin (81%), insulin (78%), glucose (67%) (10 mg/kg/day) Reduction of nicotine self-administration at 0.3 mg/kg and cue-induced nicotine seeking at 1 mg/kg in the self-administration paradigm and at 3 mg/kg in the conditioned place preference test in several animal models (see also text). Increase of the number of shocks (0.3 mg/kg) in the punished drinking test (3 mg/kg) and percentage of time spent into open arms at 10 mg/kg in the elevated plus-maze test. Decrease of immobility time from 3 mg/kg, *p.o.* in the forced swimming test.	*Obesity:* reduction of hunger, daily caloric intake and mean body weight (7-day repeated doses of 20 mg) *Antagonism of cannabis effects:* inhibition of cannabis-induced effects, *e.g.* heart rate increase (90 mg single dose, 40 mg repeated doses)
Pharmacokinetics:		
- adsorption	Oral bioavaibility low to moderate (12% male rats, 46% female rats, 18% male macaques) Extensive first pass metabolism Peak concentration after 1-3 h Stedy state PK: 1 week once-daily dosing	Rapidly absorbed upon oral administration. Decreased absorption with increased dose C_{max} reached 2 h after 20 mg dose Low solubility and high permeability Steady state: 13 days in normal-weight volunteers, 39 days in obese patients
- distribution	Volum of distribution > total body water (11.5 L/kg rats, 24.4 L/kg macaques) Tissues with greatest uptake: liver, adrenals, brown fat, kidneys, lymph nodes High binding (up to 100%) to plasma proteins	Never administered *i.v.* Extensive distribution Very high binding to plasma proteins, mainly albumin (mean-99.9%)
- metabolism	Amidohydrolysis, oxidative metabolism on the piperidine moiety, glucoronidation of the acid, hydroxy phase 1 metabolism	Metabolized by CYP3A4 and hepatic amidohydrolase Metabolites inactive against human cloned CB1 receptors
- excretion	Long terminal half life in rats (7.3 h) and macaques (20 h) and medium clearance (~1L/h/kg) Excretion via bile in the faeces (>70%), small amount in urine. Excreted in milk (rats)	Mean terminal half-life: 10 days, in normal-weight volunteers, 16 days, in obese patients. Clerance 5L/h. After a 20 mg/kg dose: 32% excretion as unchanged in faeces; 3% in urine and 61% in faeces over 312 h. Biliary excretion of metabolites

*data at http://www.emea.eu.int

TABLE 2

Overview of rimonabant effects

Pathologies	Preclinical studies		Clinical studies	Effects	References
	In vitro models	In vivo models			
Obesity, metabolic syndrome and associated cardiovascular diseases	Mouse preadipocytes			Inhibition of cell proliferation and MAPK activity; induction of adiponectin and GAPDH mRNA	Gary-Bobo et al., 2006
		Nongenetic-obese mice		Body weight loss and early reduction of food intake; weight loss maintained with normal diet	Di Marzo et al., 2001; Arnone et al., 2003; Bensaid et al., 2003; Cota et al., 2003
		C57BL/6J and CB1$^{-/-}$mice		Reduction of de novo fatty acid synthesis in mice	Osei-HYiaman et al., 2005
			RIO Lipids, RIO Europe RIO North America	Weight loss and reduced waist circumference; increased HDL levels and reduction of triglycerides, fasting insulin and insulin resistance	Despres et al., 2005 Van Gaal et al., 2005; Pi-Sunyer et al 2006
			RIO diabetes	Weight loss reduction, improvement of glucose control and metabolic parameters in type 2 diabetes	Scheen et al., 2006
Neurodegenerative disorders	Primary neurons			Blockade of neuroprotective effects of CB1 receptor agonists	Abood et al., 2001
		BDV rats		Increase of convulsive phenomena	Solbrig et al., 2005
		Reserpine treated rats		Reduction of hyperkinetic state	Segovia et al., 2003
		Quinorple treated rats		Potentiation of hyperkinesias	Giuffrida et al., 1999
		R6/2 Huntington's disease mice		Blockade of neuroprotective effects of CB1 receptor agonists	Centonze et al., 2005
		Malonate treated rat		Inhibition of neuroprotection	Lastres-Beker et al., 2003
		Wistar rats		Epileptic activity during development	Bernard et al., 2005
		Wistar rats		Analgesic properties	Naderi et al.,2005
Drug seeking (psycostimulants and opioids)		Wistar, Long-Evans, Lister Hooded rats		Cocaine: Attenuation of drug and cue-induced reinstatement Heroin: Suppression of drug induced reinstatement and attenuation of cue induced reinstatement Methamphetamine: Blockage of METH and cue induced reinstatement	De Vries et al., 2001 and 2003; Anggadiredja et al., 2004; Spano et al., 2004
Alchool dependence		Wistar, Wistar/msP rats		Alchol: reduction of cue induced reinstatement	Cippitelli et al., 2005; Economidou et al., 2006
		Wistar rats		Attenuation of cue induced reinstatement	De Vries et al., 2005
Nicotine dependence		Sprague-Dawley rats		Reduction of conditioned behavior after withdrawal	Cohen et al., 2005
			STRATUS-US	36% patients quit smoking	Anthenelli and Despres, 2004

TABLE 2 continued

Pathologies	Preclinical studies		Clinical studies	Effects	References
	In vitro models	In vivo models			
Cancer	Rat thyroid cancer KiMol cells	Thyroid cancer xenografts in athymic mice		Inhibition of thyroid tumours growth	Bifulco et al., 2004
	Human mantle cell lymphoma (MCL)			Decreased viability; additive effect with anandamide	Flygare et al., 2005
	Human breast cancer cells MDA-MB-231	Breast cancer xenografts in athymic mice		Antiproliferative effect with G1/S phase cell cycle arrest, linked to lipid rafts/caveolae localization of CB1 receptor signaling; reduced volume of xenografts tumours in mice	Sarnataro et al., 2006
Infertility	Human sperm			Antagonizing the negative effects of increased levels of anandamide on sperm motility and acrosome reaction.	Rossato et al., 2005
		Sprague-Dawley rats		Enhancement of penile erection	Da Silva et al., 2003; Melis et al., 2006
Liver fibrosis	Human and murine hepatic myofibroblasts	CD1, C57BL/6J, Cnr1$^{-/-}$ mice		Reduction of wound-healing response to acute liver injury; inhibited progression of fibrosis; growth inhibition of hepatic myofibroblasts	Teixeira-Clerc et al., 2006
Chronic inflammatory diseases: ulcer		Crl:CD BR rats C57BL/6J and CB1$^{-/-}$mice		Prevention of indomethacin-induced ulcers in rats and mice	Croci et al., 2003

TABLE 3

CB1 antagonists in clinical development

Drug	Manufacturer	Clinical phase	Study type[¶]
Rimonabant	Sanofi-Aventis	III	- Reducing the risk of major cardiovascular events in abdominally obese patients - Effects on Abdominal Obese Patients With dyslipidemia - Amount and the activity of visceral fat in abdominally obese patients with metabolic syndrome - Effects on abdominally obese patients with impaired fasting blood glucose with or without other comorbidities - Effect on high density lipoprotein kinetics in patients with abdominal obesity and additional cardiometabolic risk factors
		II	- Reduction of voluntary ethanol drinking
CP-945,598	Pfizer	III	- 2-year weight loss efficacy and safety in obese subjects - Long-term study on weight loss and safety in obese subjects - Obesity in overweight type 2 diabetic patients
MK-0364	Merck	II	- Weight maintenance in obese subjects - Weight loss in obese and overweight subjects
Ave-1625	Sanofi-Aventis	II	- Effects on abdominally obese patients with atherogenic dyslipidaemia
SR147778	Sanofi-Aventis	II	- Efficacy and safety in obese subjects
SLV-319	Solvay Pharmaceuticals Bristol-Myers Squibb	Moved to Phase II	

[¶]data at http://www.clinicaltrials.gov

Figure 1