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REVIEW

Endogenous cannabinoid system as a modulator of food intake[1]

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The ability of *Cannabis sativa* (marijuana) to increase hunger has been noticed for centuries, although intensive research on its molecular mode of action started only after the characterization of its main psychoactive component Δ^9-tetrahydrocannabinol in the late 1960s. Despite the public concern related to the abuse of marijuana and its derivatives, scientific studies have pointed to the therapeutic potentials of cannabinoid compounds and have highlighted their ability to stimulate appetite, especially for sweet and palatable food. Later, the discovery of specific receptors and their endogenous ligands (endocannabinoids) suggested the existence of an endogenous cannabinoid system, providing a physiological basis for biological effects induced by marijuana and other cannabinoids. Epidemiological reports describing the appetite-stimulating properties of cannabinoids and the recent insights into the molecular mechanisms underlying cannabinoid action have proposed a central role of the cannabinoid system in obesity. The aim of this review is to provide an extensive overview on the role of this neuromodulatory system in feeding behavior by summarizing the most relevant data obtained from human and animal studies and by elucidating the interactions of the cannabinoid system with the most important neuronal networks and metabolic pathways involved in the control of food intake. Finally, a critical evaluation of future potential therapeutical applications of cannabinoid antagonists in the therapy of obesity and eating disorders will be discussed.
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Introduction

Epidemiological reports have underlined an alarming increase in the prevalence of obesity and eating disorders, strongly indicating the necessity to counteract this trend in modern societies.[1] Many recent studies have focused on the identification of the molecular basis, the neuronal networks and metabolic pathways involved in the control of body weight and the regulation of food intake. The characterization of a number of neuropeptides present in distinct hypothalamic nuclei and the ability of signals derived from peripheral organs to modulate the activity of these neuropeptides suggest the existence of a complex hypothalamic network which contributes to the control of energy balance and food intake.[2] An intensified research on the basic mechanisms involved in feeding and appetite is also desirable because of the urgent need to provide new pharmacological and therapeutical approaches to cope obesity.

Cannabis sativa has been cultivated for more than 5000 y both to obtain fibers for manufacturing of textiles and to provide a variety of extracts for medicinal and recreational use. To the present, marijuana and other psychoactive derivatives of *Cannabis sativa* represent the most widely illegal drug consumed in the Western world. However, despite the social problems related to the abuse of these substances, scientific and social communities have recently started to be aware of the therapeutic potentials of cannabinoids and of new synthetic compounds interfering with the endogenous cannabinoid system.[3,4]

Since 300 AD, it was observed that Cannabis can stimulate hunger and increase appetite, particularly for sweet and palatable food.[5] However, only a few years ago this

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phenomenon was seriously taken into consideration in research. After the discovery of cannabinoid receptors and their endogenous ligands (endocannabinoids), the existence of an endogenous cannabinoid system has been proposed, providing a physiological basis for the biological effects induced by marijuana and its derivatives. The importance of this system is also underlined by the finding of a high degree of evolutionary conservation across species, emphasizing the fundamental physiological role played by cannabinoids in brain function.[6]

The aim of this review is to give an overview on the role of the cannabinoid system in eating behavior and in the control of food intake by summarizing the relevant data reported in human and animal studies. Moreover, starting from the present knowledge about cannabinoid pharmacology, a critical evaluation of potential therapeutical applications of cannabinoid antagonists in the therapy of obesity and eating disorders will be presented.

The endogenous cannabinoid system

Cannabinoid research was largely neglected at the beginning of the 20th century, partly because of the political antimarijuana attitude, which officially started in the United States with the Harrison Act in 1914, leading to full prohibition 20 years later. During the 1960s, the sudden increase of the recreational use of Cannabis stimulated the public concern about its negative effects on the health of the consumers. On the other hand, this renewed interest initiated a series of scientific investigations into the numerous chemical constituents of Cannabis and their mechanisms of action,[7] finally leading to the identification of the structure of Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient of marijuana.[8] However, the definitive breakthrough concerning the importance of this system was given by the discovery of cannabinoid receptors and their endogenous ligands.

Cannabinoid receptors

In 1990, the first cannabinoid receptor (CB1) was cloned,[9] followed 3 y later by the characterization of a second cannabinoid receptor (CB2).[10]

Cannabinoid receptors belong to the G protein-coupled receptor superfamily and, to the present, include CB1, CB2 and a splice variant of the CB1 (for a review see Howlett *et al*[11]). There is important pharmacological and physiological evidence suggesting the existence of other cannabinoid receptor subtypes that have not yet been cloned. Typically, the activation of cannabinoid receptors modulates adenylate-cyclase, potassium and calcium channels and signal-regulated kinases. Moreover, cannabinoid receptors are able to crosstalk with other neurotransmitter receptor systems, for example, recruiting by this way other intracellular signal transduction pathways.[11] Given its wide distribution in the central nervous system (CNS) CB1 was considered as the 'brain-type' cannabinoid receptor, whereas CB2, mainly expressed in immune cells, was considered as its 'peripheral' counterpart. However, this classification does not hold true anymore as many studies show expression of CB1 also in peripheral tissues. On the other hand, CB2 was also localized in brain-derived immune cells.[7]

In the CNS, CB1 is predominantly expressed presynaptically, modulating the release of neurotransmitters, including γ-aminobutyric acid (GABA), dopamine, noradrenaline, glutamate and serotonin.[12]

Δ^9-THC-mediated behavioral effects include ataxia, analgesia, hypothermia, euphoria, short-term memory deficits and other cognitive impairments. They are mediated by CB1 as suggested by the expression of this receptor in brain areas implicated in these functions[13] and by the lack of these effects in CB1-deficient mice.[14–16]

Endogenous cannabinoids

The presence of specific receptors mediating the actions of marijuana and its derivatives strongly stimulated the search for endogenous ligands for cannabinoid receptors. The first endogenous cannabinoid, arachidonoyl ethanolamide, was identified from the porcine brain in 1992 and was named anandamide, from the Sanskrit word 'ananda' that means internal bliss.[17] Anandamide is able to reproduce most of the typical behavioral effects of Δ^9-THC in rodents and shares the same G protein-mediated actions on adenylate cyclase and Ca^{2+} channels with Δ^9-THC (for a review see Di Marzo *et al*[4]). This substance binds both to CB1 and CB2,[18] with a higher affinity to CB1 and is present at highest concentration in hippocampus, cortex, thalamus and cerebellum of different species including humans.[19]

Since the discovery of this ligand, other polyunsaturated fatty acid derivatives, acting as functional agonists of cannabinoid receptors, have been characterized and collectively termed endocannabinoids.[20–22] As an example, Noladin ether is the most recent ether-type endocannabinoid identified only 1 y ago.[23] Among these compounds, 2-arachidonoylglycerol (2-AG), identified in canine gut in a search for endogenous ligands selective for CB2, displays a lower affinity for CB1; nevertheless, it represents the most abundant endocannabinoid in the brain.[21,22,24] In contrast to classical neurotransmitters, endocannabinoids do not appear to be stored in the interior of synaptic vesicles, because of the high lipophilicity of these ligands.[4] In fact, phospholipid molecules within the cellular membrane were shown to serve as precursors and storage depots for anandamide synthesis.[25] Anandamide is produced from such membrane phospholipids (eg *N*-arachidonoyl phosphatidyl ethanolamine), after cleavage of the phosphodiester bond by an as-yet-unidentified phospholipase D that is activated by Ca^{2+} ions.[4] Endocannabinoids, like 'classical' neurotransmitters, are released from neurons following membrane depolarization and Ca^{2+} influx into the cells, are inactivated by a reuptake mechanism, involving facilitated transport by an as-yet-unisolated anandamide

membrane transporter, and hydrolyzed by the enzyme fatty acid amide hydrolase in neurons and astrocytes[4] (Figure 1).

Recently, it has been shown that monoglyceride lipase (MGL) participates in 2-AG inactivation. The hydrolysis by means of MGL seems to be a primary mechanism for 2-AG degradation in neurons.[26]

The biochemical characteristics of anandamide and other endocannabinoids and their mode of release upon demand emphasize the notion that endocannabinoids may act primarily as neuromodulatory substances, in a different way as compared with 'classical' neurotransmitters.

Cannabinoid receptor ligands

In recent years, several synthetic and plant-derived cannabinoids have been characterized. They exhibit different affinities and potencies in activating cannabinoid receptors.[11] Owing to the chemical structures, these substances are divided into four major classes (Figure 2). The first group includes the 'classical' cannabinoids and is composed of substances containing a like-3-ring structure. The cannabinoids from Cannabis plants (more than 60 different compounds, including Δ^9-THC, Δ^8-THC, cannabinol and cannabidiol) and several synthetic compounds synthesized at the Hebrew University in Israel, named HU, are members of this group.[27] Among these substances, HU-210 induces the effects typical for cannabinoids most potently.[28] The second group includes the so-called 'nonclassical' cannabinoids and comprises bi- and tricyclic analogs of Δ^9-THC such as CP-55,940.[11] The third group comprises compounds named aminoalkylindols. The most representative member of this class is WIN-55,212, a CB1 agonist widely used in experimental models.[11] The fourth group is represented by the endocannabinoids that are able to bind to cannabinoid receptors and to mimic the effects of plant-derived and synthetic cannabinoids. Remarkably, they are structurally not related to Δ^9-THC.

Pharmacological investigations have put emphasis on the generation of substances acting as specific antagonist of cannabinoid receptors. Among the increasing number of compounds sharing antagonistic properties, SR 141716A[29] and AM 281,[30] highly specific for CB1, SR 144528,[31] a CB2-selective antagonist, and AM630,[32] that binds to both CB1 and CB2, are the most widespread substances. However, all these compounds are considered as 'inverse agonist' rather than pure antagonists. Whereas antagonists are able to block the activation induced by the stimulus of an agonist, an inverse agonist has

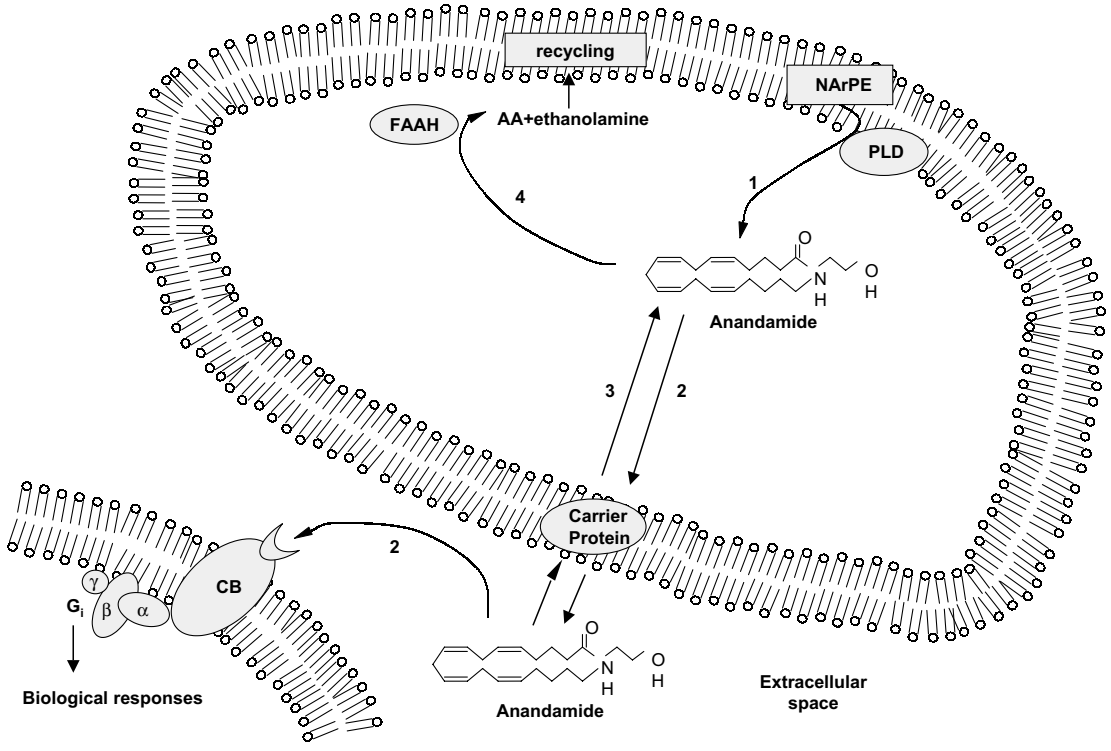

Figure 1 Biosynthesis, release and inactivation of anandamide. Anandamide is synthesized from the phospholipase d-catalyzed hydrolysis of the membrane phospholipid precursor *N*-arachidonoyl phosphatidyl ethanolamine (NArPE) in neurons (1). After synthesis, anandamide is released from neurons through facilitated transport and binds to cannabinoid receptors (CB), leading to biological responses (2). The same carrier involved in the release, probably also mediates anandamide reuptake by neurons (3). After reuptake, the endocannabinoid is degraded through the action of the membrane-bound enzyme fatty acid amide hydrolase (FAAH) (4). Arachidonic acid (AA) and ethanolamine, produced from anandamide hydrolysis, are rapidly re-esterified to membrane phospholipids.

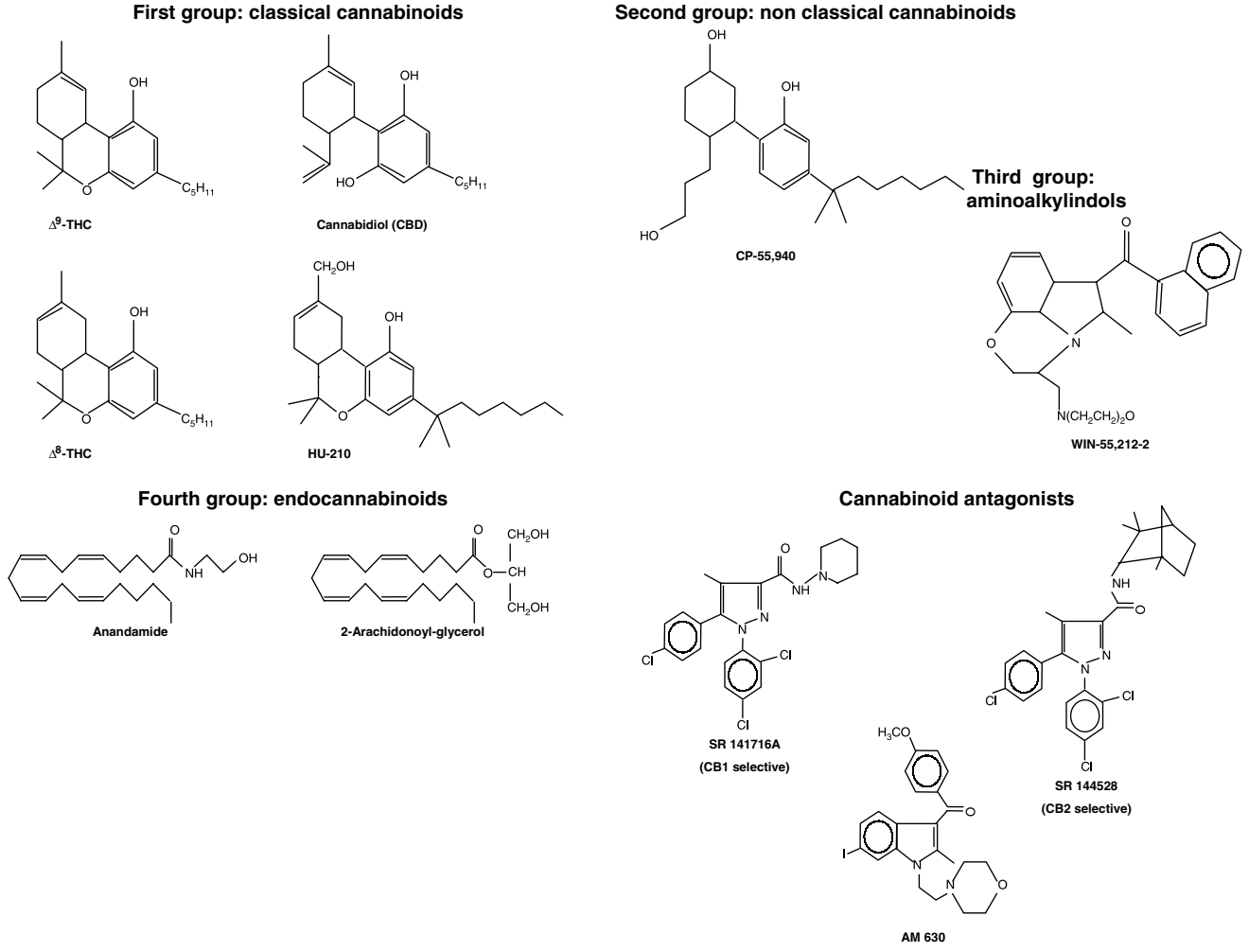

Figure 2 Chemical structures of representative cannabinoid compounds. See the text for the classification in the groups.

the ability to decrease the constitutive level of receptor activation in the absence of an agonist.[7] Studies with cannabinoid receptor antagonists indicate that there might be a continuous level of signaling by both CB1 and CB2 in the absence of an agonist and that the chronic use of substances such as SR 141716A increases CB1 protein levels on the cell membrane and sensitize it towards agonist action.[7]

Although not acting as ligands of cannabinoid receptors, inhibitors of cellular uptake of endocannabinoids, such as AM404,[33] VDM11[34] and UCM707[35] provide another interesting class of drugs interfering with the endogenous cannabinoid system. Given the 'upon demand' nature of synthesis and release of endocannabinoids, these drugs might allow to induce a targeted increase in the concentration of endogenous cannabinoids, possibly reducing some of the undesirable side effects observed when using receptor agonists.

Roles of the endocannabinoid system

The endogenous cannabinoid system, comprising specific receptors, endogenous ligands and degradation enzymes for the ligands, seems to act as a neuromodulatory system, influencing the activity of other neurotransmitter systems.[4] A plethora of effects is attributed to the action of cannabinoids.

Cannabinoids and concomitant CB1 activation are involved in antinociception, in control of movement and in inhibition of short-term memory.[36]

The cannabinoid system has an important role in the regulation of hormone secretion,[37] not only through a primary action on the hypothalamus, but also by a direct action on the pituitary gland. Expression of CB1 and the synthesis of endocannabinoids in human pituitary cells and the ability of cannabinoids to inhibit prolactin and growth hormone secretion and to increase adrenocorticotropin (ACTH) secretion were recently described.[38]

The ability to modulate the hypothalamus–pituitary–adrenal axis and the involvement in the stress-response are supported by a number of studies in which cannabinoid agonists were shown to produce anxiolytic effects in a dose-dependent manner.[39]

Moreover, the cannabinoid system is able to modulate immune and inflammatory responses and various physiological functions, such as cardiovascular (inducing changes in heart rate and output, and vasodilatation), respiratory (provoking hyper- or hypoventilation and bronchodilation), reproductive (inhibiting testosterone secretion and determining anovulation and uterus relaxation) and ocular (decreasing intraocular pressure) function.[40–44] Other fascinating properties of cannabinoids are represented by the antitumoral activity. This ability was already proposed in the 1970s[45] and recently, striking data were presented by *in vitro* and *in vivo* studies for different types of tumors.[46,47]

Moreover, cannabinoids seem to exert neuroprotective roles. In fact, endocannabinoids may protect neurons from hypoxic and traumatic injury and may represent an endogenous neuroprotective system.[48]

In conclusion, in addition to the role as modulator of the food intake, the cannabinoid system is involved in several physiological functions and might be related to a general stress-recovery system. Such a variety of effects was concisely summarized by a recent statement by Di Marzo *et al*:[4] 'Feel less pain, control your movement, relax, eat, forget, sleep and protect'. The activation of the endogenous cannabinoid system could represent a crucial and important component for each of these functions.

Cannabinoids and appetite in humans

As far back as 300 AD, marijuana was recommended in India to treat loss of appetite. During the 19th century, physicians mentioned the increased stimulation of appetite following Cannabis use.[5] The first protocol committed to study the effect of marijuana on food intake was performed by a military committee in 1933. Soldiers using this drug were described to feel hungry, eating much more than control subjects.[49] Further studies focussing on the effect of marijuana and of synthetic derivatives on healthy subjects, confirmed the appetite stimulating properties of this drug (reviewed in Abel[5]). Interestingly, in nearly all of these studies, greater quantities of food were eaten by the subjects when feeling 'high'. Moreover, subjects had a prevalence in eating sweet and tasty foods and a tendency to eat voraciously even when the feeling of hunger had vanished.[50,51]

However, these early investigations were characterized by lack of scientific thoroughness, because no standardization of dose was used and no stringent criteria of randomization of the patient recruitment were defined. Thus, not until 1971, a first study on the effect of marijuana was conducted on a strict scientific basis. In these experiments, the acute effect of oral doses of Cannabis extracts that were standardized for Δ^9-THC contents was monitored in normal young volunteers in fasted or fed conditions. In both conditions, a trend of greater food intake (milkshakes) following ingestion of marijuana as compared to placebo was described. However, this trend was significant only in fed subjects.[52] In another study, in adult volunteer subjects, a clear confirmation of the increased desire for food (marshmallows) in subjects smoking marijuana was found.[53] Unfortunately, no quantification of the amount of marijuana inhaled by the subjects was given in this study. Interestingly, a few years later, another study revealed a different effect on appetite after having monitored the Δ^9-THC content in marijuana cigarettes. In fact, higher doses caused an initial decrease in food intake followed by a subsequent increase, whereas lower doses caused only appetite stimulation.[54] In agreement with this, ancient Indian texts reported the use of Cannabis by ascetics and Indian mendicants to overcome the sensation of hunger. This effect might be explained by different potencies of Cannabis preparations. The weak preparation ('bhang') acts as a stimulant, while the potent preparations ('ganja', 'charas') inhibit appetite.[55]

This series of studies, which showed a variable potency of cannabinoids, either stimulating or inhibiting food intake depending on the preparation or dose used, were all performed by acute treatments of Cannabis. However, experiments in which chronic treatments were applied were able to demonstrate that smoked marijuana significantly increased the mean daily caloric intake in humans. In the first systematic study, Greenberg *et al*[56] studied, under research ward conditions for 1 month, long-term body weight change and energy intake in marijuana smokers, using marijuana cigarettes containing approximately 20% of Δ^9-THC. An increase of body weight and energy intake was observed during the first days. After this period, body weight continued to rise despite of the stabilization of energy intake and, during a 5-day post-drug phase, body weight and energy intake decreased significantly. Subsequently, in 1986, Foltin *et al*[57] studied the effect of smoked marijuana on feeding in a group of healthy subjects in a residential laboratory for periods up to 4 weeks. Each test day comprised three phases: a private work period, a performance task and a period of social access. The daily food intake, in comparison to placebo conditions, significantly increased with the marijuana treatment. Interestingly, the overconsumption of food primarily occurred during periods of social access. Noticing that the recreational use of Cannabis commonly is in a social group rather than in isolation, the stimulating effect on appetite might also be dependent on social facilitation and environmental familiarity. After 2 y, the effects of smoked marijuana on the intake of different foods were tested. Subjects were provided with a wide variety of different food items having the possibility to get free access to them. The drug increased the total food intake and, specifically, increased the consumption of sweeties, suggesting an interaction between the effect of the drug and the

palatability of the food.[58] Another investigation confirmed this notion showing that different routes of Δ^9-THC administration (oral, smoke inhalation or suppository) induced an effect on food selection.[59]

More controlled studies are clearly required to determine how the motivation to eat is affected by cannabinoids and to establish whether overconsumption is a phenomenon involving any kind of foods or whether it is specifically related to particular taste modalities or specific foods.[60] However, this selective action on food choice was confirmed in a number of animal studies, where a potential role of cannabinoids in modulating the interaction of different pathways involved in the brain 'reward' system was hypothesized.[61,62]

Therapeutic use of cannabinoids in the control of food intake

Despite the limitations of the human studies discussed above, the stimulating effect on appetite observed in healthy subjects suggested to assess the utility of cannabinoid treatment of clinical syndromes featured by appetite or weight loss, such as cancer or AIDS-associated anorexia.[63,64] Moreover, the antiemetic effect of Δ^9-THC and other components of marijuana with reduced psychotropic actions (ie Δ^8-THC) represents a possible additional benefit to limit nausea and vomiting symptoms often associated with most of the chemotherapeutic drugs.[65]

Anorexia and cachexia are diagnosed in the majority of cancer patients with advanced disease and are independent risk factors for morbidity and mortality, affecting response to chemotherapy and duration of survival.[66] In the 1970s, orally administered Δ^9-THC to cancer patients under treatment with chemotherapeutic drugs, was reported to possess antiemetic properties.[67] Another study investigated the effects of Δ^9-THC in 54 patients with advanced cancer in a crossover study and found significant weight gain under Δ^9-THC treatment as compared to the weight loss under placebo treatment. At the lowest Δ^9-THC dose, there was almost complete absence of subjective euphoria, a symptom commonly associated with the compound, and a few side effects, such as somnolence, dizziness and disassociation, were reported only in 25% of the patients.[68]

In 1985, the use of Δ^9-THC was approved in the United States by the Food and Drug Administration (FDA), and the compound named Dronabinol was first officially admitted for the oncological treatment of chemotherapy-induced nausea and vomiting refractory to other agents, and in 1992, the same drug has also been proposed for the treatment of patients with HIV-induced wasting syndrome. Many studies have documented that this drug is extremely safe with no fatal events and mild or moderate side effects, such as sedation and psychotropic symptoms. Moreover, the mild symptoms have been shown to resolve within hours after discontinuation of therapy.[69] In all studies concerning Dronabinol administration in HIV patients, a noticeable stimulating effect of the drug on appetite was demonstrated.[69,70] The administration of 5 mg of Dronabinol (twice/day) before meals was able to mildly increase appetite, energy intake and body weight together with a significant gain in body fat and with a marked improvement in patients' mood.[71] A further confirmation was given by a study in which Dronabinol was administered to 88 AIDS patients for 6 weeks. Stabilization of the weight or a modest weight gain were the most successful results obtained.[72] These results were later confirmed by a follow-up study involving 94 AIDS patients in which Dronabinol was daily administered for 12 months.[64] Positive effects of Dronabinol in improving body weight were recently described in patients suffering from Alzheimer's disease.[73]

There is still some controversy, as the exact mechanisms of action of this drug have not yet been completely clarified. In addition, there is a substantial ethical impediment to fully accept the psychotropic actions of this substance. Finally, it is still unclear why smoked marijuana is better tolerated and favored by the patients in comparison to the oral administration of the principal component of marijuana as Δ^9-THC.[63] One hypothesis is that marijuana contains several other cannabinoids, such as cannabidiol, which might attenuate the psychotropic effect of Δ^9-THC.[63] Altogether, the wide discussion in the media and in the scientific communities regarding this topic underlines the importance not only for the medical aspects but also for the social implications of this issue.

Cannabinoid effects on food intake in animal models

Animal models have represented an ideal tool to get further insights into the mechanism(s) involved in the cannabinoid-mediated stimulation of food intake. However, the data obtained from animals are still incomplete and not always straightforward in their conclusions.[60] Contradictory results are largely attributed to the heterogeneous response dependent on the animal species tested and to experimental procedures used. Moreover, the comparisons between various experimental data sets are extremely difficult because of the variability of the activity of substances, the dosages and the routes of administration used in each experimental model (reviewed in Abel[5]).

A study from 1965 showing an increase in food intake in rats after Cannabis administration (10 mg/kg, intraperitoneal injection) during the first hour postinjection was the first to corroborate the anecdotal literature in humans regarding the effect of Cannabis on hunger and paved the way to the powerful potential of using animals to explore the underlined mechanisms in greater details.[74] A confounding issue in studies performed during the 1970s was the high dose of Δ^9-THC used in most of the studies.[75–80] Since Δ^9-THC is known to produce a sedating effect in animals at doses above 10 mg/kg body weight, studies employing higher amount of this drug should be reviewed with caution in terms of the

effects of the drug on food intake. In reviewing the studies published between 1965 and 1975, Abel reported an increased food intake after cannabinoid administration only in three out of 25 experiments.[5] Thus, it is clear that the magnitude of the effect of exogenous cannabinoids on food intake strictly depends on the dose used and do not increase linearly with the dose. As a simple conclusion, one may derive that in analogy to what was found in human subjects, low doses of cannabinoids appear to increase food intake also in animals, whereas high doses seem to decrease it.

The route of administration of cannabinoid compounds represents another source of confusion. Phenolic compounds, such as Δ^9-THC, are highly caustic, and intraperitoneal injection has been the predominant route of Δ^9-THC administration in the first series of animal studies. Since peritonitis has been described as a side effect, the weight loss associated with cannabinoid treatment could be interpreted as a result of abdominal distress.[5]

In 1977, the study of Brown *et al*[81] suggested that low doses of Δ^9-THC (0.25 and 0.40 mg/kg) produced in rats dose- and time-dependent preference toward food and sucrose solution intake. In analogy to the 'marshmallow effect' described by Abel in human subjects, this experiment showing a greater effect of cannabinoids in choosing sweet foods, can be regarded as the milestone in the notion that cannabinoids exert a preferential effect on palatable food intake. As a consequence, very low doses of cannabinoid were used in follow-up studies. Intravenous injections of very low doses of either the l-isomer of Δ^9-THC or 9-azacannabinol (0.125, 0.250 and 0.50 mg) in sheep, induced an increase in food intake during the first 30 min after injection.[82,83] A final confirmation that a low orally administered dosage of Δ^9-THC is able to stimulate food intake was given by a recent dose–response and time-course study on spontaneous feeding in rats. In this study, oral doses of Δ^9-THC (0.5, 1.0 and 2.0 mg/kg) produced substantial hyperphagia during the first hour of testing.[84]

The development of specific compounds able to antagonize CB1 action gave a new impulse to the study of cannabinoid-dependent regulation of feeding behavior. These drugs gave strong indications that hyperphagic effects induced by Δ^9-THC were mediated by CB1 activation. A direct central action of Δ^9-THC was suggested a long time ago, when injections of this compound into the ventromedial- or latero-hypothalamic areas in rats fed *ad libitum*, produced a strong increase in food intake within the first 24 h.[85] This was confirmed by another study in which Δ^9-THC was shown to exert a facilitatory effect on feeding after electrical stimulation of the lateral hypothalamus in rats.[86]

Recent studies have found that intraperitoneal injection of the CB1 antagonist SR 141716A is able to significantly reduce sucrose or alcohol intake and craving in rats.[87–89] Moreover, SR 141716A was reported to selectively reduce sweet food intake in marmoset.[90] Repeated daily administration of the same antagonist was found to reduce both food intake and body weight in rats in a dose-dependent manner[91] and to inhibit the appetite-stimulating effect of Δ^9-THC and WIN 55,212-2.[84,92] A strong indication for an involvement of cannabinoids and CB1 in the system modulating appetite came from the observation that compounds able to antagonize CB1 negatively influenced the enhancement of sucrose ingestion that was induced by Neuropeptide Y (NPY), a peptide that has been found to be associated with abnormal feeding behavior and obesity when it is overexpressed.[87]

A direct involvement of endocannabinoids in the stimulation of food intake was suggested by a recent experiment showing that anandamide, via CB1 activation, was able to induce overeating in prefed rats.[61] Interestingly, the degree of hyperphagia was modest when compared with the effects of Δ^9-THC, but persisted for a much longer time. Even more intriguing was the finding that in the early phase of the experiment, when the satiating effect of prefeeding was apparent as an almost complete suppression of feeding, anandamide produced only very weak effects. However, when the inhibitory effects of prefeeding began to wane, the stimulatory actions of the endocannabinoid became much more evident.[61] Similar conclusions were obtained by one study reporting the role of anandamide in modulating the behavioral and neurochemical consequences of semistarvation. Diet restriction in mice caused a significant decrease of noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) in the hypothalamus and hippocampus. Decrease of dopamine and 5-HT was partially restored by anandamide administration, whereas the decrease of noradrenaline was not restored. Moreover, anandamide was shown to exert an appetite stimulating effect even at a very low dose (0.001 mg/kg).[93] Therefore, the ability of low doses of this endocannabinoid to improve food intake and to reverse some of the neurotransmitter changes caused by diet restriction, could represent a future therapeutic potential in the treatment of cachexia-associated diseases in humans.

The effects of endocannabinoids on food intake is clearly related to a direct action at the level of the hypothalamus. In fact, both CB1 and endocannabinoids are present at high levels in the hypothalamus (Figure 3), the brain region most directly involved in the regulation of appetite and food intake.[94] Recent experiments showed that pretreating of prefed rats with intrahypothalamic injection of the selective CB1 antagonist SR141716A dramatically reduced the hyperphagic effect of anandamide administered in the same way.[95]

Very convincing demonstration of a stimulating effect of endocannabinoids on food intake came from a recent work which showed that acute leptin treatment of normal mice and ob/ob mice (ie mutant mice lacking leptin) reduces anandamide and 2-AG in the hypothalamus and that defective leptin signaling is associated with elevated hypothalamic levels of endocannabinoids as demonstrated in ob/ob mice and fa/fa rats (ie mutant animals with nonfunctional leptin receptors). Moreover, when these mice with *ad libitum* access to food were treated with CB1 antagonist SR 141716A, their food intake was markedly reduced,

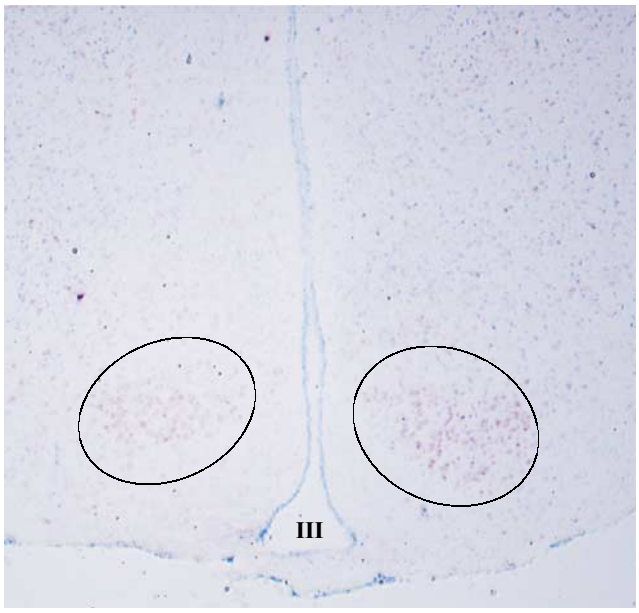

Figure 3 Expression of CB1 mRNA in mouse ventromedial hypothalamus by nonradioactive *in situ* hybridization (ISH). Strong hybridization signal for CB1 mRNA (red color) is observed in the ventromedial hypothalamus (circles). The symbol III indicates the third ventricle. ISH was performed as previously described.[118]

and chronic treatment for a week resulted in significant retardation of weight gain.[96] These experiments allow a definitive conclusion that hypothalamic endocannabinoids are under partial negative control of leptin. Furthermore, endocannabinoids may be considered to belong to the growing family of appetite-stimulating mediators.

Role of hypothalamus and reward circuitry in cannabinoid-induced hyperphagia

Ventromedial, dorsomedial and lateral hypothalamus, together with arcuate and paraventricular nuclei, are the hypothalamic areas involved in food intake control and feeding behavior.[2] These regions are interconnected with the neuronal pathways regulating the so-called 'reward' system.[97]

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 ventral tegmental area, the nucleus accumbens and the ventral pallidum. This circuit is implicated in the pleasure produced by natural rewards, such as food and sex, and it is the neural substrate of drug addiction and addiction-related phenomena, such as craving and dysphoria induced by withdrawal.[98] In such a framework, food intake acts on dopamine, opioid, serotonin and noradrenaline neuronal fibers, which connect hindbrain and midbrain to the hypothalamus to modulate the action of feeding and satiety factors.[99]

It is now clear that cannabinoids activate the brain structures mentioned above and are able to influence reward-related behaviors. The high expression of CB1 in hypothalamic nuclei and in areas involved in reward constitutes a strong indication that the cannabinoid system is directly involved in feeding regulation.[100]

The complex and redundant hypothalamic network provides high levels of adaptability of feeding behavior to endogenous and exogenous stimuli. Signals coming from the periphery and informing the brain of the state of the body's fat stores and of the gastrointestinal tracts play a fundamental role in this context. An important example of such peripheral control is the adipocyte-derived hormone leptin, which acts on receptors located in the hypothalamus. As mentioned above, leptin is also able to influence endocannabinoid levels suggesting that the endogenous cannabinoid system could be an important member of this network.[96,101,102] Although these observations clearly suggest that hypothalamic endocannabinoids are part of the leptin-regulated neural circuitry involved in the control of appetite and food intake, a possible interaction of CB1 and endocannabinoids with other feeding regulating pathways or a possible involvement of endocannabinoids at extra-hypothalamic sites cannot be excluded at present.

Redundancy in appetite stimulating signaling is conceivable in view of the vital importance of feeding for survival. Whereas defects in anorexigenic signaling pathways almost always lead to obesity, loss of NPY in mutant mice, one of the most important appetite-stimulating signals, does not result in a lean phenotype.[103] This result raised the issue of the presence of compensatory mechanisms activated when NPY signaling is lost. Endocannabinoids could be plausible candidates to replace NPY activation, as the finding that administration of CB1 antagonist SR 141716A suppressed food intake to the same extent in both NPY knockout and wild-type mice, seems to confirm that NPY and endocannabinoids promote food intake independently from each other.[96]

Concerning the reward system, some studies proposed that cannabinoids may induce overconsumption by amplifying the palatability, or orosensory reward of food.[87,89] Hyperphagia was demonstrated after the administration of Δ^9-THC or anandamide in animals given palatable food.[61,62] Furthermore, an increased motivation for sucrose intake and beer consumption was observed in rats following administration of cannabinoid CB1 agonists.[104] The selective blockade of CB1 by SR141716A reduces the motivation for sucrose, beer and alcohol intake, indicating that positive incentive and/or motivational processes could be under a tight permissive control by CB1-mediated actions.[105]

Taking these studies into account, an attractive hypothesis about the involvement of the endogenous cannabinoid system in reward circuitry was proposed. Endocannabinoids seem to have a role in the processes underlying the motivation to obtain palatable ingesta by gradually increasing during intermeal intervals to reach some critical level

when motivation to eat is triggered. Accordingly, the longer is the time lapse since the last meal, the greater is the activity in relevant endocannabinoid circuits, and the higher is the motivation to eat.[60]

The neuroanatomical basis through which the endogenous cannabinoid system acts in reward contest is still highly speculative. However, some circuits seem to be closely linked to activation of the endogenous cannabinoid system.

Dopaminergic pathway

One of the most important reward pathway is constituted by the mesolimbic dopaminergic system.[97] It is well known that a functional relation between endocannabinoids and dopaminergic activity exists involving either the reward or the hypothalamic network. Both CB1 and endocannabinoids were found in the rat limbic forebrain,[106] in which colocalization with dopamine type 1 and 2 receptors and CB1 has been described.[107,108] Psychoactive drugs such as marijuana, ethanol and also pleasant stimuli or palatable food, are thought to induce the release of dopamine in specific brain regions.[109] Therefore, a correlation between limbic endocannabinoid/dopamine levels and craving for tasty food is supposed to occur.[98] In fact, a rise in anandamide levels was observed in the limbic forebrain of Δ^9-THC-tolerant rats,[110] and infusion of endocannabinoids (anandamide and 2-AG) or cannabinoid agonists in a subregion of nucleus accumbens can induce hyperphagia in freely feeding rats.[60]

Serotoninergic pathway

The interaction of the endogenous cannabinoid system with the serotoninergic system has been also studied according to the involvement of serotonin in the control of feeding behavior.[111] However, the administration of cannabinoid antagonist in rats combined with dexfenfluramine, a drug stimulating the release of serotonin, let to additive but not synergistic effects on reducing food intake, which is consistent with the hypothesis that the two pathways are working via independent mechanisms of action.[112]

Opioid pathway

The endogenous opioid peptides are linked to central reward processes and there is increasing evidence supporting an important functional crosstalk between the opioid and the endocannabinoid system, in relation to a wide range of physiological processes, including appetite. Ledent *et al*[16] reported that the behavioral manifestations of the physical dependence on morphine were reduced in CB1 knockout mice. Similarly, Navarro *et al*[113] reported that SR 1411716A blocked heroin and morphine self-administration in rats.

Opioids are strongly implicated in the mediation of food reward. Opioid receptor agonists and antagonists are able to increase or reduce food intake, respectively,[114] and Gallate *et al*[88] found that the facilitatory effects of a cannabinoid agonist on responding for palatable solutions were not only reversed by CB1 antagonism but also by naloxone, an opioid receptor antagonist. In another study, the effect of naloxone to inhibit food intake was found to be functionally related with the effects induced by SR 141716A.[115] Firstly, the experiments confirmed the anorectic action of SR 141716A on palatable food and demonstrated also this effect on bland chow. Interestingly, when administered together with naloxone, a synergistic effect in the inhibition of feeding was observed, indicating that the cannabinoid and the opioid system appear to regulate converging pathways.[112] The notion of the existence of a coactivation of the cannabinoid and the opioid system in the control of feeding behavior is also reinforced by the observation that the hyperphagic effect of Δ^9-THC in rats was attenuated by subanorectic doses of SR 141716A and naloxone, whereas SR 1445428 (a CB2 antagonist) was unable to affect Δ^9-THC-induced feeding.[116]

The mechanism(s) of the interaction between these two systems has not clarified yet, but one could speculate about an involvement of cholecystokinin (CCK). This peptide is highly expressed in the brain and is involved in feeding, memory processing and other behavioral functions.[117] CCK is highly coexpressed with CB1 in cortical and limbic brain areas,[118] and is considered to be a satiety factor and to act as an antiopioid peptide.[119] Given these findings, cannabinoids might modulate the release of CCK,[120] and the inhibition of the negative feedback exerted by CCK neurons on opioid pathways could result in a strengthened opioid action. Overall, these observations imply that cannabinoids affect the motivation to ingest via actions on both cannabinoid and opioid systems.

A final comment should be given also to the putative functions of CB1 present in the enteric nervous system. This area of CB1 expression might suggest the existence of a putative crosstalk between central and peripheral sites in the context of the roles of the cannabinoid system. Oleylethanolamide (OEA), a monounsaturated fatty acid ethanolamide and a natural congenitor of anandamide whose pharmacological effects cannot be accounted for by the activation of any of the known cannabinoid receptors, was recently shown to be reduced in its concentration in the rat's small intestine after food deprivation.[121] Peripheral administration of OEA causes a potent and persistent decrease in food intake and in body mass gain, but is completely ineffective when administered centrally.[121] These results indicate that endocannabinoids are also involved in the peripheral regulation of feeding.

Cannabinoid antagonists in the therapy of obesity

Overall, studies both in humans and animals have highlighted the role of the endogenous cannabinoid system in feeding regulation. Indeed, it seems to be reasonable to hypothesize a therapeutic role for cannabinoid antagonists in the treatment of obesity. The CB1 antagonist SR 141716A (pharmaceutical name Rimonabant) was already tested in

humans in order to investigate the potential ability to reduce subjective intoxication and tachycardia in healthy subjects with a history of marijuana use. This study showed that SR 141716A was well tolerated by all subjects and a single oral dose of this drug produced a significant dose-dependent blockade of marijuana effects, without altering Δ^9-THC pharmacokinetics.[122] Although the drug does not seem to have remarkable side effects, more controlled studies are required to validate a general use of this CB1 antagonist due to the involvement of the cannabinoid system in memory, learning, neuroprotection and other functional behaviors. Another point not yet clarified is whether the drug will be active and useful for long periods of time, and whether it does develop tolerance. Rimonabant is now undergoing a multicenter randomized, double-blind phase III trial in order to assess the effects on weight loss in obese patients with or without comorbidities, with dislipidemia and with type II diabetes (http://www.clinicaltrials.gov). Taking into account the effect of cannabinoids on food intake, especially regarding the influence on palatability and on preferential choice of tasty and sweet ingesta, we can predict the target population for this drug. The CB1 antagonist may be an ideal drug for the type of obesity associated with specific eating disorders such as 'sweet and snack-eating'. Moreover, patients affected by eating disorders such as compulsive episodes, could benefit from this compound. However, this drug could also be proposed for the treatment of those syndromes inducing obesity characterized by genetic alterations of hypothalamic peptides signaling systems. The increased levels of endocannabinoids in mutant rodents lacking leptin or having nonfunctional leptin receptors, seem to reinforce this notion.[96] Furthermore, the functional relation between the cannabinoid system and other relevant neurotransmitter systems for food intake control, such as dopamine and endogenous opioids, could suggest a potential positive interaction between CB1 antagonists and other anorectic drugs.

In conclusion, the mechanisms by which cannabinoids influence the food intake have still to be explored in much greater detail. However, a large body of data suggest the cannabinoid system as a future important therapeutical target for the treatment of obesity.[123] The ongoing clinical trials with the CB1 antagonist SR 141716A will help us to clarify many unsolved issues.

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