REVIEW
CB₁ receptor antagonists: new discoveries leading to new perspectives

E. Kirilly,¹ X. Gonda² and G. Bagdy¹,³,⁴
¹ Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary
² Department of Clinical and Theoretical Mental Health, Kátvölgyi Clinical Center, Semmelweis University, Budapest, Hungary
³ Group of Neurochemistry, Semmelweis University and Hungarian Academy of Sciences, Budapest, Hungary
⁴ Group of Neuropsychopharmacology, Semmelweis University and Hungarian Academy of Sciences, Budapest, Hungary

Received 9 August 2011, revision requested 4 October 2011, revision received 2 December 2011, accepted 19 December 2011
Correspondence: Prof. G. Bagdy, Chairman, Department of Pharmacodynamics, Semmelweis University, Nagyvarad ter 4, 1089 Budapest, Hungary. E-mail: bag13638@iif.hu

Abstract
CB₁ receptor antagonists were among the most promising drug targets in the last decade. They have been explored and found to be effective as therapeutic agents for obesity and related cardiometabolic problems; however, use of rimonabant, the first marketed CB₁ receptor antagonist, has been suspended because of its anxiogenic and depressogenic side effects. Because some other antiobesity drugs, like dexfenfluramine or sibutramine, were also suspended, the unmet need for drugs that reduce body weight became enormous. One approach that emerged was the use of CB₁ receptor antagonists that poorly cross the blood brain barrier, the second, the development of neutral antagonists instead of inverse agonists, and the third, use of personalized medicine, namely the selection of the patient population without psychiatric side effects. In this review, we dissect the peripheral and central mechanisms involved in the effects of CB₁ receptor antagonists and argue that central mechanisms are more or less involved in most cardiometabolic therapeutic effects and thus, among patients with unsatisfactory therapeutic response to compounds with peripheral action, centrally acting antagonists may be needed. An analysis of pharmacogenetic factors may help to identify persons who are at no or low risk for psychiatric adverse effects. Here, we present the models and identify molecular mechanisms and receptors involved in the effects of stress-, anxiety- and depression-related neurocircuitries sensitive to CB₁ receptor antagonists, like the serotonergic, noradrenergic and dopaminergic systems, which are not only regulated by CB₁ receptors, but also regulate the synthesis of the endocannabinoid 2-arachidonoyl-glycerol.

Keywords anxiety, CNR1, depression, personalized medicine, serotonin transporter, 5-HTTLPR.

Obesity and its treatment

Obesity and associated cardiovascular and metabolic diseases

In the modern, industrialized world, obesity is one of the major epidemic health problems and its prevention and treatment is a major public health concern. Recently, treatment possibilities combine lifestyle modification, nutritional, pharmacological or surgical strategies (Hagmann 2008, Leite et al. 2009, de Mattos Viana et al. 2009). Overweight and obesity are defined as excessive body fat with a body mass index (BMI) of 30 or more in Caucasians and 25 or
more in Asians (Pan et al. 2011). Worldwide rates of obesity have doubled since 1980. In 2008, 200 million men and nearly 300 million women were obese in the world. In 2010, nearly 43 million children under the age of five were overweight. Overweight and obesity are the fifth leading risk for global deaths. At least 2.8 million adults die each year as a result of being overweight or obese (WHO 2011). In modern times, obesity is a consequence of lack of sufficient physical activity, car ownership, sedentary occupations (TV, video games etc.) and cheaper, more high-fat/energy dense diet (Halford 2001, Swinburn & Egger 2002).

In addition, overweight and obesity not only significantly decrease quality of life, but are also associated with severe medical complications such as number of chronic diseases, including non-insulin-dependent diabetes, hypertension and other cardiovascular diseases, sleep apnoea, various forms of cancer and pulmonary diseases, which present a high mortality/morbidity rate (Halford 2001, Leite et al. 2009, Li & Cheung 2011). Also, several medications, especially ones used in the treatment for psychiatric disorders, also significantly increase body weight.

**Current treatment options**

Treatment options for obesity and associated metabolic disorders are limited, and generally, the novel drugs have low efficacy, poor safety profile, and the long-term consequences are unclear. The effectiveness of treatments is very important. Antiobesity drugs must be effective in reducing body weight, and the side effects must be tolerable. Availability is also important because obesity especially affects the minorities and those who have a low socioeconomic status (Padwal & Majumdar 2007).

The first centrally acting appetite suppressants were noradrenergic products, e.g. amphetamine, methamphetamine and phendimetrazine, but these drugs caused severe cardiac and mental health problems, including addiction. Fenfluramine and dexfenfluramine, which inhibit serotonin reuptake, were taken off the market because of pulmonary hypertension and increased valvular heart disease (Ioannides-Demos et al. 2011). Recently approved pharmacotherapies are divided into two classes: centrally acting drugs, such as amfepramone, fenproporex and sibutramine, and drugs with peripheral action, such as orlistat. Amfepramone and fenproporex act by increasing levels of catecholamines and inhibiting appetite.

Orlistat (Xenical), first approved in 1998, is a gastrointestinal lipase inhibitor, which alters fat absorption by around 30% (Padwal & Majumdar 2007). Typically, 120 mg three times daily is prescribed with meals. In a 4-year double-blind, placebo-controlled randomized study of 3305 Swedish obese patients, orlistat reduced weight by 2.7 kg on average and decreased the incidence of type 2 diabetes from 9.0 to 6.2% (Torgerson et al. 2004). No clinically significant effects on triglycerides or HDL cholesterol were seen. The major adverse effects with orlistat are gastrointestinal, include flatulence, steatorrhea, malabsorption, faecal urgency, faecal incontinence, abdominal pain, gastric-upset, dyspepsia and reduced absorption of fat-soluble vitamins (Greydanus et al. 2011). Systemic adverse effects are minimal because of the lack of systemic absorption.

Sibutramine (Meridia) is a centrally acting agent and inhibits the reuptake of noradrenaline and serotonin (Padwal & Majumdar 2007). It was developed originally as an antidepressant. The drug was approved in the USA in 1997 and in the European Union in 1999. In three randomized double-blind, placebo-controlled weight-loss trials of 1 year, in 929 overweight or obese patients, sibutramine reduced weight by 4.6% (Robinson & Niswender 2009). Sibutramine has little effect on concentrations of LDL cholesterol and on glycaemic control and has conflicting effects (from no change to mild improvement) on concentrations of triglyceride and HDL cholesterol. Common side effects include insomnia, nausea, dry mouth and constipation and increases in blood pressure and pulse rate. Cardiovascular adverse effects, such as hypertension and tachycardia, limit the use of these agents, especially in patients with cardiac comorbidities (Robinson & Niswender 2009). In October, 2010, it was withdrawn from the market because of increased risk of cardiovascular events.

A further possibility to improve efficacy and reduce side effects is the use of combination therapy for obesity and associated cardiovascular risk (e.g. diabetes and hypertension). This combination therapy may produce robust additive or synergistic effects on weight loss (Aronne et al. 2010, Greenway & Bray 2010). The combination of the half-doses of the noradrenergic drug phentermine and the half-doses of the serotonergic drug fenfluramine caused fewer side effects, and the antiobesity effect was same as of the full doses of the individual drugs (Weintraub et al. 1984). Sari et al. found that the combination of orlistat with metformin did not result in an additional effect on weight loss and insulin resistance when compared to orlistat alone (Sari et al. 2004). Another study demonstrated that weight loss with pramlintide + phentermine combination was greater than with pramlintide alone (11.3 ± 0.9% and −3.7 ± 0.7% respectively) (Aronne et al. 2010).

Surgical procedures, e.g. gastric banding or bypass, are effective for sustained weight loss but are clearly invasive and associated with a high rate

In conclusion, there are only a few therapies available for the treatment for obesity, and thus the unmet need for drugs that reduce body weight and cure associated health problems became enormous. Therefore, after the discovery of endogenous cannabinoids, the CB₁ receptor antagonists became a preferred drug target.

The rise and fall of CB₁ receptor antagonists

Endocannabinoids

The endocannabinoids, their receptors and the enzymes for synthesis and hydrolysis constitute the endocannabinoid system. The herb Cannabis sativa has been used for medical purposes and as a drug of abuse for 4000 years (Pagotto et al. 2006). However, only in the middle of the twentieth century were its constituents identified, with the chemical characterization of Δ⁹-tetrahydrocannabinol (THC) and other substances.

The endogenous endocannabinoid system is an important regulator of several metabolic and physiological processes including energy/weight homeostasis (Pagotto et al. 2005, Despres et al. 2009). The endocannabinoid system includes two membrane receptors, the CB₁ and CB₂ receptors, and two main endogenous agonists of cannabinoid receptors, N-arachidonoyl-ethanolamine (anandamide) and 2-arachidonoyl-glycerol (2-AG). Anandamide and 2-AG can be found in the hypothalamus, a region responsible for controlling food intake. Unlike regular neurotransmitters synthesized in presynaptic neurones and stored in vesicles prior to release, endocannabinoids are synthesized from the membrane lipids of postsynaptic neurones upon calcium influx because of neuronal activation and immediately diffuse into the synaptic cleft. Furthermore, the activation of receptors with Gq/11 signal transduction, in situ synthesis and release of the endocannabinoid 2-AG occurs. Activation of two enzymes, phospholipase-Cβ and diacylglycerol lipase, is responsible for this action (Turu et al. 2007, 2009, Lazary et al. 2011). CB₁ and CB₂ receptors belong to the seven-transmembrane G-protein-coupled receptor family (Kendall & Alexander 2009). Their activation typically leads to inhibition of adenylate cyclase, consequently closing calcium channels, opening potassium channels and stimulating protein kinase A (Alger 2002, Wilson & Nicoll 2002, Freund et al. 2003, Kendall & Alexander 2009). In the central nervous system (CNS), endocannabinoid effects are mediated via the CB₁ receptor and play a role in emotional regulation, pain perception and motivation related to food intake.

CB₁ receptors

Receptor distribution. Among the two types of endocannabinoid receptors, the CB₂ receptor is expressed mainly in the periphery, and the CB₁ receptor is located in the central and peripheral nervous system in mammals (Rodgers et al. 2003), mediating various behavioural effects of endogenous and exogenous ligands. CB₁ receptors are mainly located in the CNS on presynaptic nerve terminals and their activation brings about a decrease in neurotransmission; therefore, they act as retrograde messengers (Moreira et al. 2009, Moreira & Worjka 2010). CB₁ receptors are found in several brain areas including the olfactory bulbs, some cortical brain regions, hippocampus, amygdala, basal ganglia, thalamic and hypothalamic nuclei, cerebellar cortex and brainstem nuclei (Herkenham et al. 1990, Lynn & Herkenham 1994, Moreira & Crippa 2009, Ward & Raffa 2011). In the brainstem, CB₁ receptors are expressed in a very low density, and several key medullary nuclei responsible for the organization of respiratory and cardiovascular functions seem to lack endocannabinoid signalling (Kendall & Alexander 2009).

CB₁ receptors can be located on several neurones, including GABA-ergic, glutamatergic, serotonergic and dopaminergic neurones (Freund et al. 2003, Szabo & Schlicker 2005, Pacher et al. 2006, Lazary et al. 2011, Umathe et al. 2011). Activation of CB₁ receptors inhibits the release of neurotransmitters involved in anxiety and depression. CB₁ receptors are highly abundant on GABA-ergic neurones and are in somewhat lower density on glutamatergic neurones (Moreira et al. 2009).

CB₁ receptors are also expressed in the periphery and are present in several non-neuronal tissues (e.g. the gastrointestinal system, reproductive system, cardiovascular system, adipocytes, liver, skeletal muscle cells and pancreas). The receptors are directly involved in the orexigenic effect of endocannabinoids as well as in the regulation of metabolism, body weight and insulin resistance and are, therefore, the main targets in the development of new drugs for obesity treatment (Cota et al. 2003).

CB₂ receptors can be found primarily in the immune system (Matsuda et al. 1990, Munro et al. 1993, Balazsa et al. 2008), such as the spleen, tonsils, thymus and lymphoid tissues (Galiegue et al. 1995, Kendall & Alexander 2009), but are also present in keratinocytes (Ibrahim et al. 2005), gut neurones (Wright et al. 2008) and the brainstem (Van Sickle et al. 2005). In addition, CB₂ receptors have been found to be expressed in several pathological states, e.g. in spinal cord and dorsal root ganglion tissues of animal pain models (Van Sickle et al. 2005, Jhaveri et al. 2011).
et al. 2008) and human multiple sclerosis CNS tissues (Benito et al. 2007).

Physiological functions. Neuronal activity modulated by endocannabinoids is a fundamental principle of the CNS and impacts neurodevelopment, synaptic plasticity and behaviour as well (Moreira & Wotjak 2010). Endocannabinoids play a role in a variety of physiological processes and brain functions including pain, body temperature, sleep, arousal, motor function, feeding and such emotional processes as depression or anxiety. Although the role of endocannabinoids in emotional regulation is poorly understood, the effects are thought to be mediated through the CB1 receptors, which are densely expressed in brain areas playing a role in emotional, cognitive, sensory and motor processes, such as the prefrontal and cingular cortex, basal ganglia, hippocampus, amygdala, paraventricular nucleus of the hypothalamus, the periaqueductal grey, the basal ganglia and the cerebellum (Navarro et al. 1997, Rodgers et al. 2003, Griebel et al. 2005, Moreira & Wotjak 2010).

CB1 receptors are expressed in many different brain regions involved learning and memory processes (Marsicano & Lutz 1999). Cannabinoid agonists, including marijuana in humans and synthetic agonists in animals, cause important changes mainly in short-term episodic and working memory (Ranganathan & D’Souza 2006). Williams et al. found that oral THC administration stimulates nocturnal feeding (Williams et al. 1998) and demonstrated that THC hyperphagia is mediated by CB1 receptors, being attenuated by rimonabant but not by the selective CB2 receptor antagonist SR144258 (Williams & Kirkham 2002). CB1 receptor activation in the spinal cord (Hohmann & Herkenham 1998, Kelly & Chapman 2001, Kelly et al. 2003) and in the periphery (Kelly et al. 2003) attenuates nociceptive responses in naive rats.

Some of the effects of endocannabinoids are related to food intake and energy balance control. The endocannabinoid system is an integrated physiologic system that modulates nutrient intake, transport, metabolism and storage, the dysfunction of which is associated with abdominal adiposity. Animal and clinical studies demonstrated that obesity was associated with a pathological overactivation of the endocannabinoid system revealed by an up-regulation of CB1 receptors and/or an enhancement of endocannabinoid levels (Di Marzo & Mattias 2005, Di Marzo et al. 2009). The endocannabinoid system also plays a crucial role in glucose and lipid metabolism.

Many drugs of abuse can influence the levels of endocannabinoids in the brain. It was demonstrated that the endocannabinoid system is involved in drug-seeking behaviour and the mechanisms that underlie relapse to drug use. The cannabinoid CB1 antagonist/inverse agonist rimonabant reduces the behavioural effects of stimuli associated with drugs of abuse, including nicotine, alcohol, cocaine and marijuana. Because blocking the cannabinoid CB1 receptor was shown to decrease eating and reduces the behavioural effects of stimuli associated with drugs of abuse, including nicotine, alcohol, cocaine and marijuana, and nicotine self-administration in animals (Pacher et al. 2006, Le Foll et al. 2008), there has been a great deal of interest in this novel class of drugs.

Rimonabant

Rimonabant (SR141716A, Acomplia, Sanofi-Aventis), the first selective CB1 receptor antagonist or inverse antagonist (Rinaldi-Carmona et al. 1994), was developed as an antiobesity agent on the premise that blocking central cannabionoid activity might reduce food intake (Pacher et al. 2006). Rimonabant has a 1000-fold greater affinity for the CB1 receptor than for the CB2 receptor (Padwal & Majumdar 2007).

Indications for treatment included overweight patients (BMI >27 kg m−2) with a major comorbidity (hypertension, dyslipidaemia, type 2 diabetes) or obese patients (BMI ≥30 kg m−2) with or without comorbidity (Van Gaal et al. 2008). Efficient antiobesity treatment was shown by a series of major reports (Despres et al. 2008, Hampp et al. 2008), and dyslipidaemias, diabetes and metabolic syndrome were also ameliorated (Despres et al. 2005, Van Gaal et al. 2005, Scheen et al. 2006, Rosenstock et al. 2008). The majority of the drug (99%) is bound to plasma proteins. The drug is hepatically metabolized by cytochrome P450. It may interact with other drugs metabolized by cytochrome P450, especially those biotransformed by isoforms CYP 2A6, 2C9, 2C19 and 3A4 (Ducobu & Sternon 2005). Some CYP3A4 inhibitors, like ketoconazole, lead to an increase in rimonabant blood levels, while CYP3A4 inducers decrease plasma concentrations and consequently lead to loss in rimonabant effectiveness (Wierzbicki 2006). The drug half-life is twice as long in obese patients as in non-obese people, because of a larger peripheral volume of distribution.

Rimonabant was not only proposed for the treatment for obesity and metabolic conditions but also for addictive disorder and various dependence syndromes (smoking cessation) (Cahill & Ussher 2007, Le Foll et al. 2008, Rigotti et al. 2009). In June 2006, it had been approved by the European Medicines Agency (EMA) as an obesity treatment (Moreira & Crippa 2009). However, it induced significant psychiatric side effects, namely anxiety and depression, and therefore has been withdrawn from the market in October 2008.
Clinical studies.
Therapeutic effects: Clinical studies have confirmed that, when used in combination with a low calorie diet, rimonabant promotes loss of body weight, loss in waist circumference and improvements in dyslipidaemia (Table 1) (Van Gaal et al. 2008). The first large multicenter, randomized, placebo-controlled phase III trials were the Rimonabant in Obesity and Related Metabolic Disorders Studies (RIO Studies). In all cases, patients were also prescribed a hypocaloric diet and advised on increased physical activity. Psychiatric disorders were exclusion criteria, and side effects were evaluated by the Hospital Anxiety and Depression Scale (HAD). Four double-blind trials compared rimonabant 5 mg or 20 mg daily with placebo in 5500 overweight and obese non-diabetic patients (RIO-Lipid, RIO-North America, RIO-Europe) and in 1047 patients with type 2 diabetes (RIO-Diabetes). RIO-Europe and RIO-North America were 2-year studies including overweight and obese adults. RIO-Lipid was a 1-year study and required the presence of untreated dyslipidaemia. RIO-Diabetes was also a 1-year study and involved patients with type II diabetes in the treatment for over 6 months. Results showed that patients taking one daily dose of 20 mg of rimonabant produced a significant reduction in weight (−6.3 to −6.6 kg vs. placebo −1.6 to −1.8 kg) and many cardiometabolic risk factors (Table 1). The changes observed included waist circumference (average decrease 3.6 cm), A1C, triglycerides (average decrease 13.2%) and HDL cholesterol (average increase 7.2%), glucose tolerance improvement and blood pressure decrease. Other important findings were also observed, such as an increase in adiponectin and a decrease in C-reactive protein (CRP) levels (Despres et al. 2005, Van Gaal et al. 2005, Pi-Sunyer et al. 2006, Scheen et al. 2006). The involvement of central and peripheral components to the therapeutic effects of rimonabant is shown in Table 1.

On the basis of these promising early studies, several clinical trials with rimonabant have been designed and started [Strategy To Reduce Atherosclerosis Development InVolving Administration of Rimonabant – the Intravascular Ultrasound Study (STRA-DIVARIUS)], Study Evaluating Rimonabant Efficacy in Drug-NAïve Diabetic Patients (SERENADE), Study Evaluating Rimonabant Efficacy in Insulin-Treated Diabetic Patients (ARPEGGIO), A Glycemic Control Evaluation of Glimepiride vs. Rimonabant on Top of Metformin in Type 2 Diabetes (ALLEGRO), An International Study of Rimonabant in Dyslipidaemia With AtheroGenic Risk In Abdominally Obese Patients (ADAGIO-Lipids), Atherosclerosis Underlying Development Assessed by Intima-Media Thickness in Patients on Rimonabant (AUDITOR), Comprehensive Rimonabant Evaluation Study of Cardiovascular ENDpoints and Outcomes (CRES-CENDO, RIO-ASIA) (Mach et al. 2009). The STRADIVARIUS study showed a significant effect on body weight, but no significant changes in the primary endpoint (percent atheroma value), but demonstrated normalized atheroma volume, as a secondary endpoint (Nissen et al. 2008, Rosenstock et al. 2008). The 9-month RIO-Asia study evaluated the efficacy and safety of 20 mg rimonabant in obese Asian population (643 patients), who has a greater body fat content at lower BMI compared with Caucasians (Pan et al. 2011). This study confirmed the efficacy and good tolerability of rimonabant (Pan et al. 2011).

Adverse effects: Adverse events generally occurred within the first few months of treatment and were generally mild and transient. Meta-analysis of the RIO studies provided evidence that in some patients, rimonabant was associated with the development of severe adverse psychiatric events (Table 2) (Despres 2009). In total, 13.8% of participants who received 20 mg rimonabant discontinued treatment because of adverse events vs. 7.2% of those receiving placebo (Van Gaal et al. 2008). In the pooled RIO studies, 26% of rimonabant-treated patients experienced psychiatric symptoms vs. 14% of placebo-treated subjects (Table 2). The percentage of participants reporting symptoms of depression (depressed mood, depression, depressive symptoms or major depression) was 9% in rimonabant-treated patients who received 20 mg vs. 5% of placebo-treated subjects (FDA 2007).

Furthermore, rimonabant was associated with significantly increased anxiety. These findings are especially important because people who had a history of

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Involvement of central and peripheral mechanisms in the cardiometabolic therapeutic effects of CB1 receptor antagonists (for references see chapter ‘rimonabant’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Therapeutic effects</td>
<td>Central mechanism</td>
</tr>
<tr>
<td>Body weight</td>
<td>Yes</td>
</tr>
<tr>
<td>Appetite</td>
<td>Yes</td>
</tr>
<tr>
<td>Blood pressure, hypertension</td>
<td>Yes</td>
</tr>
<tr>
<td>Waist circumference (abdominal obesity)</td>
<td>Yes</td>
</tr>
<tr>
<td>Type2 diabetes, insulin resistance</td>
<td>?</td>
</tr>
<tr>
<td>HbA1c (glycated haemoglobin)</td>
<td>?</td>
</tr>
<tr>
<td>C-reactive protein level</td>
<td>?</td>
</tr>
<tr>
<td>Triglycerides level</td>
<td>?</td>
</tr>
<tr>
<td>HDL cholesterol level increase</td>
<td>?</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>?</td>
</tr>
<tr>
<td>Alanine aminotransferase level (fatty liver disease)</td>
<td>No</td>
</tr>
</tbody>
</table>

?, unknown central mechanism.
Adverse effects

Other events

Nervous system disorders

Gastrointestinal disorders

Psychiatric disorders

Nausea, dizziness, diarrhoea and insomnia occurred 1 or psychiatric origin (Table 2). Nausea, dizziness, diarrhoea were of gastrointestinal, nervous system treated patients were of gastrointestinal, nervous system antagonists (for references see chapter Ruilope et al. 2005, FDA 2007, Moreira 2009). The results of rimonabant clinical studies prompted the questioning of the CB1 antagonism approach and the psychiatric withdrawal of other centrally acting molecules. Several other CB1 receptor antagonists/inverse agonists were withdrawn from clinical studies at the same time, including taranabant (Merck) and otenabant (Pfizer) in phase III, and ibipinabant (Solvay/Bristol-Myers Squibb) and surinabant (Sanofi-Aventis) in phase II (Jones 2008). These drugs also induced adverse psychiatric side effects in the clinical studies (Moreira et al. 2009). On the basis of the results obtained with rimonabant and peripheral CB1 receptor antagonists described in this part of the manuscript, the therapeutic (Table 1) and other, non-therapeutic effects (Table 2) may be derived to peripheral and central components.

Peripheral CB1 receptor antagonists

The new strategy is developing peripheral CB1 antagonists, which minimize or prevent CNS adverse effects.

Table 2: Involvement of central and peripheral mechanisms in the adverse and other non-therapeutic effects of CB1 receptor antagonists (for references see chapter ‘rimonabant’)

<table>
<thead>
<tr>
<th>Adverse effects</th>
<th>Central mechanism</th>
<th>Peripheral mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychiatric disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Depression</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Mood alterations with</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>depressive symptoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irritability</td>
<td>Yes</td>
<td>?</td>
</tr>
<tr>
<td>Insomnia</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Nervousness</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Vomiting</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td>Nervous system disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Vertigo</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hypoaesthesia/Paraoaesthesia</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td>Motor impairment (tremor,</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>balance disorder)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cognitive disorders (mental</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>impairment, somnolence,</td>
<td></td>
<td></td>
</tr>
<tr>
<td>disturbance of thinking/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>perception)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palpitations</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td>Other events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Upper respiratory tract</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthenia/Fatigue</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Hot flush</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Tendonitis</td>
<td>?</td>
<td>Yes</td>
</tr>
<tr>
<td>Contusion</td>
<td>?</td>
<td>Yes</td>
</tr>
</tbody>
</table>

serious depression or other psychiatric illnesses had been excluded before study entry and people with severe obesity have been shown to be at high risk of depression. Rimonabant also significantly increased risk of suicide ideation.

The most frequent adverse events in rimonabant-treated patients were of gastrointestinal, nervous system or psychiatric origin (Table 2). Nausea, dizziness, diarrhoea and insomnia occurred 1–9% more frequently in the rimonabant-treated group. Side effects leading to drug discontinuation occurred in 13–16% of patients taking the 20 mg dose (Despres et al. 2005, FDA 2007, Ruilope et al. 2008, Moreira & Crippa 2009). The involvement of central and peripheral components to the adverse effects of rimonabant is shown in Table 2.

The occurrence of paraesthesia, dyseaesthesia and hypoaesthesia was much greater in the rimonabant group (5% vs. 1.2%) but only when the studies in diabetic patients were analysed (FDA 2007) (RIO-Diabetes, SERENADE). Headache was one of the most commonly reported side effects both in rimonabant-treated subjects (10%) and in placebo-treated patients (12.7%) Pan et al. found in the RIO-Asia study that the most frequent adverse event leading to treatment discontinuation was headache in the placebo group (0.6% vs. 0.0%). Interestingly, genetic variations in the CB1 receptor gene CNR1, analysed in a recent population genetic study, failed to show association with headache in general, but significant association was found with migraine headache, and also with the symptoms of nausea and photophobia (Juhasz et al. 2009b). These data are in agreement with the role of CB1 receptors in the inhibition of neurotransmitters involved in the pathomechanisms of migraine (Bagdy et al. 2010). In the second year of treatment, the incidence of adverse events was lower, and incidence of serious adverse events was similar to that reported in the first year.

Withdrawal. Rimonabant was withdrawn from the market by the US (Food and Drug Administration) FDA and European Medicines Agency (Traynor 2007). Psychiatric (e.g. depression, anxiety and suicidal ideation) and other adverse effects (e.g. nausea, vomiting and pruritus) led to the loss of licence of rimonabant in 2008. Following this decision, Sanofi-Aventis announced on 5 November 2008 its decision to withdraw rimonabant from the market worldwide and to discontinue its ongoing rimonabant clinical trials for all indications (Sanofi-Aventis 2008). The results of rimonabant clinical studies prompted the questioning of the CB1 antagonism approach and the psychiatric safety of CB1 receptor antagonists (Christensen et al. 2007, FDA 2007, Rucker et al. 2007).
and preserve antiobesity effects. Several study found that CB₁ antagonists poorly penetrating blood–brain barrier show promising results (Table 3). However, except TM38837, these compounds have been tested only in animals and the clinical efficacy and safety of these new antiobesity compounds are yet to be seen. Questions emerged that some of these compounds still have central effects (e.g. LH-21, URB447) or the food intake and weight-loss effects are markedly reduced compared to rimonabant (Pavon et al. 2008, LoVerme et al. 2009). The most promising molecules are probably AM6545 and TM38837 (Table 3). AM6545 is a rimonabant analogue with high affinity and selectivity for CB₁ receptors but is less lipid-soluble and restricted almost completely to periphery. It has a special preclinical profile and does not affect behavioural responses mediated by CB₁ receptors in the brain of mice with genetic or diet-induced obesity (DIO-mice) but cause improvement in glucose homeostasis, fatty liver and plasma lipid profile without sustained reduction in body weight (Table 3) (Tam et al. 2010). In 2009, 7TM Pharma discovered the first second-generation CB₁ receptor antagonist, TM38837, and successfully completed a double-blind, placebo-controlled cross-over Phase I clinical trial in 24 subjects. TM38837 was ineffective at reversing well-established CNS-mediated effects of THC (Ward & Raffa 2011). This molecule had shown marked weight reduction and very favourable side effects in various animal models for obesity compared to other known CB₁ antagonists. The non-human primate positron emission tomography results demonstrated the peripheral restriction of this drug. Pavon et al. found that chronic administration of the neutral CB₁ receptor antagonist LH-21 (3 mg kg⁻¹) reduces feeding but has no effect on serum lipid levels, glucose levels or blood biochemical parameters, such as AST, ALT or creatinine in obese Zucker rats. LH-21 has lower brain

Table 3 Peripheral CB₁ receptor antagonists and their main effects

<table>
<thead>
<tr>
<th>Molecules</th>
<th>Cannabinoid receptor effect</th>
<th>Animal/human dose(s) (mg kg⁻¹)</th>
<th>Brain penetration</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM6545 (Makriyannis Lab, North-eastern University)</td>
<td>Neutral antagonist</td>
<td>10 DIO-mice</td>
<td>Markedly reduced brain penetration</td>
<td>Less efficacious to reduce body weight than rimonabant, weight-independent improvements in metabolic profile (fatty liver (TG), hepatocellular damage (ALT), glucose homeostasis)</td>
<td>Tam et al. (2010)</td>
</tr>
<tr>
<td>TM38837 (7TM Pharma)</td>
<td>Neutral antagonist</td>
<td>Non-human primate, Phase I clinical trial</td>
<td>No brain permeability</td>
<td>Marked weight reduction and very favourable side effects in various animal models for obesity</td>
<td>Ward &amp; Raffa (2011)</td>
</tr>
<tr>
<td>LH-21 (CSIC, Madrid)</td>
<td>Neutral antagonist</td>
<td>0.03; 0.3; 3 Zucker Rats</td>
<td>Poor brain penetration</td>
<td>Reduced food intake and body weight gain in obese rats (3 mg kg⁻¹); unable to modify the abnormal lipid profile, glucose levels or blood biochemical parameters such as AST, ALT and creatinine</td>
<td>Pavon et al. (2008)</td>
</tr>
<tr>
<td>URB447 (Piomelli, Tarzia Labs)</td>
<td>Mixed CB₁ neutral antagonist/CB₂ agonist</td>
<td>5; 20 Swiss mice; ob/ob mice</td>
<td>No brain permeability</td>
<td>Lowers food intake and body weight gain in mice without entering the brain or antagonizing central CB₁-dependent responses</td>
<td>LoVerme et al. (2009)</td>
</tr>
<tr>
<td>Compound-1 (Yoon Lab, Ajou University)</td>
<td>SR141716A derivative, selective CB₁ antagonist</td>
<td>10; 30; 100 DIO-mice</td>
<td>Lower brain permeability</td>
<td>Dose-dependent antiobesity effect, lowest dose completely inhibited elevated hepatic SREBP-1c expression</td>
<td>Son et al. (2010)</td>
</tr>
<tr>
<td>D4 (7TM Pharma)</td>
<td>Antagonist/ inverse agonist</td>
<td>1; 3; 10 DIO-mice</td>
<td>Lower brain permeability</td>
<td>Reduction in body weight (3 and 10 mg kg⁻¹)</td>
<td>Receveur et al. (2010)</td>
</tr>
</tbody>
</table>

DIO, diet-induced obesity.
permeability than rimonabant (Table 3) (Pavon et al. 2008).

URB447 (20 mg kg⁻¹) was the first described CB₁/-CB₂-mixed antagonist without brain permeability (LoVerme et al. 2009), which decreases food intake and body weight gain in mice (Table 3). These results suggest that this CNS-impermeant antagonist reduces food intake by blocking CB₁ receptors in the periphery (LoVerme et al. 2009). Another peripheral CB₁ receptor antagonist, Compound-1, crosses the BBB at a significantly lower rate than that of rimonabant and reduces food intake only at 100 mg kg⁻¹, which recovers to baseline levels after 12 days in HF-DIO-mice (Son et al. 2010). Son et al. concluded that total inhibition of peripheral CB₁ receptors may not be sufficient to have a complete antiobesity effect (Table 3) (Son et al. 2010). Another peripheral-acting CB₁ agonist (D4) with lower lipophilicity, higher polar surface area and improved plasma/brain ratios has been shown to have weight-reducing effects in DIO-mice (Table 3) (Receveur et al. 2010). These observations suggest that significant metabolic and perhaps antiobesity effects of the peripherally restricted CB₁ antagonists may be achieved at least in rodents (Table 3). Whether these effects are present also in humans, and whether the effect size and ratio could be comparable to central CB₁ receptor antagonists like rimonabant is still a question.

Neutral antagonists vs. inverse agonists

Inverse agonists, after binding to the receptor, have intrinsic activity and produce effects that are opposite compared to endocannabinoids or exogenous CB₁ receptor agonists. Rimonabant and other centrally acting CB₁ receptor antagonists that entered clinical phase and were withdrawn (like taranabant, otena-bant, surinabant, ibipinabant) are all inverse agonists. On the basis of this recognition, after the failure of rimonabant, the development of neutral antagonists was targeted. The main assumption was that neutral antagonists may lack psychiatric and possibly also other side effects but retain their metabolic actions. There is, however, an increasing debate whether it is feasible for a ligand to behave as a true neutral antagonist in a living system (Kenakin 2004, Giraldo et al. 2007), especially in the case of the CB₁ receptors, which have endogenous ligands, the endocannabi-noids, in ample concentration throughout the brain and body (Pacher et al. 2006, Lazary et al. 2011). Indeed, the neutral antagonist AM4113 that enters the brain produces significant anxiety-like effects, and these are comparable to those of rimonabant (Jarbe et al. 2008). Although clinical observations are not yet available, these data question the idea that neutral antagonists that enter the brain may be devoid of psychiatric side effects.

Identification of patients with low risk to adverse effects to CB₁ receptor antagonists

Centrally acting CB₁ receptor antagonists were promising new tools in the treatment for obesity and related metabolic and cardiovascular conditions; however, their anxiogenic and depressogenic capacity contributes to severe and possibly dangerous side effects in some patients. Peripheral CB₁ receptor antagonists may have significant therapeutic metabolic effects without central side effects, but according to rodent studies, therapeutic effects, at least decrease in food intake and weight gain, may be reduced compared to the centrally acting compounds, like rimonabant. Therefore, it can be assumed that among patients with unsatisfactory therapeutic response to compounds with peripheral action, centrally acting antagonists may be needed, and thus it would be crucial to obtain tools, which could identify patients who are at a low risk of developing adverse central effects during CB₁ receptor antagonist therapy. Our knowledge at this point concerning the mechanisms through which CB₁ receptors and the antagonism of these receptors are involved in depression and anxiety is far from complete. We have, however, increasing knowledge concerning the endocannabinoid system and also the interplay between this and other neurotransmitter systems. One possible tool for the identification of such patients is identifying those genes or, more precisely, genetic polymorphisms, which are involved in mediating the anxiogenic and depressogenic effects of CB₁ receptor antagonists and other pharmacological medications, and screening for them prior to therapy. Another observation that could be used in conjunction of genetic testing comes from clinical observations indicating that anxiety and depression as adverse effects during rimonabant therapy are more likely to manifest in patients who have a history of depression, which suggests that this agent is more likely to exacerbate already existing anxiety and depressive states rather than generating de novo ones (Moreira & Crippa 2009, Lazary et al. 2011) Therefore, a careful and thorough psychiatric anamnesis, also including psychiatric disorders and symptoms in first-degree relatives, and also evaluating the existence of traits and the con-founding environmental factors related to anxiety and depression, may help identifying those at high risk.

Many, and especially many of the severe and difficult-to-predict side effects of medications are genetically based, and recent advances in personalized medicine aim at identifying genotypes that can predict treatment outcome and side effects to generate
CB1 receptor regulation of pathways involved in stress and anxiety

Cannabinoids are profoundly involved in mediating fear and anxiety and exert diverse effects, probably due to their interaction with various neurotransmitter systems and action in various brain regions. Investigations indicated that endocannabinoids exert a tonic modulating effect on mood and anxiety (Moreira & Wotjak 2010). Clinical studies point to a distinctive role of the endocannabinoid system in ameliorating fear, anxiety and stress responses (Moreira & Wotjak 2010).

Results on the anxiogenic and anxiolytic effects of cannabinoids are contradicting in animal studies, and anxiogenic and anxiolytic effects of cannabinoids depend on dose, animal strain and test, and genetic and pharmacological manipulation of CB1 receptors also yield conflicting results (Navarro et al. 1997, Haller et al. 2002, Rodgers et al. 2003). In humans, CB1 receptor antagonists induce psychiatric side effects, mainly anxiety and depression in about 30% of the patients, and this side effect led to the withdrawal of rimonabant as described previously. These responses appear to be due to inhibiting the endogenous cannabinoid tone regulating emotional responses in humans similar to that in rats (Navarro et al. 1997, Haller et al. 2002, Lazary et al. 2011). The endocannabinoid system may exert an endogenous regulation on emotional responsivity, and emotional states possibly present from early developmental stages (Viveros et al. 2005). Endocannabinoids may be synthetized in the amygdala during states of anxiety or stress and influence amygdala outputs (Fig. 1) thereby controlling emotional states, which means that the endocannabinoid system is activated during anxiogenic situations and acts as a negative feedback system limiting anxiety (Viveros et al. 2005, Lazary et al. 2011). Studies indicate that CB1 receptors may restrain anxiety responses and prevent mood alterations in response to stress (Moreira & Crippa 2009, Lazary et al. 2011).

Because CB1 receptors can be found in high density on GABA neurones and in a lesser amount on glutamatergic neurones (Freund et al. 2003, Pacher et al. 2006, Lazary et al. 2011), and although CB1 antagonists may increase GABA release, and thus may mimic the action of anxiolytic drugs like benzodiazepines, in clinical practice, they are associated with increased anxiety as a side effect, which may suggest that CB1 receptors on GABA-ergic neurones are not responsible for mediating the anxiogenic effects associated with CB1 receptor antagonists. Another possibility accounting for increased anxiety as a side effect of CB1 antagonists is increased glutamate release, but there are
rather contradictory results and little support for increased glutamate release because of application of CB₁ antagonists (Krystal et al. 2010).

Serotonin–endocannabinoid interactions in the regulation of anxiety. The most likely candidate for mediating the anxiety-related effects of CB₁ receptor antagonists is the serotonergic system (Fig. 1). Several lines of evidence indicate the importance of CB₁ receptors in serotonin receptor-mediated behaviours and also in the modulation of anxiety, depression and stress response, including the presence of CB₁ receptors on serotonergic neurones (Balazsa et al. 2008), endocannabinoid release after Gq protein activation (Turó et al. 2009), which occurs also as a response to certain serotonin receptor activation like 5-HT₂C, and the usually opposite functions mediated by endocannabinoids and 5-HT₂C receptors (Fig. 1) (Umathe et al. 2011). CB₁ receptors are expressed on serotonergic neurones and are present in brain areas including the amygdala, prefrontal cortex, limbic system, striatum and the thalamus, which play a role both in depression and in anxiety (Lazary et al. 2011, Umathe et al. 2011), and an interaction effect between CNR1, the gene of the CB₁ receptor and the serotonin transporter gene on anxiety has been described previously in a human population (Lazary et al. 2009). There are several other lines of evidence to support this interplay between the serotonergic and endocannabinoid system in anxiety. Many effects of the endocannabinoid system, such as emotional regulation, motor function, feeding behaviour, sleep-wake cycle, are also associated with affective disorders and are also attributed to the serotonergic system. CB₁ receptor knock-out mice and serotonin transporter knockout mice both display increased anxiety-like behaviours (Haller et al. 2002, Lira et al. 2003). The involvement of the serotonergic system in anxiety is well known and supported by results from different fields (Fig. 1). Acute SSRI administration may lead to anxiety, e.g. agitation, irritability or insomnia, and this effect seems to be associated with 5-HTTLPR genotype, namely it appears much more frequently in persons who carry the s allele, which is associated with lower expression of serotonin transporter, and thus, with a longer duration and higher concentration of synaptic and extracellular 5-HT concentration (Murphy et al. 2008, Lazary et al. 2011). Acute SSRI treatment-induced anxiety occurs also in rodents and disappears with chronic treatment (To & Bagdy 1999, To et al. 1999). Furthermore, this effect can be blocked by 5HT₂C antagonists (Bagdy et al. 2001), indicating a profound involvement of the serotonergic tone and the 5-HT₂C receptor in mediating fear and anxiety and related stress responses, like the activation of the sympathetic adrenal system or the hypothalamic–pituitary–adrenocortical system (Bagdy et al. 1989a,b, Bagdy 1996, 1998, Kantor et al. 2000). Evidence indicates a key role for serotonin reuptake in fear and anxiety regulation in the general population and also in affective illness. Studies in humans and rodents, including imaging studies, receptor KO and overexpressing mice all suggest a direct association between synaptic/extracellular 5-HT concentration, the activation of 5-HT₂C receptors and anxiety (Bagdy et al. 2001, Hariri et al. 2005, 2006, Pezawas et al. 2005, Lazary et al. 2011).

Upon acute stress serotonergic neurones of the raphe nuclei that project to such brain areas as the amygdala, are activated and lead to anxiety via 5-HT₂C receptors (Fig. 1). However, chronically elevated extracellular serotonin levels exert an anxiodepressant effect because of a down-regulation and desensitization of serotonin receptors including the 5-HT₂C receptor. These mechanisms explain the acute anxiogenic and chronic antidepressive and anxiolytic effects of SSRI treatment, and both are associated with polymorphisms in the serotonin transporter gene (Murphy et al. 2008, Lazary et al. 2011).

On the other hand, the endocannabinoid system is also involved in the regulation of anxiety and depression. CB₁ receptors exert a tonic inhibitory action on serotonin release. Serotonin via postsynaptic 5HT₂C receptors coupled with Gq/11 causes 2-AG production and release and leads to retrograde stimulation of presynaptic CB₁ receptors (Fig. 1). By this mechanism, endocannabinoids tonically inhibits serotonin release. In contrast, CB₁ receptor antagonists abolish this tonic inhibition and lead to anxiety through the stimulation of serotonin release and the activation of 5-HT₂C receptors (Lazary et al. 2011). This mechanism is shown in Figure 1.

Neuropeptide–CB₁ receptor–serotonin interactions in stress responses. The endocannabinoid system is involved in the serotonergic regulation of fear and anxiety, but it works also in the other way, namely the serotonergic tone interferes with the endocannabinoid or neuropeptide regulation of anxiety. Lack of CB₁ receptor activity impairs autoinhibition and also the negative feedback and functionality of 5-HT₁ and 5-HT₂/₂C receptors in CB₁ receptor KO mice, suggesting developmental and/or adaptive changes in the serotonergic system because of the failure of functional CB₁ receptors (Mato et al. 2007, Aso et al. 2009, Lazary et al. 2011), which also supports the interaction between the endocannabinoid and serotonergic systems in the regulation of anxiety (Lazary et al. 2011). Chronic fluoxetine treatment leads to the elevation of synaptic serotonin levels and increased CB₁ receptor density in the cerebral cortex (Bagdy
et al. 2001, Umathe et al. 2011), and 5-HT_{1A} and 5-HT_{2C} receptor activation releases endocannabinoids (Lazary et al. 2011). Stress responses, including the activation of the hypothalamic-pituitary-adrenocortical axis including CRH neurenes of the hypothalamic paraventricular nucleus, and also the activation of the sympahtoadrenal system, are regulated by 5-HT_{1A}, 5-HT_{1A} and 5-HT_{2C} receptors (Bagdy et al. 1989a,b, Bagdy & Makara 1994, Bagdy 1996). Cannabinoids induce a CB1 receptor-mediated activation of the HPA axis via a central pathway, probably via increased CRH release (Viveros et al. 2005), indicating a functional relationship between the endocannabinoid system and the HPA axis in stress-related responses (Viveros et al. 2005). The serotonergic system also seems to be involved in the effects of cannabinoids on adrenocortical activity, with a functional relationship between CB1 and 5-HT_{1A} receptors (Viveros et al. 2005). CB1 KO mice show impaired action of buspirone, a 5HT_{1A} partial antagonist (Viveros et al. 2005). Furthermore, besides CRH, serotonin is also involved in other neuropeptide-endocannabinoid-anxiety circuits. CB1 receptors are found on CCK-containing GABA-ergic inhibitory interneurons, and cannabinoids influence CCK release, suggesting an interplay between endocannabinoids and CCK in the mediation of anxiety (Viveros et al. 2005). GABA_{A} receptors seem to be involved both in anxiogenic and in anxiolytic effects of endocannabinoids (Viveros et al. 2005). Chronic SSRI treatment, generally used as antidepressant or anxiolytic, has been shown to attenuate both CRH- and CCK-mediated anxiety, suggesting the possible involvement of serotonin in the aforementioned interactions (To & Bagdy 1999, To et al. 1999).

CB1 receptor regulation of pathways involved in depression

Serotonin–noradrenaline–endocannabinoid interactions in the regulation of depression. The classic theory about the biological aetiology of depression hypothesizes that depression is because of a deficiency of monoamine neurotransmitters, namely serotonin, noradrenaline and dopamine, and almost all antidepressants boost the synaptic action of one or more of these three monoamines. Although several problems and difficulties were discovered later, the introduction of adaptive changes in receptors and downstream molecular events kept this model alive and still useful (Stahl 2000). As described earlier, the extracellular concentration of serotonin in the terminal fields like the prefrontal cortex is elevated in CB1 receptor KO animals, and in control animals, an increase in serotonin concentration occurs as a result of treatment with CB1 receptor antagonists as shown in Fig. 1 (Aso et al. 2009, Lazary et al. 2011). A functional desensitization of the 5-HT_{1A} autoreceptors in CB1 mutant mice and also in wild-type animals receiving chronic treatment with the CB1 receptor antagonist rimonabant has been described (Valverde & Torrens 2011). In CB1 receptor mutant mice, decrease in 5-HTT binding site densities has been found in the frontal cortex and hippocampus, and reduction in 5-HT_{2C} receptors in the dorsal raphe (Aso et al. 2009). Furthermore, the phenotype of CB1 receptor-deficient mice shows several features of depressive-like disorder (Valverde & Torrens 2011). Clinical studies with CB1 receptor antagonists that enter the brain, like rimonabant, may cause depression as described previously. In addition, low concentration of endocannabinoids has been associated with depression (Hill et al. 2009). All these data suggest that attenuated serotonergic activity is unlikely in depression associated with CB1 receptor antagonists; moreover, a higher than normal extracellular serotonin concentration both in the raphe and in the terminal regions including the prefrontal cortex, the amygdala or the hippocampus could be expected (Aso et al. 2009, Lazary et al. 2011, Valverde & Torrens 2011). Higher than normal serotonergic activity in addition with attenuated CB1 receptor function could, however, attenuate the firing of noradrenergic and dopaminergic neurons, and thus, account for symptoms of depression.

The noradrenergic system, namely the decrease of the noradrenergic tone is a possible suspect in the mediation of endocannabinoid effects on mood (Fig. 2). Locus coeruleus (LC) noradrenergic neurons are under strict control of GABA-ergic neurons (Osmanovic & Shefner 1990, Gervasoni et al. 1998). The main source of afferent GABA input to the LC derives from two populations of neurons in the medulla: the nucleus paragigantocellularis and nucleus prepositus hypoglossi (Osmanovic & Shefner 1990). Prepositus hypoglossi-evoked inhibition of LC is mediated by GABA, acting primarily at the GABA_{A} receptor subtype (Ennis & Aston-Jones 1989). Increase in the activity of these neurons causes a marked decrease in the noradrenergic activity. There are at least three types of CB1 receptors that suppress the release of GABA (Fig. 2). First, those that are present at GABA-ergic terminals, second, those that are present on the terminals of other neurons that terminate at these GABA-ergic neurones and exert an excitatory activity on GABA-ergic neurones. These latter terminals that activate the GABA-ergic tone could be glutamatergic or serotonergic (Boothman et al. 2006, Cinar et al. 2008). Thus, the above described increase in extracellular 5-HT concentration because of the lack of normal CB1 receptor function and/or the decreased endocannabinoid synthesis or blockade of CB1 recep-

© 2011 The Authors
CB1 receptors that maintain and serotonin receptors that suppress noradrenergic tone relevant for depression

**Figure 2** Schematic model showing some key components of the innervation of LC neurons relevant for depression. The model shows the localization of CB1 receptors that maintain and the serotonin receptors that suppress the firing of noradrenergic neurons. Note that synthesis of endocannabinoids and extracellular 5-HT concentration also affect the activity of noradrenergic neurons, and the former depends on calcium signal generation through the excitatory neurotransmitter glutamate and also through the activation of Gq proteins by the increase of monoaminergic activity as shown in Figure 1. Around these synapses endocannabinoids, especially 2-arachidonoyl-glycerol may act as a retrograde transmitter. For details of Gq protein-mediated molecular mechanism see Lazary et al. (2011) and Turu et al. (2009).

Serotonin–dopamine–endocannabinoid interactions in the regulation of anhedonia. Anhedonia is a typical symptom of depression. Decreased motivation and a general decrease in energy levels are also among the possible symptoms of depression. It is generally accepted that endocannabinoids play a major modulatory role in the control of reinforcement, reward and motivation. The reinforcing properties of most prototypic drugs of abuse were absent or impaired in mice lacking CB1 receptors. The final common pathway of reward is the mesolimbic dopamine pathway that terminates on the nucleus accumbens (Stahl 2000). The dopaminergic neurons of the mesocorticolimbic pathway are under control of excitatory and inhibitory inputs that are modulated by CB1 receptors (Fig. 3). CB1 receptors that are relevant for the reward process are present on terminals of GABA-ergic inhibitory neurons terminals, and on terminals of glutamatergic and serotonergic neurons (Fig. 3). All these CB1 receptors inhibit the activation of dopaminergic neurons, and thus contribute to the reward process. Inhibition of these CB1 receptors as well as the activation of 5-HT2A/2C receptors contributes to anhedonia associated with depression. Furthermore, like in the case of the serotonergic system, there is an interaction between endocannabinoids and the dopaminergic neurons. Release of endocannabinoids has been shown after the depolarization of nucleus accumbens neurons, and from dopaminergic neurons in the ventral tegmental area (Valverde & Torrens 2011). In conclusion, mood-altering psychiatric side effects of rimonabant may be associated with the inhibition of these CB1 receptors and concomitant increase in serotonergic output (Fig. 3).

**Figure 3** Schematic figure showing some key components of the innervation of dopaminergic neurons of the ventral tegmental area (VTA) relevant for anhedonia. The model shows the localization of CB1 receptors that maintain and the serotonin receptors that suppress the firing of dopaminergic neurons. Note that synthesis of endocannabinoids and extracellular 5-HT concentration also affect the activity of dopaminergic neurons, and the former depends on calcium signal generation through the excitatory neurotransmitter glutamate and also through the activation of Gq proteins by the increase of monoaminergic activity as shown in Figure 1. Around these synapses endocannabinoids, especially 2-arachidonoyl-glycerol may act as retrograde transmitter. For details of Gq protein-mediated molecular mechanism see Lazary et al. (2011) and Turu et al. (2009).
Interaction of the serotonin transporter gene with drug-induced anxiety and depression

The serotonin transporter is a major player of serotonergic function by determining the termination of the serotonergic signal in the synapse, and the serotonin transporter gene is of particular interest in psychiatry, because of associations described between its variants and psychiatric disorders including affective and anxiety disorders, and psychopharmacogenetics (Gonda et al. 2007). The serotonin transporter is encoded by a single gene (SLC6A4), which is located on chromosome 17q11.1–0.12, and of the polymorphisms described within this gene, the serotonin transporter–linked promoter region (5-HTTLPR) attracted the majority of interest (Lesch et al. 1993). Different variants of this gene lead to differences in transcriptional activity and therefore different basal activity of the serotonin transporter, with the l allele showing greater transcriptional activity compared to the short variant (Heils et al. 1996). In the initial studies, the 5-HTTLPR s allele was associated with affective disorders (Collier et al. 1996) and anxiety-related traits (Lesch et al. 1996, Gonda et al. 2007), and in spite of conflicting results from earlier studies, the fact that the 5-HTTLPR plays a role in the development of depression by mediating reaction to stressful life events was replicated in meta-analyses (Caspi et al. 2003, Lazary et al. 2008, Karg et al. 2011).

Besides affective and anxiety disorders, the 5-HTTLPR also seems to be associated with therapeutic response to SSRIs used in the treatment of these conditions. Higher frequency in anxiety-related side effects, reduction in treatment compliance and/or in therapeutic response to SSRIs and interferon-alpha treatment are associated with the s allele of 5-HTTLPR, the functional, most frequently studied polymorphism of the SERT gene in studies in European and North American studies. In an initial study in 53 patients receiving fluvoxamine, the ss genotype was associated with poorer response rates (Smiraldi et al. 1998), while in a subsequent study, the similar association was found for the s allele (Zanardi et al. 2001). Similarly, slower response to paroxetine was found to be associated with the s allele (Pollock et al. 2000) and ss genotype (Zanardi et al. 2000), and the s allele was also associated with poorer response to paroxetine (Zanardi et al. 2000). Studies with fluoxetine indicate poor response in s allele carrier and ss genotype subjects (Rausch et al. 2002, Joyce et al. 2003), while other studies found no association (Peters et al. 2004, Kraft et al. 2005). In support of the aforementioned data, the ll genotype was associated with earlier response to sertraline (Durham et al. 2004). In case of citalopram, in some studies, the ss genotype was associated with non-remission (Arias et al. 2003) and the s allele in women and younger subjects was associated with less favourable response to treatment (Smits et al. 2008), although other studies reported no association between response to citalopram treatment and SSRI genotype (Hu et al. 2007, Kraft et al. 2007). Similarly, escitalopram was associated in men with worse treatment outcome in one study (Huezo-Diaz et al. 2009), while other studies found no association (Maron et al. 2009). Therefore, although individual studies yielded many positive results concerning the association of 5-HTTLPR genotype with poorer response to SSRI treatment, the results are conflicting, and meta-analyses do not show a consistent conclusion. While one meta-analysis found evidence for the involvement of this polymorphism in predicting both response and remission because of SSRI treatment (Serretti et al. 2007), another one concluded that 5HTTLPR genotype is not associated with response to SSRIs but found a weak association in case of remission (Taylor et al. 2010),
In the previous meta-analysis where a robust and significant association was described, the authors also suggested that the s allele is related to an acceleration of treatment effect and not to the overall response rate (Serretti et al. 2007). In a recent study, the II carriers were represented in significantly higher rate among those patients who achieved remission compared to non-remitters (Illi et al. 2011). The contradiction between studies and meta-analyses may in part be explained by heterogeneity of studies, including study samples among other factors. Also, functional effects of individual genes may only be revealed in interaction with polymorphisms of other genes, or with interaction with important life events (Taylor et al. 2010).

Some studies indicate that the 5-HTTLPR is associated with discontinuation of antidepressants not only because of lack of response but also because of adverse effects (Schwab et al. 2010). In one study, it was reported that compared to II subjects, patients carrying the ss and sl genotype in a geriatric population were at a greater risk of discontinuing paroxetine therapy because of such adverse effects as gastrointestinal discomfort, fatigue, agitation, sweating and dizziness. SS patients also had a greater severity of adverse events. In the same study, discontinuation of treatment with mirtazapine, an antidepressant with a different mechanism of action, was associated with the l allele (Schwab et al. 2010), and side effects included drowsiness, dizziness and anxiety (Schwab et al. 2010). In another study in 37 patients treated with fluoxetine, the ss genotype showed association with increased frequency of insomnia and agitation (Perlis et al. 2003). In the STAR*D study, although no association between SSRI efficacy and 5-HTTLPR was found, they found a significant association with side effects, diarrhoea being more common in S allele carriers (Hu et al. 2007). In another study in SSRI-treated patient, agitation showed a 9.5-fold greater incidence in ss genotype patients compared to those with the sl and II genotype (Murphy et al. 2008). In case of SSRIs, the 5-HTTLPR seems to be an important underlying factor in the induction of antidepressant-induced mania (Mundo et al. 2001, Daray et al. 2010) and serotonin syndrome (Daray et al. 2010).

These studies indicate that 5-HTTLPR genotype is associated with a disturbance of circadian rhythms and levels of alertness as side effects of SSRI therapy, which are related to anxiety (Schwab et al. 2010), in addition to its direct relationship to agitation and anxiety as side effects of SSRI therapy. Taking together the results from the available individual studies, increased anxiety-related side effects can be observed significantly more often in subjects with the ss genotype (Murphy et al. 2008).

The 5-HTTLPR in interaction with the gene of the CB1 receptor is a strong candidate also for mediating anxiety and depression in response to rimonabant (Lazary et al. 2011). This is supported by findings mentioned previously, indicating that the effect of CB1 genotype on the emergence of anxiety is exaggerated in interaction with the serotonin transporter gene (Lazary et al. 2009), which may suggest that those subjects who carry the lower expressing genotype of the 5-HTTLPR polymorphism, namely the ss genotype, may be at an especially high risk of such side effects as anxiety and depression during CB1 receptor antagonist treatment (Juhasz et al. 2009a, Lazary et al. 2011).

The 5-HTTLPR was also shown to be associated with the development of psychiatric side effects in case of such other pharmacotherapies as alpha interferon and ribavirin (Bull et al. 2009, Lotrich et al. 2009). In a large, multicentre study of HCV patients receiving pegylated interferon-α and ribavirin, the II genotype was associated with significantly less depressive symptoms during treatment, which is an important finding because depression induced by this treatment significantly worsens life quality of patients (Bull et al. 2009). In the same study, the CC genotype of the IL-6 gene was protective against the development of depressive symptoms, and an interaction between the two genes was also found. The authors concluded that patients with the CC and LL genotypes are the less likely to develop depressive symptoms during treatment (Bull et al. 2009). In another study, in interferon-α-treated HCV patients, during interferon-alpha treatment, the l allele of the 5-HTTLPR was associated with less depressive symptoms during treatment, and the ll genotype showed the least depressive symptomatology (Lotrich et al. 2009). In this study, the ll genotype was particularly associated with better sleep quality, which may be an important mediator of its protective effect against depression. Although the depressogenic mechanism of interferon-alpha treatment is unknown, an influence on frontal lobe and anterior cingulated function as well as the involvement of such neurotransmitter systems as the dopaminergic, serotonergic and glutamatergic is likely to play a role (Lotrich et al. 2009).

The 5-HTTLPR polymorphism therefore possibly contributes to anxiogenic and depressogenic side effects of several medications. Our existing knowledge concerning the role of the 5-HTTLPR and the CNR1 gene in depression and anxiety raises attention to the importance of these gene variants in side effects emerging during CB1 receptor antagonist treatment, and it also points to the possibility of screening for those polymorphic variants, which convey an increased susceptibility for these side effects.
Conclusion

After the suspension of rimonabant and other centrally acting CB1 receptor antagonists, there are still promising approaches in this field. The development of CB1 receptor antagonists that poorly cross the blood–brain barrier produced molecules with proven metabolic effects and devoid of anxiety-like effects in rodents. The development of neutral antagonists instead of inverse agonists to avoid side effects is debated, but final conclusion has not been reached yet. An analysis of genetic, phenotypic and environmental factors may help to identify those persons who are at no or low risk for psychiatric adverse effects after treatment with brain penetrant CB1 antagonist. Our existing knowledge concerning the role of the serotonin transporter and the CB1 receptor genes in depression and anxiety points to the possibility for screening for those polymorphic variants, which convey an increased susceptibility for these side effects. Genetic screening in combination with a thorough anamnestic including psychiatric symptoms and possible anxiety and depressive traits not only in the patient but also in their first-degree relatives, as well as the exploration of life stressors and environmental factors may provide a useful tool for predicting and identifying those patients who are at a high risk for side effects associated with CB1 antagonist therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

The preparation of this review was supported by the Sixth Framework Program of the EU, LSHM-CT-2004-503474, TAMOP-4.2.1.B-09/1/KMR-2010-0001 and ETT 318/04/2009.

References

Bagdy, G., Graf, M., Anheuer, Z.E., Modos, E.A. & Kantor, S. 2001. Anxiety-like effects induced by acute fluoxetine, sertraline or m-CPP treatment are reversed by pretreatment with the 5-HT2C receptor agonist SB-242084 but not the 5-HT1A receptor agonist WAY-100635. Int J Neuropsychopharmacol 4, 399–408.


susceptibility gene for affective disorders and the response of the human amygdala. *Arch Gen Psychiatry* 62, 146–152.


