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Pharmacological comparison of traditional and non-traditional cannabinoid receptor 1 blockers in rodent models in vivo☆,☆☆

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ABSTRACT

Cannabinoid receptor 1 (CB1R) antagonists have been proven to be effective anti-obesity drugs; however, psychiatric side effects have halted their pharmaceutical development worldwide. Despite the emergence of next generation CB1R blockers, a preclinical head to head comparison of the anti-obesity and psychiatric side effect profiles of the key compounds has not been performed. Here, we compared classical CB1R antagonists (rimonabant, taranabant, otenabant, ibipinabant, and surinabant) and non-traditional CB1R blockers (the partial agonist O-1269, the neutral antagonists VCHSR and LH-21 and the peripherally acting inverse agonist JD-5037) using an in vivo screening cascade. First, the potencies of these compounds to reduce CB1R agonist-induced hypothermia and decrease fasting-induced food intake were determined. Then, equipotent doses of the non-toxic compounds were compared in a diet-induced obesity (DIO) test, which includes measurements of metabolic syndrome markers. Psychiatric side effects were assessed by measuring anxiogenicity in an ultrasonic vocalization test. All classical CB1R blockers were centrally acting appetite suppressants and decreased body weight and food intake in an obesity-dependent manner, with only slight effects on metabolic syndrome markers. In addition, all classical CB1R blockers increased ultrasonic vocalization. Surprisingly, none of the non-classical CB1R blockers was eligible for the DIO comparison and side effect profiling. O-1269 and LH-21 induced convulsive behavior, whereas VCHSR and JD-5037 were devoid of any in vivo activity. The classical CB1R blockers displayed similar therapeutic and side effect profiles in vivo, whereas the available non-traditional CB1R blockers were not appropriate tools for testing the therapeutic potential of alternative CB1R inhibitors.

1. Introduction

The development of cannabinoid receptor 1 (CB1R) antagonists was one of the most important pharmaceutical competitions in the first years of the 21st century (Le Foll et al., 2009). Although the main focus was on obesity (Bellocchio et al., 2006), CB1R antagonists also had promising potential in treating other disorders, such as nicotine dependence or liver cirrhosis (Pacher et al., 2006). Nearly a dozen compounds were in various stages of clinical trials when the FDA unanimously refused the application for the new drug rimonabant (Heal et al., 2009) because of the increased incidence of severe psychiatric adverse events, such as depression and anxiety [FDA rimonabant briefing document, (FDA, 2007)]. Within a year, all major pharmaceutical companies announced the discontinuation of their CB1R antagonist programs.

Since then, many research groups initiated preclinical programs to develop CB1R blockers that did not display the psychiatric side effects of the "classical" CB1R inverse agonists (Sharma et al., 2014). The majority of these second generation or "non-traditional" programs relies on one of two main theories: the concepts of "neutral antagonism" and "peripheral antagonism" (Silvestri and Di Marzo, 2012). The first concept asserts that CB1 receptors that may play an important role in

Abbreviations: CB_1R, cannabinoid receptor 1; BW, body weight; DIO, diet-induced obesity; ED50, the dose where the predicted effect reaches 50%; Emax, the dose where the predicted effect reaches 100%; LED, least statistically effective dose; USV, ultrasonic vocalization; FDA, Food and Drug Administration; N.D., no data

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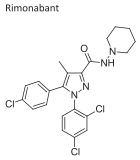
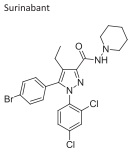
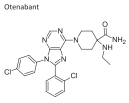
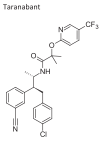
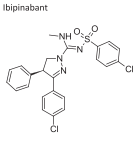
the suppression of anxiety and depression may be constitutively active and exert inhibitory effects, even in the absence of any endogenic agonist. In contrast, the CB1 receptors that are involved in obesity and stimulated by the high endocannabinoid levels are not constitutively active. According to this hypothesis, neutral antagonists may induce fewer side effects, as constitutive activity is solely inhibited by inverse agonists (Pertwee, 2005). The principle of peripherally restricted antagonists posits that a CB1R blocker, which does not pass the blood-brain barrier, would be devoid of psychiatric effects but would still decrease body weight (Chorvat, 2013). Currently, more than a dozen new compounds have been synthesized and studied (Sharma et al., 2014). However, neither an elaborated model system testing the therapeutic and side effects of the CB1R blockers nor a direct preclinical comparison with the classical CB1R antagonists have been published to date.

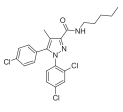
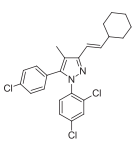
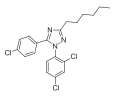
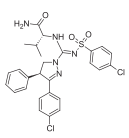
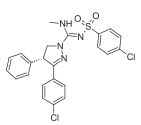
Here, we directly compare the in vivo pharmacodynamic profiles of representative CB1R blockers using an in vivo preclinical assay cascade designed to model the most important therapeutic and side effects of human obesity studies and to investigate if any CB1 blocker displays a better risk/benefit ratio than rimonabant.

We tested five "classical" and four "non-traditional" CB1R blockers that possessed diverse structures and mechanism of actions, such as partial agonism, neutral antagonism or peripheral inverse agonism, as shown in Table 1. Our set of "classical" compounds consisted of five CB1R ligands developed by major pharmaceutical companies. In addition to the two diarylpyrazole structured analogues, the first in class rimonabant (Rinaldi-Carmona et al., 1994) and its successor surinabant (Doggrell, 2005), three structurally different molecules, ibipinabant (Lange et al., 2005), taranabant (Fong et al., 2007) and otenabant (Griffith et al., 2009), all of which are putative centrally acting inverse agonists (Ward and Raffa, 2011), were selected. Three of the "non-traditional" CB1R blockers are also rimonabant analogues: a putative centrally acting neutral antagonist, VCHSR (Manca et al., 2013); a putative centrally acting partial agonist, O-1269 (Wiley et al., 2001); and the putative peripherally acting neutral antagonist LH-21 (Pavon et al., 2006). However, the peripherally acting inverse agonist JD-5037 (Chorvat et al., 2012) is an ibipinabant analogue. The non-CB1 binding enantiomer of ibipinabant (+)SLV-319 (Lange et al., 2004) and the monoaminergic reuptake blocker Sibutramine (Buckett et al., 1988) were used as negative and positive reference compounds in some appetite and obesity-related tests, respectively.

We administered these compounds using a common formula and investigated their central antagonist and appetite suppressive potential in vivo to assess their basic pharmacodynamic properties. The first feature was assessed by measuring CB1R agonist-induced hypothermia (Pério et al., 1996; Son et al., 2010), and the latter feature was assessed using the "fasting-induced food intake test" in mice (Colombo et al., 1998; Gómez et al., 2002; Kirkham et al., 2002). Compounds that proved to be ineffective (up to the highest dose applied, 30 mg/kg) or toxic (inflicting severe side effects) in these assays were discarded. The remaining compounds were compared in the diet-induced obesity (DIO) model at equipotent doses that were determined based on their effects on mice in the first two tests. Body weight and food intake were measured daily and the levels of obesity-associated blood markers (triglyceride, cholesterol and glucose levels) were assessed at the end of the two week treatment. Obese mice have been suggested to be more susceptible to the nutritional effects of rimonabant, (Jbilo et al., 2005); therefore, we simultaneously compared the effects of all CB1R blockers

Table 1
Names, structures, descriptors and main published data for the compounds studied.

Name	Rimonabant	Surinabant	Otenabant	Taranabant	Ibipinabant
Structure					
Structural group	Diaryl-pyrazole	Diaryl-pyrazole	Non diaryl-pyrazole	Non diaryl-pyrazole	Non diaryl-pyrazole
Receptor interaction	Inverse agonist	Inverse agonist	Inverse agonist	Inverse agonist	Inverse agonist
Site of action	Central/peripheral	Central/peripheral	Central/peripheral	Central/peripheral	Central/peripheral
CB1R Ki (nM)	5.6 ± 0.5*	3.5 ± 0.29*	0.7 ± 0.1*	0.13 ± 0.0*	7.8 ± 1.4*
Hypothermia (mg/kg)	0.38 p.o. (50%)*	0.4p.o. (LED)*	10 s.c. (73%)*	3i.v. (100%)*	3 p.o. (LED)*
Food intake (mg/kg)	**2.5 i.p. (LED)	3 p.o. (LED)*	10 p.o. (54%)*	1 i.p. (LED)*	3 i.p. (LED)**
References	*Rinaldi-Carmona 1994, **Colombo 1998	*Rinaldi-Carmona 2004	*Griffith 2009	* Fong 2008	*Lange 2004; **Need 2005

Name	O-1269	VCHSR	LH-21	JD-5037	(+)SLV 319
Structure					
Structural group	Diaryl-pyrazole	Diaryl-pyrazole	Diaryl-pyrazole	Non diaryl-pyrazole	Non diaryl-pyrazole
Receptor interaction	Partial agonist	Neutral antagonist	Neutral antagonist	Inverse agonist	Non-CB1 ligand
Site of action	central/peripheral	Central/peripheral	Peripheral	Peripheral	Central/peripheral
CB1R Ki (nM)	32 ± 5*	11.2 ± 2*	855.6 ± 296*	0.35*	894 ± 324*
Hypothermia (mg/kg)	1 i.v. (LED)*	N.D.	< 1 i.p. (LED)*	N.D.	> 30 p.o.*
Food intake (mg/kg)	N.D.*	10 i.p. (LED)*	0.3 i.p. (LED)**	3 p.o. (DIO)*	N.D.
References	*Wiley et al., 2001	*Manca et al., 2013	*Jagerovic et al., 2004; **Pavon et al., 2006	*Tam et al., 2012	*Lange et al., 2004

We used three descriptors (structural group, receptor interaction, and site of action) to categorize the different CB1R blockers. "Central/peripheral" site of action means that the compound is known to penetrate the blood brain barrier, whereas "peripheral" compounds have been shown to be devoid of central activity. The CB1R binding, hypothermia and food intake results collected here represent data from the papers referenced under each compound (data and references are linked by the appropriate asterisks). For the in vivo tests, the least effective doses (LEDs) – or the percent effect at the dose indicated – are presented. N.D. means that no data have been reported in the literature.