

TRENDS IN CLINICAL PRACTICE

The endocannabinoid system and the treatment of obesity

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Abstract

The endocannabinoids are endogenous lipids capable of binding to both cannabinoid receptors (CB) CB1 and CB2. These receptors belong to the G protein-coupled family receptors and they were discovered while investigating the mode of action of Δ^9 -tetrahydrocannabinol, a component of *Cannabis sativa*, to which they bind with high affinity. Among many other brain sites, CB1 is present in the hypothalamic nuclei involved in the control of energy balance and body weight, as well as in neurons of the mesolimbic system which is believed to mediate the incentive value of food. At central nervous system level, CB1 activation is necessary to induce food intake after a short period of food deprivation, and when CB1 is activated by endocannabinoids produced *in situ*, a stimulation of the ingestion of palatable food has been described. CB1 stimulation leads to modulation of the release of some hypothalamic anorexigenic and orexigenic mediators, as well as of dopamine in the nucleus accumbens shell. Recent evidence has proved that CB1 is also present in the peripheral organs, such as the adipose tissue and gastrointestinal system, key organs in the regulation of energy metabolism. Animal models have provided solid evidence that genetically induced obesity leads to long-lasting overstimulation of endocannabinoid system synthesis resulting in permanent overactivation of CB1, which may then contribute to the maintenance of this disease. Importantly, at peripheral level, CB1 activation has been shown to stimulate lipogenesis in adipocytes. CB1 blockers increase adiponectin production in adipocytes, which leads to increased fatty acid oxidation and free fatty acid clearance. Moreover, CB1 has been shown to be up-regulated in adipocytes derived from obese rodents. These results support the role of endocannabinoids in the development and maintenance of obesity, paving the way for the development of a new class of drugs such as the CB1 blockers as a therapy for tackling obesity and the associated major cardiovascular risk factors.

Key words: *CB1 receptor, endocannabinoid system, obesity, rimonabant*

Introduction

Several centuries ago it was observed that *Cannabis sativa* is able to stimulate appetite (see review in (1)). In the 1960s many studies were therefore initiated to evaluate the orexigenic properties of Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the most important component derived from *Cannabis sativa* (2), in more detail. Only with the advent of molecular biology techniques, were the Δ^9 -THC binding sites finally cloned and consequently named CB1 (3,4) and CB2 (5). These receptors belong to the G protein-coupled superfamily characterized by a seven transmembrane loop domain. CB1 is widely distributed within the central nervous system; its expression is mainly localized at the level of the hippocampus, ganglia, cerebral cortex, cerebellum, limbic system and hypothalamus (6). CB1 is also

expressed in peripheral tissues (7–11). On the other hand, CB2 is mainly expressed in immune cells (9,12) and does not play a part in the regulation of food intake. Interestingly, at the same time as CB1 and CB2 cloning, CB1 endogenous ligands, named endocannabinoids, were identified and synthesized. The most important are anandamide and 2-arachidonoylglycerol (2-AG) (13–15). The discovery of specific receptors and their endogenous ligands suggested the existence of an endogenous cannabinoid system. Endogenous cannabinoids are lipids and they are synthesized and released from neurons in response to membrane depolarization; after their release their rapid inactivation is induced by specific enzymes (16,17). Therefore, endocannabinoids do not appear to be stored in synaptic vesicles but are produced and released ‘on demand’.

The endogenous cannabinoid system is involved in feeding behaviour, in antinociception, in short-term memory regulation and in movement control. It also seems to have an important role in neuro-protection; endocannabinoids have antiproliferative effects, they are also able to modulate hormone secretion, immune and inflammatory responses (see reviews in (18–20)).

In conclusion, the endocannabinoid system is a general stress-recovery system and is silent overall. It becomes transiently activated to help re-establish the normal steady state of cells or tissues that have been subjected to stress. Therefore, the stimulus to eat provided by the endocannabinoids is a response, among others, to help the organism to recover from a transient unbalanced situation like acute starvation (21).

Cannabinoids and feeding behaviour

By the mid 1980s many clinical studies had been initiated, based on the observations of the hyperphagia induced by assumption of the *Cannabis sativa*. Δ^9 -THC (known as Dronabinol) has been shown to exert an anti-emetic effect and to induce appetite stimulation in some clinical syndromes featured by appetite and weight loss such as cancer or AIDS-associated anorexia. Consequently, the use of Δ^9 -THC has been approved by the Food and Drug Administration for the treatment of AIDS wasting syndrome (22–24). The development of rimonabant, an exogenous compound able to antagonize CB1 (25), and the recent generation of genetically modified mice lacking CB1 confirmed the relevant role of the endocannabinoid system in the modulation of food intake and energy balance, paving the way for the first experimental clinical trials in which a novel CB1 blocker, named rimonabant, has been used to tackle obesity and its related metabolic abnormalities.

Central mechanisms of endocannabinoids in promoting food intake

The mesolimbic dopaminergic system is involved in the motivation to search for palatable food (26). CB1 is highly expressed in mesolimbic structures (27) and the exogenous and endogenous cannabinoids interact with neuronal dopaminergic and opioid pathways in order to promote the properties of food (28–30). It is still under discussion whether the hyperphagia induced by cannabinoids may only increase the incentive value of food stimuli or whether these substances may also stimulate the

Key messages

- The endocannabinoid system is a physiological system, comprising the cannabinoid receptors, CB1 and CB2, and their natural endogenous ligands, such as anandamide and 2-arachidonoyl glycerol. For many years *Cannabis sativa* through the action of its component Δ^9 -tetrahydrocannabinol, an exogenous cannabinoid, has been known to stimulate hunger and appetite and these effects have prompted research into its mechanism of action.
- The endocannabinoid system, acting both centrally and peripherally, is positively regulating appetite and energy balance.
- Modulation of the endocannabinoid system by specifically blocking the CB1 receptor in both the brain and periphery can provide a novel target for the treatment of visceral obesity.

orosensory reward given by palatable food (31). In favour of the first hypothesis, a number of reports demonstrated that CB1 activation may restore feeding in satiated animals (see review in (32)), whereas the CB1 blockade decreases the rates of responding for food (33,34). On the other hand, the selective CB1 blockade by rimonabant has been shown to reduce the motivation for sucrose or alcohol intake, giving emphasis to the rewarding properties of endocannabinoids toward palatable food (35–37). Endocannabinoids have been shown to be modulated by food restriction or feeding not only in the rat limbic forebrain but also, at least 2-AG, at level of hypothalamus (21,38). In this later cerebral area the main control of food intake is exerted by a series of neurons that provide high levels of adaptability of feeding behaviour to endogenous and exogenous stimuli (see review in (39)). The state of the relative energy balance coming from a variety of signals of peripheral origin is known to modulate the neurochemical activation of the hypothalamic neurons. Peptides such as leptin and adiponectin secreted by adipocytes as well as signals from the gastrointestinal tract, such as ghrelin, may directly target hypothalamic neurons. CB1 and endocannabinoids are present in the hypothalamic areas involved in food intake and they are able to cross-talk to signals of peripheral origin. Injection of anandamide into the ventromedial hypothalamus of pre-satiated rats resulted in CB1 dependent hyperphagia (40). A single intravenous

injection of leptin (a satiety factor) leads to a decrease of hypothalamic endocannabinoid release; on the other hand, several animal models of obesity such as *ob/ob* or *db/db* mice or *fafa* Zucker rats (all models of impairment of leptin or leptin receptor) have been characterized by increased hypothalamic endocannabinoid levels (41). Therefore, we can conclude that, at least in genetically modified animals, obesity is linked to a chronic activation of the endocannabinoid system. Our recent paper shows a link between CB1 and hypothalamic neurons involved in feeding regulation. Hypothalamic CB1 mRNA has been demonstrated to be co-expressed in corticotropin releasing hormone (CRH), cocaine-amphetamine related transcript (CART), melanin concentrating hormone (MCH) and prepro-orexin neurons. Moreover, in CB1^{-/-} mice (animals lacking CB1 expression) we showed an increased CRH expression in paraventricular nuclei and a reduced CART expression in the dorsomedial and lateral hypothalamus (10).

CB1 peripheral location and its metabolic actions

As mentioned above, our studies on food intake and body weight showed that CB1^{-/-} mice were leaner and lighter than control mice (wild-type). As we demonstrated by nuclear magnetic resonance, the body weight reduction was attributable to a significant decrease in fat mass in comparison to the wild-type animals. Moreover, in 'pair-feeding' experiments on CB1^{-/-} and control mice we found that CB1^{-/-} young mice were leaner because of a food-intake reduction, whereas the leaner phenotype of adult mice was partially due to a food intake-independent mechanism, probably related to an activation of a metabolic process (10).

Similar results were independently obtained by another group by using CB1^{-/-} mice undergoing a high fat calorie diet. In this case, CB1 knock-out mice were resistant to diet-induced obesity. In the same publication, Ravinet-Trillou et al. showed that animals lacking CB1 were characterized by a dramatic amelioration of many metabolic parameters that were partially independent of a reduction in food intake (42).

Rimonabant administration in diet-induced obesity (DIO) mice has been shown to induce a transient anorectic effect which was overcome by a more significant and sustained reduction in body weight, due to a decrease in body fat mass (43). These data confirmed that CB1 antagonists may act as an anti-obesity drug by a dual mechanism of action that is initially targeting neuronal sites and

thereafter peripheral organs involved in energy storage and expenditure.

Our *in vitro* studies showed that CB1 is functionally active in white adipocytes stimulating lipogenesis. Their stimulation enhances lipoprotein lipase activation and this effect has been shown to be specifically blocked by rimonabant (10). Another group showed that CB1 is mostly expressed in mature adipocytes when compared to preadipocytes and that CB1 expression is increased in adipocytes from obese mice compared to those derived from lean ones (11), confirming the notion that an overaction of the endocannabinoid system is associated with obesity. Importantly, rimonabant induces adiponectin release from adipocytes *in vitro* (11). Adiponectin is an adipose tissue-specific circulating protein, playing a key regulatory role in fat and glucose metabolism (see review in (44)). This protein exhibits anti-atherogenic and anti-diabetic properties. It is associated with an increased insulin sensitivity, and in the liver adiponectin decreases hepatic glucose production and regulates free fatty acid metabolism, via suppression of lipogenesis and activation of free fatty acid oxidation. Human obesity and diseases associated with insulin resistance or type 2 diabetes are characterized by a reduced tissue and haematic adiponectin concentration (see review in (45)). We can hypothesize that rimonabant may act as an anti-obesity peripheral drug by directly influencing the adipose tissue, targeting some enzymes involved in lipogenesis and stimulating adiponectin production.

However, peripheral mechanisms of action of endocannabinoids may be even more complex than previously described. CB1 is also expressed in the liver; in this organ CB1 activation has a vasoactive function (7). At present, we cannot exclude the possibility that CB1 may also play a role in the important hepatic regulatory functions like those in lipid and carbohydrate metabolism. Moreover very recently, a new putative site of action of endocannabinoids has been found in organs like skeletal muscles regulating metabolic processes. Intriguingly, in *ob/ob* treated animals when compared to placebo treated mice, it has been shown that 7 days of rimonabant treatment activated thermogenesis as demonstrated by a 37% increase in basal oxygen consumption (46). To explain this finding the authors speculated that rimonabant may act by stimulating efferent sympathetic activity as shown in the past by another group (47). However, the authors also hypothesized that the increase in thermogenesis may also be due to an increase of free fatty acid oxidation promoted by the adiponectin increment directly stimulated by

rimonabant. Furthermore, in the same manuscript the authors found that rimonabant treatment caused a significant soleus muscle glucose uptake (46). This increase may significantly contribute to the improved hyperglycemia observed in DIO mice treated with CB1 antagonists in other experimental settings.

CB1 and endocannabinoids are also highly expressed in the gastrointestinal tract neurons. In the small intestine starvation determines a 7-fold increase in anandamide release. Intriguingly, this effect is reversed on re-feeding (48). Vagal afferent neurons expressing cholecystinin (CCK) receptor type1 also express CB1. CCK is known to display an important satiety role. These neurons project to the stomach and duodenum. Interestingly, the expression of CB1 in the ganglia was increased by food deprivation and after re-feeding there was a rapid loss of CB1. This effect has been shown to be blocked by CCK antagonists and mimicked by administration of CCK itself. Therefore, it can be hypothesized that the endocannabinoid system may also influence food intake by a gastroduodenal cross-talk with CCK signaling (49).

In conclusion, a varied and increasing number of organs may be the site of action of the endocannabinoid system in influencing food intake and metabolic processes (Figure 1). The detailed definition of each individual contribution and the reciprocal interactions between organs will be mandatory in future studies on this topic.

CB1 antagonists: a brand new class of drugs

The discovery of rimonabant and the impressive amount of data obtained in experimental models of obesity have substantially accelerated the search for

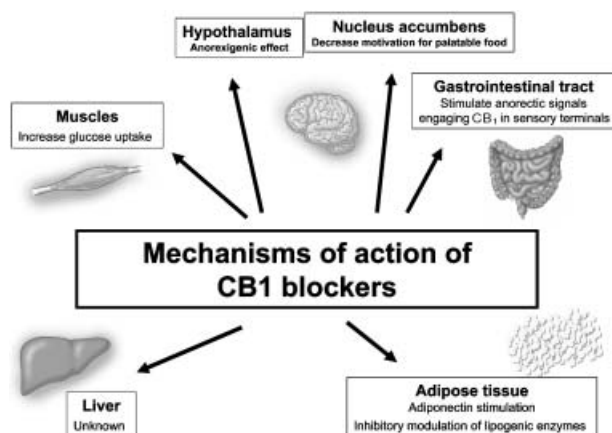


Figure 1. Sites of action of drugs having CB1 blocker activity.

alternative compounds sharing similar properties. However, there have been only a few peer-review publications on these new compounds. Beside rimonabant, AM-251 (50–52) and SR147778, a new compound from the Sanofi laboratory (53), have up to now been the most characterized in their anti-obesity action in animals. However, it should be noted that a large series of CB1 antagonists from the Solvay laboratory have recently been developed and hypothesized for future clinical use as drugs targeting obesity (54,55).

Clinical trials using rimonabant: a new CB1 blocker

In 1994 Sanofi-Synthelabo discovered rimonabant, the first specific CB1 blocker (25). This discovery made it possible to understand many facets of the endocannabinoid system and paved the way for the development of novel pharmacotherapies to tackle obesity. A double-blind, placebo-controlled, phase II trial using 4 months of rimonabant treatment in a group of obese patients with a body mass index ranging from 30 to 40 showed an average weight loss of 3.5 kg and 4.4 kg at the 5 mg and 20 mg doses, respectively; whereas in the placebo group the average weight loss was limited to 1.1 kg. Importantly, the weight loss did not plateau during the 16-week study period. In this study the drug demonstrated a good safety profile (56).

Another phase II, double-blind, placebo-controlled study involved 20 obese patients with a short-term rimonabant treatment period (7 days) to investigate hunger, calorie and fat intake. A significant decrease in all these three parameters was found (56).

A large phase III trial was initiated in August 2001 including more than 6,600 obese or overweight patients. All studies were concluded in 2004. These studies, named RIO-North America and RIO-Europe respectively, recruited obese or overweight patients with or without comorbidities who were treated for two years with 5 mg or 20 mg rimonabant *versus* placebo. The primary endpoints of RIO North America were the absolute change in weight from baseline at 1 year and the prevention of weight regain after re-randomization (2nd year), whereas the RIO-Europe main endpoint was the assessment of weight reduction by using the same dosages. Secondary endpoints of both studies were the number of weight responder patients, and the changes in waist circumference, in metabolic and lipidic parameters, and the changes in the number of patients affected by the metabolic syndrome as defined by National Cholesterol Education Program's Adult Treatment Program III (NCEP

ATPIII) criteria. RIO-Lipids and RIO-Diabetes have been proposed in order to investigate the amelioration after rimonabant treatment of specific comorbidity factors associated with obesity or overweight such as hyperlipidemia and diabetes, respectively (56). To date no data have been published in peer-reviewed journals.

Conclusions

Overall, the endocannabinoid system seems to play a key role in the development and maintenance of obesity (see previous reviews (32,56–59)). The blockade of CB1 at both central and peripheral level helps to normalize the dysfunction of the system. Novel classes of drugs targeting the endocannabinoid system, such as rimonabant, may definitively prove in the near future to be effective in managing obesity and metabolic risk factors by contributing in this way to develop a brand new approach to address emerging world problems like obesity and its associated cardiovascular risk factors.

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References

1. Abel EL. Cannabis: effects on hunger and thirst. *Behav Biol.* 1975;15:255–81.
2. Gaoni Y, Mechoulam R. Isolation, structure and partial synthesis of an active constituent of hashish. *J Am Chem Soc.* 1964;86:1646–7.
3. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature.* 1990;346:561–4.
4. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science.* 1992;258:1946–9.
5. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature.* 1993;365:61–5.
6. Howlett AC, Glass M, Dragunow M, Faulk RL. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience.* 1997;77:299–318.
7. Batkai S, Jarai Z, Wagner JA, Goparaju SK, Varga K, Liu J, et al. Endocannabinoids acting at vascular CB1 receptors mediate the vasodilated state in advanced liver cirrhosis. *Nat Med.* 2001;7:827–32.
8. Pagotto U, Marsicano G, Fezza F, Theodoropoulou M, Grübler Y, Stalla J, et al. Normal human pituitary gland and pituitary adenomas express cannabinoid receptor type 1 and synthesize endogenous cannabinoids: first evidence for a direct role of cannabinoids on hormone modulation at the human pituitary level. *J Clin Endocrinol Metab.* 2001;86:2687–96.
9. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, et al. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev.* 2002;54:161–202.
10. Cota D, Marsicano G, Tschoep M, Gruebler Y, Flachskamm C, Schubert M, et al. The endogenous cannabinoid system affects energy balance via central orexigenic drive and peripheral lipogenesis. *J Clin Invest.* 2003;112:423–31.
11. Bensaid M, Gary-Bobo M, Esclangon A, Maffrand JP, Le Fur G, Oury-Donat F, Soubrié P. The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. *Mol Pharmacol.* 2003;63:908–14.
12. Pertwee RG. Pharmacology of cannabinoid CB1 and CB2 receptors. *Pharmacol Ther.* 1997;74:129–80.
13. Hanus L, Gopher A, Almog S, Mechoulam R. Two new unsaturated fatty acid ethanolamides in brain that bind to the cannabinoid receptor. *J Med Chem.* 1993;36:3032–4.
14. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, et al. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun.* 1995;215:89–97.
15. Mechoulam R, Ben Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol.* 1995;50:83–90.
16. Di Marzo V, Fontana A, Cadas H, Schinelli S, Cimino G, Schwartz JC, et al. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. *Nature.* 1994;372:686–91.
17. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc Natl Acad Sci U S A.* 2002;99:10819–24.
18. Di Marzo V, Melck D, Bisogno T, De Petrocellis L. Endocannabinoids: endogenous cannabinoid receptor ligands with neuromodulatory action. *Trends Neurosci.* 1998;21:521–8.
19. Piomelli D, Giuffrida A, Calignano A, Rodriguez De Fonseca F. The endocannabinoid system as a target for therapeutic drugs. *Trends Pharmacol Sci.* 2000;21:218–24.
20. De Petrocellis L, Cascio MG, Di Marzo V. The endocannabinoid system: a general view and latest additions. *Br J Pharmacol.* 2004;141:765–74.
21. Hanus L, Avraham Y, Ben-Shushan D, Zolotarev O, Berry EM, Mechoulam R. Short-term fasting and prolonged semistarvation have opposite effects on 2-AG levels in mouse brain. *Brain Res.* 2003;983:144–51.
22. Plasse TF, Gorter RW, Krasnow SH, Lane M, Shepard KV, Wadleigh RG. Recent clinical experience with dronabinol. *Pharmacol Biochem Behav.* 1991;40:695–700.
23. Gorter R, Seefried M, Volberding P. Dronabinol effects on weight in patients with HIV infection. *AIDS.* 1992;6:127.
24. Beal JE, Olson R, Lefkowitz L, Laubenstein L, Bellman P, Yangco B, et al. Long-term efficacy and safety of dronabinol for acquired immunodeficiency syndrome-associated anorexia. *J Pain Symptom Manage.* 1997;14:7–14.
25. Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, et al. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* 1994;350:240–4.

26. Berridge KC. Food reward: brain substrates of wanting and liking. *Neurosci Biobehav Rev.* 1996;20:1–25.
27. Herkenham M, Lynn AB, Johnson MR, Melvin LD, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci.* 1991;11:563–83.
28. Kirkham TC, Williams C. Synergistic effects of opioid and cannabinoid antagonists on food intake. *Psychopharmacology (Berl).* 2001;153:267–70.
29. Verty ANA, Singh ME, McGregor IS, Mallet PE. The cannabinoid receptor antagonist SR141716 attenuates overfeeding induced by systemic or intracranial morphine. *Psychopharmacology (Berl).* 2003;168:314–23.
30. Verty ANA, McGregor IS, Mallet PE. The dopamine receptor antagonist SCH 23390 attenuates feeding induced by Δ^9 -tetrahydrocannabinol. *Brain Res.* 2004;1020:188–95.
31. Higgs S, Williams CM, Kirkham TC. Cannabinoid influences on palatability: microstructural analysis of sucrose drinking after delta(9)-tetrahydrocannabinol, anandamide, 2-arachidonoyl glycerol and SR141716. *Psychopharmacology (Berl).* 2003;165:370–7.
32. Kirkham TC, Williams CM. Endogenous cannabinoids and food appetite. *Nutr Res Rev.* 2001;14:65–86.
33. Freedland CS, Poston JS, Porrino LJ. Effects of SR141716, a central cannabinoid receptor antagonist, on food-maintained responding. *Pharmacol Biochem Behav.* 2000;67:265–70.
34. Perio A, Barnouin MC, Poncelet M, Soubrié P. Activity of SR141716 on post-reinforcement pauses in operant responding for sucrose reward in rats. *Behav Pharmacol.* 1998;12:641–6.
35. Arnone M, Maruani J, Chaperon F, Thiebot MH, Poncelet M, Soubrié P, et al. Selective inhibition of sucrose and ethanol intake by SR 141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology (Berl).* 1997;132:104–6.
36. Simiand J, Keane M, Keane PE, Soubrié P. CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. *Behav Pharmacol.* 2001;9:179–81.
37. Sanchis-Segura C, Cline BH, Marsicano G, Lutz B, Spanagel R. Reduced sensitivity to reward in CB1 knockout mice. *Psychopharmacology (Berl).* 2004;176:223–32.
38. Kirkham TC, Williams CM, Fezza F, Di Marzo V. Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br J Pharmacol.* 2002;136:550–7.
39. Flier JS. Obesity wars: molecular progress confronts an expanding epidemic. *Cell.* 2004;116:337–50.
40. Jamshidi N, Taylor DA. Anandamide administration into the ventromedial hypothalamus stimulates appetite in rats. *Br J Pharmacol.* 2001;134:1151–4.
41. Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, Jarai Z, et al. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature.* 2001;410:822–5.
42. Ravinet Trillou C, Delgorge C, Menet C, Arnone M, Soubrié P. CB1 cannabinoid-receptor knockout in mice leads to leanness, resistance to diet-induced obesity and enhanced leptin sensitivity. *Int J Obes.* 2004;28:640–8.
43. Ravinet Trillou C, Arnone M, Delgorge C, Gonalons N, Keane P, Maffrand JP, et al. Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol.* 2003;284:R345–53.
44. Gil-Campos M, Canete R, Gil A. Adiponectin, the missing link in insulin resistance and obesity. *Clin Nutr.* 2004;23:963–74.
45. Chandran M, Phillips SA, Ciaraldi T, Henry RR. Adiponectin: more than just another fat cell hormone? *Diabetes Care.* 2003;26:2442–50.
46. Liu YU, Connoley IP, Wilson CA, Stock MJ. Effects of the cannabinoid CB1 receptor antagonist SR141716 on oxygen consumption and soleus muscle glucose uptake in Lep^{ob}/Lep^{ob} mice. *Int J Obes.* 2005;29:183–7.
47. Tzavara ET, Perry KW, Rodriguez DE, Bymaster FP, Nomikos GG. The cannabinoid CB1 receptor antagonist SR141716A increases norepinephrine outflow in the rat anterior hypothalamus. *Eur J Pharmacol.* 2001;426:R3–4.
48. Gomez R, Navarro M, Ferrer B, Trigo JM, Bilbao A, Del Arco I, et al. A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. *J Neurosci.* 2002;22:9612–7.
49. Burdyga G, Lal S, Varro A, Dimaline R, Thompson DG, Dockray GJ. Expression of cannabinoid CB1 receptors by vagal afferent neurons is inhibited by cholecystokinin. *J Neurosci.* 2004;24:2708–11.
50. Hildebrandt AL, Kelly-Sullivan DM, Black SC. Antiobesity effects of chronic cannabinoid CB1 receptor antagonist treatment in diet-induced obese mice. *Eur J Pharmacol.* 2003;462:125–32.
51. Zhou D, Shearman LP. Voluntary exercise augments acute effects of CB1-receptor inverse agonist on body weight loss in obese and lean mice. *Pharmacol Biochem Behav.* 2004;77:117–25.
52. Chambers AP, Sharkey KA, Koopmans HS. Cannabinoid (CB) 1 receptor antagonist, AM 251, causes a sustained reduction of daily food intake in the rat. *Physiol Behav.* 2004;82:863–9.
53. Rinaldi-Carmona M, Barth F, Congy C, Martinez S, Oustric D, Perio A, et al. SR147778, a new potent and selective antagonist of the CB1 cannabinoid receptor. Biochemical and pharmacological characterization. *J Pharmacol Exp Ther.* 2004;310:905–14.
54. Lange JHM, Coolen HKAC, von Stuienburg HH, Dijkman JAR, Herremans AHJ, Ronken E, et al. Synthesis, biological properties, and molecular modeling investigations of novel 3,4-diarylpyrazolines as potent and selective CB1 cannabinoid receptor antagonists. *J Med Chem.* 2004;427:627–43.
55. Lange JHM, Kruse CG. Recent advances in CB1 cannabinoid receptor antagonists. *Curr Opin Drug Discov Devel.* 2004;7:498–506.
56. Fernandez JR, Allison DB. Rimonabant. *Curr Opin Invest Drug.* 2004;5:430–5.
57. Cota D, Marsicano G, Lutz B, Vicennati V, Stalla GK, Pasquali R, Pagotto U. Endogenous cannabinoid system as a modulator of food intake. *Int J Obes.* 2003;27:289–301.
58. Cota D, Genghini S, Pasquali R, Pagotto U. Antagonizing the cannabinoid receptor type 1: a dual way to fight obesity. *J Endocrinol Invest.* 2003;26:1041–4.
59. Marsicano G, Cota D, Stalla GK, Pasquali R, Pagotto U, Lutz B. Cannabinoids in energy balance and prospectives in the therapy of obesity. *Curr Med Chem.* 2003;3:81–7.