

The Emerging Role of the Endocannabinoid System in Endocrine Regulation and Energy Balance

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During the last few years, the endocannabinoid system has emerged as a highly relevant topic in the scientific community. Many different regulatory actions have been attributed to endocannabinoids, and their involvement in several pathophysiological conditions is under intense scrutiny. Cannabinoid receptors, named CB1 receptor and CB2 receptor, first discovered as the molecular targets of the psychotropic component of the plant *Cannabis sativa*, participate in the physiological modulation of many central and peripheral functions. CB2 receptor is mainly expressed in immune cells, whereas CB1 receptor is the most abundant G protein-coupled receptor expressed in the brain. CB1 receptor is expressed in the hypothalamus and the pituitary gland, and its activation is known to modulate all the endocrine hypothalamic-peripheral endocrine axes. An increasing amount of data highlights the role of the system in the stress response by influencing the hypothalamic-pituitary-adrenal axis and in the control of reproduction by modifying gonadotropin release, fertility, and

sexual behavior.

The ability of the endocannabinoid system to control appetite, food intake, and energy balance has recently received great attention, particularly in the light of the different modes of action underlying these functions. The endocannabinoid system modulates rewarding properties of food by acting at specific mesolimbic areas in the brain. In the hypothalamus, CB1 receptor and endocannabinoids are integrated components of the networks controlling appetite and food intake. Interestingly, the endocannabinoid system was recently shown to control metabolic functions by acting on peripheral tissues, such as adipocytes, hepatocytes, the gastrointestinal tract, and, possibly, skeletal muscle. The relevance of the system is further strengthened by the notion that drugs interfering with the activity of the endocannabinoid system are considered as promising candidates for the treatment of various diseases, including obesity. (*Endocrine Reviews* 27: 73–100, 2006)

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I. Introduction

THE FIRST STEPS in the discovery of the endocannabinoid system date back almost 4000 yr, when the therapeutic and psychotropic actions of the plant *Cannabis sativa*

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Abbreviations: AEA, Arachidonoyl ethanolamide; 2-AG, 2-arachidonoyl-glycerol; CB1^{-/-}, mice lacking cannabinoid receptor type 1; CB1 receptor, cannabinoid receptor type 1; CB2 receptor, cannabinoid receptor type 2; CCK, cholecystokinin; CNS, central nervous system; D1, dopamine receptor type 1; D2, dopamine receptor type 2; DIO, diet-induced obesity; FAAH, fatty acid amide hydrolase; GABA, γ -aminobutyric acid; HDL, high-density lipoprotein; HPA, hypothalamus-pituitary-adrenal; IHC, immunohistochemistry; ISH, *in situ* hybridization; MCR4, melanocortin receptor type 4; OEA, oleoylethanolamide; PPAR, peroxisome proliferator-activated receptor; PRL, prolactin; PVN, paraventricular nucleus; RIO, rimonabant in obesity; Δ^9 -THC, Δ^9 -tetrahydrocannabinol; UCP, uncoupling protein; VTA, ventral tegmental area.

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were first documented in India (1). Over the last 40 yr, after Gaoni and Mechoulam (2) purified the psychoactive component from hemp, a stunning amount of research has revealed the endocannabinoid system as a central modulatory system in animal physiology.

Elements of the endocannabinoid system comprise the cannabinoid receptors, the endogenous lipid ligands (endocannabinoids), and the machinery for their biosynthesis and metabolism (3, 4). Despite public concern related to the abuse of marijuana and its derivatives, the research on the endocannabinoid system has recently aroused enormous interest not only for the physiological functions, but also for the promising therapeutic potentials of drugs interfering with the activity of cannabinoid receptors. This review aims to provide an overview on the pivotal role of the endocannabinoid system in the modulation of the neuroendocrine and peripheral endocrine systems. Moreover, in the context of the recently proposed therapeutic applications of cannabinoid receptor antagonists in the treatment of obesity, the key role of the endocannabinoid system in the control of eating behavior, food intake, and energy metabolism will be discussed in the light of the recent data obtained from human and animal studies.

II. The Endocannabinoid System

The large and widespread medical, religious, and recreational use of marijuana throughout the ages was apparently not sufficient to initiate careful and extensive research on cannabinoids until the last few decades of the 20th century. Conversely, the political antimarijuana attitude in the United States and the consequent prohibition in the 1930s did not help to encourage scientific interest on this topic. In the 1960s, the growing public concern regarding the potential negative healthy effects of cannabinoids associated with the exponential increase in its recreational use forced governmental institutions to invest resources to understand the modes of action of marijuana and the pathophysiological implications of its use in more detail. Cannabinoid research received a pivotal boost from the characterization of the chemical structure of Δ^9 -tetrahydrocannabinol (Δ^9 -THC), the main psychoactive constituent of marijuana (2). This finding paved the way to the understanding of marijuana's mechanisms of action and, many years later, to the cloning of the two receptor subtypes that are able to bind exogenous cannabinoids, named cannabinoid receptor type 1 (CB1 receptor) and type 2 (CB2 receptor), respectively, and to the identification of their endogenous ligands: the endocannabinoids (5–9). Cannabinoid receptors, endocannabinoids, and the machinery for their synthesis and degradation represent the elements of a novel endogenous signaling system (the so-called endocannabinoid system), which is involved in a plethora of physiological functions (3, 4). During the last few years, an overwhelming amount of data has been acquired to understand the biological roles of this system in more detail. However, many questions are still open, and promising new discoveries await us in the near future.

In general, the endocannabinoid system is involved in many different physiological functions, many of which relate

to stress-recovery systems and to the maintenance of homeostatic balance (10). Among other functions, the endocannabinoid system is involved in neuroprotection (11–13), modulation of nociception (14), regulation of motor activity (15), and the control of certain phases of memory processing (16–18). In addition, the endocannabinoid system is involved in modulating the immune and inflammatory responses (19–21). It also influences the cardiovascular and respiratory systems by controlling heart rate, blood pressure, and bronchial functions (22). Finally, yet importantly, endocannabinoids are known to exert important antiproliferative actions in tumor cells (23). A full discussion of the plethora of functions of the endocannabinoid system in maintaining homeostasis is beyond the scope and space of the present review. However, the reviews cited in this article will further help to obtain a broad insight into the physiological roles of the endocannabinoid system.

A. Cannabinoid receptors

Two cannabinoid receptors have been identified and molecularly characterized so far, namely the seven transmembrane G protein-coupled cannabinoid receptor type 1 (CB1 receptor) (6) and type 2 (CB2 receptor) (7). CB1 receptor was originally described as the "brain type" cannabinoid receptor, because its levels of expression were high in the brain (24). However, recent studies attribute new sites of action of endocannabinoids to many peripheral organs through CB1 receptor activation. The generalization for CB1 receptor being the eminent "brain type" receptor is therefore no longer appropriate. Conversely, CB2 receptors are present almost exclusively in immune and blood cells, where they may participate in regulating immune responses (25). However, CB2 receptors also exert functions in nonimmune cells such as keratinocytes (26). Pharmacological evidence exists for the presence of other cannabinoid receptors, which, however, have not yet been cloned (27). The endocannabinoid anandamide is also able to bind to and activate vanilloid receptors, transient receptor potential vanilloid type 1 (28), and to inhibit TASK-1 K^+ channels (29). Moreover, pharmacological studies indicate that still unidentified additional cannabinoid receptors might exist in the hippocampus, modulating the release of glutamate (30), and on endothelial cells (31). Two patents have been recently published claiming that a number of cannabinoid ligands also bind to GPR55, an orphan G protein-coupled receptor, suggesting that this receptor might represent a novel target of cannabinoid action (32). CB1 receptor, however, is the best characterized target of exogenous and endogenous cannabinoids in the modulation of neuroendocrine and metabolic responses, and this review will focus mainly on this receptor.

1. *CB1 receptor expression in the brain.* Cannabinoid receptor distribution was studied by means of autoradiography of ligand-receptor binding on slide-mounted rat brain sections (24, 33), by *in situ* hybridization (ISH) (34–36), by autoradiography in human brain (37), by immunohistochemistry (IHC) (38–41), and by agonist-stimulated [35 S]GTP γ S binding to slide-mounted sections (42, 43). Expression studies showed very early that CB1 receptor is one of the most

abundant G protein-coupled receptors in the mammalian brain (24). CB1 receptors are widely expressed in the brain, including the olfactory bulb, cortical regions (neocortex, pyriform cortex, hippocampus, and amygdala), several parts of basal ganglia, thalamic and hypothalamic nuclei, cerebellar cortex, and brainstem nuclei. The levels of expression vary considerably among the various brain regions and neuronal subpopulations. For instance, agonist-mediated receptor binding revealed high densities of CB1 receptor protein in the cornu ammonis pyramidal cell layers of the hippocampus (24), which was later shown by IHC to be due to a dense plexus of immunoreactive fibers deriving from γ -aminobutyric acid (GABA)-ergic interneurons and surrounding the cell bodies of pyramidal cells, which appear *per se* to be devoid of CB1 receptor protein (38, 41, 44). However, pyramidal cells of the hippocampus and other cortical regions do express low but significant levels of CB1 receptor mRNA (34, 36), indicating the possibility that CB1 receptor protein in these cells is localized on distal projections and/or is expressed at low levels, which are below the limit of detectability with currently available immunohistochemical methods. A similar situation is present also in other cortical regions, such as the amygdala, neocortex, entorhinal cortex, and pyriform cortex.

In subcortical regions, CB1 receptor is present at relatively high levels in the septal region (lateral and medial septum, and vertical and horizontal nuclei of the diagonal band). Lower levels of expression are present in hypothalamic regions, such as the medial and lateral preoptic nucleus, magnocellular preoptic nucleus, and paraventricular nucleus (PVN) (36). In the caudal hypothalamus, CB1 receptor is expressed in the premammillary nucleus. In the lateral hypothalamus, CB1 receptor is present in scattered cells (34, 36). In the PVN, CB1 receptor mRNA coexpresses with CRH mRNA (45). In the thalamus, CB1 receptor is present in the lateral habenula, reticular thalamic nucleus, and zona incerta. Midbrain dopaminergic neurons are generally considered to lack CB1 receptor expression. However, recent observations indicate that very low levels of CB1 receptor might be present in tyrosine hydroxylase-expressing neurons in the ventral tegmental area (VTA) (46) and in dopaminergic terminals in the striatum (47). In the hindbrain, apart from the molecular and granular layers of cerebellum expressing high levels of the receptor, CB1 receptor is present at low levels in some nuclei of the brain stem, such as the periaqueductal gray (34, 38). Functional mapping by agonist-stimulated [35 S]GTP γ S binding using different CB1 receptor agonists revealed that cannabinoid activation of G proteins occurs with the same regional distribution as the receptors (43, 48). However, in some regions, the ratio between the estimated amount of CB1 receptor and G protein activation is not always constant, thus indicating regional differences in receptor-coupling efficiencies (43). This is important to consider, because sometimes the endocannabinoid system seems to influence functions involving regions where the density of CB1 receptor is relatively low (*e.g.*, modulation of food intake in the hypothalamic area). Therefore, the activity of cannabinoids on CB1 receptor cannot be predicted based solely on the relative receptor density, but other factors, such as receptor coupling efficiency, should be taken into account. For

instance, by using conditional mutagenesis in mice, the relatively low levels of CB1 receptor expression in cortical pyramidal neurons were recently shown to play a central role in the endocannabinoid-mediated protection against excitotoxic seizures (12). In conclusion, CB1 receptor is widely expressed in the brain and is present at different levels in different neuronal subpopulation and brain regions, and there is apparently no strict correlation between levels of expression and receptor functionality.

2. CB1 receptor expression in the pituitary. Early studies showed a scattered presence of CB1 receptor in both lobes of the rodent pituitary (33). Recent studies examined the distribution of CB1 receptor mRNA in the anterior pituitary lobe in more detail. In 1999, the abundant CB1 receptor presence in the rat adenohypophysis was associated with the ability of this gland to synthesize endocannabinoids (49). CB1 receptor was also shown to be present in prolactin (PRL)- and LH-secreting cells of the rat pituitary (50). CB1 receptor expression was also detected by means of double-immunofluorescence in the pituitary gland of *Xenopus laevis*, where the receptor was found in lactotrophs, gonadotrophs, and thyrotrophs (51). The expression of CB1 receptor in the human pituitary appears to be substantially different from the localization of the same receptor described in rodents and frogs (52). By using ISH and double IHC, CB1 receptor was localized in the majority of corticotrophs and somatotrophs of the normal human anterior lobe; only a small percentage of the PRL-secreting cells are positive for CB1 receptor, whereas no immunoreactivity was found in LH-, FSH-, or TSH-positive cells. The neural lobe is devoid of CB1 receptor immunoreactivity (52). Interestingly, folliculo-stellate cells are also positive for CB1 receptor, although functional data have not yet been associated with this expression (52). CB1 receptor was also found in human pituitary adenomas, such as ACTH-producing adenomas (which give rise to Cushing's syndrome), GH-producing tumors (leading to acromegaly), and in prolactinomas, whereas no CB1 receptor staining was found in so-called nonfunctioning pituitary adenomas, tumors expressing LH and/or FSH, and/or α -subunit being devoid of any hormonal staining (52). These data were confirmed by a study in which cDNA microarray analysis was used to compare gene expression pattern in pituitary adenomas *vs.* normal pituitary (53). Among other genes differentially expressed, ACTH- and GH-producing tumors express higher levels of CB1 receptor compared with the normal pituitary (53). Notably, the human normal anterior pituitary gland and pituitary tumors were shown to be capable of synthesizing endocannabinoids (52).

In rodents, CB1 receptor expression in the pituitary is under the influence of circulating sex hormones, as demonstrated by the ability of androgens and estrogens to up- and down-regulate CB1 receptor, respectively (49). In agreement with these findings, decreased CB1 receptor expression has been found in estrogen-induced pituitary hyperplasia in rats (49). Accordingly, in rats, the male pituitary displays higher levels of CB1 receptor mRNA than the female one (49). In contrast, the human pituitary does not show this gender difference (52).

Exogenous cannabinoids can modulate the expression of

CB1 receptor in the pituitary. After a transient down-regulation of the receptor (first 1–3 d), chronic administration of CB1 receptor agonists is able to produce a consistent increase of CB1 receptor expression in the anterior pituitary lobe (after 14 d) (54). This finding seems to be in contrast with the level in the ventromedial hypothalamic nucleus, where CB1 receptor mRNA was down-regulated by chronic CB1 receptor agonist treatment (54).

3. CB1 receptor expression in the peripheral organs

a. CB1 receptor in the thyroid gland. CB1 receptor expression during the late embryological stages of the rat thyroid was found to be very high (55), whereas lower but still detectable levels of CB1 receptor mRNA and protein were present in the adult rat gland distributed in both follicular and parafollicular cells as demonstrated by IHC (56).

b. CB1 receptor in the adrenal gland. A faint signal for CB1 receptor was detected in the human adrenal glands by quantitative RT-PCR method (57). However, ISH or IHC studies are needed to clearly localize CB1 receptor in the different areas that make up the gland.

c. CB1 receptor in the peripheral organs involved in metabolic control. In 2003, two independent groups found the presence of CB1 receptor in adipocytes of mice and humans (58–60). In both species, this expression is more evident in mature adipocytes than in preadipocytes (59, 60), indicating that the full cellular machinery of the fat cell is needed to exert cannabinoid action. Little is known about CB1 receptor expression in the muscle. Recently, the CB1 receptor antagonist SR141716 was shown to directly affect glucose uptake in the isolated soleus muscle of genetically obese mice (61). Consistently, CB1 receptor is present in the murine soleus muscle as shown by RT-PCR (Fig. 1). Additional investigations are needed to fully understand the importance of this expression site.

Recently, an elegant study by Kunos' group (62) localized CB1 receptor in the mouse liver. CB1 receptor mRNA was detected by ISH with strong labeling in Kupffer cells, whereas lower levels of expression were found in hepatocytes and endothelial cells. Interestingly, CB1 receptor expression was more prominent in hepatocytes surrounding

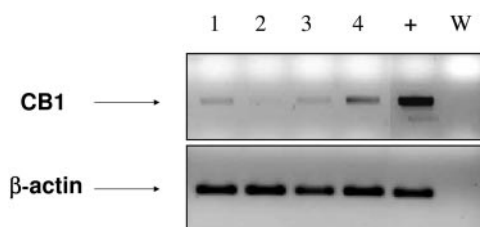


FIG. 1. Expression of CB1 mRNA in soleus muscle of mice. RT-PCR was performed using 1 μ g of total RNA extracted by phenol-chloroform method from soleus muscle as shown in Ref. 58. β -actin and CB1 mRNA expression. Lane 1, Wild-type 12-wk-old mice undergoing standard diet; lane 2, CB1^{-/-} littermate mice undergoing standard diet; lane 3, C56BL/6 mice used as control undergoing standard diet; lane 4, C56BL/6 under high-fat diet for 2 months; lane +, positive control (hypothalamus); and lane W, negative control (PCR blank). Note the increased CB1 signal in muscle derived from mice on high-fat diet in comparison to the muscle derived from mice on standard diet.

the central veins. Human hepatic stellate cells also have been shown to express CB1 receptor (63).

At present, nothing is known about CB1 receptor in the exocrine and endocrine cells of the pancreas.

d. CB1 in the gastrointestinal tract. The endocannabinoid system is present in the gastrointestinal tract where it modulates several functions, including motility, inflammation, and secretion (64). Interestingly, CB1 receptor is expressed in vagal nerve terminals innervating the gastrointestinal tract (64), which are involved in gut-brain signaling, modulating food intake. They express cholecystokinin (CCK) receptor type 1 whose activation is known to play a very important role in mediating satiety. Vagal neurons are known to express receptors for leptin and orexin-A (65, 66), whose ligands activate and reduce the anorectic effect of CCK on vagal afferent nerve discharge, respectively. Importantly, CB1 receptor is also present in these neurons, and its expression is decreased after feeding and enhanced in fasting conditions (67). CCK was shown to mediate the effect of food in down-regulating vagal CB1 receptor expression (67). CB1 receptor was also found in the fundus of the stomach, but the cellular localization is not yet known. However, a single SR141716 administration is able to reduce the levels of ghrelin (68), whose production takes place in the gastric endocrine (X-) cells (69).

e. CB1 receptor in the reproductive organs. CB1 receptor has been known for a long time to be expressed in the testis (57, 70). In particular, it seems to be localized in Leydig cells (71), whereas Sertoli cells that are able to inactivate arachidonoyl ethanolamide (AEA) do not express CB1 receptor (72). Sea urchin sperms, an ideal model for studying fertilization processes, express cannabinoid binding sites (73). Human sperms possess functional binding sites for cannabinoids (74). Very recently, Rossato *et al.* (75) elegantly showed that CB1 receptor is present in the head and the middle piece of human sperm.

CB1 receptor is also expressed in the ovary (57), probably located in the granulosa cell layer where Δ^9 -THC was shown to inhibit cAMP accumulation (76). CB1 receptor is present in the mouse uterus (77) and in the human myometrium (78), and is associated with the relaxant effect of cannabinoid receptor agonists (78). Importantly, CB1 receptor is coexpressed with β -adrenergic receptors in the oviduct muscularis, where the endocannabinoid system regulates motility and embryo transport (79). Both CB1 and CB2 receptors are located in the mouse preimplantation embryos (80) as well as in all layers of human placenta; particularly high levels are detectable in the amniotic epithelium and in the maternal decidua layer (81).

4. Signal transduction of CB1 receptor. The signal transduction of cannabinoid receptors has been extensively described in many excellent reviews (3, 4, 25, 82–85), and its detailed description is beyond the scope of the present article. It is important to note, however, that CB1 receptor activation might lead to the stimulation of different intracellular pathways, depending on the cell type involved and the experimental conditions. For instance, CB1 receptor, which normally inhibits adenylate cyclase, can also stimulate the cAMP

pathway in particular conditions (86, 87). Moreover, recent results suggest the possibility of functional interactions of CB1 receptors with other receptors, for instance, with type 1 orexin receptors (88), 5HT₂ serotonin receptors (89), and dopamine receptor type 2 (D2) (87). The possibility that such interactions depend on heterooligomerization processes might represent a very interesting novel aspect (87), which will expand the view of the pharmacology and physiology of the endocannabinoid system. These considerations should also be borne in mind to understand the roles of the endocannabinoid system in regulating the endocrine systems. Figure 2 summarizes the best-described intracellular effects of CB1 receptor stimulation, including the regulation of the

cAMP cascade, modulation of ion channels, stimulation of kinase pathways, and induction of immediate early genes.

B. Endocannabinoids

1. *Structure.* In 1992, the first endogenous cannabinoid, AEA, also called anandamide, was identified (8). Subsequently, a second endocannabinoid, 2-arachidonoyl glycerol (2-AG), was discovered (5, 9). Both these compounds are derivatives of arachidonic acid and are able to bind to CB1 and CB2 receptors, although with differences in affinities and activation efficacies (90). During the last few years, several other bioactive lipid mediators have been described; they appear

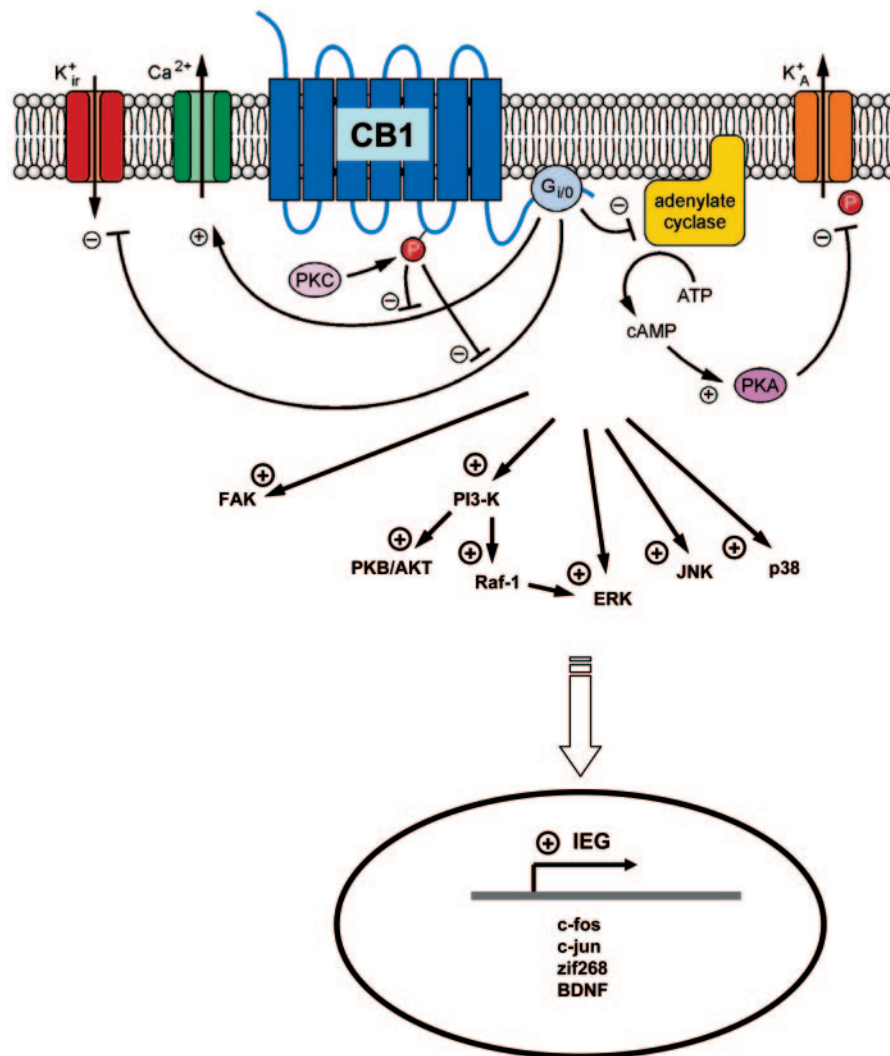


FIG. 2. Schematic representation of the main effects of CB1 on intracellular signaling cascades. Activation of CB1 leads to the stimulation of G_{v/o} proteins that, in turn, inhibits the adenylate cyclase-mediated conversion of ATP to cAMP. cAMP molecules can bind the regulatory subunits of protein kinase A (PKA) and cause the liberation of the catalytic subunits. Activated PKA can phosphorylate A-type potassium (K_A⁺) channels, causing a decrease of the current. Given the negative effect of CB1 on adenylate cyclase, the final result is an activation of K_A⁺ channels. G_{v/o} activated by CB1 can also directly inhibit N- or P/Q-type Ca²⁺ channels and activate inwardly rectifying potassium (K_{ir}⁺) channels. These last two effects are controlled by protein kinase C (PKC), which, after activation, can phosphorylate CB1 in the third cytoplasmic loop and uncouple the receptor from the ion channels. Activation of CB1 can also stimulate several intracellular kinases, such as focal adhesion kinase (FAK), phosphatidylinositol-3-kinase (PI3-K) and its downstream effector protein kinase B (PKB)/AKT, ERKs, c-Jun N-terminal kinase (c-JNK), and p38 MAPK (p38). Stimulation of cytoplasmic kinases could also mediate the CB1-induced expression of the immediate early genes (IEG), such as the transcription factors c-fos, c-jun, and zif268, and the brain-derived neurotrophic factor (BDNF). Note that these events were described in different cellular systems and, therefore, they might not occur in the same cell types.

to act, at least in part, through CB1 and/or CB2 receptors and confer specific pharmacological effects *in vivo* (91). Specifically, these compounds are 2-arachidonoyl-glycerol ether (noladin ether) (92), O-arachidonoyl-ethanolamine (virodhamine) (93), N-arachidonoyl-dopamine (94), and possibly oleamide (95). However, the endogenous function in physiological processes for all these latter compounds have not yet been established in detail and need further investigation (4). Furthermore, there are several additional putative lipid mediators that might have cannabinomimetic actions, but whose exact mechanism of action is not known in detail (91). In some cases, their cannabinomimetic effects may be partially attributed to interference with the endocannabinoid-inactivating enzymes (91). These lipids might, therefore, be able to enhance the activity of cannabinoid receptors by increasing the concentration of the endocannabinoids such as AEA and/or 2-AG.

2. Synthesis, release, uptake, and degradation of endocannabinoids: on demand activation of the endocannabinoid system. Endocannabinoids are very lipophilic and thus cannot be stored in vesicles like other neurotransmitters. Consequently, the regulation of endocannabinoid signaling is tightly controlled by their synthesis, release, uptake, and degradation (3). Several different stimuli, including membrane depolarization and increased intracellular Ca^{2+} and/or receptor stimulation, can activate complex enzymatic machineries, which lead to the cleavage of membrane phospholipids and eventually to the synthesis of endocannabinoids. Importantly, different enzymes are involved in the synthesis of distinct endocannabinoids, indicating an independent involvement of endocannabinoids in different conditions. After synthesis, endocannabinoids can activate cannabinoid receptors, either after previous release into the extracellular space or directly moving within the cell membrane. Endocannabinoid signaling is limited by very efficient degradation processes, involving facilitated uptake from the extracellular space into the cell and enzymatic catabolism mediated by specific intracellular enzymes. The molecular nature of the carrier protein(s) involved in endocannabinoid uptake has not yet been elucidated. However, the enzymes able to degrade endocannabinoids are quite well characterized. They are fatty acid amide hydrolase (FAAH) for anandamide and related compounds (96) and monoglycerol lipase for 2-AG (97), although other enzymes might be partially involved in the degradation of this last compound (98). A detailed description of the biochemical mechanisms leading to the synthesis, release, uptake, and degradation of endocannabinoids is beyond the scope of the present article, and we refer the reader to several excellent and exhaustive reviews recently published on the subject (3, 4, 30, 82, 99–101). An interesting aspect of endocannabinoid activity is the rapid induction of their synthesis, receptor activation, and degradation (3, 102). The endocannabinoid system has thus been suggested to act on demand, with a tightly regulated spatial and temporal selectivity. The system exerts its modulatory actions only when and where it is needed. This fact poses an important distinction between the physiological functions

of the endocannabinoid system (selective in time and space) and the pharmacological actions of exogenous cannabinoid receptor agonists, which lack such selectivity. In the context of endocrine regulation, it is interesting to mention here that hormonal stimulation with glucocorticoids can lead to the synthesis of endocannabinoids in the hypothalamus through rapid nongenomic mechanisms (103). It was also recently shown that phospholipase $\text{C}\beta$ represents an intracellular coincidence detector of membrane depolarization and receptor stimulation leading to the synthesis and, possibly, the release of endocannabinoids in the hippocampus (104). These data reveal a novel mechanism for activation of the endocannabinoid system, which could also be involved in the regulation of endocrine systems. Concerning degradation of endocannabinoids, which represents an important regulatory aspect of the activity of the endocannabinoid system, it should also be mentioned that a recent study investigated whether endocytic processes are involved in the uptake of endocannabinoids and found that about half of the AEA uptake occurs via a caveola/lipid raft-related process (105).

3. Endocannabinoid-mediated inter- and intracellular signaling. Several mechanisms underlying endocannabinoid-mediated signaling have been reported. 1) In the central nervous system (CNS), endocannabinoids can act as neurotransmitters transferring information from one neuron to the next. Here, postsynaptically released endocannabinoids travel to the presynaptic site where they activate CB1 receptors. They thus mediate a retrograde signal (30, 106, 107). The overall effect is a decrease in the release of neurotransmitters such as glutamate and GABA. This phenomenon is present in synaptic connections of many brain regions, thus representing an important modulatory mechanism of neuronal transmission. With respect to the aims of the present review, it is noteworthy that this function has also been shown in the VTA (108, 109), where the modulation of reward properties of food presumably occur, and in the hypothalamus, where endocannabinoids and CB1 receptor mediate the acute glucocorticoid-dependent depression of glutamatergic transmission (103). 2) Endocannabinoids can mediate an autocrine signaling that induces a self-inhibitory effect on neuronal activity. This was shown for GABAergic neurons in the cerebral cortex (110). 3) Endocannabinoids may act in a paracrine or autocrine manner, not involving synaptic transmission. This is presumably applicable for glial cells (111) and in nonneuronal cells such as the adipocytes and the hepatocytes. 4) Because endocannabinoids and CB1 receptor are also present within the cell, it cannot be excluded that endocannabinoids may act as intracellular signaling molecules. Importantly, AEA and 2-AG do not appear as interchangeable mediators. For instance, electrophysiological and biochemical evidence shows that 2-AG is mostly involved in retrograde control of synaptic activity in the VTA (109), or the hippocampus (112), whereas AEA appears to play an important role in other regions, such as the basal ganglia (113) and the amygdala (114).

In summary, endocannabinoids appear to be very versatile signaling mediators, involved in a broad spectrum of physiological regulatory processes.

C. Cannabinoid agonists

1. *Plant-derived cannabinoids.* The isolation and characterization of the psychoactive component of *C. sativa* represented a challenging research task. This was due to the fact that the extracts from *Cannabis* plants contain more than 60 different, chemically closely related terpeno-phenols that are difficult to separate and purify. This prevented the isolation of pure crystals for determination of the structure. The breakthrough was achieved using improved column chromatography. As mentioned above, in the early 1960s, Gaoni and Mechoulam (2) succeeded in isolating and pharmacologically characterizing various plant-derived cannabinoids. In hemp, the major psychoactive compound is represented by Δ^9 -THC, whereas Δ^8 -tetrahydrocannabinol is only present in very low amounts. The majority of terpeno-phenols in hemp lack psychoactivity. They include cannabidiol, cannabinol, cannabigerol, and cannabichromene. Although psychoactive cannabinoids bind to and activate both CB1 and CB2 cannabinoid receptors, nonpsychoactive cannabinoids are also able to exert various pharmacological effects *in vivo*, although only at rather high concentrations and not by activation of CB1 or CB2 receptors. Cannabidiol has recently gained additional attention due to its anticonvulsive, neuroprotective, and antiemetic activities (115–117). The underlying mechanisms of actions of this plant-derived cannabinoid have not yet been elucidated.

2. *Classification of exogenous and endogenous cannabinoids.* Based on structural features, plant-derived and synthetic cannabinoids are divided into different classes (25). In brief: 1) For “classic” cannabinoids, the main psychoactive constituent of *Cannabis*, Δ^9 -THC, encompasses tricyclic dibenzopyran compounds and serves as the lead structure. Δ^9 -THC is a partial agonist of CB1 and CB2 receptors. The synthetic derivative HU210 shows the highest potency among the known CB1 receptor agonists and also activates CB2 receptors (25). HU308, another synthetic Δ^9 -THC derivative, was found to be a selective CB2 receptor agonist (118). 2) So-called “nonclassic” cannabinoids are synthetic Δ^9 -THC derivatives that lack the dihydropyran ring. The most famous one is represented by CP-55,940, a potent and complete agonist of CB1 and CB2 receptors, synthesized by Pfizer. It was originally pivotal for the molecular identification of CB1 receptor (25). 3) Finally, aminoalkylindoles, represented by R-(+)-WIN-55,212-2, are compounds structurally unrelated to Δ^9 -THC but with strong cannabimimetic activities (25). They bind to both CB1 and CB2 receptors (25).

All endocannabinoids are structurally rather distinct from plant-derived and most synthetic cannabinoids. Prototypically, they belong to the eicosanoids, fatty acid derivatives containing a chain with 20 carbon atoms. The synthetic AEA derivative arachidonyl-2'-chloroethylamide represents a selective CB1 receptor agonist with very low activity on CB2 receptor (25).

The quest for specific ligands for either of the cannabinoid receptors represents an important research topic. In particular, if CB2 receptor is targeted with a specific agonist, with no activity on CB1 receptor, the psychotropic side effects of the agonist are avoided. This may be very relevant for alleviating peripheral pain where CB2 receptor is involved (26,

119). Further important progress may also be achieved by the development of cannabinoid receptor agonists that do not pass the blood-brain barrier. Such compounds would focus on the receptors in the periphery and would thus prevent undesirable side effects originating from the CNS.

Although not acting as ligands of cannabinoid receptors, inhibitors of cellular uptake of endocannabinoids, such as AM404 (120), VDM11 (121), and UCM707 (122) provide another interesting class of drugs interfering with the endocannabinoid system. Given the on demand nature of the synthesis and release of endocannabinoids, these drugs make it possible to induce a targeted increase in the concentration of endocannabinoids, likely reducing some of the undesirable side effects observed by using receptor agonists.

D. Cannabinoid type 1 receptor antagonists

Pharmacological investigations have placed emphasis on the generation of substances acting as specific antagonists of cannabinoid receptors. Among the increasing number of compounds sharing CB1 receptor antagonistic properties (123, 124), the compounds most characterized are SR141716 (125), SR14778 (126), AM251 (124), AM281 (127), LY320135 (128), and SLV319 (129). The CB1 receptor antagonists known so far are diarylpyrazoles, or aminoalkylindoles, or triazole derivatives. Diarylpyrazoles include SR141716, which is the first selective CB1 receptor antagonist reported. It was discovered approximately a decade ago, and it has been the compound most studied so far. Pharmacologically, SR141716 shows a K_i value of binding to rat brain synaptosome of 1.98 ± 0.36 nM (125). Few data on the metabolism and pharmacokinetics of SR141716 are available in humans (130). The dose of SR141716 that produced a 50% antagonism of agonist effect in the mouse was 0.23 mg/kg, and a dose of 3 mg/kg produces a long-lasting (18 h) blockade of the effect of WIN-55212-3 (131).

There are different possible mechanisms by which CB1 receptor antagonists produce their effects on the CB1 receptor (132). The ligands can be competitive antagonists of CB1 receptor activation by endogenously released endocannabinoids, or they can act as inverse agonists and modulating constitutive CB1 receptor activity by shifting it from an active “on” to an inactive “off” state (133). They may also act by CB1 receptor independent mechanisms (132). These mechanisms are not mutually exclusive.

III. Exogenous and Endogenous Cannabinoids and Their Role in Endocrine Regulation

It has been known for a long time that exogenous cannabinoids are able to affect secretion of pituitary hormones, thus having a strong effect on peripheral target organ functions. Notably, in 1972 the first report of an induction of gynecomastia due to marijuana consumption led to a dramatic acceleration of studies on this topic (134). The hypothalamus is generally considered as the main site of cannabinoid action on neuroendocrine functions. This view is elegantly supported by a recent publication showing that endocannabinoids act as retrograde messengers activating CB1 receptors expressed at presynaptic glutamatergic ter-

minals in the hypothalamus (103). The subsequent activation of the CB1 receptor signaling cascade leads to the inhibition of the release of the excitatory neurotransmitter glutamate onto the neuroendocrine cells of the PVN and the supraoptic nucleus (103). This leads to a general suppressive effect on neuroendocrine cells and a final inhibitory effect on neuroendocrine function.

However, it was recently proposed that the endocannabinoid system might control hormonal balance also through a direct effect at the level of the peripheral target organs. An overview of the cannabinoid actions on endocrine axes is given in Table 1.

A. Cannabinoids and the hypothalamic-pituitary-adrenal axis

Stimulation of the hypothalamic-pituitary-adrenal (HPA) axis is a crucial neuroendocrine response to stress. Psychological or physiological stressors are known to induce CRH production in the PVN of the hypothalamus, eventually leading to a release of this hypothalamic peptide onto the anterior pituitary gland. In turn, this leads to increased circulating levels of ACTH and, finally, to an increase of corticosteroids secreted by the adrenal gland.

Until a few years ago, the impact of the cannabinoids on the HPA axis was considered as an exception. Whereas the commonly accepted view attributes the cannabinoid system with a general inhibitory role on neuroendocrine functions, it was suggested that cannabinoids are, on the contrary, able to stimulate the HPA axis. In fact, many studies in animals point to a CB1 receptor-dependent (135) increase of circulating ACTH and glucocorticoid levels after pharmacological administration of plant-derived (136), synthetic (137, 138), or endogenous cannabinoid agonists (139, 140). In agreement with this, a simultaneous elevation of CRH in the PVN and of proopiomelanocortin in the anterior pituitary after chronic treatment (18 d) with the CB1 receptor agonist CP-55,940 was observed in rats (138). Cannabinoids were proposed to act exclusively at hypothalamic sites after the finding that Δ^9 -THC did not induce hyperactivation of the HPA axis in hypophysectomized rats (141), and that Δ^9 -THC or WIN 55,212-2 was unable to stimulate ACTH release from basal and CRH-stimulated dispersed pituitary cells or isolated pituitary slices, respectively (135, 142).

However, this concept was recently challenged by several reports showing a different function of endocannabinoids on the HPA axis. In fact, some studies showed that administration of the CB1 receptor antagonist SR141716 in rats is able to induce ACTH and corticosterone release and to produce anxiety-like behavior (143, 144). It is well known that this behavior represents part of the physiological response to stressful stimuli and is, indeed, associated with the hyperactivation of the HPA axis (145). Moreover, compounds able to increase endocannabinoid tone by inhibiting FAAH activity were recently proposed as treatment for anxiety-related disorders because they were shown to reduce restraint-induced corticosterone release (146) and to diminish the anxiety-like response in different tasks (147). In addition, mice lacking CB1 receptor (CB1^{-/-}) are resistant to some actions of anxiolytic drugs (148). In support of the existence of a close interaction between the endocannabinoid system and CRH, it is important to mention that CB1 receptor and CRH mRNAs are coexpressed in PVN neurons, and that CB1^{-/-} mice present increased CRH mRNA levels in this region, indicative of a possible basal alteration of the HPA axis activity due to the disruption of CB1 receptor signaling (58). Therefore, a novel view seems to attribute the endocannabinoid system with a critical inhibitory action on HPA functions. A recent elegant report by Patel *et al.* (146) shed light on this issue. The authors confirmed previous studies showing that systemic treatment with SR141716 is able to increase serum corticosterone concentrations in basal conditions; more importantly, they found that pretreatment of mice with the same CB1 receptor antagonist before acute restraint stress provokes a potentiation of the restraint-induced rise in serum corticosterone concentrations. In addition, endogenous cannabinoids and, in particular 2-AG, were found to be decreased after a short period of restraint stress, whereas a condition of prolonged stress was associated with an increase in 2-AG concentrations (146). Accordingly, they concluded that endocannabinoid signaling negatively modulates the stress-induced activation of the HPA axis, confirming the notion that a pharmacological increase in endocannabinoid signaling activity may constitute a novel approach to the treatment of anxiety-related disorders (146). These findings reinforce the general concept that the pharmacological administration of cannabinoids may lead to a

TABLE 1. Overview of the cannabinoid action by CB1 activation in the various endocrine axes

Endocrine axis	Cannabinoid actions	Ref.
HPA axis	Acute stimulation of CRH by CB1 agonists	135–140
	Stimulation of the HPA by CB1 antagonists inducing a potentiation of stress-induced rise of the axis	143, 144, 146–148
	Direct stimulation at the level of ACTH-producing cells (controversial data)	52, 135, 142
	No studies on the direct effect at adrenal gland	
Hypothalamus-pituitary-GH axis	Inhibitory action through somatostatin activation	150–154
	Hypothalamus-pituitary-thyroid axis	Inhibition of T ₃ and T ₄ secretion by direct action at level of the thyroid
Hypothalamus-PRL axis		Action at the level of PRL-producing cells
	Hypothalamus-pituitary-gonadal axis	Inhibitory action through dopamine activation
No effect on FSH		172
Inhibition of LH pulse through a multiple action on neuronal systems regulating GnRH secretion		54, 165, 172–176, 183–185
Inhibition of testosterone and ovarian androgens		76, 202–204
Inhibition of achrosome reaction		75, 208, 209
	Inhibition of sperm fertilization and capacity	75, 210

completely different action when compared with the physiological functions of the endocannabinoid system as shown by experiments using CB1 receptor antagonist or CB1^{-/-} mice.

Besides the hypothalamus, peripheral sites of action, such as pituitary and adrenal glands, could participate in the endocannabinoid modulation of the HPA functions. In cultured human ACTH-producing tumors, WIN 55,212-2 was found to be ineffective in influencing basal ACTH secretion. However, the simultaneous application of WIN 55,212-2 and CRH caused a synergistic action, which was abolished by SR141716, indicating that the activation of CB1 receptor might play a role during CRH-induced activation of ACTH-secreting cells (52). Therefore, in the corticotroph cells, an endocannabinoid tone could interfere with the normal regulation of the adenylate cyclase activity and, thus, with the secretion of ACTH. As mentioned above, a pending question regards CB1 receptor expression and endocannabinoid production at the level of cortical adrenal gland and their putative role in the secretive function of this gland. Further efforts are needed to solve this important issue. Interestingly, our recent unpublished studies indicate that CB1^{-/-} have higher plasma levels of corticosterone but normal levels of ACTH, suggesting a putative regulation of adrenal activity by the endocannabinoid system (our unpublished results).

B. The role of cannabinoids in GH secretion

GH secretion is mainly stimulated by hypothalamic GHRH and by the recently discovered peptide ghrelin (69), whereas somatostatin is the most important negative regulator. Other neurotransmitters such as serotonin, dopamine, and catecholamines can affect GH secretion through modulation of GHRH release. Few data are available concerning the effects of marijuana on GH in humans. Four days of marijuana consumption were shown to inhibit the GH-counteracting response provoked by insulin-induced hypoglycemia (149). Δ^9 -THC and synthetic cannabinoids were shown to inhibit GH secretion in rodents (150–152). However, compared with other hormones, it is still questionable whether cannabinoids are able to decrease GH secretion acting exclusively at the hypothalamic level or whether they also directly influence GH pituitary output. Rettori *et al.* (153) observed that only intracerebroventricular Δ^9 -THC administration was able to reduce GH secretion, whereas no effect was observed in cultured rat pituitary cells. Interestingly, by incubating fragments of median eminence with Δ^9 -THC, a significant stimulation of basal somatostatin was found (154); this finding makes it possible to speculate that the inhibitory action of Δ^9 -THC on GH secretion could be mediated by somatostatinergic activation (154). Recent data point to a functional cross-talk between CB1 receptor and the ghrelinergic system. In fact, hyperphagia associated with intracerebroventricular administration of ghrelin is blocked by pre-treating the rats with SR141716 (155). Unfortunately, no data have been provided concerning GH release in this experimental setting. Altogether, these data seem to indicate that the effect of exogenous cannabinoids on GH secretion is located at a suprapituitary level. However, the cannabinoid antagonist WIN 55,212-2 inhibited GH secretion in human GH-

producing adenomas in culture, and this effect was reversed by the specific CB1 receptor antagonist SR141716, suggesting that cannabinoids are able to directly influence basal GH secretion through CB1 receptor activation, at least in tumoral tissues (52). No data are available on the physiological modulation made by the endocannabinoid system on GH secretion.

C. Cannabinoids and the hypothalamic-pituitary-thyroid axis

Pioneer studies showed that marijuana is able to decrease TSH and thyroid hormones in rats (156, 157) and iodine accumulation in the isolated rat thyroid (158). The lack of changes in TRH secretion in the hypothalamus led the authors to conclude that the cannabinoid effect could be attributed to a direct action at the level of the pituitary or the thyroid gland (157). Recently, Porcella *et al.* (56) found a CB1 receptor-dependent decrease (30%) in both free T₃ and free T₄ 4 h after the administration of the synthetic cannabinoid agonist WIN 55,212-2 in rats. TSH levels were unaffected, indicating that the thyroid gland itself may be the direct target of cannabinoid action (56). On the other hand, the lack of TSH changes may also be explained by an action of cannabinoids on the levels of thyroid binding protein or on the metabolism of thyroid hormones. More studies are needed to verify these hypotheses. Concerning the physiological roles of the endocannabinoid system, an inhibitory action on TRH neurons through a glucocorticoid-induced inhibition of glutamate transmission was recently proposed (103).

D. The role of cannabinoids in prolactin secretion

There is no general consensus regarding the effect of exogenous cannabinoids on PRL secretion. Early studies in rodents and primates favor an inhibitory role of cannabinoids on PRL release (153, 159–162) through a CB1 receptor-mediated effect (163). Conversely, some reports showed that cannabinoids may also have either a stimulatory effect (164, 165) or no effect (166) on PRL release. As often occurs in the field of cannabinoids, this controversy may be largely due to the different experimental settings used. The conflicting data may also originate from the biphasic profile of PRL observed after Δ^9 -THC administration, with an initial increase followed by a marked decrease after time (167). In the same study, the antagonist SR141716 was only able to block the inhibitory effect, whereas no effect was seen toward the cannabinoid stimulatory effect on PRL (167). There is a general agreement that cannabinoid activation of the tuberoinfundibular dopaminergic neurons controlling PRL secretion is the main mechanism responsible for the inhibition of this pituitary hormone (168, 169). When Δ^9 -THC was chronically administered to ovariectomized or hypophysectomized female rats or to dispersed pituitary cells in culture, no effect was seen on PRL release, suggesting that the inhibitory cannabinoid effect targets the CNS directly (161). Similar conclusions were drawn from similar models by other authors (153). Recently, exogenous AEA was shown to inhibit PRL release from male rats by acting on the CB1 receptor on dopaminergic neurons located in the medial basal hypothal-

amus (162). However, like other hormones, it has also been hypothesized that cannabinoids may also affect PRL secretion directly in the pituitary. Indeed, Δ^9 -THC was able to prevent estrogen-induced PRL secretion *in vivo* (170) and *in vitro* (170). The direct effect of cannabinoids at pituitary level was also confirmed by the demonstration that WIN 55,212-2 does not affect basal secretion, but inhibits vasoactive intestinal peptide- and TRH-stimulated PRL release in tumoral pituitary GH₄C₁ cells (171). WIN 55,212-2 was also able to inhibit PRL secretion in a single case of prolactinoma in culture (52). In conclusion, we can assume that the biphasic action on PRL secretion of exogenous cannabinoids is mediated by an initial activation of CB1 receptor located at the level of the pituitary and followed by a persistent inhibitory action mediated by the activation of the release of dopamine from hypothalamic structures.

E. The role of cannabinoids in modulation of the hypothalamic-pituitary-gonadal axis and fertility

1. In females. While FSH secretion seems to be unaffected by administration of exogenous or endogenous cannabinoids (172), several pieces of evidence attribute cannabinoids with a strong ability to down-regulate blood LH levels (49, 165, 172, 173). This effect is due to a complete suppression of the secretory pulse of LH (174, 175). In monkeys, chronic administration (18 d) of Δ^9 -THC was shown to block estrogen and LH surges and the consequent elevation in progesterone (176). However, the same animals developed tolerance to the antireproductive effect of the drug after a few months of treatment (177). In women smoking a single marijuana cigarette with a fixed content of Δ^9 -THC, a decrease of LH was observed during the luteal phase, whereas no effect was seen on the same hormone in the follicular phase and in the postmenopausal state (178, 179). The sustained use of marijuana (at least four times per week) may cause alterations of the menstrual cycle, such as oligomenorrhea; however, no changes were shown in hormonal parameters in a group of 13 pregnant women who continued to smoke marijuana during pregnancy (180). An excess of cannabinoids may also impair regular ovulation, not only acting at the hypothalamic level but also directly affecting ovarian granulosa layers (76).

A general consensus attributes the LH-inhibitory action of cannabinoids to a suprapituitary site of action. In fact, administration of gonadotropins or GnRH was able to induce ovulation or LH release, respectively, even in the presence of high levels of Δ^9 -THC (174, 175). However, a report showed that cannabinoids are not able to block the basal GnRH secretion from hypothalamus *in vitro* (165). This last finding suggests that cannabinoids indirectly modify GnRH secretion by negatively modulating the activity of neurotransmitters known to facilitate GnRH secretion, such as norepinephrine (165) and glutamate (181), and by stimulating those modulators known to down-regulate GnRH secretion, such as dopamine (182), GABA (183), opioids (184), and CRH (185). The stimulatory effect of cannabinoids on dopaminergic neurons is well known (186), however their impact on the brain dopaminergic activity varies as a function of the gonadal status, as demonstrated by several lines of evidence (187). In particular, it has been shown that steroid hormone

receptors mediate the well known Δ^9 -THC-facilitation on sexual behavior (188) exerted, as recently shown, by CB1 receptor activation (189). Moreover, in the same study Mani *et al.* (189) reported that an interaction between progesterone and dopamine receptor type 1 (D1) is required for Δ^9 -THC-facilitated sexual receptivity in female rats.

However, although pharmacological studies have helped to explain the relevant role of the cannabinoids in modulation of the hypothalamus-pituitary-gonadal axis and sexual behavior, it is not yet known how, where, and under what circumstances the endocannabinoids are produced to do so. The recent findings of fluctuation during the ovarian cycle of AEA in both hypothalamus and pituitary (49) allowed some authors to speculate that endocannabinoids may influence hormonal secretion and sexual behavior by directly targeting the CB1 receptor (190). Furthermore, an important production of endocannabinoids was found in the ovary, in particular at the time of ovulation, making it possible to hypothesize that the endocannabinoids may help to regulate follicular maturation and development of the ovary (74).

The uterus contains the highest level of AEA detected so far in mammalian tissues, and it is the only tissue where AEA is the main component (up to 95%) of *N*-acylethanolamides (191). This observation, together with the expression of CB1 receptors in preimplantation embryos (80), recently prompted strong efforts focused on the role of the endocannabinoid system during early pregnancy and in the modulation of embryo-uterine interactions. High levels of AEA adversely affect embryo development and implantation through CB1 receptor activation (192), whereas low levels of AEA promote embryonic growth and differentiation (193–195). It is therefore evident that the degradation of AEA by FAAH is a crucial enzymatic checkpoint in the control of reproduction. Notably, a strong inverse correlation was described between levels of FAAH activity in maternal peripheral blood mononuclear cells and spontaneous miscarriage in women (196). In addition, FAAH activity is lower, and consequently AEA higher, in patients who fail to achieve pregnancy during *in vitro* fertilization embryo transfer in comparison to patients who become pregnant (197). Furthermore, AEA levels in the mouse uterus are inversely related to uterine receptivity for implantation, being higher with uterine refractoriness to blastocyst implantation (191, 198, 199) and lower at implantation sites (194). We can therefore conclude that high levels of maternal AEA are detrimental to early placental and fetal development. In favor of this hypothesis, it was recently shown that high levels of FAAH are present in the cytotrophoblast, presumably to prevent the transfer of AEA from maternal blood to the embryo (200). A series of studies by Maccarrone *et al.* (72, 195) showed that the activity of FAAH is under the strict regulation of several hormones, such as progesterone, leptin, and FSH, very well-known modulators of fertility. Importantly, by using genetic or pharmacological blockade of the CB1 receptor, it was very recently demonstrated that an impairment in endocannabinoid signaling leads to a retention of a large number of embryos in the mouse oviduct, leading to pregnancy failure. This is due to a profound impairment of a coordinated oviductal smooth muscle contraction and relaxation (79). The authors propose that their findings may

have strong implications for ectopic pregnancy in women because one major cause of tubal pregnancy is embryo retention in the fallopian tube (79). Consistently, both endogenous and exogenous cannabinoids exert a CB1 receptor-mediated relaxant effect, not only on the oviductal smooth muscle but also on the human pregnant myometrium, highlighting a possible role of endocannabinoids during human parturition and pregnancy (78). In fact, pregnancy also seems to be tightly controlled by the endocannabinoid system (200). In summary, all the steps starting with fertilization up to pregnancy seem to be tightly modulated by endocannabinoids, reinforcing the concept that the endocannabinoid system should be considered not only as a central neuromodulator but also as a physiological actor in a wider scenario.

2. *In males.* Cannabinoids also were shown to decrease LH in males (201, 202). Although there is still no general consensus, chronic cannabinoid use in several species seems to decrease testosterone production (203) and secretion (201, 202), to suppress spermatogenesis, and to reduce the weight of testes and accessory reproductive organs (204). The important effects of cannabinoids on the gonadal system are mainly attributed to CB1 receptor activation, as demonstrated by using specific CB1 receptor agonists and antagonists (151, 205). Definitive confirmation was provided by a recent study showing that AEA injected ip is able to lower LH and testosterone in wild-type mice but not in CB1^{-/-} mice (71). Interestingly, the testis is known to express CB1 receptor (70) and to synthesize endocannabinoids (206). The cannabinoid effect in down-regulating testosterone circulating levels may explain the reduced copulatory behavior in male rodents exposed to Δ^9 -THC (207).

The finding that male genital tract fluids contain significant concentrations of endocannabinoids (74) suggests that these lipid-signaling molecules may influence important processes controlling sperm/egg functions and gamete interactions. Studies with sea urchin gametes provided the first evidence that cannabinoids, in particular AEA, are able to directly inhibit achrosome reaction and sperm fertilization capacity (208). On the other hand, seminal plasma contains high amount of AEA, and this may contribute to maintaining sperms in a quiescent metabolic condition (74). The content of AEA decreases progressively in the uterus, oviduct, and follicular fluid, and this change in endocannabinoids may render sperms suitable for capacitation and fertilizing ability (74, 209). Furthermore, as shown in sea urchin, the eggs may have the capacity to release AEA after activation by the fertilizing sperm (210), inducing a CB1 receptor activation that might be able to prevent polyspermic fertilization by blocking the acrosome reaction in other sperm (209).

In humans, CB1 receptor activation by AEA was also shown to reduce sperm mobility by affecting mitochondrial activity, and to inhibit capacitation-induced acrosome reaction. Importantly, these effects are inhibited by the CB1 receptor antagonist SR141716 (75). It is therefore reasonable to hypothesize that AEA levels might be increased in different pathological conditions of the male reproductive tract. In these cases, the pharmacological blockade of the endocannabinoid system might be helpful in the treatment of some forms of male infertility (75).

In conclusion, it appears that the endocannabinoid system plays an important role in the regulation of the hypothalamus-pituitary-gonadal axis both in females and in males, and fertility may be affected by cannabinoid drugs. This evidence may represent an important issue in clinical endocrinological praxis. In the light of the widespread use of marijuana as a recreational drug among young people, subtle alterations of the gonadal hormonal profile or in fertility may therefore be attributed to a concomitant use of cannabis derivatives. On the other hand, the results of human epidemiological studies have not always been clear in confirming this negative impact (211), and more detailed research on this topic is needed in the future before drawing definitive conclusions.

IV. Endocannabinoid System in the Modulation of Energy Balance

Two notions highlight the importance of the endocannabinoid system in the regulation of food intake and energy metabolism. The first is the finding of a high degree of evolutionary conservation of the role of this system in the regulation of feeding responses (212). The second is the observation that high levels of endocannabinoids in maternal milk are critically important for the initiation of the suckling response in newborns (213).

A. Animal studies before the discovery of endocannabinoids

Animal models are ideal tools for elucidating the putative mechanism(s) of cannabinoids in the control of energy metabolism. The studies performed in different species to test the orexigenic properties of Δ^9 -THC up to the discovery of endocannabinoids are summarized in Table 2 (214–244). From a general point of view, one can say that rather contradictory results were obtained in these experiments. The ambiguous data could likely be attributed to differences in the animal model and in the experimental procedures used. Moreover, in early studies using marijuana extracts, comparisons between various experimental data sets are extremely difficult due to the variability of the activity of cannabis derivatives, the dosages, and the routes of administration. In general, early studies using low doses of cannabinoids showed a reliable increase in food intake. When doses of Δ^9 -THC above 10 mg/kg were used, a concomitant decrease in food intake was observed due to the confounding factors given by the sedative effect of the drug. Studies employing high amounts of Δ^9 -THC should thus be viewed with caution in terms of effects on appetite and body weight. This is also the reason why, in reviewing the studies published between 1965 and 1975, Abel reported an increased food intake after cannabinoid administration only in 3 of 25 experiments (245). In 1998, Williams *et al.* (246) provided a very convincing and well-performed experiment to characterize the orexigenic property of Δ^9 -THC. The authors maximized the ability to detect hyperphagia by adopting a prefed paradigm in which the animals were characterized by low baseline food intake before drug administration. In this experimental setting, Δ^9 -THC was given orally at increasing dosage before unrestricted access to a standard diet. The authors

TABLE 2. Summary of the effects of exogenous cannabinoids on food intake

Animal model	Compound	Dose	Route of administration	Effects	Ref.
Nonrodent species					
Chick	Δ^9 -THC	1–10 mg/kg	im	↓ FI	214
Dog	Δ^9 -THC	0.5–32 mg/kg	iv	↓ FI	215
	Cannabis extract		Smoke	↑ FI	216
	Δ^9 -THC	225–3600 mg/kg	p.o.	↓ FI	217
	Δ^8 -THC	225–3600 mg/kg	p.o.	↓ FI	217
	Cannabis extract	225–3600 mg/kg	p.o.	↓ FI	217
	Δ^9 -THC	2 and 8 mg/kg	sc	↑ FI	218
Guinea pig	Δ^9 -THC	3 mg/kg	ip	↓ BW	219
Hamster	Cannabis extract	200, 300 mg/kg	sc	↓ FI	220
Monkey	Δ^9 -THC	4, 8 mg/kg	ip	↓ FI	221
	Δ^9 -THC	100 μ g	iv	↓ FI	222
	Δ^9 -THC	225–3600 mg/kg	p.o.	↓ FI	217
	Δ^8 -THC	225–3600 mg/kg	p.o.	↓ FI	217
	Cannabis extract	225–3600 mg/kg	p.o.	↓ FI	217
Pigeon	Δ^9 -THC	36 mg/kg	im	↓ FI	223
Rabbit	Cannabis extract	25, 50 mg/kg	sc	↓ FI	220
	Δ^9 -THC	3, 10, 30, 100 mg/kg	sc	↓ FI	224
Sheep	I- Δ^9 -THC	0.125, 0.250, 0.50 mg	iv	↑ FI	242
	9-Aza-cannabinol	0.25 and 0.50 mg	iv	↑ FI	242
	9-Aza-cannabinol	5.5 μ g/kg	iv	↑ FI	243
Rat	Cannabis extract	10 mg/kg	ip	↑ FI	225
	Δ^9 -THC	0.01–200 mg/kg	sc	↓ FI	226
	Cannabis extract	10 mg/kg	ip	↓ FI	227
	Δ^8 -THC	10 mg/kg	ip	↓ FI	228
	Cannabis extract	10 mg/kg	ip	↓ FI	229
	Cannabis extract	50 mg/kg	ip	↓ FI	229
	Δ^9 -THC	5–25 mg/kg	p.o.	↓ FI	230
	Cannabis extract	5–25 mg/kg	p.o.	↓ FI	230
	Δ^9 -THC	225–3600 mg/kg	p.o.	↑ FI	217
	Δ^8 -THC	225–3600 mg/kg	p.o.	↑ FI	217
	Cannabis extract	225–3600 mg/kg	p.o.	↑ FI	217
	Δ^9 -THC	5–80 mg/kg	ip	↓ FI	231
	Δ^9 -THC	110 mg/kg	p.o.	↑ FI	232
	Δ^9 -THC		Smoke	↑ FI	244
	Δ^9 -THC	2.5–5.0 mg/kg	ip	↓ FI	233
	Δ^9 -THC	1.25, 2.5, 5 mg/kg	ip	↓ FI	234
	Δ^9 -THC	2.5 and 5 mg/kg	ip	↓ FI	235
	Cannabinol	50 mg/kg	ip	↓ FI	235
	Cannabidiol	50 mg/kg	ip	↓ FI	235
	Δ^9 -THC	2.5 and 5 mg/kg	ip	↑ Sucrose	235
	Cannabinol	50 mg/kg	ip	↑ Sucrose	235
	Cannabidiol	50 mg/kg	ip	↑ Sucrose	235
	Δ^9 -THC	1, 4, 8 mg/kg	ip	↓ FI	236
	Δ^9 -THC	4 mg/kg	ip	↓ FI, BW	237
	Δ^9 -THC	0.25 μ g	Intrahypothalamic	↑ FI	238
	Δ^9 -THC	4 mg/kg	Intragastric	↑ FI	238
	Δ^9 -THC	20 mg/kg	ip	↓ FI, BW	239
	Δ^9 -THC	1 mg/kg	p.o.	↑ Sweet solution	240
	Δ^9 -THC	0.4 mg/kg	ip	↑ FI	241

↓, Decrease; ↑, increase; FI, food intake; BW, body weight; p.o., per os.

observed that the maximum effect of the drug (1.0 mg/kg) was far greater than previously reported results, showing a 4-fold increase in food consumption over 1 h. Importantly, this hyperphagic effect was largely attenuated by pretreatment with the CB1 receptor antagonist SR141716, strongly supporting the notion that CB1 receptor activation mediates the hyperphagic effect of Δ^9 -THC (247). In this experiment, it was also reported that at doses of Δ^9 -THC higher than 1.0 mg/kg, the rats become unable to overeat due to the presence of motoric and sedative side effects (246). These results strongly suggest that the anorectic effect of Δ^9 -THC shown by many previous reports

was indirectly due to the sedated state induced by high doses of the drug.

B. Studies in humans with exogenous cannabinoids before the discovery of endocannabinoids

Abel (245) also critically reviewed the studies aimed at proving the stimulating effect of cannabis on hunger in humans. However, the lack of scientific thoroughness of these earlier studies led Abel to conclude that the putative cannabis-induced hunger effect was still far from being proven (245). Greenberg *et al.* (248) were the first to systematically

assess, under rigorous experimental conditions, the effect of a well-defined amount of Δ^9 -THC in terms of changes in feeding behavior and in body weight in humans. Both parameters increased after the first few days of the experiment. However, after this period, body weight continued to rise, averaging 2.3 kg across the whole 21-d period study, whereas a stabilization of energy intake was observed. This pioneer study already suggested that the ability of cannabinoids to stimulate hunger may vanish with time, whereas a possible metabolic effect of the drug may remain active longer (248). Nonetheless, later studies did not investigate the metabolic idea further, preferring to concentrate interest on the ability of cannabis to stimulate hyperphagia and overconsumption of highly palatable food at the central level. In 1986, Foltin *et al.* (249) noted a relevant increase in frequency and consumption of snack foods induced by marijuana only in the periods of social facilitation and environmental familiarity and not when the subjects were alone, indicating on the one hand a strong link between recreational use of the drug and its orexigenic properties and, on the other hand, the ability of marijuana to drive the tendency for palatable food. This hypothesis was further substantiated by the same group a few years later when increased total food intake particularly related to consumption of palatable food (sweet solid snacks) was observed as a main effect of smoked marijuana (250).

The stimulating effect of cannabinoids on appetite observed in healthy subjects promoted assessment of the efficacy of a cannabinoid treatment for clinical syndromes featuring loss of appetite or weight, such as cancer or AIDS-associated anorexia (251–253), or as adjuvant therapy to limit nausea and vomiting symptoms associated with most chemotherapeutic drugs (254). In 1985, the U.S. Food and Drug Administration officially approved the use of Δ^9 -THC (commercially named Dronabinol) for the treatment of chemotherapy-induced nausea and vomiting refractory to other drugs. In 1992, Dronabinol was approved for the treatment of patients with HIV-induced wasting syndrome. Recently, Dronabinol was also proposed as an orexigenic drug in patients suffering from Alzheimer's disease (255).

The most comprehensive data are those obtained when Dronabinol was administered in HIV patients with wasting syndrome (252, 256–259). To varying degrees, the drug was able to mildly increase appetite and energy intake in all studies. However, a marked improvement in mood was also documented, raising the question of whether the positive effect in energy balance may derive from a specific action of cannabinoids in the brain areas controlling food intake or may be simply due to a generalized change in the sense of well-being. Intriguingly, in some reports, a significant gain was found in body fat mass associated with minimal changes in appetite rating and food intake (255, 258). At that time, this finding remained unexplained. However, with the current knowledge of CB1 receptor expression at the level of the adipose tissue (58, 59), we can hypothesize that the increase in fat mass of HIV patients was probably due to a direct lipogenic action of Δ^9 -THC. In this context, it is still unknown, and it would be of great relevance to investigate whether the administration of Dronabinol can improve the pathological changes in fat

distribution induced by the concomitant retroviral therapy in patients with AIDS (260).

C. Endocannabinoid functions at mesolimbic level to regulate rewarding properties of food

After the finding of the hyperphagic effect of Δ^9 -THC mediated by CB1 receptor activation, Williams and Kirkham (261) reported that endocannabinoids were also able to stimulate hunger in a dose-dependent manner. The degree of overeating induced by 1 mg/kg AEA was only a 2-fold increase over a 3-h test, therefore less than that obtained with the same dosage of Δ^9 -THC. However, Δ^9 -THC-induced hyperphagia was restricted to the first hour of testing, whereas the AEA effect was evident later when the inhibitory effects of the prefeed started to wane (261). The authors speculated that administration of AEA may represent an amplification of endocannabinoid activity associated with the normal, episodic pattern of meal-taking in rats (261).

Importantly, the effect of AEA was completely blocked by pretreating the animals with SR141716, confirming the pivotal role of CB1 receptor activation in the hyperphagic effects of endocannabinoids (247, 262). Similar conclusions were derived from other studies in which AEA was able to exert an appetite-stimulating effect even at very low doses in mice (0.001 mg/kg) (263) and 2-AG was capable of promoting feeding behavior (264). These data therefore make it possible to attribute the endocannabinoid system with an important role in the processes underlying the motivation to obtain food. It is suggested that endocannabinoids gradually increase during intermeal intervals, reaching a critical level where motivation to eat is triggered. Accordingly, the longer the time since the last meal, the greater the activity in relevant endocannabinoid circuits, and consequently the higher the motivation to eat (265). The findings of increased levels of AEA and 2-AG in the fasting condition in the nucleus accumbens and a decline of 2-AG concomitant with the feeding state strongly support this hypothesis (264). Interestingly, unchanged levels of endocannabinoids were shown in the cerebellum, a region not involved in the control of feeding, further confirming the notion that endocannabinoids are produced *in situ* and on demand (264).

With the advent of CB1 receptor-specific antagonists (Table 3), it became clear that, even when injected alone, these compounds are able to modify ingestive behavior. An ip injection of SR141716 was found to significantly reduce sucrose or alcohol intake and craving in rodents (266–268) and in marmosets (269), leading to the hypothesis that the activation of the endocannabinoid system may alter the appetitive value of ingested substances. This idea is consistent with the evidence in favor of a facilitatory function of the endocannabinoid system on brain reward circuits (266, 269). Evidence therefore suggests that endocannabinoids bring forward the onset of eating in satiated animals and increase the incentive value of the food regardless of the quality of the macronutrients (“incentive hypothesis”) (270). Other findings, however, resembling the “marshmallow effect” in marijuana smokers (245), have been interpreted in terms of an endocannabinoid action toward a preference to eat highly palatable food (“orosensory reward hypothesis”) (271). In

TABLE 3. Summary of the effect of CB1 antagonist treatment on food intake in different rodent models

CB1 antagonist	Dosage and route	Animal model	Length of treatment	Diet	Effect	Ref.
SR141716	2.5 mg/kg-d ip	Wistar rats	14 d	SD	-3.3% BW <i>vs.</i> vehicle	305
SR141716	10 mg/kg-d ip	Wistar rats	14 d	SD	-6.9% BW <i>vs.</i> vehicle	305
SR141716	10 mg/kg-d ip	Zucker rats	14 d	SD	-20% BW <i>vs.</i> vehicle	59
SR141716	10 mg/kg-d oral	Mice	5 wk	HFD	-20% BW <i>vs.</i> HFD-vehicle	306
SR141716	3 mg/kg-d oral	Mice	40 d	HFD	-10% BW <i>vs.</i> HFD-vehicle	306
SR141716	10 mg/kg-d oral	Mice	40 d	HFD	-18% BW <i>vs.</i> HFD-vehicle	306
SR141716	10 mg/kg-d oral	Mice	3 d	Caloric restriction	-20% BW <i>vs.</i> pairfed	306
AM 251	3 mg/kg-d oral	Mice	14 d	HFD	-10% BW <i>vs.</i> HFD-vehicle	311
AM 251	30 mg/kg-d oral	Mice	14 d	HFD	-20% BW <i>vs.</i> HFD-vehicle	311
SR141716	10 mg/kg-d oral	Mice	10 wk	HFD	-22% BW <i>vs.</i> HFD-vehicle	309

BW, Body weight; SD, standard diet; HFD, high-fat diet.

favor of this latter hypothesis, there are several reports indicating the ability of CB1 receptor blockade to decrease the rewarding properties of addictive drugs (186, 272–274). It is now clear that the endocannabinoid system participates in the modulation of “reward/reinforcement” circuitries and its manipulation is able to influence reward-related behaviors (275). The high expression of CB1 receptor in areas involved in reward constitutes a strong indication that the endocannabinoid system is directly involved in various physiological functions controlled in these brain regions, including feeding (43). The reward/reinforcement circuitry of the mammalian brain consists of a series of synaptically interconnected brain nuclei associated with the medial forebrain bundle, linking the VTA, the nucleus accumbens, and the ventral pallidum (275). This circuit is implicated in the pleasure produced by natural rewards, such as food, addictive drugs, and sex, and it is the neural substrate of drug addiction and addiction-related phenomena, such as craving and dysphoria induced by withdrawal (275). In such a framework, food intake acts on dopamine, opioid, serotonin, and noradrenergic neuronal fibers, which connect the hindbrain and midbrain to the hypothalamus to modulate the action of feeding and satiety factors (276).

The most relevant reward pathway is represented by the mesolimbic dopaminergic system. It has been shown that increased levels of extracellular dopamine and its metabolites are found within the nucleus accumbens after ingestion of highly palatable food (277). Moreover, administration of a dopamine D1 agonist reduces food intake (278). Both CB1 receptor and endocannabinoids were found in the rat limbic forebrain (279), in which colocalization with dopamine D1 and D2 and CB1 receptor were described (280). Psychoactive drugs such as marijuana, ethanol, and also pleasant stimuli or palatable food are known to induce the release of dopamine in specific brain regions (281). A correlation between limbic endocannabinoid/dopamine levels and craving for tasty food is thus presumed to occur (275). Verty *et al.* (282) recently substantiated the hypothesis of the existence of cannabinoid-dopamine interactions in feeding behavior, demonstrating that the dopamine D1 antagonist SCH 23390 attenuated feeding induced by Δ^9 -THC. The endocannabinoid system also provides retrograde control of synaptic transmission onto the VTA dopaminergic neurons, where the postsynaptic synthesis of endocannabinoids is under the control of somatodendritically released dopamine (108).

A relevant interplay also exists between the endocannabinoid system and the endogenous opioid peptides (283). Both

systems are linked to central reward processes, and there is increasing evidence supporting an important functional cross-talk between the two systems, in relation to a wide range of physiological processes, including appetite. Several reports indicate that opioid receptor agonists increase food intake (284–286), whereas opioid antagonists induce anorectic effects (287). Gallate and McGregor (267) found that the facilitatory effects of a cannabinoid agonist on responding to palatable solutions were reversed not only by CB1 receptor antagonism but also by naloxone, an opioid receptor antagonist. The existence of cross-talk between the endocannabinoid and opioid systems in controlling food intake was also confirmed by several studies in which naloxone and SR141716 synergistically depress food intake at doses that do not alter food intake on their own (287, 288). However, a recent finding seems to localize the interaction between opioids and endocannabinoids involved in feeding behavior not at the mesolimbic system level but, preferentially, at the level of the PVN of the hypothalamus. In fact, SR141716 was able to attenuate morphine-induced feeding only when the opioid was directly injected in the PVN and not in the nucleus accumbens. According to this last finding, the endocannabinoid system appears to participate in the opioid-mediated enhancement of rewarding properties of food in the hypothalamus and not in the nucleus accumbens (286).

According to the involvement of serotonin in the control of feeding behavior (289), the interaction of the endocannabinoid system with the serotonergic system has also been investigated. However, the administration of a CB1 receptor antagonist in rats combined with dexfenfluramine, an anorectic drug stimulating the release of serotonin, led to additional but not synergistic effects on reducing food intake, which is consistent with the hypothesis that the two pathways work via independent mechanisms of action (288). This notion is important, because it makes it possible to exclude a synergistic effect in a possible future combination of anti-obesity drugs such as those inhibiting serotonin reuptake, like sibutramine (290) and CB1 receptor antagonists.

D. The endocannabinoid system as a new hypothalamic player in the regulation of food intake

A complex and redundant neuronal hypothalamic network provides high levels of adaptability of feeding behavior to various central and peripheral stimuli (291). Redundancy in appetite-stimulating signaling is conceivable in view of the vital importance of feeding for survival (291). Whereas de-

fects in anorexigenic signaling pathways almost always lead to obesity, loss of orexigenic signals rarely results in a lean phenotype. An example of this redundancy in orexigenic hypothalamic signaling systems is provided by mice lacking neuropeptide Y (one of the most important appetite-stimulating neuropeptides) where compensatory mechanisms are likely to be activated (292). Signals coming from various peripheral organs, such as the liver, gastrointestinal tract, and adipose tissue, are conveyed mainly at the hypothalamic level to constantly inform the brain about the state of nutrition (291, 293). An example of such peripheral control is the adipocyte-derived hormone leptin, which acts on receptors located in the hypothalamus (291). A milestone in the identification of the endocannabinoid system as a new player in the regulation of food intake at hypothalamic level was the finding that leptin is a strong modulator of hypothalamic endocannabinoid levels (294). Di Marzo *et al.* showed that acute leptin treatment reduced AEA and 2-AG not only in the hypothalamus of normal mice but also in mice lacking leptin signaling. They also described the defect in leptin signaling as being constitutively associated with elevated hypothalamic levels of endocannabinoids. In these animals, SR141716 was able to reduce food intake, confirming the anorectic properties of the compound (294). These findings suggest that, at least in genetically modified animal models, obesity is associated with a chronic hypothalamic overactivation of the endocannabinoid system, which may in turn explain the hyperphagic behavior of the animals having leptin signal impairment. However, before giving a general value to this assumption, the intrahypothalamic amount of endocannabinoid levels during the development of obesity in normal rodents eating a high-fat diet must be investigated. Nevertheless, endocannabinoids are variably produced in the hypothalamus of normal animals. In fact, 2-AG levels increase during acute fasting, decline as the animals are refed, and return to normal values in satiated animals (264, 295). However, a long period of diet restriction (12 d) was found to be associated with reduced levels of 2-AG in the hypothalamus (295). The authors interpreted these data observing that the decrease of 2-AG levels in mice after a prolonged diet may represent a general psychobehavioral strategy for intermittent starvation when food is scarce (295).

As mentioned above, the hypothalamus is not the cerebral area where the highest levels of CB1 receptor expression are found (24, 36, 38). However, studies using [³⁵S]GTPγS binding indicated that the hypothalamic CB1 receptor coupling to G proteins is more efficient than in other cerebral areas known to be a site of high CB1 receptor expression, such as the hippocampus or the entopeduncular nucleus (43). On the other hand, it is also evident that CB1 receptors are present at a very high density in the brain compared with other receptors. Therefore, even regions with a relatively lower density of CB1 receptors, such as the hypothalamus, contain a significant number of receptors. Both these factors thus probably explain the ability of hypothalamic CB1 receptors to strongly affect the functions of this brain region. Interestingly, no changes in CB1 receptor expression have been shown at the level of hypothalamus after diet modification (296). The direct involvement of the hypothalamus in the modulation of food intake operated by endocannabinoids

was also demonstrated by the significant hyperphagic effects of AEA directly administered into the ventromedial nucleus and by the inhibition of this effect obtained by the injection of SR141716 via the same route (297).

It was only during the last few years that the interaction of CB1 receptor and endocannabinoids in feeding-regulating pathways started to be elucidated in detail. The CB1 receptor is expressed in key hypothalamic peptidergic systems, such as those producing CRH in the PVN, cocaine-amphetamine-related transcript in the dorsomedial nucleus, and melanin-concentrating hormone and orexins in the lateral hypothalamus-perifornical area (58). Importantly, these data were recently confirmed by the demonstration that CB1 receptor activation strongly augments the orexin-A-stimulated intracellular pathway (88). CB1^{-/-} mice also possess increased CRH and reduced cocaine-amphetamine-related transcript expression, indicating that the genetic impairment of the endocannabinoid system may affect the pattern of gene expression of peptides involved in the regulation of food intake (58). Conversely, the neuropeptide Y/agouti-related protein system in the arcuate nucleus does not seem to be directly targeted by endocannabinoid action (58, 294). This fact confirms that orexigenic pathways are less critical (or at least functionally more redundant) in the chronic maintenance of energy balance (298). Functional cross-talk between CB1 receptor and melanocortin receptor type 4 (MCR4) has been recently highlighted by the finding of the synergistic action of subanorectic doses of SR141716 and of a MCR4 agonist administered together (299). Furthermore, the same authors showed that the orexigenic impulse given by the administration of CB1 receptor agonists is not blocked by the co-stimulation with MCR4 agonists, whereas CB1 receptor antagonists are able to inhibit the stimulation of food intake induced by MCR4 antagonists. Consequently, the authors hypothesized that the melanocortin receptor signaling in the hypothalamic regulation of food intake is upstream of the activation of the endocannabinoid system (299).

The mechanism(s) of action of the endocannabinoids at hypothalamic synaptic level are still a matter of debate. Great progress has recently been made by the finding 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 (103). This release of endocannabinoids from the parvocellular neurons is stimulated by a nongenomic effect of glucocorticoids. Therefore, it is conceivable that the well-known regulation of food intake by glucocorticoids may partly derive from functional cross-talk with the endocannabinoid system (300). The same inhibitory mechanism mediated by glucocorticoids through an activation of the endocannabinoid system has also been proposed for other hormones and neuropeptides such as oxytocin and vasopressin (103). In this sense, we may speculate that the recently described interaction between endocannabinoid and the oxytocin system in modulating food intake (301) may derive from the same fast feedback mechanism mediated by nongenomic glucocorticoid inhibition.

Despite the dogma that neurons do not utilize fatty acids for energy, a growing body of evidence points to a critical role for both fatty acid production and utilization in regu-

lating hypothalamic neurons that regulate food intake (302). In fact, inhibitors of fatty acid synthase are capable of greatly affecting appetite in an anorexigenic manner (303, 304). In such a scenario, it has recently been proposed that via CB1 receptors, endocannabinoids may modulate the fatty acid synthetic pathway in the hypothalamus, and the inhibition of the hypothalamic expression by rimonabant may explain the anorexigenic properties of cannabinoid antagonists (62).

E. The peripheral effect of the endocannabinoid system in the modulation of metabolic functions

Several lines of evidence are currently converging, indicating that the effects of CB1 receptor blockade on food intake and body weight are not limited to a central mode of action. An early report describing the effect of CB1 receptor blockade on changes in food intake and in body weight was, in this sense, highly predictive of a mechanism of action not limited to the mesolimbic or hypothalamic circuits. In fact, Colombo *et al.* (305) were the first to demonstrate, in lean rats fed with a standard diet, that the tolerance to the anorectic effects of two different doses of SR141716 (2.5 and 10 mg/kg) develops rather rapidly (5 d). Nevertheless, the body weight loss in SR141716-treated rats persisted for 14 d, well beyond the drug effect on food intake. At that time, the authors were not able to explain this body weight loss that was not related to a decrease in food intake, and they merely hypothesized a stimulatory action of SR141716 on the energy expenditure (305). However, in the last 2 yr, the use of CB1^{-/-} mice has represented an important tool to substantiate further the hypothesis of an additional effect of endocannabinoids in peripheral organs. Indeed, the lack of CB1 receptor in mutant mice causes hypophagia and body fat reduction. Importantly, pair-feeding experiments showed that in young CB1^{-/-} mice, the lean phenotype is predominantly caused by decreased caloric intake, whereas in adult CB1^{-/-} mice metabolic factors appear to be the major cause of the lean phenotype. These experiments therefore suggested that the endocannabinoid system might regulate central food intake-related mechanisms at young ages, but that this function diminishes with age (58). These observations converge on the idea that additional peripheral food intake-independent metabolic functions may participate, or even predominate, in the control of energy balance exerted by the endocannabinoid system (58). Even more prominent differences in terms of body weight regulation are obtained when a high-fat diet is administered to adult CB1^{-/-} mice and wild-type littermates. In contrast to wild-type littermates, CB1^{-/-} mice do not display hyperphagia or reduction of their relative energy intake and were resistant to diet-induced obesity (DIO) (306). Importantly, the obesity-prone diet induced a significant increase of fasting glycemia in the two genotypes, but the sensitivity to insulin remained unchanged in CB1^{-/-} mice, whereas it was significantly reduced in the wild-type animals (306).

The expression of CB1 receptor in adipocytes and the ability of SR141716 to block lipogenesis stimulated by cannabinoids represent a first important step forward in understanding the peripheral mechanisms of action of the endocannabinoid system in regulating metabolic processes (58).

Moreover, the presence of CB1 receptor is increased in mature adipocytes compared with preadipocytes (59, 60), indicating that CB1 receptor activation is likely needed more for metabolic processes than for differentiation. Importantly, a recent study shed further light on the mechanisms of action of the endocannabinoid system on adipose tissue. By using SR141716 in DIO mice, Jbilo *et al.* (307) were able to reverse the phenotype of obese adipocytes at both macroscopic and genomic levels. They showed that a major restoration of white adipocyte morphology similar to lean animals occurred in adipocytes derived from obese animals after CB1 antagonist treatment. More importantly, they found that the major alterations in gene expression levels induced by obesity in white adipose tissue were mostly reversed in SR141716-treated obese mice. Importantly, the transcriptional patterns of treated obese mice were similar to those obtained in the CB1^{-/-} mice fed with a high-fat diet, supporting a CB1 receptor-mediated process. Functional analysis of these modulations indicated that the reduction of adipose mass by the drug was due to enhanced lipolysis through the induction of enzymes of the β -oxidation and tricarboxylic acid cycle; increased energy expenditure, mainly through futile cycling (calcium and substrate); and a tight regulation of glucose homeostasis. In particular, in this last context the SR141716-induced increased expression of glucose transporter 4, the insulin-responsive glucose transporter, appears very important (307). This finding makes it possible to hypothesize that cannabinoid antagonists may also be attractive drugs in fighting diabetes. Altogether, these data confirmed that the endocannabinoid system has a major role in the regulation of energy metabolism in adipocytes. Importantly, CB1 receptor expression has been found to be higher in adipocytes derived from obese animals compared with lean controls (59). Similar to the finding of higher levels of endocannabinoids in the hypothalamus derived from obese animals, the overexpression of CB1 receptor in adipocytes of obese rats seems to confirm the notion that hyperactivity of the endocannabinoid system is associated with the obesity state. However, this up-regulation of CB1 receptor expression in fat pads derived from rodents has not been confirmed in adipocytes derived from sc fat of obese women (60); on the other hand, a partial limitation of this study is that CB1 receptors have not been measured in visceral fat tissue that is supposed to be more prone to the endocannabinoid action. Finally, the increase in levels of adiponectin in Zucker obese rats chronically treated with SR141716 *in vivo* (59) and in 3T3 F442A adipocytes acutely stimulated with the CB1 receptor antagonist *in vitro* (59) points to a close relationship between CB1 receptor blockade and the production of this antiatherogenic and antidiabetic adipocyte-derived protein (308). The quick and strong improvement of hyperinsulinemia detected after a very short-term treatment with SR141716 (4 d) in obese Zucker rats was also attributed to an increase in adiponectin (59). However, the well-known reduction in food intake and the consequent body weight loss displayed at the beginning of SR141716 treatment may be the most obvious explanation for the changes in adiponectin levels. The ability of long-term treatment with SR141716 to enhance the circulating levels of adiponectin was further confirmed in DIO mice (309).

In the last few years, several studies using different CB1 receptor antagonists confirmed the hypothesis that a potential peripheral mode of action of pharmacological CB1 receptor blockade may play a relevant role in the final weight loss effect. Ravinet-Trillou *et al.* (310) found that long-term (40 d) treatment with two different dosages of SR141716 (3 and 10 mg/kg, respectively) produces a marked acute hypophagia in DIO mice only in the first few days of treatment, followed by the development of tolerance to the anorectic effect of the drug. However, the effect on body weight was sustained until the end of the 5-wk experiment compared with DIO mice treated with the vehicle. The significant difference in weight of white adipose pads between SR141716- and vehicle-treated animals confirmed that weight loss was accompanied by a decrease in adipose tissue. Similar data showed a rapid tolerance to the anorectic action despite a sustained and prolonged effect on body fat loss also being obtained when obese Zucker rats were treated for 14 d with SR141716 (59). Importantly, another CB1 receptor antagonist, AM-251, produced similar effects in DIO mice (311). Very recently, Poirier *et al.* (309) monitored weight and metabolic marker changes in three groups of mice after establishing a condition of obesity by a 5-month high-fat diet. Two groups of animals were maintained on a high-fat diet, but one was treated for 10-wk with 10 mg/kg SR141716 and the other one with a vehicle. A third group received a dietary switch to standard food after the 5 months on a high-fat diet. SR141716 induced a weight loss of approximately of 78% in comparison to the weight of the animals receiving the vehicle. More importantly, the antiobesity effect of the drug was equivalent (both in terms of time course and maximum effect) to that achieved by switching obese mice to a normal diet (309). Again, the authors demonstrated that the anorectic effect of the CB1 receptor antagonist vanished with time because the energy intake in the SR141716-treated animals was equivalent to animals on a high-fat diet during the last 6 wk of the experiment and significantly greater than in the group receiving standard diet. Consistent with a previous report (310), the SR141716-induced weight loss was accompanied by normalization of leptin, insulin, and glucose levels (309). Notably, SR141716 also normalized triglycerides and low-density lipoprotein-cholesterol. Moreover, the high-density lipoprotein (HDL)-cholesterol/low-density lipoprotein-cholesterol ratio after SR141716 treatment was significantly higher than in the other two groups (309). Whether this effect on lipid metabolism is indirectly related to an elevation of adiponectin is still a matter of debate.

Shearman *et al.* (312) recently showed that a 9-d treatment of DIO mice with the CB1 receptor antagonist AM251 increases uncoupling protein (UCP)-1 and UCP-3 mRNA expression level in brown adipose tissue, suggesting that CB1 receptor blockade may contribute to increased thermogenesis. Moreover, Liu *et al.* (61) found that a 7-d treatment with SR141716 induces an increase in basal oxygen consumption compared with the vehicle in ob/ob mice. The authors were not able to identify the mechanism by which SR141716 treatment is able to affect energy expenditure. A start on clarifying the molecular mechanism by which treatment with SR141716 may favor thermogenesis has been made with the microarray experiment performed by Jbilo *et al.* (307). These data suggest

that the cannabinoid antagonist treatment is able to stimulate the expression of genes favoring energy dissipation through mitochondrial heat production in brown adipose tissue (307). However, it should be mentioned that *in vivo* microdialysis studies showed that SR141716 increases noradrenaline outflow in rat anterior hypothalamus, suggesting a possible central stimulation of efferent sympathetic activity (313). Importantly, Liu *et al.* (61) also showed that a 7-d treatment of SR141716 induces a significant increase in glucose uptake in isolated soleus muscle. This activity might contribute to the improved hyperglycemia seen after SR141716 treatment in previous studies. As shown in Fig. 2, we found that the soleus muscle derived from obese mice contains increased levels of CB1 receptor compared with lean controls, further confirming the hypothesis of a hyperactivity of the endocannabinoid system associated with a condition of obesity.

Hepatocytes, key players in the metabolic processes, were not considered as a target of endocannabinoid action for a long period of time. However, substantial amounts of 2-AG are present in the liver (1.15 nmol/g tissue), and this quantity is nearly double the amount detected in other peripheral organs (295). These observations suggested the idea that the liver might be a new target of endocannabinoid action. Very recently, Osei-Hyiaman *et al.* (62) strongly substantiated this hypothesis by a series of experiments in which they identified the liver as a primary site for endocannabinoid-mediated modulation of lipogenesis. In fact, probably via inhibition of adenylate cyclase, the cannabinoid agonist HU210 stimulates the expression of several genes involved in the *de novo* synthesis of fatty acids, such as lipogenic transcription factor SREBP-1c and its targets acetyl-CoA carboxylase-1 and fatty acid synthase. The inhibition of this lipogenic response by SR141716 and its absence in CB1^{-/-} mice confirms the lipogenic role of CB1 receptors localized in hepatocytes. However, more importantly, the authors found that the marked increase in the basal rate of hepatic fatty acid synthesis as well as the development of hepatic steatosis observed after the administration of high-fat diet were blunted by SR141716 and absent in CB1 receptor knockout mice. High-fat diet also induces an increase in the number of CB1 receptors and in hepatic levels of AEA, strongly suggesting that the blockade of the endocannabinoid system plays an important protection against the pathological consequences of a fat diet in the liver (62). These data pave the way to hypothesize the clinical use of CB1 antagonists in preventing or reversing the development of fatty liver. Another recent report showed that cannabinoids inhibit AMP-activated protein kinase activity in the liver (314). A decrease of AMP-activated protein kinase activity is known to lead to increased storage of energy, particularly in the form of fat, in hepatocytes. This mechanism may contribute to explaining the role of endocannabinoids in promoting the development of hepatic steatosis. Based on the whole body result of these data, it has been hypothesized recently that the hepatic endocannabinoid system may represent a target for the treatment of nonalcoholic fatty liver disease (315).

A considerable amount of evidence suggests that the endocannabinoid system may regulate food intake by also acting in the gastrointestinal tract. Importantly, the concentration of AEA in intestinal tissue increases during food

deprivation in rats, reaching levels that are 3-fold greater than those needed to halve maximally activated CB1 receptor and 7-fold higher than the amount detected after refeeding. This surge in AEA levels may, together with the increase in the CNS, be another hunger signal to promote feeding (316). In general, we can conclude that through multiple interactions, endocannabinoids may modulate food intake also at the level of the gastrointestinal tract.

F. Oleoylethanolamide: a new anorectic fatty acid amide

Another endogenous lipid, a monounsaturated fatty acid ethanolamide, named oleoylethanolamide (OEA), was recently proposed as an important modulator of food intake (317). OEA is an analog of AEA, but the activation of any of the known cannabinoid receptors cannot explain its pharmacological effects. Recently, Piomelli's group elucidated that its action is through an activation of the nuclear receptor peroxisome proliferator-activated receptor (PPAR)- α (318).

Peripheral administration of OEA causes a potent and persistent decrease in food intake, but this compound is completely ineffective when administered centrally (316–318). OEA-induced anorexia is not caused by nonspecific behavioral effects, because no aversion or illnesses have been reported after the peripheral administration of the compound (319). Interestingly, similar to the effects described after the administration of capsaicin (vanilloid type 1 receptor agonist) and of the PPAR- α agonist Wy-14643, a short-term reduction in heat expenditure and locomotor activity has been observed after the peripheral administration of OEA (319). However, the mechanisms underlying the reduction in motor activity remain unclear (319). OEA not only acts as a satiety signal, but also reduces body weight gain and serum lipid levels in genetically obese rats and in DIO mice (318). Through the direct activation of PPAR- α , OEA may stimulate lipolysis and fatty acid oxidation (320). However, when administered orally (321), its tissue distribution is mainly at gastrointestinal levels rather than in other visceral organs controlling metabolism, supporting the hypothesis that OEA acts on PPAR- α present in the initial segment of the gastrointestinal tract, such as stomach, duodenum, and jejunum. Importantly, these data were recently independently confirmed by another group (322). In conclusion, OEA is a new orally active anorectic agent that may possess potential as a new antiobesity drug.

V. Cannabinoid Receptor Antagonists as New Pharmacological Tools to Tackle Obesity and Obesity-Related Diseases

A. Emerging issues in the treatment of obesity and related diseases by cannabinoid antagonists

The whole body of data mentioned above highlights the role of the endocannabinoid system in feeding and energy balance regulation. Indeed, it was reasonable to hypothesize a therapeutic role for cannabinoid antagonists in the treatment of obesity. SR141716, also named rimonabant (commercialized as Acomplia), is now undergoing multicenter randomized, double-blind phase III trials to assess the effects

on weight loss in obese patients with or without comorbidities with dyslipidemia and with type 2 diabetes (323). Moreover, the multitude of patents filed over the last few years claiming the synthesis of novel CB1 receptor antagonists reflects the intense competition in this area (123). Other compounds are under development, such as SLV-319 (Solvay, Weesp, The Netherlands) (129), which is undergoing phase I trials (323). However, at present, little is known about the results of these trials.

B. Clinical trial studies with rimonabant, the first CB1 receptor antagonist in clinical use to tackle obesity and obesity-related diseases

The CB1 receptor antagonist rimonabant was initially tested in humans not as an antiobesity drug but for its potential ability to reduce subjective intoxication and tachycardia in healthy subjects with a history of marijuana use or as an antipsychotic agent in schizophrenic patients. The first study showed that rimonabant was well tolerated by the participants even at a 90-mg dose (single oral dose). A significant dose-dependent blockade of marijuana effects was shown. However, the ability to reduce the intoxication induced by marijuana was very mild (130).

The results derived from the clinical trial in which rimonabant was tested to treat schizophrenia and schizoaffective disorders were not very satisfactory, because the effects of the drug in ameliorating clinical symptoms were not different from those obtained by placebo (324). However, in this trial, rimonabant treatment at 20 mg/d dosage was very well tolerated.

Bearing in mind the function of the endocannabinoid system in the mesolimbic rewarding system, rimonabant is also undergoing clinical trials as an aid to preventing the relapse of smoking cessation (323). Preliminary data from the STRATUS-US trial (smoking cessation in smokers motivated to quit) were recently presented at the 53rd Annual Scientific Session of the American College of Cardiology. The clinical study enrolled 787 smokers who received rimonabant at a dose of 5 or 20 mg or a placebo in a randomized fashion. The clinical trial lasted 10 wk, and the smokers were permitted to smoke during the first 2 wk but were asked to abstain from smoking after this period. The quit rate for subjects in the 20-mg rimonabant group was double that of the placebo group. In particular, the smokers characterized by overweight and obesity showed a relevant reduction in weight gain over the 10-wk treatment (325).

The most promising data seem to derive from rimonabant as a treatment for obesity. A phase II, 4-month, double blind, placebo-controlled study examined the effect of three different dosages of rimonabant (5, 10, or 20 mg/d) in obese patients with a body mass index between 30 and 40 kg/m². Patients taking the 20-mg dose reported a weight loss of 4.4 kg in comparison to the 1.1-kg average in the placebo group. No significant adverse effects were noted. At the end of the treatment, weight loss was not maintained. However, the rebound in weight did not reach the pretreatment values (323). Another phase II, 7-d treatment, double-blind, placebo-controlled study was performed to evaluate hunger, calorie and fat intake. All these parameters were significantly re-

duced at the end of the short treatment, and the resulting average loss in body weight was 0.72 kg. The drug showed a good safety profile (323).

A large phase III trial named as RIO (rimonabant in obesity) was initiated in August 2001 including more than 6600 overweight or obese patients (323). All studies have already been concluded, and some of them are already reported in the literature (326, 327). Two of these studies, named RIO-North America and RIO-Europe, recruited obese and overweight patients with or without comorbidities who were treated for 2 yr with 5 or 20 mg rimonabant *vs.* placebo. The primary endpoints of the RIO-North America study were the absolute change in weight from baseline to 1 yr and the prevention of weight regain after rerandomization (second year), whereas the main endpoint of the RIO-Europe study was the assessment of weight reduction by using the same dosages. Secondary endpoints of both studies were the number of weight responders and the changes in waist circumference, metabolic and lipid parameters, and the number of patients affected by the metabolic syndrome as defined by National Cholesterol Education Program's Adult Treatment Program III (NCEP-ATP III) criteria (328). RIO-Lipids and RIO-Diabetes are the other two clinical trials with rimonabant aimed at investigating the amelioration, after treatment with the CB1 receptor antagonist, of specific comorbidity factors associated with obesity or overweight such as hyperlipidemia and diabetes. In the RIO-Lipids study, presented by the American College of Cardiology in New Orleans in March 2004, 1036 obese patients characterized by lipid profile alterations and body mass index of 27–40 kg/m² were randomized to double-blind treatment with either placebo or rimonabant 5 or 20 mg/d (326). All patients were required to follow a reduced calorie diet. After 1 yr of therapy, patients in the 20-mg dose group showed a loss of 8.8 kg compared with the 2-kg reduction in the patients treated with placebo. Rimonabant was associated with an important and significant reduction in waist circumference, triglycerides, and C reactive protein, whereas a significant increase in HDL-cholesterol was found in the 20-mg treatment group compared with the group of patients undergoing placebo treatment. Forty-three percent of patients in the 20-mg treatment cohort lost more than 10% of their initial body weight compared with the 10.3% observed in the placebo group. The number of patients in the 20-mg rimonabant group classified as having metabolic syndrome (according to NCEP-ATP III criteria) decreased from 52.9 to 25.8% after 1 yr. Rimonabant was generally well tolerated, and the most frequently reported side effects were gastrointestinal and upper respiratory tract symptoms (326).

Similar data have been obtained by the *ad interim* analysis of the first year treatment in the RIO-Europe study (327, 329). More than 67% of patients who completed treatment with 20 mg rimonabant achieved 5% or more weight loss, whereas 39% achieved 10% or more weight loss. The pattern of weight loss appeared to be sustained for up to 36–40 wk. A concomitant reduction in waist circumference of about 9 cm was observed in patients treated with 20 mg rimonabant. A significant improvement of lipid and glycemic profile was also observed in this study in patients with 20 mg rimonabant, with a significant increase in HDL-cholesterol (22% *vs.* 14%

in placebo-treated patients) and a concomitant reduction of triglycerides (6.8% *vs.* an increase of 8.3% in placebo-treated patients). As expected by studies in the animals described above, the study of Van Gaal *et al.* (327) demonstrated that rimonabant adds a further important and significant weight-independent effect on lipid parameters to the positive effects derived from weight loss and waist reduction. In fact, as determined by statistical analysis, the effect of 20 mg rimonabant on both HDL-cholesterol and triglycerides at 12 months has been shown to be partly independent of weight loss, being 60% of the increase in HDL-cholesterol and 45% of the reduction in triglyceride accounted for by weight loss, and the remainder due to reasons not related to body weight changes (327). Although Van Gaal *et al.* (327) proposed that a rise in adiponectin might be responsible for these relevant positive changes in lipid profile, other mechanisms might enter into play. Full understanding of these still unknown modes of action is urgently needed to better characterize the ideal phenotype of obese patients to be targeted with CB1 receptor antagonist drugs.

Rimonabant treatment was well tolerated, and the most common adverse events experienced with 20 mg rimonabant were gastrointestinal symptoms such as nausea and diarrhea and mood disorders such as anxiety and depression. However, the effects were found to be mild, and the discontinuation rate due to these events was similar between patients taking 20 mg rimonabant or placebo. The genesis of these adverse events might be explained by bearing in mind that, as explained above, CB1 receptor plays a role in gastrointestinal motility and in HPA axis activation. Nausea and diarrhea on the one hand and anxiety and depression on the other hand might be due to CB1 receptor pharmacological blockade.

Concerning studies in humans, a very recent report (330) confirms, on a genetic basis, the possible association between the chronic pathological overactivation of the endocannabinoid system and the development of obesity. In fact, in a large cohort of Caucasian and black subjects, overweight and obesity have been found to be associated with a polymorphism in FAAH. This genetic variant predicts a substitution of threonine for a highly conserved proline residue (P129T). It has been observed that patients carrying this polymorphism may have approximately half the enzymatic activity of FAAH. This may lead to a reduced inactivation of AEA and, eventually, to an inappropriate chronic increase of endocannabinoid tone (330). In such a context, a recent work (60) showed increased circulating levels of AEA and 2-AG in obese women when compared with a lean control group. Moreover, in the same study, a marked down-regulation of FAAH gene expression in adipose tissue of obese women has been found, suggesting that the increased endocannabinoid levels may be secondary to decreased enzymatic degradation (60).

VI. Summary and Perspectives

A number of studies show that the endocannabinoid system profoundly influences both hormone secretion and metabolic processes. Animal models have represented the ideal tool for advancing the understanding of the mechanisms of these functions. However, the data derived from early studies were not

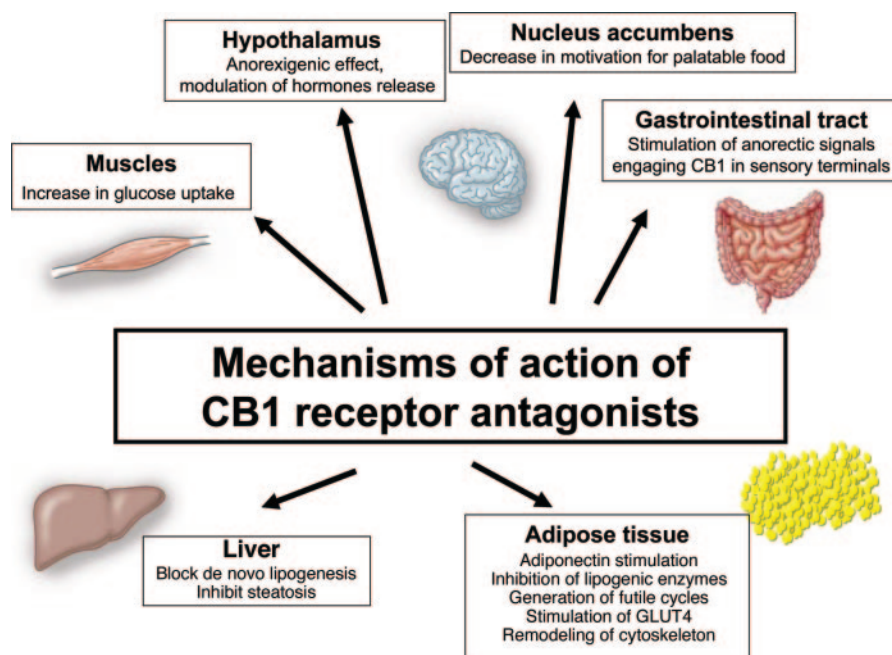


FIG. 3. Actions of CB1 antagonists on the target organs involved in food intake and metabolic control. Schematic drawing illustrating the main sites of action of CB1 antagonists in the control of energy balance

always straightforward in the conclusions. The contradictory results had to be largely attributed to the heterogeneous variety of substances, dosages, and routes of administration used in each experimental model. Studies in humans with marijuana or Δ^9 -THC were even more contradictory in their conclusions, because no standardization of dose was used and no stringent criteria (*i.e.*, randomization) of patient recruitment were defined in nearly all the experimental models.

However, the generation of CB1^{-/-} mice and the introduction of CB1 receptor antagonists initially in animal models and later in humans provided a remarkable stimulus to better characterize the functions of the endocannabinoid system in the regulation of hormone secretion and metabolic processes (Fig. 3).

As a general conclusion, the endocannabinoid system appears to play a very important regulatory role in the secretion of hormones related to reproductive functions and to stress responses. These observations have led to some important clinical considerations. High levels of endocannabinoids seem to negatively affect reproduction by acting at different sites. It is therefore possible to speculate about a clinical use of CB1 receptor antagonists to ameliorate gonadotropin pulsatility or to improve fertilization capability. On the other hand, endocannabinoids are important modulators in the physiological response of the HPA axis during repetitive stress conditions and in pathological conditions, such as anxiety, phobias, depression, and posttraumatic stress disorders (16, 147). Moreover, the endocannabinoid system has been proposed as playing an important role in protection against neurotoxicity and, possibly, certain forms of epilepsy (115, 331, 332). Drugs presumed to increase endocannabinoid tone are therefore currently proposed as a new therapeutic frontier to treat anxiety-related disorders and neurodegenerative diseases (82). The use of drugs acting as antagonists of CB1 receptor should thus be carefully monitored when administered, for instance, to patients with anxiety traits, epilepsy, or neurodegenerative disorders.

The anecdotes regarding the orexigenic properties of marijuana have nowadays been substantiated by an impressive number of reports that make it possible to definitively include cannabinoids in the large family of orexigenic signals. This large body of data provided the basis to establish a novel approach to tackle obesity and related disorders by means, as strongly suggested by the clinical trials with rimonabant, of a CB1 receptor antagonist.

During the last few years, it has become evident that multiple mechanisms of action, not solely limited to the CNS, are involved in the endocannabinoid-mediated control of food intake and energy balance. The full understanding of these modes of action may lead to the identification of the particular types of obesity where treatment with CB1 receptor antagonists work most efficiently. The potential clinical use of rimonabant will also help us to clarify how the endocannabinoid system affects the physiological functions and the pathological diseases related to hormonal secretion and energy balance.

Note Added in Proof

After this manuscript was accepted, the complete RIO-Lipids study was published (see Refs. 326 and 333).

Acknowledgments

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