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The cannabinoid system: a role in both the homeostatic and hedonic control of eating?

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Knowledge of the cannabinoid system and its components has expanded greatly over the past decade. There is increasing evidence for its role in the regulation of food intake and appetite. Cannabinoid system activity in the hypothalamus is thought to contribute to the homeostatic regulation of energy balance, under the control of the hormone leptin. A second component of cannabinoid-mediated food intake appears to involve reward pathways and the hedonic aspect of eating. With the cannabinoid system contributing to both regulatory pathways, it presents an attractive therapeutic target for the treatment of both obesity and eating disorders.

Anandamide: Endocannabinoids: 2-Arachidonoyl glycerol: SR 141716: Food intake: Obesity: Reward

The regulation of energy homeostasis and feeding behaviour is highly complex. It depends on the brain being able to read, interpret and integrate a wide range of signals and to make appropriate changes in food intake and energy expenditure as a result of the information. Responsibility for this control is shared between several brain regions, spanning both higher and lower centres (cortex to brainstem), within which are located numerous neurochemical transmitters. Regulatory activities of this complexity are likely to be controlled by a number of transmitters operating at a variety of levels. Novel information regarding the neuronal circuits that control food intake continues to extend our understanding of energy homeostasis. The present review will focus on one neuronal system, the cannabinoid system.

In the past decade, cannabinoid receptors and their putative ligands have been discovered within the central nervous system and linked to a number of aspects of feeding behaviour, including a potential role in the regulation of food intake. Recently, interest has revived in the effects on appetite of the plant-derived cannabinoids and analogous molecules. The present article will discuss current advances in this area and will also consider the potential of the cannabinoid system as a therapeutic target in the control of body weight.

Cannabinoid system components

The cannabinoid system consists of two receptors (termed CB1 and CB2), their endogenous ligands (the endocannabinoids) and the uptake mechanisms and hydrolysing enzymes that regulate ligand levels.

The cannabinoid receptors belong to the 7-transmembrane G-protein coupled receptor family. CB1 is known as the central receptor subtype and is expressed at particularly high levels in brain regions including the cortex, basal ganglia, cerebellum and hippocampus (Glass *et al.* 1997; Harrold *et al.* 2002). However, the distribution of CB1 is not limited to brain circuitry, with receptors recently identified on nerve terminals innervating the gastrointestinal tract (Croci *et al.* 1998; Hohmann & Herkenham, 1999). By contrast, expression of CB2, the peripheral receptor, is restricted to sites at the periphery, mostly within immune cells. There is evidence for the existence of a further centrally located cannabinoid receptor, as certain effects of centrally administered cannabinoid ligands are not inhibited by the CB1-specific antagonist SR 141716 (Welch *et al.* 1998). It is also possible that the endocannabinoids may exert some of their pharmacological actions by non-receptor-mediated mechanisms, e.g. membrane perturbations and gap junction inhibition (Boger *et al.* 1999).

The psychoactive ingredient of marijuana, Δ^9-tetrahydrocannabinol (THC) is known to interact with CB1 receptors (Ledent *et al.* 1999). It mimics the effects of the endogenous cannabinoids, the first of which was identified in porcine brain in 1992 and termed anandamide from 'ananda' meaning 'bliss' (Devane *et al.* 1992; Di Marzo *et al.* 1998*a*). To date, three endocannabinoids have been identified, with the inclusion of 2-arachidonoyl glycerol (AG) and very recently nolodin ether (Hanus *et al.* 2001). Anandamide is widely distributed within the brain. However, its basal levels are low compared with most neurotransmitters, with the lipophilic compound being synthesised on demand and immediately released from nerve terminals by a Ca^{2+} dependent mechanism. Anandamide is inactivated by reuptake via the anandamide membrane transporter (Day *et al.* 2001) and rapid degradation by fatty acid amide hydroxylase (FAAH)-mediated hydrolysis (Giuffrida *et al.* 2001). 2-AG is thought to be similarly regulated by the anandamide membrane transporter and FAAH, both of which are distributed in brain areas in a pattern corresponding to that of CB1 receptors. It is too early to apply these principles to nolodin ether.

Endocannabinoids are implicated in a variety of physiological functions including pain reduction, motor regulation, learning and memory, appetite stimulation and reward. In some of these functions the cannabinoids play a modulatory role, whilst in others they are essential system components.

Feeding and appetite

Cannabinoids and food intake

There is increasing evidence for a role of the cannabinoid system in the regulation of food intake and appetite. Both exogenous cannabinoids, e.g. Δ^9-THC and the endogenous cannabinoids, anandamide and 2-AG, are reliably reported to stimulate feeding (Williams *et al.* 1998; Williams & Kirkham, 1999; Hao *et al.* 2000). The hyperphagia induced is powerful; peripheral administration of Δ^9-THC stimulates feeding as potently as central injection of neuropeptide Y (Corp *et al.* 1990). As the hyperphagia is selectively blocked by the CB1 receptor antagonist SR 141716, but not by an antagonist of the peripheral CB2 receptors (SR 144258), this suggests that the actions are mediated by the central receptors. This is further supported by the observation that mice with genetically impaired CB1 receptors eat less than their wild type littermates in response to food deprivation (Di Marzo *et al.* 2001).

These observations suggest that tonic cannabinoid release may be crucial to the normal regulation of feeding. Direct measurements of brain endocannabinoid levels in response to fasting, feeding and satiation further support this observation. Fasting increases levels of anandamide and 2-AG in the nucleus accumbens, and to a lesser extent the hypothalamus, where 2-AG levels also declined with feeding (Kirkham *et al.* 2002). No changes were detected in satiated rats and levels in the cerebellum, a control region not directly involved in the control of feeding, were unaffected regardless of nutritional state (Kirkham *et al.* 2002).

The mechanisms of cannabinoid-induced hyperphagia remain to be elucidated. However, there is a body of evidence that points towards an involvement of both reward processes and established homeostatic pathways, many of which are regulated by the hormone leptin and operate within hypothalamic nuclei (Table 1).

Table 1. Summary of the cannabinoid system-mediated regulation of energy homeostasis, indicating the known influence of perturbations of energy balance and drug administration on receptor density and endocannabinoid levels

		Input	Receptor density	Ligand levels	Output
CNS: CB1 receptors		Fasting	?	↑ In nucleus accumbens	–
	Cortex Forebrain Hippocampus	Dietary obesity	Down-regulation = increased activity	?	↑ Intake of palatable food
		SR 141716	?	?	↓ Intake of palatable food ↑ Thermogenesis?
			(Sparse expression)		
		Fasting	?	↑	–
		Fasting – refed	?	↑ – ↓	–
		Satiated	?	–	–
	Hypothalamus	Dietary obesity	Unchanged	?	–
		Anandamide (VMH)	?	↑	Hyperphagia
		↑ Leptin (dosing)	?	↓	Hypophagia
		↓ Leptin (e.g. *ob/ob*)	?	↑	Hyperphagia
Peripheral CB1 receptors	Gastrointestinal tract	Fasting	?	↑	↓ Gastric emptying ↓ Gastric peristalsis

CNS, central nervous system; VMH, ventromedial hypothalamic nucleus.

1 Subpopulations of cannabinoid receptor containing neurones appear to exist, playing roles in both the hedonic and homeostatic control of food intake. Whether these represent functional independent populations is unclear. It has been shown that cannabinoid receptor-containing neurones in the hypothalamus are all intrinsic to this brain region (Romero *et al.* 1998). However, modulation of brain reward circuitry by leptin has also been reported (Fulton *et al.* 2000).

2 Peripheral anandamide may promote feeding by acting on specific hypothalamic areas important in the control of food intake. However, endocannabinoids are rapidly hydrolysed in the intestine and may not reach the brain in sufficient quantities to interact with central CB1 receptors (Di Marzo *et al.* 1998*b*). Alternatively, signals from the viscera indicating cannabinoid-mediated alterations of gastric activity may converge on the nucleus of the solitary tract in the medulla, from where inputs are relayed to the hypothalamus.

Hedonic mechanisms for regulating food intake

Brain reward systems allow the reinforcement of responses that have no homeostatic value. Motivation and reward have been studied most extensively in the context of drug addiction. However, a number of studies suggest that food reward and drug reward pathways may share some common components, including evidence that the cannabinoid system plays roles in both feeding and reward (Table 1).

Association of the cannabinoid system with reward processes is indicated by a number of lines of evidence. SR 141716 antagonises the hunger induced by anandamide and 2-AG. However, the compound also produces changes in ingestive behaviour when administered alone. SR 141716 selectively inhibits consumption of palatable food and drink, with decreased intake of sucrose, alcohol and a sweet diet observed in rats, mice and marmosets respectively (Arnone et al. 1997; Simiand et al. 1998). However, it has little effect on bland food consumption. These results suggest that the central cannabinoid system may act to amplify reward indices.

This is further supported by the observation that CB1 receptors are expressed particularly in areas of the brain such as the nucleus accumbens, the hippocampus and the entopeduncular nucleus; these areas are either directly involved in hedonic aspects of eating or are connected to reward-related brain areas (Finkelstein et al. 1996; Gorbachevskaia, 1999; Pecina & Berridge, 2000). In addition, the cannabinoids appear to interact with known opioidergic reward pathways, indicated by synergistic actions of SR 141716 and the opioid receptor antagonist, naloxone, on food intake (Welch & Eads, 1999; Kirkham & Williams, 2001).

Evidence in human subjects also supports specific cannabinoid involvement in food (orosensory) reward. For example, hyperphagic effects of marijuana in human volunteers were principally attributed to an increase in the consumption of highly palatable sweet foods such as chocolate and biscuits (Iverson, 2000).

Cannabinoids, leptin and the hypothalamus

Several lines of evidence suggest that the cannabinoids are modulated by leptin and this may be involved in the control of feeding (Table 1). First, leptin administration decreases hypothalamic levels of anandamide and 2-AG; endogenous cannabinoid levels in the only extrahypothalamic site examined, the cerebellum, were reportedly unaffected (Di Marzo et al. 2001). Curiously, CB1 receptor density, as determined by autoradiography, is relatively sparse within the hypothalamus (Harrold et al. 2002), although studies using $[^{35}S]$guanylyl $5'$-$[\gamma$-$[^{35}S]$thio]-triphosphate binding indicate that receptor coupling to G proteins is more efficient in the hypothalamus than in areas with a higher receptor density (Breivogel et al. 1997). In addition, studies have shown that anandamide increases Fos expression in the paraventricular nucleus of the rat hypothalamus, which plays an important role in the regulation of energy balance (Wenger et al. 1997; Patel et al. 1998). Furthermore, anandamide administration into the ventromedial hypothalamic nucleus of

satiated rats induces significant hyperphagia (Jamshidi & Taylor, 2001). Finally, defective leptin signalling in ob/ob and db/db mice and fa/fa Zucker rats is associated with elevated hypothalamic endocannabinoid levels, with these levels being reduced in ob/ob mice following leptin treatment (Di Marzo et al. 2001).

Genetically obese rodents exhibit a continuous motivation to eat and thus demonstrate extreme hyperphagia. The evidence presented earlier implicates the endocannabinoid system in this mechanism, possibly acting as a component of a leptin-sensitive regulatory pathway. Interestingly, a deficient leptin function has been reported in dietary-obese animals, with the development of leptin resistance (Widdowson et al. 1997a), which could modulate the relationship between plasma leptin levels and cannabinoid system activity. It is tempting to speculate that the hyperphagia demonstrated by dietary obese animals may also arise from an increased hypothalamic endocannabinoid system activity occurring as a consequence of reduced leptin regulation. However, evidence has recently come to light that refutes this argument. Hypothalamic 2-AG levels have been found to increase with food deprivation and decline with feeding (Kirkham et al. 2002), suggesting that once initiated, eating no longer depends on hypothalamic endocannabinoids for maintenance. Furthermore, no relationship has been identified between CB1 receptor binding density and leptin in dietary obese animals (as discussed later).

A role for the cannabinoid system in common human obesity?

Unselected Wistar rats given a palatable diet overeat to a variable degree, with approximately half the animals becoming significantly obese (Harrold et al. 2000). This dietary-induced obesity, attributable to voluntary hyperphagia, is the closest approximation to common lifestyle-related obesity in man, in which overconsumption of palatable food is an important contributing factor. Recent evidence points to the conclusion that the endogenous cannabinoids, acting on discrete extrahypothalamic populations of CB1 receptors, may drive appetite for palatable food and thus lead to the development of dietary-induced obesity. Rats fed a palatable diet for 10 weeks demonstrated reduced CB1 receptor density in the forebrain and hippocampus, consistent with increased activation of the receptors by endogenous cannabinoids. By contrast, CB1 receptor binding in the hypothalamus was low and unaltered. Furthermore a lack of correlation between receptor density and plasma leptin suggests that this receptor activity is not regulated by the circulating hormone (Harrold et al. 2002).

The anatomical localisation of these changes is notable, drawing attention away from the hypothalamus. The unaltered hypothalamic receptor density argues against a role for hypothalamic cannabinoids in driving appetite in dietary obesity. It is possible that hypothalamic cannabinoids act to stimulate feeding under particular circumstances, e.g. starvation, when falling leptin and insulin levels are known to activate other orexigenic systems such as neuropeptide Y. Unlike the cannabinoid system, these pathways are reported to be switched off under conditions of excess intake of palatable food (Widdowson et al. 1997b).

Therefore, pharmacological targeting of the cannabinoid system may prove particularly useful in the treatment of human obesity. This is supported by the recent observation that SR 141716-treated dietary obese mice demonstrate transient reductions in food intake. Sustained falls in body weight and adiposity were also reported. These were attributed to the hypophagia, potentially in conjunction with a thermogenic or metabolic effect, as treated animals demonstrated significantly greater weight loss following a 24 h fast than vehicle-treated controls (Ravinet Trillou *et al.* 2003).

Peripheral cannabinoid actions

Despite the existence of central mechanisms for the regulation of food intake by the endocannabinoids, evidence suggests that they may also promote feeding by acting at peripheral sites (Table 1). Indeed, CB1 receptors are located on nerve terminals innervating the gastrointestinal tract, which are involved in mediating gut-derived satiety signals (Croci *et al.* 1998; Hohmann & Herkenham, 1999). In addition, capsaicin-induced deafferentation prevents changes in feeding elicited by the administration of cannabinoid drugs (Gómez *et al.* 2002). Moreover, the peripheral administration of CB1 agonists and antagonists and the acute administration of peripherally acting satiety factors or feeding inhibitors, such as gastrointestinal hormones and the non-cannabinoid anandamide analogue oleamide, induce similar patterns of c-fos expression in hypothalamic and brainstem areas regulating food intake (Rodríguez de Fonseca *et al.* 1997). Finally, central administration of SR 141716 has no effect on food intake in food-deprived animals. SR 141716 is active only after intraperitoneal or oral administration, but not after subcutaneous injection, further supporting the hypothesis of peripheral actions of cannabinoids on food intake (Gómez *et al.* 2002).

There is some controversy as to whether peripheral anandamide also promotes feeding by acting on specific hypothalamic areas involved in energy homeostasis. For example, diets containing polyunsaturated non-esterified fatty acids are known to enhance anandamide levels in some brain structures of newborn pigs and mice (Berger *et al.* 2001). Furthermore, food deprivation for 24 h increases intestinal anandamide concentrations 7-fold, reaching levels that are 3-fold greater than those needed to half-maximally activate CB1 receptors (Devane *et al.* 1992). However, as only $1.6–5.0\%$ of orally administered cannabinoids survive their passage through the digestive system and enter the bloodstream (Di Marzo *et al.* 1998*b*), probably due to the high levels of the enzyme that degrades the compounds in the gastrointestinal tract (FAAH), this suggests that levels are too low to cause considerable central effects. This is supported by the observation that no increases in brain levels of anandamide occur after 24 h of food deprivation (Gómez *et al.* 2002).

It is hypothesised that the raised gut anandamide levels following food deprivation may serve as a short-range hunger signal to promote feeding. Elevated anandamide may also play a role in regulating gastric emptying and intestinal peristalsis, both processes being inhibited by the endocannabinoids (Calignano *et al.* 1997; Izzo *et al.* 1999). Interestingly, intestinal levels of anandamide and oleoylethanolamide (the oleic acid analogue of anandamide) are inversely correlated: oleoylethanolamide increases after a meal in conjunction with reductions in anandamide (Rodríguez de Fonseca *et al.* 2001; Gómez *et al.* 2002). It is possible that both act in a coordinated way to control food intake and gastric motility via opposing actions on gut nerve terminals.

Recently, a peripheral role for CB1 receptors in metabolic regulation has been indicated by the observation that SR 141716 increases Acrp30 (more commonly known as adiponectin) mRNA expression in adipose tissue of obese *fa/fa* rats and in cultures of adipocytes (Bensaid *et al.* 2003). Adiponectin induces non-esterified fatty acid oxidation, decreases hyperglycaemia and hyperinsulinaemia and reduces body weight. This regulation may play a role in the body weight reduction induced by SR 141716, with metabolic regulation contributing to its anti-obesity effects.

Future perspectives

The ability of marijuana to increase hunger has been noticed for centuries. Despite the public concern related to the abuse of marijuana and its derivatives, scientific studies have highlighted their ability to stimulate appetite, especially for sweet and palatable food, and point to the future therapeutic potentials of cannabinoid compounds in the treatment of obesity and eating disorders.

Cannabinoids and cachexia

Application of cannabinoid effects include the treatment of wasting diseases in which patients are unable or unwilling to eat. Indeed, Δ^9-THC is used clinically for this purpose, particularly in AIDS and cancer patients (Mechoulam & Fride, 2001). Anorexia also develops with old age in man. This is analogous to the decline in food intake observed in old mice. An age-dependent decline in alcohol preference has also been observed (Wang *et al.* 2003). This is absent in CB1 receptor knockout mice, independent of their age, suggesting that the decline is related to loss of cannabinoid signalling in relevant brain areas (Wang *et al.* 2003). No age-dependent change in anandamide, 2-AG or CB1 receptor density have been detected in wild type mice, suggesting that a decrease in ligand or receptor number is unlikely to account for the decline. In fact, a reduction in agonist stimulated guanylyl $5'$-$[\gamma$-$[^{35}S]$thio]-triphosphate labelling in old mice suggests that a localised decline in the coupling of CB1 receptors to G-proteins may account for reductions in food intake and alcohol preference. Accordingly, treatment with low doses of anandamide is able to cause a small but significant increase in voluntary alcohol intake in old mice (Wang *et al.* 2003).

Although anandamide binds and activates the CB1 receptor *in vitro*, the compound produces only weak and transient cannabinoid effects *in vivo*, thus limiting its effectiveness as a means of treatment. This probably arises as a result of anandamide's rapid catabolism (Adams *et al.* 1998). Indeed, the half-life of anandamide appears to be in the order of minutes (Willoughby *et al.* 1997). One candidate enzyme for regulating anandamide activity is

FAAH. Mice lacking FAAH are severely impaired in their ability to degrade anandamide, and when treated with the CB1 receptor ligand exhibit intense CB1 mediated effects that are inhibited by SR 141716 (Cravatt *et al.* 2001). Thus, FAAH may represent an attractive pharmacological target, with inhibitors of the enzyme (whose actions would only be evident as sites where endocannabinoid production and release is taking place) serving as therapeutic agents for the treatment of cachexia. To this end, several exceptionally potent inhibitors of FAAH are being investigated (Boger *et al.* 2000).

Another potential compound is oleamide, a lipid found in cerebrospinal fluid which causes similar pharmacological effects to anandamide in mice. Only anandamide binds to the CB1 receptor, but by inhibiting FAAH and thus increasing the concentration of anandamide, oleamide potentiates the endocannabinoid binding and enhances its effects (Mechoulam *et al.* 1997).

A similar entourage effect has been observed for 2-AG with actions of the endocannabinoid being enhanced *in vitro* and *in vivo* by co-administration of other fatty acid glycerol esters that coexist with 2-AG in the brain (Ben-Shabat *et al.* 1998). These compounds (e.g. 2-linoleoylglycerol and 2-palmitoylglycerol) have no intrinsic activity at the CB1 receptor, but amplify both the binding of 2-AG and its post-receptor signalling, probably by inhibiting the degradation of 2-AG by FAAH. These compounds have been shown to enhance certain central actions of 2-AG (motor activity and analgesia) when given intraperitoneally with the endocannabinoid, in the ratios at which they are found in the brain. As yet, the effects of these endogenous enhancers on feeding have not been investigated, but it is predicted that they would enhance the ability of endogenous 2-AG to increase palatable food intake.

Cannabinoids and obesity

Unlike the cannabinoid system, other appetite stimulating systems such as neuropeptide Y/agouti gene-related peptide are reportedly switched off under conditions of excess intake (Widdowson *et al.* 1997*b*). Therefore, pharmacological targeting of the cannabinoid system may prove useful in the treatment of lifestyle-related obesity in human subjects. It is not yet clear to what extent pharmacological agents acting on this system may have sustained actions and applicability to different feeding regimens. For CB1 antagonists to be useful anorectic drugs, their effects would have to be sustained over days and apply to more than just sweet food. It has been reported that the anorectic actions of SR 141716 disappear within 3–6 d of treatment in rats, suggesting that tolerance develops, but weight loss is sustained (Colombo *et al.* 1998). However, the dosing protocol used in this study was not optimal for sustained effects. Early results from clinical trials on the experimental drug Rimonabant (SR 141716) in the USA and Europe have been promising, with patients losing up to 4 kg over a 16-week period. However, more research is needed to determine the long-term effects of the drug. Phase III clinical trials are currently underway, being due for completion in August 2003.

The location of the anorectic actions of SR 141716 is not clear. As the receptor antagonist is able to cross the blood–brain barrier, it has been assumed that the effects have a central origin. However, CB1 receptors are not exclusive to the brain. High effectiveness of intraperitoneal and oral administration suggests that further studies of gut mechanisms of action are warranted. Furthermore, it may prove useful to combine cannabinoid antagonists with agents acting at other neurotransmitter systems implicated in the control of food intake, e.g. opioid systems.

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