Cannabinoid Receptor Antagonists and the Metabolic Syndrome: Novel Promising Therapeutical Approaches

C. Cervino, R. Pasquali and U. Pagotto*

Endocrinology Unit, Department of Internal Medicine and Gastroenterology and C.R.B.A., S.Orsola-Malpighi Hospital, Alma Mater Studiorum University of Bologna, 40138 Bologna, Italy

Abstract: Recent findings in animals and in humans have shown that cannabinoid type 1 receptor antagonists are suitable to become the most promising validated class of drugs to tackle obesity and related disorders. This mini-review will provide a concise and updated revision of the state of art on this topic.

Key Words: Endocannabinoid, cannabinoid type 1 receptor, cannabinoid type 1 receptor antagonist, rimonabant, metabolic syndrome.

INTRODUCTION

Obesity has increased at a striking rate over the last three decades in the Western countries. This negative trend dramatically impacts on physical health and on the relative cardiovascular risk. In fact, particularly when judged at visceral level, obesity is strongly associated with an increased risk of life-threatening conditions such as diabetes, arterial hypertension, dyslipidemia and cardiovascular diseases [1]. Waist circumference, both in women and men, provides a convenient measure of visceral obesity. Therefore, waist circumference reduction should be the target of clinical intervention in obese patients [2]. Although lifestyle adjustments, such as nutritional changes and physical activity, are commonly thought of as the milestones of the treatment of obesity, it is now evident that it is necessary to support obese patients with a pharmacological approach for almost two reasons: to further reduce the metabolic risk profile and to avoid regaining lost weight.

Among the various pharmacological targets explored in recent years, the endocannabinoid system nowadays constitutes the most promising and the most intriguing one proposed so far.

1. THE ENDOCANNABINOID SYSTEM

Cannabinoid research received an important boost by the identification of the chemical structure of Δ^9-tetrahydrocannabinol (THC) (Fig. **(1)**), the best characterized cannabinoid component of *Cannabis Sativa*, among several other cannabinoids present in the plant [3]. The second remarkable success in this field of research was provided by the discovery of the CB1 receptor, the binding site of exogenous cannabinoids and of the synthetic analogs synthesized thereafter [4].

Together with the characterization of another cannabinoid receptor named CB2 receptor, anandamide (N-arachidonoylethanolamine) (AEA) [5] and 2-arachidonoylglycerol (2-AG) [6, 7], the first endogenous ligands for CB1 and CB2 receptors, derivatives of arachidonic acid, were identified (Fig. **(1)**). Over the last few years, several other derivatives of long-chain polyunsaturated fatty acids have been described [8-10]; they appear to act, at least in part, through CB1 and CB2 receptors. Their functions are, however, less characterized when compared to AEA and 2-AG.

Although the CB1 receptor was originally described as the "brain type", because it is the most abundant G protein-coupled receptor in the central nervous system of mammalians [11], recent studies highlighted its presence in various peripheral organs including those involved in the control of metabolism such as adipocytes [12-14], hepatocytes [15], endocrine pancreatic cells [16] and skeletal muscle cells [17]. On the other hand, the CB2 receptor is mainly expressed in immune cells and does not seem to play a role in the regulation of metabolic processes, so far [11]. However, as was the case with CB1 receptors, recent evidence has demonstrated that the CB2 receptor is not only limited to immune and hematopoietic cells, but is also present in the brain [18], in the liver [19], in the bone [20] and in the pancreas [16].

Endocannabinoids are lipophilic substances and their synthesis derives from phospholipid precursors. Unlike other neurotransmitters or hormones, they are not stored in vesicles but released "on demand". This peculiar characteristic needs, therefore, a strict regulation of the different phases of their release, uptake and degradation. In general, the synthesis of endocannabinoids is triggered by elevated intracellular concentrations of Ca^{2+}, such as during membrane depolarization [see review in 21]. However, Ca^{2+}-independent processes have also been proposed to induce endocannabinoid synthesis [22]. Very interestingly, it has recently been shown that non-genomic actions of glucocorticoids can also stimulate the synthesis of endocannabinoids [23].

Briefly, the formation of AEA occurs in two steps (see review in [21]). Initially, the precursor phosphatidyl ethanolamine, an abundant lipid present in the cell membrane, exchanges the ethanolamine moiety with an arachidonic acid

*Address correspondence to this author at the Endocrinology Unit and C.R.B.A, Dept. of Internal Medicine and Gastroenterology, S. Orsola-Malpighi Hospital, *Via* Massarenti, 9, 40138 Bologna, Italy; Tel: +39-051-6363009; Fax: +39-051-636080; E-mail: pagube@med.unibo.it

1389-5575/07 $50.00+.00

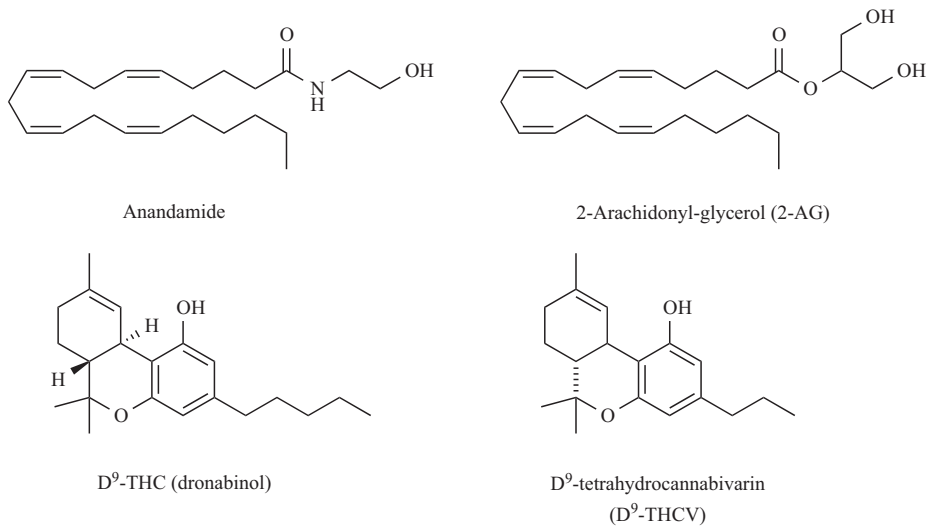


Fig. (1). Endocannabinoids and exogenous CB1 receptor ligands derived from *Cannabis Sativa* plants.

moiety to yield N-arachidonoyl phosphatidyl ethanolamine using the enzyme N-acyltransferase. There after, AEA is synthesized from this intermediate by a recently cloned N-acylphosphatidylethanolamine-selective phospholipase D (NAPE-PLD) [24]. In addition, AEA can also be synthesized from N-arachidonoyl phosphatidyl ethanolamine *via* an additional intermediate, N-arachidonoyl phosphatidyl lysoethanolamine. It is obvious that NAPE-PLD is close to becoming a promising pharmacological target in the near future, whenever a tissutal reduction of AEA production is required [25].

The second major endocannabinoid, 2-AG, is synthesized in two steps from phosphatidyl inositol, another lipid precursor abundantly present in membranes (see review in [21]). Two different pathways have, however, been described for the synthesis of 2-AG: 1,2-diacylglycerol and lysophosphatidyl inositol were found to be intermediate products. A phospholipase C and a phospholipase A1, respectively, are involved in the generation of the two intermediate compounds. Two diacylglycerol lipase (DAGL) enzymes (DAGLα and DAGLβ) catalyzing the synthesis of 2-AG from 1,2-diacylglycerol have recently been cloned [26]. The other enzymes involved have not yet been clearly identified.

In conclusion, although significant progress has been achieved regarding the biosynthetic enzymes for endocannabinoids, a great effort is still needed to characterize all the components of these pathways.

In a similar way to "classical" neurotransmitters, it is proposed that endocannabinoids – after exerting their effects in the extracellular space – are taken up into the cell by a transport mechanism. Transport is not driven by transmembrane ion gradients, but it appears to be facilitated by membrane diffusion of these lipophilic compounds [21]. The molecular identity of such a putative endocannabinoid transporter(s) has not yet been identified. However, it has become clear that this process is complex and likely involves several proteins and mechanisms [27].

The degradation of endocannabinoids is better understood than their biosynthesis. AEA is hydrolyzed to arachidonic acid and ethanolamine by the enzyme fatty acid amide hydrolase (FAAH) [28]. AEA is not the only substrate of FAAH, as it also degrades other bioactive lipids [28]. Several FAAH inhibitors will be tested *in vivo* for their ability to significantly enhance AEA levels [25].

The degradation of 2-AG likely involves at least two enzymes. Monoacylglycerol lipase appears to be responsible for about half the 2-AG-hydrolyzing activity in the tissue, suggesting that additional 2-AG-hydrolyzing enzymes may exist [29].

2. CANNABINOID AGONISTS

As mentioned above, together with THC, the extracts from *Cannabis* plants contain more than other 60 different, chemically closely related phytocannabinoids [30]. The majority of terpeno-phenols in hemp lack psychoactivity, but exert various pharmacological effects *in vivo*, although these effects are present only at a rather high concentration and do not seem to be mediated by CB1- or CB2 receptors (see review in [25]). Among them, cannabidiol has recently obtained further attention due to its anti-convulsive, anti-inflammatory and anti-tumoral properties (see review in [25] and [31]). The underlying mechanisms of action of this plant-derived cannabinoid have not yet been elucidated, but it has been hypothesized that its actions could probably derive from an interaction with an unidentified cannabinoid receptor or from an inhibition of AEA degradation. Tetrahydrocannabivarin, another component of marijuana, has recently been proposed as a new phytocannabinoid having CB1 receptor antagonist properties (Fig. (**1**)) [32].

Based on structural features, phyto- and synthetic cannabinoids can be divided into four classes [11]:

1) "Classical" cannabinoids. The leading structure is represented by THC. This class encompasses tricyclic dibenzopyran compounds. Among its members, the synthetic derivative HU210 shows the highest potency among the known CB1 receptor agonists and also activates the CB2 receptor [11]. This class also includes nabilone and HU-

211 (dexanabinol); the latter is a compound developed by Pharmos that is undergoing phase III clinical trials as a neuroprotective agent for head injury [33].

2) "Non-classical" cannabinoids. These are synthetic THC derivatives, which lack the dihydropyran ring. The best known member of this class is represented by CP-55,940, a potent agonist of CB1 and CB2 receptors, which was pivotal for the molecular identification of the CB1 receptor (Fig. (**2**)) [34].

3) Aminoalkylindoles, represented by *R*-(+)-WIN-55,212-2, are compounds structurally unrelated to THC, but with strong cannabimimetic activities [11]. They bind to both CB1 and CB2 receptors [11]. WIN 55,212–2 (Fig. (**2**)) was discovered by accident in a program directed at the development of non-steroidal anti-inflammatory drugs [35]. The members of this class are able to bind different parts of the CB1 receptor compared to ligands such as CP-55,940 and anandamide [36].

4) Endocannabinoids, which are structurally distinct from plant-derived cannabinoids. Prototypically, they belong to the eicosanoids, fatty acid derivatives containing a chain with 20 carbon atoms.

Bearing in mind the disparate actions of the endocannabinoid system, the non-selective cannabinoid receptor agonists such as CP-55,940, WIN 55,212–2 and THC (Fig. (**1**) and (**3**)) are thought to exhibit a practical use as appetite stimulants, anti-emetics, analgesics, antiglaucoma agents, tumor growth inhibitors and for the treatment of neurodegenerative disorders, including multiple sclerosis (see review in [25]).

3. CB1 RECEPTOR ANTAGONISTS

The CB1 receptor antagonists known so far are diarylpyrazoles, or aminoalkylindoles or triazole derivatives [37]. Diarylpyrazole compounds include SR141716 (named rimonabant), which was discovered by Sanofi-Synthélabo (now Sanofi-Aventis) in 1994 [38]. It represents the first reported selective CB1 receptor antagonist (Fig. (**3**)) and it has been the most studied compound so far [39]. The structure-activity relationship of rimonabant has been reported [40, 41] based on receptor binding affinities, functional antagonistic activities and other pharmacological assays [42]. Optimal binding at the CB1 receptor involves a para-substituted phenyl ring at the pyrazole 5-position and a 2-chloro- or 2,4-dichloro-phenyl substitution pattern at the pyrazole 1-position. Interestingly, rimonabant and some other analogs have been shown, in some experimental models, to act as inverse agonists on a constitutively active CB1 receptor, rather than neutral receptor antagonists [43].

Sanofi-Aventis has recently generated a second-generation antagonist, SR147778 (Fig. (**3**)) [44], which has a longer action period than rimonabant and this property may be due to an ethyl group at the 4-position of its pyrazole ring, which is more metabolically resistant; SR147778 is currently in phase I clinical development.

Other CB1 receptor antagonists have been described, such as CP-272,871 (Fig. **3**) by Pfizer, however, it is a significantly less potent and less CB1 receptor selective antagonist [45].

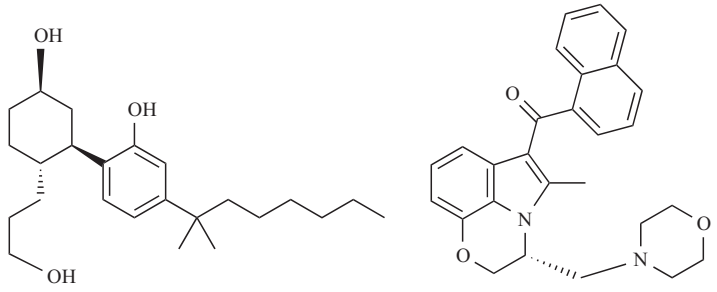
The NIDA Institute recently disclosed NIDA-41020 (Fig. (**3**)), a less potent CB1 receptor antagonist, but with significantly reduced lipophilicity compared to rimonabant [46].

Besides rimonabant, AM-251 [47-49] and SR147778 [44] have so far been the most characterized in their anti-obesity action in animals. However, it should be noted that a large series of CB1 receptor antagonists from Solvay, as SLV319 [50], has recently been developed and hypothesized for future clinical use [37].

4. CANNABINOIDS AND THE CONTROL OF FOOD INTAKE: AN EVERGREEN STORY

The idea that cannabinoids can stimulate hunger has a historical tradition; in fact, in an Indian pharmacopoeia, *Cannabis sativa* use was proposed for patients in whom it was necessary to promote feeding and to reduce vomiting (see review in [51]). The orexigenic properties of Cannabis were also noted for the 'munchies' caused after marijuana smoking and afterward, widely studied in various animal models. However, rather contradictory results were obtained in these initial experiments due to the variability of the dosages, of the routes of administration and of the purity of the extracts. Great caution should therefore be taken in interpreting the results derived from most of the experiments performed with phytocannabinoids (see review in [51]).

Only during the 1980's, when THC, also known as Dronabinol (Fig. (**1**)), became available for studies in humans, was the ability of this compound was known to stimulate



CP 55,940 WIN 55,212-2

Fig. (2). Cannabinoid mixed CB1/2 receptor agonists.

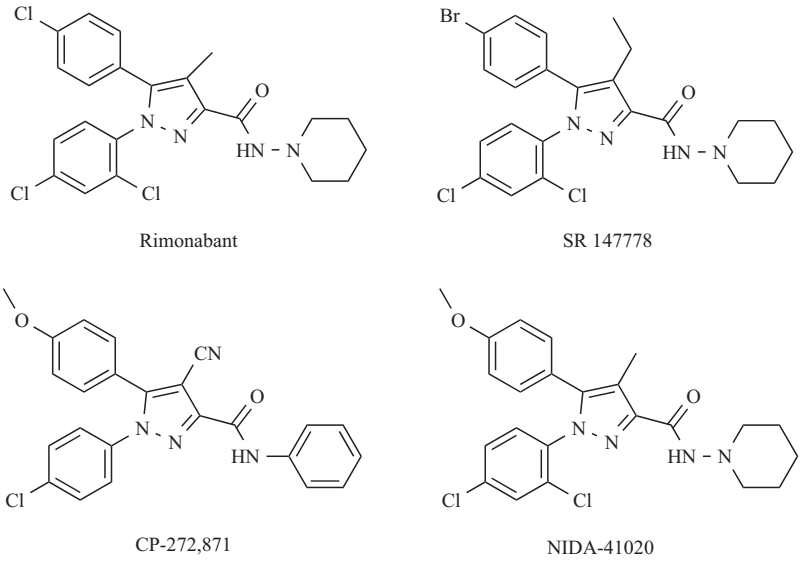


Fig. (3). First and second generation of CB1 receptor antagonists.

appetite substantiated in a more scientific and rigorous manner, in particular when it was positively tested in syndromes characterized by wasting such as cancer or AIDS-associated anorexia (see review in [51]).

4.1. Central Effects of the Endocannabinoids System to Promote Appetite

Several lines of evidence strengthen the notion that through activation of the CB1 receptor, the endocannabinoid system is deeply implicated in the control of food intake. Briefly, (i) there is evidence that both AEA and 2-AG are able even at low doses to stimulate food intake regardless of the site of administration (central or peripheral), (ii) the mesolimbic dopaminergic system [52] and hypothalamus [51] are sites of expression of CB1 receptors, (iii) endocannabinoids can interact with neuronal dopaminergic and opioid pathways in order to increase the value incentive of food [53-55], (iv) and last but not the least, the selective CB1 receptor blockade by rimonabant has been shown to reduce the incentive for sucrose or alcohol intake, reinforcing the concept of the endocannabinoids rewarding properties toward palatable foods [56-58], and to block the AEA-induced hyperphagia when this compound is administered in the ventromedial hypothalamus of pre-satiated rats [59].

4.2. Endocannabinoids and the Appetite-Related Neural Circuit

In view of the well known ability of the endocannabinoid system to be activated "on demand" after typical stressful circumstances occurring in life, it was not surprising to detect an increase in endocannabinoid production at the hypothalamic and at the limbic forebrain level in a typical stress situation such as starving [60-61].

Specific hypothalamic neuronal populations exert a strategic control on food intake [62]. The homeostasis of energy balance originates from a multiplicity of peripheral signals, known to control the activation of these hypothalamic neurons [62]. A recent paper described the anatomical link between the CB1 receptor and hypothalamic neurons involved in feeding control network [12]. The interaction between endocannabinoids and leptin (a satiety factor) has been the most studied so far [63, 64]. Pivotal in this sense were the experiments in which several animal models of impaired leptinergic signal resulting in obese and hyperphagic phenotype, such as that exhibited by *ob/ob* or *db/db* mice or *fa/fa* Zucker rats, were characterized by increased pathological intrahypothalamic endocannabinoid levels [63]. Moreover, as further demonstration of the interaction between leptin and endocannabinoids, a single intravenous injection of leptin in *ob/ob* mice reduced the pathological overproduction of endocannabinoids [63]. A putative site for an interaction between leptin and endocannabinoids is the lateral hypothalamus [64]. We have demonstrated that the CB1 receptors present in this area are co-localized with Melanin Concentrating Hormone (MCH) and prepro-orexin neurons [12]. Both populations of these latter neurons have been implicated in the hedonic or motivational aspects of food intake, acting *via* projections to limbic areas [65]. A recent paper shed light on the interaction between endocannabinoids, MCH neurons and leptin signaling [64]. In fact, MCH neurons (promoting an increase in food intake) are tonically inhibited by GABAergic inputs coming from the limbic system. On one hand, by decreasing GABA release, endocannabinoids may enhance the excitability of MCH neurons leading to an increased feeding behavior, while on the other, by inhibiting the voltage-gated calcium currents in the same neurons, the leptin signal may result in less synthesis and release of endocannabinoids that lead to a reduced excitability of MCH neurons and to a decrease in appetite as a final effect. Therefore, the lateral hypothalamus may represent the site of converging signals between hypothalamic and mesolimbic structures to mediate the orexigenic activity of endocannabinoids [64].

CB1 receptor mRNA has also been shown to be co-expressed with Corticotropin Releasing Hormone (CRH).

Recently, it has been found that postsynaptically released endocannabinoids acting at presynaptic CB1 receptors are able to decrease glutamatergic transmission onto CRH-producing neurons, resulting in an inhibition of CRH release [23]. This release of endocannabinoids from the parvocellular neurons is stimulated by a non-genomic effect of glucocorticoids. Therefore, it is conceivable that the well-known regulation of food intake by glucocorticoids may partly derive from a functional cross-talk with the endocannabinoid system [66].

Endocannabinoids also interact with other neuropeptides including Cocaine Amphetamine Related Transcript and α-melanocyte-stimulating hormone (α-MSH), but their cross-talk is not yet fully elucidated [12, 67, 68]. On the contrary, the neuropeptide Y (NPY)/agouti related protein system in the arcuate nucleus does not seem to be directly targeted by the endocannabinoid system, because no CB1 receptor expression has been found in this area [12]. Nevertheless, recent evidence has shown that AEA is able to increase NPY release by hypothalamic explants *via* an activation of CB1 receptor depolarization, suggestive of a cross-talk between endocannabinoids and NPY orexigenic pathways [69].

4.3. Blockade of CB1 Receptors Affect Several Functions of Peripheral Organs Involved in Control of the Metabolism

By using the genetic ablation of CB1 in rodents as a tool, we found that CB1 receptor knock-out male mice (CB1-/-) were leaner and lighter than control littermates. The body weight decrease was due to a reduction in fat mass and to a proportional increase in lean mass as demonstrated by Nuclear Magnetic Resonance [12]. Importantly, "pair-feeding" experiments in adult CB1-/- *vs.* control mice showed that leanness was due to a mechanism partially independent of food intake, related to an activation of unknown metabolic processes [12]. Even more robust differences in body weight have been further obtained by another group, when a high-fat diet was administered to CB1-/- mice and control mice [70].

The evidence indicating that the effects of CB1 receptor blockade on body weight was not limited to a central mode of action has also been proved by pharmacological blockade of the CB1 receptor in different obese animal models. The whole body of these studies demonstrated that the anorectic effect of different CB1 receptor antagonists vanished with time, whereas body weight reduction persisted well beyond the drug effect on food intake [47, 71, 72].

All these data confirmed that CB1 receptor antagonists may act as anti-obesity drugs by a dual mechanism of action that initially targets neuronal sites controlling food intake and thereafter peripheral organs involved in energy storage and expenditure (see review in [17]).

Recent studies performed by several independent laboratories shed light on the peripheral model of action of the endocannabinoid system. Indeed, after the first demonstration of the presence and the functional role of the CB1 receptor on the adipocytes, it is now becoming clear that endocannabinoids are able to interact with all the organs involved in the control of metabolic functions.

Adipocytes

Our *in vitro* studies showed that CB1 receptors are functionally active in white adipocytes stimulating lipogenesis. In fact, their activation enhances lipoprotein lipase activity and this effect can be specifically blocked by rimonabant [12]. At the same time, another group showed that CB1 receptors are predominantly expressed in murine mature adipocytes rather than in preadipocytes [13], meaning that the putative role of the CB1 receptor is not related to the differentiating processes of the fat cells, but is likely associated with some metabolic function. Similar data were subsequently confirmed in humans [14]. More importantly, rimonabant has been shown to induce adiponectin release from adipocytes *in vitro* [13]. Adiponectin is a circulating adipokine secreted by adipose tissue, playing a key regulatory role in fat and glucose metabolism [see review in 73]. This protein exhibits anti-atherogenic and anti-diabetic properties. It is associated with 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. Obesity and type 2 diabetes are characterized by a reduced tissutal and hematic adiponectin concentration (see review in [73]).

Further progress has been provided by a recent study by Jbilo *et al.* who showed that 40 days of rimonabant treatment was able to reverse the phenotype of adipocytes derived from obese mice at both macroscopic and genomic level [74]. Chronic pharmacological CB1 receptor blockade restored the white adipocyte morphology of obese animals similar to that in lean animals. More importantly, the same authors were able to demonstrate that rimonabant treatment reversed the alterations in gene expression obtained by a prolonged high fat diet. In detail, they found that the reduction of adipose mass favored by CB1 receptor antagonist treatment was induced by a series of events such as: (i) an enhanced lipolysis through the induction of enzymes of the β-oxidation and tricarboxylic acid cycle [74], (ii) an increase in energy expenditure through futile cycle stimulation and (iii) an improvement in the regulation of glucose homeostasis as demonstrated by the stimulation of glucose-transporter 4, a key-player of glucose metabolism [74].

Gastrointestinal Tract

The CB1 receptor and endocannabinoids are also highly expressed in the gastrointestinal tract neurons. In the small intestine, starvation induces a 7-fold increase in AEA release but, intriguingly, this effect is reversed on re-feeding [75]. Cholecystokinin (CCK) is known to display an important satiety role and the CCK type 1 receptor is expressed in the same vagal afferent neurons, which also express the CB1 receptor [76]. These neurons project to the stomach and duodenum. In addition, the CB1 receptor expression in the ganglia is increased by food deprivation and decreased after refeeding; this effect has been shown to be blocked by CCK antagonists and mimicked by administration of CCK itself. Therefore, the endocannabinoid system could also affect food intake acting through CCK signaling [76].

Skeletal Muscles

The CB1 receptor is present in skeletal muscles and seems to be over-expressed in the soleus muscle of obese

mice as compared to lean controls [17]. Liu *et al.* found that rimonabant treatment in *ob/ob* mice generated a considerable glucose uptake in soleus muscle in comparison with placebo treated animals [77]. This increase may significantly contribute to the improved hyperglycemia observed in diet-induced obese mice treated with CB1 antagonists in other experimental settings [17]. It has also been shown that 7 days of rimonabant treatment can activate thermogenesis, as demonstrated by a 37% increase in basal oxygen consumption [77]. To explain this finding, the authors speculated that rimonabant may act by stimulating efferent sympathetic activity, as also shown by another group [78]; however, the authors also hypothesized that the enhanced thermogenesis may also be due to an intensification of free fatty acid oxidation promoted by the adiponectin rise, directly stimulated by rimonabant.

Liver

The CB1 receptor is also expressed in hepatocytes, as recently demonstrated by Osey-Hyiaman *et al.* [15]. At the hepatic level, activation of the CB1 receptor increases the gene expression of the lipogenic transcription factor sterol regulatory element binding protein-1 c (SREBP-1c) and of its targets, fatty acid synthase (FAS) and acetyl-CoA carboxylase-1 (ACC-1) [15], leading to a stimulation of *de novo* lipogenesis. The stimulation in cannabinoid-induced hepatic lipogenesis is CB1 receptor dependent, as demonstrated by the ability of rimonabant to reduce the fatty acid synthesis rate [15]. Moreover, a prolonged high-fat diet in mice increases hepatic levels of AEA and induces an upregulation of CB1 receptor expression that in turn may promote the development of steatosis [15]. Importantly, CB1-/- mice are resistant to develop hepatic steatosis. Interestingly, in the hypothalamus, where FAS inhibitors elicit anorexia, SREBP-1c and FAS expression are stimulated by CB1 receptor agonists and inhibited by rimonabant [15]. Therefore, we can presume that the modulation of the FAS pathway may be a novel additional target of CB1 receptor antagonists at both central and peripheral level.

Endocrine Pancreas

Using quantitative real-time PCR and immunocytochemical tools, Juan-Picò *et al.* recently demonstrated the existence of both CB1 and CB2 receptors in the endocrine pancreas [16]. The CB1 receptor was mainly visualized in glucagon-containing α-cells, while the CB2 receptor was found in both α- and β-cells. They found, however, that endocannabinoids are able to decrease insulin secretion regulating Ca^{2+} signaling [16].

In conclusion, the endocannabinoid system may target a large variety of peripheral organs while modulating metabolic processes; the detailed characterization of each individual contribution and the reciprocal interactions among the organs are thus mandatory in future studies approaching this issue.

5. OBESITY IS ASSOCIATED WITH AN OVERACTIVATION OF THE ENDOCANNABINOID SYSTEM

The majority of the studies conducted up to now regarding the regulation of the endocannabinoid system on metabolic processes have been performed on lean animals and not in animal models of obesity. This, in our opinion, represents one of the biases that did not allow a definitive conclusion to be reached on the mode of action by which the endocannabinoid system may contribute to the development of obesity. In fact, a recent series of reports seems to identify a close association between the formation of a state of obesity with a simultaneous over-activation of the endocannabinoid system expressed as an over-production of endocannabinoids or an over-expression of CB1 receptors. In fact, there is now evidence that the CB1 receptor is over-expressed in tissues derived from obese animals when compared to lean controls such as the liver [15], the skeletal muscles [17], and the adipose tissue [13]. Nothing is at present known in humans, with the exception of the human white adipose tissue in which, at variance to mice, this finding has not been confirmed [14]. In fact, in subcutaneous adipose tissue derived from obese postmenopausal women, when compared to a lean control population, CB1 receptor expression was not increased [14]; on the other hand, a limitation of this study is that CB1 receptors were not measured in visceral fat tissue, which is hypothesized to be more sensitive than subcutaneous fat tissue to the endocannabinoid action.

An increased production of endocannabinoids has been proposed in hepatocytes [15], adipocytes and pancreatic cells [79] derived from fat mice in comparison to lean controls. Intriguingly, increased levels of plasma endocannabinoids have been found in obese postmenopausal women, when compared to lean controls [14].

The possible explanation of the mechanisms leading to a hyperactivation of the endocannabinoid tone in obesity is not completely understood and further studies are needed to confirm this hypothesis. A recent study has begun to shed light on this issue, demonstrating a missense polymorphism in a population of obese subjects, that predicts a substitution of threonine for a highly conserved proline residue (P129T) in the sequence of the FAAH, the enzyme that quickly degrades anandamide after its action. Patients with this polymorphism have approximately half the enzymatic activity and this physiological reduction of function may influence the clearance of endocannabinoids, leading to a sustained and possibly pathological tone as found in animal models [80].

5.1. Rimonabant in Humans: A New Pharmacological Perspective in Tackling Obesity and Related Disorders

The discovery of rimonabant not only made it possible to understand many aspects of the endocannabinoid system, but it also very soon appeared to be a promising tool for various diseases in which a pathological increased endocannabinoid tone was presumed to occur. The CB1 receptor antagonist was thus proposed to reduce subjective intoxication and tachycardia in subjects with a history of Cannabis use [81] or as an anti-psychotic agent in schizophrenic patients [82]. Both these applications failed when tested in clinical trials in humans. Moreover, bearing in mind the function of the endocannabinoid system in the mesolimbic rewarding system, rimonabant is also undergoing clinical trials as an aid to prevent the relapse of smoking cessation [83]. The results of the clinical trials from the STRATUS program (smoking cessation in smokers motivated to quit) have not yet been pub-

lished in peer-reviewed journals, and there are therefore still unsolved questions regarding its efficacy in helping to resolve nicotine addiction.

Undoubtedly, the most promising data concerning the use of rimonabant in humans are emerging from the clinical trials on tackling obesity.

A double-blind, placebo-controlled, phase II trial based on 4 months of rimonabant treatment in a group of obese patients (body mass index (BMI): between 30 and 40 kg/m^2) showed an average weight loss of 3.5 kg and 4.4 kg (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 [84]. Another phase II, double-blind, placebo-controlled study involving 20 obese patients with a short-term rimonabant treatment period (7 days) to investigate hunger, calorie and fat intake showed a significant decrease in all three parameters [84].

A worldwide phase III trial named RIO (rimonabant in obesity) was initiated in 2001 including more than 6,600 obese or overweight patients with or without concomitant comorbidities. This study consisted of four different clinical trials. Two of them named RIO-Europe and RIO-North America recruited obese or overweight patients with or without comorbidities who were treated for two years with 5 mg or 20 mg rimonabant *vs.* placebo. The other two trials were named RIO-Lipids and RIO-Diabetes and were set up in order to investigate the amelioration after rimonabant treatment of specific comorbidity factors associated with obesity such as hyperlipidemia and diabetes, respectively.

The primary objective of the four RIO studies was the loss of body weight, associated in the RIO-North America trial with the prevention of weight regain after re-randomization (second year) and in the RIO-Europe trial with the assessment of weight reduction by using the same dosages after two years of treatment. Although the clinical characteristics of the selected patients differed from trial to trial, the secondary endpoints of all four studies were similar, as represented by the number of weight responders and the changes in waist circumference. Secondary measures also included changes from baseline in levels of high-density lipoprotein cholesterol, triglycerides, glucose and insulin during an oral glucose-tolerance test and the prevalence of the metabolic syndrome as defined by the criteria of the National Cholesterol Education Program's Adult Treatment Program III (NCEP-ATP III) [85].

5.2. The Results of the RIO-Europe and the RIO-Lipids Trials

Two studies have been published up to now: the RIO-Europe *ad interim* analysis of the first year [86] and the RIO-Lipids study [87]. Importantly, the results provided by both trials were very similar in both primary and secondary endpoints and this result highlights the relevance of the studies. As a general assumption, we can say that, unlike the 20 mg dose, the treatment with 5 mg rimonabant often did not provide statistically significant changes when compared to placebo; to simplify, therefore, only the data concerning the 20 mg rimonabant treatment will be mentioned when compared to placebo treatment. It has to be borne in mind that a hypocaloric diet was recommended to all subjects recruited in the four trials. Basal metabolic rate was estimated with the Harris Benedict formula, and 600 Kcal were subtracted to calculate a recommended daily energy intake for each patient. Moreover, at each visit, patients received dietary counselling and were encouraged to increase their physical activity.

In the RIO-Europe study, a weight reduction of –8.6 kg was observed in the 20 mg treatment group of patients completing the study compared to the –3.6 kg detected in the placebo treated group [86]. In the RIO-Lipids study, similar data were observed: -8.6 kg in the 20 mg rimonabant treatment group *vs.* –2.3 kg in the placebo group completing the study [87]. As expected, a concomitant reduction in waist circumference of about 9 cm was observed in patients treated with rimonabant 20 mg in both studies, whereas the reduction in placebo treated patients ranged from 3 to 4 cm [86, 87]. The proportion of those who had a weight loss equal to or greater than 10% in the 20 mg treatment group was 39% in the RIO-Europe study and 32% in the RIO-Lipids study when compared to 12.4% and 7.2% in the placebo group of the two studies, respectively. The proportion of patients who had a weight loss equal to or greater than 5% was similar in the two studies, with RIO-Europe showing 67% and RIO-Lipids 58% of the completers of the studies receiving 20 mg treatment *vs*. 30.5 and 19.5% in the placebo groups of both studies [86, 87]. The pattern of weight loss showed a sustained profile for up to 36-40 weeks, followed by a plateau phase.

A significant increase of HDL-cholesterol and a decrease of triglyceride concentrations in patients treated with 20 mg rimonabant was detected in both studies. In the RIO-Europe study, HDL-cholesterol significantly increased by 22.3% after 20 mg rimonabant compared to 13.4% shown in placebo treated patients [86]. In the RIO-Lipids study, the fall of triglycerides was -15.8% in 20 mg rimonabant treated patients *vs*. -3.6% in the placebo cohort in the completer population and -12.6% *vs.* -0.2% in the ITT analysis [87].

Values for plasma glucose and insulin were measured before the 75 g oral glucose tolerance test and 30, 60 and 120 minutes afterward. Both studies showed a significant reduction in 2-hr plasma insulin from baseline in 20 mg rimonabant treated patients *vs.* placebo; in addition, the RIO-Lipids study showed that the 1-hr and 2-hr plasma glucose, the 1-hr insulin, and the glucose and insulin areas under the curve decreased significantly in the group receiving 20 mg rimonabant [86, 87].

The RIO-Lipids study also examined the variation of leptin and adiponectin, both hormones implicated in the regulation of metabolic functions. Plasma leptin levels decreased significantly in the group receiving 20 mg rimonabant *vs*. placebo, whereas plasma adiponectin significantly increased when compared to placebo in the group treated with 20 mg rimonabant [87].

In the RIO-Europe study, the systolic and the diastolic blood pressure was reduced after one year of 20 mg rimonabant treatment; these changes were not, however, signifi-

cantly different from placebo [86]. On the contrary, the decreases in systolic and diastolic blood pressure with 20 mg rimonabant were statistically significant when compared to placebo in the RIO-Lipids study. Importantly, the decrease was greater among patients with hypertension at baseline [87].

The prevalence of the metabolic syndrome in the patients before and after one year of treatment was analyzed in the RIO-Lipids study. Interestingly, at baseline, 54% of the patients met the criteria for the syndrome, whereas the prevalence fell to 25.8% in the 20 mg rimonabant group *vs.* 41% in the placebo group [87].

Great care is taken with regard to the issues of safety and tolerability of any new drug tackling obesity proposed for clinical praxis. Both RIO studies showed a slightly higher rimonabant-treatment adverse or serious adverse event number when compared with placebo. The most common events occurring more frequently with 20 mg rimonabant were nausea, vomiting, diarrhea, dizziness, depression and anxiety. However, they were for the most part mild to moderate in intensity and considered to be transient, based on the occurrence mainly during the first months of the studies [86, 87].

Metabolic Effects Partially Independent of Weight Loss

Interestingly, by means of logistic regression models and/or ANCOVA using weight loss as a covariate in the RIO-Europe study, it was found that nearly half the changes in HDL-cholesterol and triglycerides were independent of weight loss, as reflected by the last weight measurement [86, 87].

Similarly, 57% of the increase in adiponectin levels observed in the RIO-Lipids group receiving 20 mg rimonabant could not be attributable to weight loss [87].

Particular attention should be paid to patients with previous evidence of psychiatric diseases when treatment with CB1 receptor antagonists is proposed. As expected, in both trials, a percentage of patients developed depression or anxiety and this led to discontinuation of the treatment in a moderate but significant number of subjects in comparison to placebo. As mentioned before, this event could be largely anticipated by remembering two facts: the endocannabinoid system is known to down-regulate the hypothalamic-pituitary-adrenal (HPA) axis in stress conditions [17], and, for many years, visceral obesity was presumed to be a disease sometimes associated with a pathological overactivation of the HPA axis [89]. In fact, obese patients often report traits of anxiety or depression and these symptoms could be considered as terminal effects of this axis derangement. So it has to be carefully weighed up whether obese patients with depression or other psychiatric disorders could be treated with this drug.

6. SUMMARY AND CONCLUSION

The promising data of the RIO-EUROPE and RIO-Lipids studies published so far need to be substantiated by the results of the other two ongoing clinical trials with rimonabant and to be definitively confirmed by the data obtained by future trials in which specific questions will be asked and hopefully solved.

However, rimonabant and related upcoming drugs of the same class may be proposed not only to tackle visceral obesity, but also to face the variety of alterations related to the pathological fat increase in abdominal depots. Intriguingly, CB1 receptor antagonists seem to work not only as anorectic drugs but also, and even more importantly, as positive modulators of crucial metabolic steps at peripheral level. Therefore, we can now look towards the future more optimistically because a new pharmacological approach aimed at tackling obesity and related metabolic diseases in a better way, when dietary counseling and change of lifestyle have failed, is close to being included in general clinical praxis.

The entry of rimonabant and its class of drugs on the market may allow us to begin considering the possibility of individually targeting the therapeutic strategies according to phenotype characteristics and to the pathophysiological mechanisms inducing the disease [89].

The line of attack to obesity has significantly changed in the last few years with the awareness of the crucial role of adipose tissue. We can thus hypothesize that CB1 receptor antagonists may be useful for a selective treatment of visceral obesity, when considerable amounts of visceral fat are simply associated with a single altered metabolic parameter such as low HDL cholesterol and/or mildly high triglycerides and/or high fasting glucose level (the s.c. fasting glucose intolerance state).

ACKNOWLEDGEMENTS

This study was supported by a Grant from the Sixth EC Program (LSHM-CT-2003-503041). We thank Miss Susan West for English revision of the manuscript.

REFERENCES

[1] Haslam, D. W. ; James, W.P. *Lancet* **2005**, *366*, 1197.
[2] Després, J. P. ; Lemieux, I ; Prud'homme, D. *BMJ* **2001**, *322*, 716.
[3] Gaoni, Y.; Mechoulam, R.; *J. Am. Chem. Soc.* **1964**, *86*, 1646.
[4] Matsuda, L.A.; Lolait, S.J.; Brownstein, M.J.; Young, A.C.; Bonner, T.I. *Nature* **1990**, *346*, 561.
[5] Devane, W.A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science* **1992**, *258*, 1946.
[6] Sugiura, T.; Kondo, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89.
[7] Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N. E.; Schatz, A. R.; Gopher, A.; Almog, S.; Martin, B. R.; Compton, D. R.; Pertwee, R.G.; Griffin, G.; Bayewitch, M.; Barg, J.; Vogel, Z. *Biochem. Pharmacol.* **1995**, *50*, 83.
[8] Hanus, L.; Abu-Lafi, S.; Fride, E.; Breuer, A.; Vogel, Z.; Shalev, D. E.; Kustanovich, I; Mechoulam, R. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 3662.
[9] Porter, A. C.; Sauer, J. M.; Knierman, M. D.; Becker, G. W.; Berna, M. J.; Bao, J.; Nomikos, G. G.; Carter, P.; Bymaster, F. P.; Leese, A. B.; Felder, C. C. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 1020.
[10] Huang, S. M.; Bisogno, T.; Trevisani, M.; Al-Hayani, A.; De Petrocellis, L.; Fezza, F.; Tognetto, M.; Petros, T. J.; Krey, J. F.; Chu, C. J.; Miller, J. D.; Davies, S. N.; Geppetti, P.; Walker, J. M.; Di Marzo, V. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 8400.
[11] Howlett, A. C.; Barth, F.; Bonner, T. I.; Cabral, G.; Casellas, P.; Devane, W. A.; Felder, C. C.; Herkenham, M.; Mackie, K.; Martin, B. R.; Mechoulam, R.; Pertwee, R. G. *Pharmacol. Rev.* **2002**, *54*, 161.
[12] Cota, D.; Marsicano, G.; Tschoep, M.; Grubler, Y.; Flachskamm, C.; Schubert, M. D.; Yassouridis, A.; Thone-Reineke, C.; Ortmann, S.; Tomassoni, F.; Cervino, C.; Nisoli, E,.; Linthorst, A. C.;

Pasquali, R.; Lutz, B.; Stalla, G.K.; Pagotto, U. *J. Clin. Invest*. **2003**, *112*, 423.
[13] Bensaid, M.; Gary-Bobo, M.; Esclangon, A.; Maffrand, J.P.; Le Fur, G.; Oury-Donat, F.; Soubrié, P. *Mol. Pharmacol.* **2003**, *63*, 908.
[14] Engeli, S.; Bohnke, J.; Feldpausch, M.; Gorzelniak, K.; Janke, J.; Batkai, S.; Pacher, P.; Harvey-White, J.; Luft, F. C.; Sharma, A. M.; Jordan, J. *Diabetes* **2005**, *54*, 2838.
[15] Osei-Hyiaman, D.; DePetrillo, M.; Pacher, P.; Liu, Y.; Radaeva, S.; Batkai, S.; Harvey-White, J.; Mackie, K.; Offertaler, L.; Wang, L.; Kunos, G. *J. Clin. Invest.* **2005**, *115*, 1298.
[16] Juan-Pico, P.; Fuentes, E.; Bermudez-Silva, F.J.; Diaz-Molina, F.J.; Ripoll, C.; Rodriguez de Fonseca, F.; Nadal, A. *Cell Calcium* **2006**, *27*, 73.
[17] Pagotto, U.; Marsicano, G.; Cota, D.; Lutz, B.; Pasquali, R. *Endocr. Rev.* **2006**, *27*, 73.
[18] Van Sickle, M.D.; Duncan, M.; Kingsley, P.J.; Mouihate, A.; Urbani, P.; Mackie, K.; Stella, N.; Makriyannis, A.; Piomelli, D.; Davison, J. S., Marnett, L. J.; Di Marzo, V.; Pittman, Q. J.; Patel, K, Q. D.; Sharkey, K. A. *Science* **2005**, *310*, 329.
[19] Julien, B.; Grenard, P.; Teixeira-Clerc, F.; Van Nhieu, J. T.; Li, L.; Karsak, M.; Zimmer, A.; Mallat, A.; Lotersztajn S. *Gastroenterology* **2005**, *128*, 742.
[20] Idris, A. I.; van't Hof, R. J.; Greig, I.R.; Ridge, S. A.; Baker, D.; Ross, R. A.; Ralston S. H. *Nat. Med.* **2005**, *11*, 774.
[21] Piomelli, D. *Nat. Rev. Neurosci.* **2003**, *4*, 873.
[22] Kim, J.; Isokawa, M.; Ledent, C.; Alger, B. E. *J. Neurosci.* **2002**, *22*, 10182.
[23] Di, S.; Malcher-Lopes, R.; Halmos, K. C.; Tasker, J. G. *J. Neurosci.* **2003**, *23*, 4850.
[24] Okamoto,Y.; Morishita, J.; Tsuboi, K.; Tonai, T.; Ueda, N. *J. Biol. Chem.* **2004**, *279*, 5298.
[25] Di Marzo, V.; Bifulco, M.; De Petrocellis, L. *Nat. Rev. Drug Discov.* **2004**, *3*, 771.
[26] Bisogno, T.; Howell, F.; Williams, G.; Minassi, A.; Cascio, M. G.; Ligresti, A.; Matias, I.; Schiano-Moriello, A.; Paul, P.; Williams, E. J.; Gangadharan, U.; Hobbs, C.; Di Marzo, V.; Doherty, P. *J. Cell. Biol.* **2003**, *163*, 463.
[27] McFarland, M. J.; Barker, E. L. *Pharmacol. Ther.,* **2004**, *104*, 117.
[28] McKinney, M. K.; Cravatt, B. F. *Annu. Rev. Biochem.* **2005**, *74*, 411.
[29] Dinh, T. P.; Carpenter, D.; Leslie, F. M.; Freund, T. F.; Katona, I.; Sensi, S. L.; Kathuria, S.; Piomelli, D. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10819.
[30] Dewey, W.L. *Pharmacol. Rev.* **1986**, *38*, 151.
[31] Mechoulam, R.; Parker, L.A.; Gallily, R. *J. Clin. Pharmacol.* **2002**, 42, 11S.
[32] Thomas, A.; Stevenson, L.A.; Wease, K. N.; Price, M. R.; Baillie, G.; Ross, R.A.; Pertwee, R.G. *Br. J. Pharmacol.* **2005**, *146*, 917.
[33] Pop, E. *Curr. Opin. Investig. Drugs* **2000**, *1*, 494.
[34] Devane, W. A.; Dysarz, F. A. III; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. *Mol. Pharmacol.* **1988**, *34*, 605.
[35] Huffman, J. W. *Curr. Med. Chem*. **1999**, *6*, 705.
[36] Song, Z. H.; Bonner, T. I. *Mol. Pharmacol.* **1996**, *49*, 891.
[37] Lange, J. H.; Kruse, C. G. *Curr. Opin. Drug Discov. Devel.* **2004**, *7*, 498.
[38] Rinaldi-Carmona, M.; Barth, F.; Heaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Neliat, G.; Caput, D.; Ferrara, P.; Soubrié, P.; Brelière, J. C.; Le Fur, G. *FEBS Lett*. **1994**; *350*, 240.
[39] Fernandez, J. R.; Allison, D. B. *Curr. Opin. Investig. Drugs* **2004**, *5*, 430.
[40] Lan, R.; Liu, Q.; Fan, P.; Lin, S.; Fernando, S.R.; McCallion, D.; Pertwee, R.; Makriyannis, A. *J. Med. Chem.* **1999**, *42*, 769.
[41] Francisco, M.E.; Seltzman, H. H.; Gilliam, A. F.; Mitchell, R. A.; Rider, S. L.; Pertwee, R. G.; Stevenson, L. A.; Thomas, B. F. *J. Med. Chem.* **2002**, *45*, 2708.
[42] Nakamura-Palacios, E. M.; Moerschbaecher, J. M.; Barker, L. A. *CNS Drug Rev.* **1999**, *5*, 43.
[43] Pertwee, R. G. *Life Sci.* **2005**, *76*, 1307.
[44] Rinaldi-Carmona, M.; Barth, F.; Congy, C.; Martinez, S.; Oustric, D:; Perio, A.; Poncelet, M.; Maruani, J.; Arnone, M.; Finance, O.; Sobrie, P.; Le Fur, G. J. *Pharmacol. Exp. Ther.* **2004**, *310*, 905.
[45] Meschler, J. P.; Kraichely, J.P.; Wilken, G.H.; Howlett, A. C. *Biochem. Pharmacol.* **2000**, *60*, 1315.
[46] Katoch-Rouse, R.; Pavlova, O. A.; Caulder, T.; Hoffman, A. F.; Mukhin, A. G.; Horti, A. G. *J. Med. Chem.* **2003**, *46*, 642.
[47] Hildebrandt, A. L.; Kelly-Sullivan, D. M.; Black, S. C. *Eur. J. Pharmacol.* **2003**, *462*, 125.
[48] Zhou, D.; Shearman, L. P. *Pharmacol. Biochem. Behav.* **2004**, *77*, 117.
[49] Chambers, A. P.; Sharkey, K. A.; Koopmans, H. S. *Physiol. Behav.* **2004**, *82*, 863.
[50] Lange, J. H.; Coolen, H. K.; van Stuivenberg, H. H.; Dijksman, J. A; Herremans, A. H.; Ronken, E.; Keizer, H. G.; Tipker, K.; McCreary, A. C.; Veerman, W.; Wals, H. C.; Stork, B.; Verveer, P. C.; den Hartog, A. P.; de Jong, N. M.; Adolfs, T. J.; Hoogendoorn, J.; Kruse, C. G. *J. Med. Chem.* **2004**, *47*, 627.
[51] Cota, D.; Marsicano, G.; Lutz, B.; Vicennati, V.; Stalla, G. K.; Pasquali, R.; Pagotto, U. *Int. J. Obes. Relat. Metab. Disord.* **2003**, *27*, 289.
[52] Herkenham, M.; Lynn, A. B.; Johnson, M. R.; Melvin, L. D.; de Costa, B. R.; Rice, K. C. *J. Neurosci.* **1991**, *11*, 563.
[53] Kirkham, T. C.; Williams C. *Psychopharmacology (Berl.)* **2001**, *153*, 267.
[54] Verty, A. N. A.; Singh, M. E.; McGregor, I. S.; Mallet, P. E. *Psychopharmacology (Berl.)* **2003**, *168*, 314.
[55] Verty, A. N. A.; McGregor, I. S.; Mallet, P. E. *Brain. Res.* **2004**, *1020*, 188.
[56] Arnone, M.; Maruani, J.; Chaperon, F.; Thiebot, M. H.; Poncelet, M.; Soubrié. P.; Le Fur, G. *Psychopharmacology (Berl.)* **1997**, *132*, 104.
[57] Simiand, J.; Keane, M.; Keane, P. E.; Soubrié, P. *Behav. Pharmacol.* **2001**, *9*, 179.
[58] Sanchis-Segura, C.; Cline, B. H.; Marsicano, G.; Lutz, B.; Spanagel, R. *Psychopharmacology (Berl.)* **2004**, *176*, 223.
[59] Jamshidi, N.; Taylor, D. A. *Br. J. Pharmacol*. **2001**, *134*, 1151.
[60] Hanus, L.; Avraham, Y.; Ben-Shushan, D.; Zolotarev, O.; Berry, E. M.; Mechoulam, R. *Brain Res.* **2003**, *983*, 144.
[61] Kirkham, T. C.; Williams, C. M.; Fezza, F.; Di Marzo, V. *Br. J. Pharmacol.* **2002**, *136*, 550.
[62] Flier, J. S. *Cell* **2004**, *116*, 337.
[63] Di Marzo, V.; Goparaju, S. K.; Wang, L.; Liu, J.; Batkai, S.; Jarai, Z.; Fezza, F.; Miura, G. I.; Palmiter, R.D.; Sugiura, T.; Kunos, G. *Nature* **2001**, *410*, 822.
[64] Jo, Y. H.; Chen, Y. J. J.; Chua, S. C. Jr.; Talmage, D. A.; Role, L. W. *Neuron* **2005**, *48*, 1055.
[65] Fulton, S. ; Woodside, B. Shizgal, P. *Science* **2000**, *27*, 125.
[66] Dallman, M. F.; Akana, S. F.; Strack, A. M.; Scribner, K.S.; Pecoraro, N.; La Fleur, S. E.; Houshyar, H.; Gomez, F. *Ann. N. Y. Acad. Sci.* **2004**, *1018*, 141.
[67] Osei-Hyiaman, D.; DePetrillo, M.; Harvey-White, J.; Bannon, A. W.; Cravatt, B. F.; Kuhar, M. J.; Mackie, K.; Palkovits, M.; Kunos, G. *Neuroendocrinology* **2005**, *81*, 273.
[68] Verty, A. N.; McFarlane, J. R.; McGregor, I. S.; Mallet, P. E. *Endocrinology* **2004**, *145*, 3224.
[69] Gamber, K. M.; MacArthur, H.; Westfall, T. C. *Neuropharmacology* **2005**, *49*, 646.
[70] Ravinet Trillou, C.; Delgorge, C.; Menet, C.; Arnone, M.; Soubrié, P. *Int. J. Obes.* **2004**, *28*, 640.
[71] Colombo, G.; Agabio, R.; Diaz, G.; Lobina, C.; Reali, R.; Gessa, G. L. *Life Sci.* **1998,** *63*, L113.
[72] Ravinet Trillou, C.; Arnone, M.; Delgorge, C.; Gonalons, N.; Keane, P.; Maffrand, J. P.; Soubrié, P. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2003**, *284*, R345.
[73] Chandran, M.; Phillips, S. A.; Ciaraldi, T.; Henry, R. R. *Diabetes Care* **2003**, *26*, 2442.
[74] Jbilo, O.; Ravinet-Trillou, C.; Arnone, M.; Buisson, I.; Bribes, E.; Péleraux, A.; Pénarier, G.; Soubrié, P.; Le Fur, G.; Galiègue, S.; Casellast, P. *FASEB J.* **2005**, *19*, 1567.
[75] Gomez, R.; Navarro, M.; Ferrer, B.; Trigo J. M.; Bilbao, A.; Del Arco, I.; Cippitelli, A.; Nava, F.; Pomelli, D.; Rodriguez de Fonseca, F. *J. Neurosci.* **2002**, *22*, 9612.
[76] Burdyga, G.; Lal, S.; Varro, A.; Dimaline, R.; Thompson, D. G.; Dockray, G. J. *J. Neurosci.* **2004**, *24*, 2708.
[77] Liu, Y. U.; Connoley, I. P.; Wilson, C. A.; Stock, M. J. *Int. J. Obes.* **2005**, *29*, 183.
[78] Tzavara, E. T.; Perry, K. W.; Rodriguez, D. E.; Bymaster, F. P.; Nomikos, G. G. *Eur. J. Pharmacol.* **2001**, *426*, R3.

[79] Matias, I.; Gonthier, M.P.; Orlando, P.; Martiadis, V.; De Petrocellis, L.; Cervino, C.; Petrosino, P.; Pasquali, R.; Maj, M.; Pagotto, U.; Di Marzo, V. *J. Clin. Endocrinol. Metab.* **2006**, *91*, 3171.

[80] Sipe, J. C.; Waalen, J.; Gerber, A.; Beutler, E. *Int. J. Obes. Relat. Metab. Disord.* **2005**, *29*, 755.

[81] Huestis, M. A.; Gorelick, D. A.; Heishman, S. J.; Preston, K. L.; Nelson, R. A.; Moolchan, E. T.; Frank, R. A. *Arch. Gen. Psychiatry* **2001**, *58*, 322.

[82] Meltzer, H. Y.; Arvanitis, L.; Bauer, D.; Rein, W. *Am. J. Psychiatry* **2004**, *161*, 975.

[83] Cleland, J. G.; Ghosh, J.; Freemantle, N.; Kaye, G. C.; Nasir, M.; Clark, A. L.; Coletta, A. P. *Eur. J. Heart Fail.* **2004**, *6*, 501-508.

[84] Fernandez, J.R.; Allison, D. B. *Curr. Opin. Invest. Drug* **2004**, *5*, 430.

[85] National Institute of Health: Third Report of the National Cholesterol Educational Program Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) Executive Summary. Bethesda, MD, National Institute of Health, National Heart, Lung and Blood Institute. **2005**.

[86] Van Gaal, L. F.; Rissanen, A. M.; Scheen, A. J.; Ziegler, O.; Rossner, S. *Lancet* **2005**, *365*, 1389.

[87] Despres, J. P.; Golay, A.; Sjostrom, L. for the Rimonabant in Obesity-Lipids Study Group. *N. Engl. J. Med*. **2005**, *353*, 2121.

[88] Pasquali, R.; Vicennati, V.; Pagotto, U. In Bray GA; Bouchard C; eds. *Handbook of Obesity*. New York: Marcel Dekker Inc; **2004**; 671.

[89] Pagotto, U.; Pasquali, R. *Lancet* **2005**, *365*, 1363.

Received: June 12, 2006 **Revised: July 24, 2006** **Accepted: July 26, 2006**