Endocannabinoids in the regulation of appetite and body weight

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The discovery of cannabinoid receptors, together with the development of selective cannabinoid receptor antagonists, has encouraged a resurgence of cannabinoid pharmacology. With the identification of endogenous agonists, such as anandamide, scientists have sought to uncover the biological role of endocannabinoid systems; initially guided by the long-established actions of cannabis and exogenous cannabinoids such as Δ⁹-tetrahydrocannabinol (THC). In particular, considerable research has examined endocannabinoid involvement in appetite, eating behaviour and body weight regulation. It is now confirmed that endocannabinoids, acting at brain CB₁ cannabinoid receptors, stimulate appetite and ingestive behaviours, partly through interactions with more established orexigenic and anorexigenic signals. Key structures such as the nucleus accumbens and hypothalamic nuclei are sensitive sites for the hyperphagic actions of these substances, and endocannabinoid activity in these regions varies in relation to nutritional status and feeding expression. Behavioural studies indicate that endocannabinoids increase eating motivation by enhancing the incentive salience and hedonic evaluation of ingesta. Moreover, there is strong evidence of an endocannabinoid role in energy metabolism and fuel storage. Recent developments point to potential clinical benefits of cannabinoid receptor antagonists in the management of obesity, and of agonists in the treatment of other disorders of eating and body weight regulation.

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Cannabinoids, endocannabinoids and cannabinoid receptors

The appetite-stimulating action of the cannabis plant (Cannabis sativa) and its extracts has been documented for many centuries (Abel, 1975; Kirkham and Williams, 2001a). This effect appears to be largely attributable to Δ⁹-tetrahydrocannabinol (THC), one of a large family of related ‘cannabinoid’ molecules first characterized in the 1960s (Gaoni and Mechoulam, 1964; Mechoulam et al., 1970). The physiological basis for the efficacy of plant-derived cannabinoids in people has now been explained by the discovery within mammalian systems of cannabinoid receptors and their endogenous ligands, the ‘endocannabinoids’ (Pertwee, 2005a). The psychoactive effects of the exogenous cannabinoids are thus explained by their ability to mimic the neural actions of the endogenous agonists. It is increasingly apparent that the changes in eating motivation associated with cannabis intoxication, or the administration of THC, reflect a crucial role for these endocannabinoid systems in the normal processes governing appetite, ingestive behaviour, energy metabolism and body weight. This review will examine primarily the evidence supporting endocannabinoid involvement in the behavioural and motivational aspects of these processes.

Two main cannabinoid receptors subtypes have been identified. The CB₁ receptor is widely distributed within the central nervous system and many peripheral tissues (Devane et al., 1988; Matsuda et al., 1990). The CB₂ receptor was until recently considered not to be significantly expressed in the central nervous system, being localized primarily in immune cells (Munro et al., 1993; Mackie, 2005); however, there is new evidence for its expression within brain neurons and glia (Onaivi et al., 2005). Both of these are cell membrane receptors belonging to the G-protein-coupled family of receptors (GPCR; Howlett, 2005). There is also growing evidence to support at least one other, non-CB₁/non-CB₂ cannabinoid receptor subtype, but genes encoding other receptors have not been identified (Fride et al., 2003; Begg et al., 2005; Pertwee, 2005a). It is generally accepted that the predominant behavioural effects of cannabinoids are mediated by brain CB₁ cannabinoid receptors. However, these receptors are also expressed in gastrointestinal, adipose and hepatic tissues, so linking the endocannabinoids to important peripheral processes related to energy storage and metabolism that may also affect appetite (Cota et al., 2003; Osci-Hyiamen et al., 2005). The availability of selective CB₁ receptor antagonists has been the prime mover in driving research in this area.
The endocannabinoids are eicosanoid compounds, synthesized from arachidonic acid. The first of these substances to be identified was arachidonoylthanolamine, now known as anandamide (Devane et al., 1992). Subsequently, 2-arachidonoylglycerol (2-AG) was isolated (Mechoulam et al., 1995; Sugiura et al., 1995), followed by the identification of the putative endocannabinoids noladin ether (2-arachidonyl glyceryl ether), N-arachidonoylethanolamine (NADA), and α-arachidonoylthanolamine (virodhamine) (Hanus et al., 2001; Porter et al., 2002). These substances are agonists at CB1 receptors, while virodhamine is apparently a partial agonist that also has antagonist activity at the CB1 receptor (Porter et al., 2002; Howlett, 2004; Pertwee, 2005a). The neuromodulatory actions of the endocannabinoids involve novel mechanisms that, as with the discovery of the endogenous opioid peptides, are likely to revolutionize thinking about neural regulation. Thus, endocannabinoids are thought to act primarily as retrograde signals: released by postsynaptic neurons, they bind to presynaptic heteroceptors to modulate the release of inhibitory and excitatory neurotransmitters through multiple GPCR-linked effector mechanisms (Schlicker and Kathmann, 2001; Howlett et al., 2004; Vaughan and Christie, 2005). The metabolic pathways of anandamide and 2-AG have now been characterized in great detail, and we can anticipate that these pathways – together with endocannabinoid uptake mechanisms – will complement receptors as targets for the pharmacological analysis of the physiological functions of these substances (Fowler et al., 2001; Di Marzo et al., 2005).

Cannabinoids and human appetite

The established behavioural actions of cannabis and the exogenous cannabinoids invited speculation that the newly discovered endocannabinoids might be of importance to appetite processes. To date only anandamide and 2-AG have been investigated to any significant extent with respect to their actions on feeding behaviour, but it is clear that endocannabinoids do play a significant role in behavioural and metabolic aspects of energy regulation.

Despite recent discoveries, much of our theorizing about cannabinoid function is still based on historical accounts of the actions of cannabis and its derivatives in people. Unfortunately, political considerations have made it notoriously difficult to conduct the necessary scientific studies in humans (Robson, 2005), and a great deal remains to be determined about the precise psychological and physiological actions of cannabinoids that underlie their hyperphagic effects. The limited literature in relation to cannabinoids and human appetite has been reviewed extensively elsewhere (Kirkham and Williams, 2001a, c, 2004; Kirkham, 2004), and in this review I shall focus on findings from the animal literature. However, the principal effects of exogenous cannabinoids in humans may be summarized briefly in relation to a few key studies.

For example, in an early study, Hollister (1971) examined acute THC effects on the consumption of chocolate milk shakes and found significantly increased intake, elevated hunger ratings and enhanced food appreciation. Over several years Foltin, Haney and colleagues have conducted the most in-depth analyses, using a residential laboratory setting (e.g. Foltin et al., 1986, 1988; Haney et al., 1999, 2005; Hart et al., 2002). Early studies demonstrated substantial increases in daily caloric intake after cannabis smoke inhalation, primarily through an increase in the frequency and consumption of sweet snack foods. It is likely, however, that these apparently selective actions may be partly attributed to the prevailing test conditions. For example, increased food intake was predominantly observed during social access periods, suggesting a degree of social facilitation. By contrast, we have found that oromucosal application of THC will increase intake, but this effect is not restricted to sweet foods, as the drug also significantly increased the size of savoury set meals. Moreover, these effects were obtained when volunteers were tested individually, suggesting that cannabinoids can have broad effects on appetite that are not limited to specific flavours, food types or the context in which food is available (Townson and Kirkham, unpublished observations).

It is probable, although so far untested, that these actions are CB1 receptor-mediated, since the broader psychological actions of cannabis in people are reversed by the selective CB1 antagonist rimonabant (SR141716; Huestis et al., 2001). Finally, in addition to studies in healthy individuals, a small number of clinical trials have been conducted to assess the possible benefits of cannabinoids in the treatment of conditions involving wasting and appetite loss, such as cancer cachexia and AIDS. Although these trials were hampered by the nature of the seriously ill populations in which they were tested, beneficial effects were uncovered. Notably, treatment with THC (generally as the synthetic form, dronabinol) leads to improvement in both appetite ratings and actual consumption levels; while weight loss is attenuated – with weight gain in some instances (Regelson et al., 1976; Plasser et al., 1991; Beal et al., 1995). Consequently, THC, in the form of dronabinol, has been approved for the amelioration of appetite and weight loss (and for its anti-emetic action; Kirkham, 2004).

Cannabinoids and feeding in animal models

Since the 1970s THC has been shown to stimulate feeding in a variety of animal models, after systemic or central administration (Brown et al., 1977; Anderson-Baker et al., 1979; Williams et al., 1998), although several laboratories initially reported hyperphagic actions through...
the use of very high, sedative doses. With the discovery of
the endocannabinoids, the pace of this aspect of feeding
research has accelerated. In this regard it is important
that the hyperphagic action of THC has been shown to
be mediated by CB1 cannabinoid receptors: THC-
induced feeding is reversed by the selective CB1
agonist rimonabant, but not the CB2-selective antago-
nist SR144258 (Williams and Kirkham, 2002a). The
hyperphagic effect of THC in rats is also remarkably
potent, causing animals to overconsume even when
replete (Williams et al., 1998). Interestingly, low doses
of Δ9-THC, have been reported to have significantly
greater hyperphagic potency than Δ2-THC, and to exert
fewer cannabimimetic side-effects (Avraham et al., 2004).
Such data indicate the importance of exploring the
behavioural actions of other, largely un-investigated
phytocannabinoids.

The hyperphagic actions of THC have been replicated
following administration of the endocannabinoids anan-
damide and 2-AG. These substances increase food intake
in rodents following systemic or central injection and
their actions are CB1 receptor-mediated, being blocked
by rimonabant but not the CB2 antagonist SR144528
(Williams and Kirkham, 1999; Hao et al., 2000; Jamshidi
and Taylor, 2001; Gómez et al., 2002; Kirkham et al., 2002).
Importantly, anandamide and 2-AG will promote feeding
when administered into hypothalamic nuclei and into the
shell of the nucleus accumbens (Jamshidi and Taylor,
2001; Kirkham and Williams, 2001c; Kirkham et al., 2002).
These regions are firmly associated with eating motiva-
tion, and their sensitivity to the hyperphagic actions of
anandamide and 2-AG strongly supports a key role for
endocannabinoids in the control of eating.

Recently, a third putative endogenous agonist, noladin
ether, has been shown to increase food intake after
systemic treatment in food-restricted mice (Avraham
et al., 2005). This action is again CB1-mediated, being
reversed by rimonabant, and is obtained with very low
doses (0.001 mg/kg, i.p.). Although there has been some
debate about whether noladin ether is naturally present
in the brain (Oka et al., 2003), its relative stability
compared to anandamide or 2-AG (which are metabolized
rapidly) may yet provide a useful tool for probing
endocannabinoid function in appetite. Clearly, more work
is required to assess the feeding actions of each
endogenous cannabinoid: given the topicality of this area
of research, there are surprisingly few studies addressing
the behavioural actions of these agonists.

**Anorectic actions of cannabinoid receptor
antagonists**

Complementing the feeding actions of CB1 agonists is
the ability of CB1 receptor blockade to suppress eating in
laboratory species. Experiments with the prototypic CB1
antagonist rimonabant (Rinaldi-Carmona et al., 1994) and
its sister compounds have largely driven research in this
field. Indeed, a role for endocannabinoids in feeding was
largely posited on the basis of the intake suppressant
actions of rimonabant, which were reported long before
any actions of the endogenous CB1 agonists on feeding
had been demonstrated. An anorectic action of rimon-
abant was first reported by Arnone and colleagues at Sanofi
in 1997. The ability of the drug to suppress ingestion was
not restricted to food, as sucrose and ethanol drinking
were also reliably affected (although water drinking in
thirsty animals was unaffected). Consequently, it was
argued that the ability of the antagonist to exert an
anorectic action was evidence for tonic endocannabinoid
activity in feeding-related systems (Arnone et al., 1997).
An apparently greater effect of rimonabant on sweet food,
together with the effects on alcohol, led to the notion
that the drug exerted its effects through reward pathways
to modify the appetitive value of ingesta (Simiand
et al., 1998). Reliable anorectic actions of rimonabant, or its
analogues (e.g. AM281, AM251, O-3259 and O-3257),
have since been reported (typically at doses above 3 mg/
kg, i.p. or p.o.). Intake suppression has been observed
following systemic or central administration in satiated or
food-deprived animals, and after acute or chronic treat-
ments (Colombo et al., 1998; Shearman et al., 2003;
Werner and Koch, 2003; Chen et al., 2004; Rutkowski,
2004; Wiley et al., 2005). Sanofi have recently reported
the ability of a new CB1 antagonist, SR147778, to dose-
dependently reduce food intake in fasted or non-deprived
rats, and to reduce sucrose consumption in rats and mice
(Rinaldi-Carmona et al., 2004). A number of studies have
also investigated the effects of CB1 blockade in genetic
models of obesity. Thus rimonabant and AM251 have
been shown to exert reliable effects on food intake and
body weight in obese fa/fa rats, and ob/ob and agouti
yellow A(y) mice, often with greater effects than in lean
littermates (e.g. Vickers et al., 2003; Zhou and Shearman,
2004).

The ability of CB1 antagonists to suppress food intake
soon led to assessment of their potential as anti-obesity
treatments. Consequently, there has been considerable
interest in the chronic effects of these drugs on intake
and body weight. In an early study, Colombo et al. (1998)
demonstrated that daily administration of rimonabant
suppressed intake of lab chow and induced persistent
weight loss in rats. Although the anorectic action of the
drug dissipated after 5 days, suppression of body-weight
gain was evident across the full course of a 14-day
experiment. More recently, Vickers et al. (2003) demon-
strated that sub-chronic oral treatment with rimonabant
dose-dependently decreased food (chow) intake and
body weight gain in both lean and genetically obese
Zucker (fa/fa) rats. Once again, anorectic effects were no
longer evident after 4 days in lean animals, but reduction
of body weight gain was maintained over 28 days. These
The effects of chronic CB₁ blockade have also been addressed in mice made obese through the provision of a high-fat diet. This diet-induced obesity is a good model for the most common form of human obesity and its consequences, including visceral obesity and diabetes. Ravinet-Trillou et al. (2003) reported that daily rimonabant administration reduced food intake by almost 50% during the first week of a 5-week study. This initial anorectic effect gradually diminished, but intake remained suppressed compared to vehicle-treated controls throughout the whole test period. Overall, body weights were reduced substantially after 1 week and stabilized at that lower level until the end of the experiment. Carcass analysis showed that rimonabant significantly reduced adipose stores, halving the proportion of body fat seen in controls fed the same high-fat diet, while preserving lean mass. A follow-up study confirmed these effects and demonstrated that the drug-induced changes in intake and body composition were dose-dependent. Additionally, elevated plasma levels of insulin, leptin and insulin resistance that accompany the development of obesity, were substantially reduced by antagonist treatment.

Hildebrandt et al. (2003) confirmed the general effectiveness of chronic CB₁ receptor blockade in dietary obesity using the rimonabant analogue, AM251. The drug dose-dependently suppressed intake, initially by as much as 60% of control levels. Significant, dose-related weight loss was evident after 3 days of treatment and was maintained over an initial 2-week period of daily oral administration; with significant intake suppression evident until day 12. Rebound hyperphagia was apparent during a subsequent drug-free inter-treatment phase, but body weight gain was negligible until after 4–5 drug-free days. Reliable anorexia and weight loss were reinstated when AM251 treatment was given for a further 2 weeks – again with very marked initial intake suppression. In this second drug stage, attenuation of the intake-suppressing actions of the drug was evident somewhat earlier, but weight loss was maintained with marked reductions in adiposity. Plasma leptin and cholesterol levels were also significantly reduced at the highest dose, with an additional tendency for plasma insulin to be reduced (Hildebrandt et al., 2003). The ability of AM251 to attenuate eating and facilitate weight loss with an interrupted dosing regime suggests that tolerance to the effects of CB₁ antagonists may not be an obstacle to the long-term application of these drugs in the treatment of obesity.

The mechanism underlying the rapid loss of CB₁ antagonist anorexia with repeated treatment is not understood, nor well-explored. This loss of effect, often described as tolerance, has been reported with respect to other effects of these compounds (e.g. Carai et al., 2004). The differential change in efficacy on food intake and body weight is presumably unrelated to metabolic tolerance, but may reflect behavioural tolerance in response to persistent intake suppression (see below), or pharmacodynamic differences between brain and the peripheral cannabinoid systems, which may be more directly linked to weight change (discussed in a later section). Rubino et al. (2000) proposed that desensitization to the initial behavioural effects of chronic rimonabant treatment may involve, variously: uncoupling of the CB₁ receptor from its transduction system; homeostatic elevation of endocannabinoid synthesis; or tolerance to increased endocannabinoid levels resulting from disinhibition of their normally negative control over specific neural pathways.

It is notable that one group has reported that a single dose of AM251 can lead to intake suppression for up to 6 days post-treatment (Chambers et al., 2004), despite the reported half-life in mouse brain for this compound being of the order of several hours (Gatley et al., 1996). McLaughlin et al. (2003) have also reported that the motivational effects of rimonabant and AM251 have a long duration, with significant suppression of food-motivated behaviour evident for up to 22h after administration. Although such a persistent effect has to be separated clearly from non-specific actions (such as the induction of conditioned taste aversion; see below), the advantages to therapeutic regimes of intermittent, long-lasting antagonist treatments and the avoidance of tolerance are obvious.

**CB₁ receptor knockout mice**

The availability of CB₁ receptor knockout mice (CB₁⁻/⁻) has provided important supporting evidence for endocannabinoid involvement in appetite regulation. Ravinet-Trillou et al. (2003) characterized the phenotype of these animals when fed either standard chow or a high-fat diet. When maintained on chow, CB₁⁻/⁻ mice were leaner and slightly hypophagic compared to the wild-type (CB₁⁺/⁺) animals. When fed the palatable, high-fat diet, CB₁⁻/⁻ mice did not display the hyperphagia characteristic of the wild-type mice and did not develop obesity. Additionally, although CB₁⁻/⁻ mice did display a preference for the high-fat diet, this was acquired more slowly than...
in CB1/ mice. Knockout mice also show reduced consumption of sucrose compared to the wild type (Poncelet et al., 2003). Additionally, Di Marzo et al. (2001) have shown that CB1 animals display a reduced hyperphagic response to fasting, eating less than wild-type littersmates.

**Non-specific effects of antagonists**

Although antagonist-anorexia has been widely replicated, there has been some debate about the mode and specificity of action of these drugs on eating motivation. For example, rimonabant and its analogues have inverse agonist properties in some assays, suggesting that suppression of food intake may reflect this ‘inverse cannabimimetic’ action rather than competitive blockade of endocannabinoid tone (Pertwee, 2005b). Arguably, these compounds are neutral antagonists at low doses and inverse agonists at higher concentrations. It is certainly the case that relatively high systemic doses of these drugs are required to reliably inhibit feeding (typically > 3 mg/kg).

However, behaviourally silent rimonabant doses effectively attenuate the feeding actions of exogenously administered endocannabinoids, which would tend to support the active role of CB1 agonists in driving food intake. A recent suggestion is that rimonabant-like drugs may act as neutral antagonists to displace agonists from one site on the CB1 receptor, but exert inverse agonist effects by acting at a separate allosteric site, so modulating the constitutive activity of the receptor (Pertwee, 2005b). The new availability of potent neutral CB1 antagonists such as NESS 0327, will help resolve these issues (Riu et al., 2003). An additional concern may be that at high doses rimonabant can bind to CB2 receptors (Pertwee, 2005b). However, the inability of the selective CB2 antagonist SR144528 to block cannabimimetic-induced feeding (Williams and Kirkham, 2002a), or to suppress feeding in its own right (Wiley et al., 2005) supports the key involvement of CB1 receptors in the behavioural actions of rimonabant.

Another important issue in relation to the mechanisms whereby antagonists suppress food intake relates to the possibility of induction of non-specific malaise, or of behaviours that are incompatible with the expression of eating. At doses that overlap those found to suppress food intake, CB1 antagonists have been reported to have anxiogenic activity, and to promote behaviours such as wet-dog and head shakes, forepaw fluttering and facial rubbing that might interfere with feeding (Navarro et al., 1997; Rubino et al., 2000). It should be noted that these effects are not universally reported in feeding studies (although, of course, they may not be routinely assessed). However, there is some evidence for the possibility that antagonists may induce conditioned taste aversion (CTA) at anorectic doses, and that this effect may partially account for intake suppression. For example, McLaughlin et al. (2005) reported that AM251 dose-dependently reduced consumption of a novel, flavoured solution with which it had been paired 4 days previously. Additionally, the antagonist induced aversive gaping responses to saccharine in taste reactivity tests. Such effects are clearly of concern in trying to interpret the anorectic actions of CB1 antagonists (and particularly in relation to their possible application in human obesity).

Aversive or illness-inducing effects of the antagonists may still be independent of any ability to specifically suppress appetite, since the endocannabinoids are separately associated with the complex mechanisms that influence nausea and vomiting (Darmani et al., 2003, 2005; Kirkham, 2004). Notably, cannabinoid CB1 receptors are widely expressed in the brain stem dorsal vagal complex associated with triggering emetic responses. And, using vomiting species, it has been demonstrated that rimonabant can induce vomiting at high systemic doses (> 10 mg/kg, i.p.; Darmani, 2001). Such effects might be anticipated given the accepted medicinal use of dronabinol and nabilone for the suppression of nausea and emesis, and the demonstration in animals that anandamide and other CB1 agonists will suppress vomiting and conditioned rejection responses to flavours associated with illness (Limebeer and Parker, 1999; Parker et al., 2003). It should be noted that, conversely, 2-AG has been shown to have emetic actions (Darmani, 2002). More analyses are required to resolve these issues, but we presume that, in most instances, cannabinoid-induced feeding does not involve the suppression of nausea.

Ultimately, of course, confirmation of the role of endocannabinoids in the stimulation of eating is not dependent on the demonstration of behaviourally specific intake suppression by CB1 antagonists. None the less, rimonabant and its analogues have helped confirm the selectivity of agonist actions at CB1 receptors by blocking their hyperphagic effects at doses that are much lower than those required to produce overt behavioural effects.

**Endocannabinoids and eating motivation**

Having established that agonist and antagonist interactions at CB1 receptors can respectively stimulate or suppress feeding, we are left the task of ascertaining the underlying mechanisms through which these changes are effected. Again, we are faced with a relative paucity of data – particularly of detailed psychometric analyses of agonist and antagonist effects in humans. Animal data are also generally restricted to measures of changing food intake levels, which of themselves may not impart any clear indication of motivational specificity. Nevertheless, we shall summarize the available findings here and argue for a dual role of central endocannabinoids in the initiation and maintenance of eating.
A common theme in hypotheses about how cannabinoids affect eating motivation reflects the kinds of effects described earlier: an increased sensitivity to the sensory properties of foods and apparently preferential effects on preferred, highly palatable foods. As already noted, the earliest studies of the effects of rimonabant on feeding were specifically couched in terms of reward processes. Arnone et al. (1997) compared the effects of repeated oral treatments in rats fed either normal, bland maintenance diets or more palatable supplements (45 mg sucrose pellets). After 1 week of daily administration, rimonabant was reported to suppress intake of the more palatable food selectively, with intake of chow being unaffected. However, on closer inspection the data reveal that rimonabant had suppressed chow intake in the initial stages of testing, but this effect disappeared over several days (a phenomenon subsequently reported by other groups). Recalling our earlier discussion, it is possible that aversive effects contributed to the initial suppression of both normal and palatable food intake. In this study animals had only restricted access to food, suggesting that behavioural tolerance developed in response to cumulative caloric deficit, through which animals overcame deleterious effects of the drug to restore consumption of the more familiar chow. However, the authors specifically ruled out any action of rimonabant to promote neophobic responses to the sweet food. A more clear-cut demonstration of selective suppression of palatable foods was subsequently reported by Simiand et al. (1998), who reported that in marmosets (Callithrix jacchus) low doses of rimonabant reduced intake of a cane sugar mix without suppressing intake of standard pellets.

These early findings were interpreted generally in terms of antagonist actions on orosensory reward processes, or on palatability responses to food. There is now strong evidence to support specific involvement of endocannabinoids in both the appetitive and consummatory aspects of eating motivation (Freedland et al., 2001; Thornton-Jones et al., 2005). Indeed, direct evidence in support of endocannabinoids in general reward processes has recently been obtained through the demonstration that anandamide is self-administered by squirrel monkeys (Saimiri sciureus) (Justinova et al., 2005). We shall briefly discuss the evidence that endocannabinoids are linked to the processes that instigate food seeking and meal initiation, as well as those that actively maintain ingestion during meals. The data indicate that endocannabinoids contribute both to incentive processes, and to hedonic evaluation of food during eating – what Berridge (2000) has respectively described as ‘wanting’ and ‘liking’ processes.

Early evidence for a role of cannabinoids in incentive processes came from studies examining drug effects on operant responding. It was found that rats will work harder to obtain palatable ingesta after administration of CB1 receptor agonists, while antagonist treatments attenuate responding (Gallate and McGregor, 1999; Gallate et al., 1999). In line with the antagonist studies are reports that CB1 knockout mice have reduced sensitivity to the motivating properties of food. Thus, CB1−/− animals show lower levels of responding for sucrose in operant situations, exhibiting lower break points than wild-type mice (Sanchis-Segura et al., 2004). Interestingly, antagonist effects on operant responding are evident with bland as well as palatable foods (Freedland et al., 2000; Pério et al., 2001), and rimonabant has proved equi-anorectic when tested with foods of differing macronutrient content (Verty et al., 2004a). This suggests that endocannabinoids modulate appetitive processes per se, to provide a general gain in the incentive value of food. Moreover, our more detailed observational analyses and monitoring of meal patterns in freely fed rats treated with cannabinoids reveal that stimulation of CB1 receptors directly increases the salience of food, irrespective of need or energetic status. Thus, exogenous and endogenous cannabinoids can induce feeding almost as soon as chow becomes available; even when animals are tested under conditions in which motivation to eat is normally minimal – for example, after pre-satiation by overconsumption of a highly palatable food (Kirkham et al., 2002; Williams and Kirkham, 2002b). Typically, in our experiments, elevated food intake after cannabinoid treatment primarily involves the advance of meal onset, rather than a marked increase in meal duration or meal size. Crucially, once initiated, the subsequent pattern of cannabinoid-induced feeding behaviour is identical to that of untreated rats feeding spontaneously under home cage conditions (Kirkham and Williams, 2001a; Williams and Kirkham, 2002b). The latter finding indicates that cannabinoids provoke feeding through adjustments to natural feeding processes rather than, for example, by inducing stereotyped behaviour (Wiley et al., 2005). Overall, the ability of agonists to increase responding for food and specifically to reduce eating latency implies that stimulation of CB1 receptors directly activates eating motivation.

Of the various brain loci linked to feeding, the shell subregion of the nucleus accumbens (AcbSh) has particularly strong associations with appetitive processes. Neural activity here is believed to signal incentive salience and to facilitate the generation of motor patterns orienting an animal toward potentially rewarding stimuli – such as food in a hungry animal (Kelley, 1999). Importantly, the AcbSh contains a relatively high density of CB1 receptors (Herkenham et al., 1991; Breivogel and Childers, 1998) and is a particularly sensitive site for the induction of endocannabinoid-induced feeding. Bilateral administration of 2-AG into this region induces substantial short-term, rimonabant-reversible hyperphagia (Kirkham et al., 2002). In line with the notion that cannabinoids increase the incentive value of food,
intra-accumbens 2-AG also significantly advances the onset of feeding without markedly affecting other meal parameters. Moreover, the rapidity of onset and magnitude of 2-AG hyperphagia injected into the AcbSh are greater than the effects of anandamide or noladin ether seen after peripheral administration or injection into other brain sites (Williams and Kirkham, 1999; Jamshidi and Taylor, 2001; Avraham et al., 2005). Importantly, and in accordance with the proposed action of CB1 agonists to promote feeding by increasing the ‘wanting’ of food, we have also shown that acute food deprivation provokes significant increases in anandamide and 2-AG levels within the limbic forebrain (which incorporates the AcbSh). Interestingly, there is indirect evidence from antagonist experiments for elevated endocannabinoid activity during fasting. Specifically, we found that the anorectic action of rimonabant was significantly enhanced in food-deprived rats compared to non-deprived animals (Kirkham and Williams, 2001a).

Mesolimbic dopamine neurons, arising in the ventral tegmental area (VTA) and projecting to the nucleus accumbens, are regarded as being key to incentive processes and food wanting (Berridge, 2000, 2004). These pathways play an important role in the generation of emotional arousal and behavioural activation in response to stimuli that predict reward (Ikemoto and Panksepp, 1999; Spanagel and Weiss, 1999). Food stimuli cause dopamine release in the nucleus accumbens after deprivation, or if the food is novel or palatable. Importantly, there is growing support for an influence of endocannabinoids on mesolimbic dopaminergic activity (Hermann et al., 2002; van der Stelt and Di Marzo, 2003; Lupica et al., 2004). For example, doses of THC within the hyperphagic range stimulate dopamine release in the nucleus accumbens (Gardner, 2005; Gardner and Vorel, 1998; Malone and Taylor, 1999). Verty et al. (2004b) have reported that THC hyperphagia is attenuated by a behaviourally silent dose of the D1 antagonist SCH23390, suggesting that mesolimbic dopamine signalling may be crucial to the appetitive actions of cannabinoids.

However, dopamine–endocannabinoid interactions are likely to be complex, since SCH23390 has been shown to increase anandamide levels in limbic forebrain, while the D2 antagonist reticlopride elevates 2-AG levels (Patel and Hillard, 2003). Rimonabant can also prevent the enhancement of food incentive value induced by dopamine agonists. Thus, the D2 receptor-prefering agonist quinelorane reinstates responding for food unexpectedly delivered during extinction, and this action is blocked by low doses of rimonabant (Duarte et al., 2004). By contrast, rimonabant had no effect on the response priming effect of food alone, indicating that CB1 receptors subserve the ability of dopamine to enhance the motivational valence of food stimuli.

As the differential actions of dopamine receptor-selective drugs indicate, the precise relationship between endocannabinoids and dopamine activity in these systems is still uncertain (for a more comprehensive discussion see Lupica et al., 2004). However, it is likely that these interactions may involve coordination of the activity of ventral tegmental dopamine neurons. Specifically, it has been proposed that stimulation of accumbens CB1 receptors may suppress glutaminergic activity, with consequent inhibition of the GABAergic medium spiny neurons that normally constrain the firing of VTA dopamine neurons (van der Stelt and Di Marzo, 2003). Inhibitory prefrontal cortex-VTA afferents also appear to undergo similar regulation by endocannabinoids. The finding that VTA dopamine neurons themselves release 2-AG, which then acts to fine-tune their activity, strengthens the potentially key role for these neuromodulators in the attribution of incentive salience and reward anticipation. Endocannabinoids, therefore, may be essential for the orientation to motivationally significant stimuli, and the elicitation of appropriate behavioural responses, such as food seeking (Melis et al., 2004; Lupica and Riegel, 2005). It should be noted that serotonin (5-HT) has been linked to reward processing (Higgins and Fletcher, 2003). However few data are available on possible endocannabinoid-5-HT interactions in this regard. Current information is confined to the reported failure of the indirect serotonergic agonist dexfenfluramine to affect THC-induced feeding (Rowland et al., 2001; Williams and Kirkham, 2002a).

The hypothalamus is also considered to play a key role in integrating the multiple chemical and behavioural components of feeding and weight regulation. Moreover, the accumbens and hypothalamus are functionally linked components of feeding regulatory pathways (Stratford and Kelley, 1999; Stratford, 2005). It is therefore significant that not only will cannabinoid administration into hypothalamic nuclei induce eating, but that hypothalamic endocannabinoid activity apparently changes with nutritional status and the expression of eating behaviour. For example, levels of 2-AG (but not anandamide) are increased in the hypothalamus after 24-h food deprivation in rats (Kirkham et al., 2002) and mice (Hanus et al., 2003). Interestingly, 2-AG levels decline as animals eat, falling to control levels as animals satiate (Kirkham et al., 2002). These changes are wholly compatible with the behavioural actions of cannabinoids (i.e. an increased urgency to feed, but only marginal changes in meal size), and indicate the key involvement of hypothalamic endocannabinoids in food wanting or craving (Kirkham and Williams, 2001c; Williams and Kirkham, 2002b). Accordingly, the decline of 2-AG levels during feeding would also indicate a minor, or indirect, role for this agent in the maintenance of feeding once it has been initiated.
It should be noted that in contrast to the effects of acute deprivation, Hanus et al. (2003) found that hypothalamic 2-AG decreased after chronic (12 days) food restriction. These discrepancies were hypothesized to reflect different adaptive strategies in response to acute or chronic food deprivation. In the short term, energy needs resulting from environmentally imposed fasting, such as might arise from behavioural adaptations to an animal’s particular ecological niche, could involve periodic upregulation of endocannabinoids to instigate food seeking. Conversely, during periods of famine, it may aid survival to conserve energy by reducing the motivation to engage in foraging – perhaps by reducing the conscious experience of hunger (Hanus et al., 2003). It is the case that circadian rhythms in brain endocannabinoids have been demonstrated in the rat, which is primarily a nocturnal feeder. Specifically, Valenti et al. (2004) found that anandamide levels in the nucleus accumbens, prefrontal cortex, striatum and hippocampus were significantly higher in the dark phase than during daytime. The story is complicated somewhat by the finding that 2-AG levels in these regions displayed the converse pattern, being higher during the light phase. These differences, and the differential regional variation in anandamide and 2-AG noted in relation to fasting and feeding, emphasize the limitations of current knowledge about the specific role of individual endocannabinoids, and the urgent need to establish more precisely the neuroanatomical, motivational and contextual specificity of the behavioural actions of CB₁ agonists.

We should also note here that CB₁ receptors located in feeding-relevant hindbrain areas, such as the dorsal motor nucleus of the vagus (DMV) and the nucleus tractus solitarius (NTS), may also be subject to cannabinoid regulation (Derbenev et al., 2004). Thus, the cannabinoid receptor agonist CP55,940 has been reported to enhance milk intake with greater potency when administered into the fourth ventricle than when injected into the third ventricle (Miller et al., 2004).

The data discussed above link endocannabinoids to appetitive processes in feeding. However, their role may be extended to involvement in food ‘liking’. Such a role is evident in anecdotal reports of cannabis users (Tart, 1970), and recent animal studies have provided support for a specific interaction of endocannabinoids with food palatability. As we have seen, CB₁ receptor blockade was reported to preferentially attenuate the intake of palatable, sweet foods (Arnone et al., 1997; Simiand et al., 1998) and reduce operant responding for sweet food (Perío et al., 2001); while CB₁ knockout mice consume less sucrose than wild types (Poncelet et al., 2003). We have examined the actions of CB₁ receptor ligands on the microstructure of sucrose drinking and found that alterations to licking behaviour induced by exogenous and endogenous CB₁ agonists are reminiscent of those observed in drug-free animals when the palatability of the test solution is increased (Higgs et al., 2003). Conversely, rimonabant alters drinking in a way that is consistent with a reduction in the palatability of the sucrose solution. Direct comparison of the effects of changing palatability and CB₁ agonists or antagonists on ingestive behaviour reinforces these conclusions. Thus, we found that in simple drinking tests, the effect of THC on ingestion of a 20% sucrose solution directly mimics the behavioural alterations following presentation of a more palatable, 30% sucrose solution: increasing the duration (but not frequency) of drinking bouts. In rats freely drinking 30% sucrose, rimonabant exerts an opposite action (Rogers et al., unpublished observations). Additionally, CB₁⁻⁻ mice are less responsive to sweet taste, consistently drinking less of a range of sucrose solutions than the wild type (Sanchis-Segura et al., 2004). Moreover, these differences were abolished when sucrose solutions were adulterated with bitter quinine, indicating that they arise from differences in the rewarding consequences of palatable ingesta rather than from any sensory impairment. Confirming our antagonist data, CB₁⁻⁻ mice drinking sucrose display reduced bout size but normal bout frequency. These findings thus support the hypothesis that endocannabinoid activity contributes significantly to the hedonic evaluation of ingesta, and that CB₁ stimulation or blockade/deletion can respectively render food more or less pleasurable.

Consistent with this notion is the fact that key components of the neural mechanisms underlying food palatability lie within the AcbSh and, as already noted, 2-AG administered into this site produces a profound hyperphagic response (Kirkham et al., 2002). Fasting, which would be expected to elevate the reward value of food, also increases levels of anandamide and 2-AG in the limbic forebrain (Kirkham et al., 2002). Moreover, Harrold et al. (2002) have shown that accumbens CB₁ receptors are down-regulated in rats that overconsume palatable food supplements. This latter effect is consistent with increased activation of these receptors by endocannabinoids, and again suggests they mediate the hedonic evaluation of palatable foods.

There are, of course, some contradictory aspects of our data that do not fully support this account. For instance, we noted the lack of effect of intra-accumbens 2-AG on meal duration (while eating latency was reduced): enhancement of palatability would be anticipated to extend meal duration. Possibly, the use of a relatively bland test food (chow) limited our ability to detect such effects in these experiments. Additionally, the precise location of drug infusion within the AcbSh may be critical to the exact behavioural profile of drug-induced changes to ingestive behaviour (Reynolds and Berridge, 2002). However, in support of the palatability/accumbens hypothesis are recent data from Berridge’s group. Using
taste reactivity tests, they found that intra-accumbens administration of anandamide specifically increases the number of positive ingestive responses to intra-oral infusions of sweet solutions, indicating that anandamide can indeed enhance the hedonic impact of sweet taste (Mahler et al., 2004). Finally, it is important to emphasize that the role of endocannabinoids in palatability responses is modulatory, since CB1 antagonists or CB1 deletion in knockout animals does not wholly remove the rewarding properties of ingesta (Sanchis-Segura et al., 2004).

**Endocannabinoid interactions with orexigenic/anorexigenic systems**

Given these associations between endocannabinoid palatability, it is noteworthy that the endocannabinoids may have important functional relationships with the endogenous opioid systems that mediate orosensory reward (Cooper and Kirkham, 1993; Kelley et al., 2002; Bodnar, 2004). It is of interest in this regard that CB1 knockout mice are not only unresponsive to cannabinoids, but display a reduced sensitivity to the rewarding effects of opiate drugs (Ledent et al., 1999). In rats, the hyperphagic action of THC is reversed by the general opioid receptor antagonist, naloxone (Williams and Kirkham, 2002a). Importantly, the facilitatory effects of a CB1 agonist on responding for palatable solutions were reversed by both a CB1 antagonist and naloxone (Gallate and McGregor, 1999). Moreover, low doses of rimonabant and opioid antagonists that are behaviourally inactive when administered singly, combine synergistically to produce a profound anorectic action when co-administered (Kirkham and Williams, 2001b; Rowland et al., 2001; Chen et al., 2004). Given the established ability of opioid antagonists to reduce the hedonic evaluation of foods and to reverse CB1 agonist-stimulated ingestion, the marked anorexia induced by combined CB1 and opioid receptor blockade again strengthens the proposition that endocannabinoids also contribute to orosensory reward processes.

In opposition to this hypothesis is a report that feeding induced by intra-accumbens morphine administration was not reversed by rimonabant (Verty et al., 2003). However, intra-accumbens administration of either morphine or anandamide increases the liking of sweet solutions (Pecina and Berridge, 2000; Mahler et al., 2004), and there is ultrastructural evidence that cannabinoid–opioid interactions are mediated by activation of CB1 and μ-opioid receptors within the same, or synaptically linked, reward-relevant neurons in the AcbSh (Pickel et al., 2004). Moreover, systemic administration of THC has been shown to stimulate β-endorphin release in both the VTA and AcbSh (Solinas et al., 2004), and CB1 agonist-induced DA release in the accumbens is blocked by intra-VTA infusion of the μ1-selective antagonist, naloxonazine (Tanda et al., 1997).

There is evidence elsewhere in the brain for interactions between endocannabinoids and opioids; notably within the hypothalamus, and particularly the paraventricular nucleus (PVN) in which orexigenic and anorexigenic pathways converge. Importantly, both CB1 and opioid receptors are expressed in the PVN, and it is a sensitive site for the hyperphagic actions of both cannabinoid and opioid receptor agonists. It is of interest then, that feeding induced by injection of morphine into this site can be reversed by rimonabant (Verty et al., 2003).

Although we have so far considered feeding motivation in terms of discrete wanting and liking components, it may be that the borders between these are less distinct than currently conceptualized. Previously, we have argued that the synergy between endogenous cannabinoid and opioid systems made apparent when CB1 and opioid receptors are simultaneously blocked is indicative of some priming function of the endocannabinoids: endocannabinoid activity in the prelude to feeding may facilitate the positive, opioidergic hedonic response to the orosensory properties of food during ingestion (Kirkham and Williams, 2001b). In essence, the two systems might co-operate to ensure that effort expended in obtaining food is optimized by rendering the food palatable and so encouraging its actual consumption. To stand aside from the data briefly, and raise a debating point: imagine for a few moments the subjective experience of eating one’s favourite food. Think how good it will taste, how pleasurable and satisfying it will be, how your mouth will water, and the lip-smacking that will accompany the first mouthful. I think it is fair to say that anticipation of food can involve at least a trace of the pleasure that will ultimately be derived from the physical experience of tasting and eating it. Is it possible that this subjective experience reflects the overlap of endocannabinoid and opioid function?

Historically, the consequences of independent interventions in endocannabinoid or opioid processes have been operationally distinguishable. For example, cannabinoids primarily reduce eating latency without affecting meal duration (i.e. actions on appetitive processes); while opioids typically do not alter eating latency but extend meal duration by enhancing palatability (i.e. actions on consummatory processes). A closer temporal relationship, or merging, of direct, primary cannabinoid influences on appetitive motivation and their secondary facilitation of opioid consummatory components may be revealed by some recent work by Solinas and Goldberg (2005). They reported that THC and morphine dose-dependently increased break points for food reinforcement, while rimonabant and naloxone dose-dependently decreased break points. Confirming our findings in free-feeding rats, the effects of THC were blocked by naloxone. But more surprisingly, the effects of morphine were blocked by rimonabant. These data potentially demonstrate
reciprocal cannabinoid–opioid mediation of appetitive behaviour. The authors concluded that ‘activation of both endogenous cannabinoid and opioid systems appears to jointly facilitate motivational effects of food… and this facilitatory modulation appears to critically depend on interactions between these two systems’ (Solinas and Goldberg, 2005). Given the extensive evidence for cannabinoid–opioid interactions in many other aspects of neural function, more research into these interactions in relation to appetite is clearly warranted.

As might be expected, functional interactions between endocannabinoids and systems implicated in feeding are not restricted to the opioids. Indeed, there is a growing body of evidence to link endocannabinoids with a wide range of other factors currently implicated in the regulation of appetite (Cota et al., 2003; Di Marzo and Matlas, 2005; Sharkey and Pittman, 2005). The following overview is largely phenomenological: despite the promulgation of substantive models for the place of endocannabinoids in appetite and energy homeostasis, it is a matter of some concern that the number of review articles in this area now seriously outweighs the volume of original research articles – not least in relation to the specific behavioural actions of the endocannabinoids themselves.

Given its pre-eminence in current accounts of energy regulation, much emphasis has been given to the demonstration of apparent interactions between the adipokine leptin and the endocannabinoids. Leptin, which originates in adipose tissue and affects a number of appetite-related factors in the hypothalamus, has been proposed to be a core component in the regulation of long-term food intake and weight control (Friedman, 2002). It is therefore of interest that functional relationships between cannabinoids and leptin are indicated by recent research, and that endocannabinoid synthesis may be regulated by leptin. Thus, Di Marzo et al. (2001) reported that exogenous leptin administration, which exerts an anorectic action, suppresses hypothalamic endocannabinoid levels in normal rats; while genetically obese, chronically hyperphagic rats (fa/fa) and mice (ob/ob/ or 2-AG levels. Unfortunately, this study provided no indication of the extent to which these changes reflect altered leptin signalling alone, rather than being a consequence of differences in eating behaviour. However, rimonabant has been shown to be more effective in suppressing food intake in obese Zucker (fa/fa) rats, which may be partly due to a greater sensitivity to the drug as a consequence of the upregulation of central cannabinoids. Additionally, CB1−/− mice have an enhanced sensitivity to the intake-suppressing actions of intracerebroventricular (i.c.v.) leptin (Ravinet-Trillou et al., 2003). We should note that data contradicting a simple relationship between leptin and cannabinoid function have been obtained using the more representative model of diet-induced obesity (DIO), induced by overconsumption of palatable foods. Thus, Harrold et al. (2002) found that although rats with DIO were hyperleptinaemic, there was no correlation between plasma leptin levels and hypothalamic CB1 expression (while CB1 receptors were upregulated in the nucleus accumbens in these animals).

In the first report of the anorectic action of rimonabant, Arnone et al. (1997) also reported that the drug could block the ability of neuropeptide Y (NPY) to increase intake of a palatable sucrose solution. Despite this finding, the considerable potency of NPY as an orexigen, and its key interactions with other hypothalamic neuropeptides regulating feeding, subsequent analysis of potentially important relationships between this peptide and the endocannabinoids has been limited. Further investigation of functional interactions is warranted however, since Poncelet et al. (2003) reported that rimonabant can prevent NPY hyperphagia, and that the ability of NPY to stimulate feeding is abolished in CB1−/− mice, although rimonabant is as effective in reducing food intake in NPY knockout mice as in the wild type (Di Marzo et al., 2001).

An important component of appetite regulation – through the co-ordination and integration of neural, metabolic and hormonal signals, is the hypothalamic melanocortin system. Verty et al. (2004c) reported that in rats sub-anorectic, i.c.v. doses of the melanocortin MCR4 receptor agonist α-melanocyte stimulating hormone (α-MSH) and rimonabant combined synergistically to suppress feeding. Feeding stimulated by the MCR4 antagonist JKC-363 was dose-dependently attenuated by rimonabant, whereas THC-induced eating was unaffected by α-MSH. These results were interpreted as indicating that CB1 receptors are located downstream from melanocortin receptors, and that endocannabinoids exert an inhibitory action on the ability of the melanocortin system to inhibit feeding.

The same group of researchers also reported functional interactions between endocannabinoids and another appetite-inhibiting peptide, oxytocin. Again, subthreshold doses of i.c.v. rimonabant and oxytocin produced supra-additive suppression of feeding. Although THC hyperphagia was unaffected by oxytocin, rimonabant dose-dependently attenuated feeding induced by the oxytocin receptor antagonist tocinoic acid. These data indicate that endocannabinoids can negatively modulate the inhibitory actions of oxytocin (Verte et al., 2004d).

Very little is presently known about the interaction of the endocannabinoids with other neuropeptides involved in energy control and food intake. However, there are indications from histological analyses suggesting that further research would be fruitful. For example, Cota et al.
Peripheral cannabinoids and feeding stimulation

In addition to the central nervous system, cannabinoids and CB1 receptors are also present in gastro-intestinal, adipose and hepatic tissues, suggesting a variety of possible influences of peripheral endocannabinoids on the regulation of appetite and body weight (Izzo et al., 2001; Cota et al., 2003; Osei-Hyiamen et al., 2005). A role for peripheral endocannabinoids in the control of feeding was indicated by observations that anandamide is synthesized within gut tissues, with small intestine concentrations increasing in 24-h fasted rats (Gómez et al., 2002). Moreover, the respective hyperphagic or anorectic actions of intraperitoneal anandamide and rimonabant were attenuated by capsaicin-induced de-afferentation of peripheral sensory nerves. These findings suggested that stimulation or blockade of peripheral CB1 receptors may influence central motivational processes, and have been interpreted as indicating a possible role for intestinal anandamide as a ‘hunger signal’. Unfortunately, this notion was originally posited on the basis that the authors could only obtain anandamide hyperphagia and rimonabant anorexia after systemic but not central administration, despite the fact that anandamide, 2-AG, THC and CB1 antagonists had already been reported by different groups to exert reliable effects on intake via intracerebral or i.c.v. injection (Jamshidi and Taylor, 2001; Koch and Matthews, 2001; Kirkham et al., 2002).

Nevertheless, subsequent study revealed that vagal afferents originating in the stomach and duodenum, which express receptors (CCK1) for the putative satiety hormone cholecystokinin (CCK), also express CB1 receptors. CB1 expression was increased by prolonged food deprivation and rapidly reduced on re-feeding. Interestingly, these respective effects were blocked by the CCK1 receptor antagonist, lorglumide, and mimicked by CCK injection in fasted animals. It was concluded that CCK may naturally inhibit the ability of peripheral anandamide to stimulate feeding via vagal activity, and so fasting may release vagal cannabinoid signals from CCK inhibition. CB1 receptors are also expressed by CCK-containing neurons within the brain (McDonald and Mascagni, 2001), although the implications of this association to feeding have not been studied. Given the contradictory data referred to above, more research is clearly required to assess the relative importance to normal feeding regulation of gut-derived endocannabinoids, and to determine whether changing gut endocannabinoid levels reflect nutritional status directly, or are related to their role in regulating gut motility and gastrointestinal enzyme secretion (Izzo et al., 2001; Coutts and Izzo, 2004).

Endocannabinoid regulation of fat metabolism and adiposity

Several findings raise the possibility of important cannabinoid influences on adiposity/body weight that may be distinct from their direct actions on appetite (Cota et al., 2003). Indeed, rimonabant and analogues have been shown to reduce adiposity in diet-induced obese mice and genetically obese rodents, independently of their primary anorectic actions (Ravinet-Trillou et al., 2003; Vickers et al., 2003). Thus, chronic CB1 blockade only transiently suppresses food intake, while weight loss persists for the duration of treatment. Moreover,
pair-feeding tests in which control animals receive the same amount of food voluntarily consumed by antagonist-treated animals, showed that weight loss was greater with rimonabant. Additionally, when deprived of food for 24h, rimonabant-treated obese mice lose more weight than similarly deprived controls (Ravinet-Trillou et al., 2003).

As we have noted, CB1 knockout mice are resistant to diet-induced obesity, and do not exhibit the insulin resistance normally occurring in high-fat-fed mice (Ravinet-Trillou et al., 2004). Additionally, rimonabant lowers plasma free fatty acid levels in dietary obese mice, corrects hyperglycaemia, reduces plasma insulin levels and counters insulin resistance (Ravinet-Trillou et al., 2003). Antagonist treatments may therefore cause direct interference with cannabinoid-mediated processes that normally regulate fat deposition in adipose tissues, fatty acid oxidation, and glucose homeostasis. It is therefore of great significance that CB1 receptors are expressed by adipocytes in normal, but not CB1 receptor-deficient mice, and that stimulation of these receptors can induce lipogenesis (Cota et al., 2003). In this context, it is noteworthy that exercise (voluntary wheel-running) has been shown to augment the actions of AM251 on weight loss in lean and obese agouti A‘ mice, with lower doses of the drug required to reduce weight than when animals were not given access to exercise (Zhou and Shearman, 2004). This augmentation was attributed to possible complementary effects on sympathetic activity, which in turn stimulates lipolysis. Jbilo et al. (2005) have also reported that rimonabant enhances lipolysis through induction of enzymes in adipose tissue that catalyse the β-oxidation and tricarboxylic acid (TCA) cycles. Critical enzymatic regulators of glucose metabolisms are also upregulated by rimonabant, supporting a proposed action of the drug to increase energy expenditure (Liu et al., 2005; Jbilo et al., 2005).

Interestingly, there are parallels between the effects of rimonabant and the adipokine adiponectin, suggesting a possible mechanism whereby body weight is reduced by the antagonist. Adiponectin expression varies inversely with adiposity in animals and humans and, like rimonabant, regulates hyperglycaemia, hyperinsulinaemia and fatty acid oxidation, and can reduce the body weight of obese animals through a food intake-independent mechanism (Berg et al., 2001; Fruebis et al., 2001; Wolf, 2003). Importantly, chronic rimonabant treatment increases adiponectin expression through a CB1-mediated mechanism, with greater effects in obese than lean rats (Bensaid et al., 2003).

As a major site of lipogenesis, the liver has also received attention as a possible site of cannabinoid activity. Thus, in one of the most intriguing recent developments, Kunos’ group has discovered that not only do hepatocytes express CB1 receptors, but that agonist stimulation exerts a lipogenic action: increasing fatty acid synthesis by upregulating expression of the lipogenic gene transcription factor sterol response element-binding protein 1c (SREBP-1c), and its target enzymes acetyl-coA carboxylase-1 (ACC1) and fatty acid synthase (FAS). Moreover, obesity induced by overconsumption of fat leads to elevated anandamide levels in the liver (through reduction of its enzymatic breakdown), upregulation of hepatic CB1 receptors, and increased hepatic fatty acid synthesis (Osei-Hyiamen et al., 2005). These changes are prevented by rimonabant and are absent in CB1−/− animals, suggesting a key role for liver endocannabinoids in the development of obesity.

Osei-Hyiamen et al. (2005) have also suggested that CB1-mediated regulation of FAS within the brain may provide evidence of a common pathway for central endocannabinoid regulation of appetite and peripheral metabolic regulation. In support of this notion is the fact that lipid metabolism in feeding-related hypothalamic neurons is sensitive to nutrient availability, and that inhibition of FAS can suppress eating (Loftus et al., 2000; Kim et al., 2002). Osei-Hyiamen et al. (2005) found that hypothalamic FAS is upregulated by CB1 agonist treatment, which would be compatible with the hyperphagic actions of these drugs. The story is complicated, however, by the finding that fasting, which is known to increase hypothalamic cannabinoid activity and to stimulate eating, had no effect on FAS expression in this region: marked changes were only detected after re-feeding.

These data may be complemented by some of our recent data in relation to AMP-activated protein kinase (AMPK). AMPK is an enzyme proposed to function as a fuel sensor, contributing to energy balance regulation at both cellular and whole-body levels (Hardie et al., 2004; Kahn et al., 2005). We found that both THC and 2-AG stimulate AMPK activity in the hypothalamus, while inhibiting AMPK activity in the liver and adipose tissue (Kola et al., 2005). Given the proposed role of AMPK, these observations may provide important evidence of interactions between this enzyme and the orexigenic actions of cannabinoids. Thus, cannabinoids could potentially increase appetite by central AMPK stimulation, or by facilitating the restorative actions of AMPK as the hypothalamus senses fuel deprivation. By contrast, peripheral inhibition of AMPK by cannabinoids may lead to fat storage. The combined effect of both central and peripheral AMPK activation could therefore be both increased food intake and lipid deposition. Although such energetic models are likely to considerably more complex, these kinds of data are once again indicative of the rapid development in the study of endocannabinoid mechanisms, and of the strengthening support for their contribution to the combined regulation of appetite and energy utilization.
Cannabinoids in the pathology and treatment of obesity and eating disorders

The involvement of endocannabinoids in appetite regulation raises two important questions: do these systems play a significant role in disorders of eating and body weight; and can pharmaceutical interventions targeting endocannabinoids have useful therapeutic applications?

The short answer to the first question is that there is little information currently available. However, three recent studies suggest that further enquiry may be fruitful. Monteleone et al. (2005) examined plasma levels of anandamide and 2-AG in women with anorexia nervosa, bulimia nervosa or binge-eating disorder. They found that plasma levels of anandamide were significantly elevated in both anorexics and women with binge-eating disorder, but not in bulimic patients. No significant alterations to 2-AG levels were detected in any of the groups. Anandamide and 2-AG levels were not reliably correlated with the severity of psychological symptoms or duration of illness; nor was there any significant relationship between levels of the two endocannabinoids. Additionally, circulating anandamide levels showed a significant inverse correlation with plasma leptin concentrations in healthy controls, anorexics and women with binge-eating disorder. These findings suggest the possibility of some derangement in the production of anandamide in women with these particular disorders, and the authors tentatively proposed that this reflected endocannabinoid mediation of the rewarding aspects of aberrant eating behaviors. The full significance of these findings will depend on verifying the source of anandamide that was measured, and the extent to which plasma levels reflect altered endocannabinoid regulation in the brain. Particularly difficult to interpret is the elevation of anandamide in both restricting, underweight anorexics and obese, overconsuming binge-eaters; although the authors suggested a possible link to leptin deficiency in the anorexic group and to leptin insensitivity in the binge eaters.

These complexities are multiplied by the finding that there may be genotypic differences between restricting and binge-purging subtypes of anorexia nervosa. Specifically, Siegfried et al. (2004) examined the frequency of a polymorphism of the human CB1 receptor gene, CNR1, and reported preferential transmission of different alleles in the patient subgroups. These authors suggested that the specific alleles do not necessarily increase an individual's susceptibility to developing anorexia nervosa, but may modify the form of its expression.

Finally, Sipe et al. (2005) recently reported obesity-related variations in a naturally occurring mis-sense polymorphism in the gene encoding fatty acid amide hydrolase (FAAH), the primary enzyme for inactivation of endocannabinoids. A homozygous FAAH 385A/A genotype was significantly associated with overweight and obesity in individuals with European or African ancestry, but not in a small group of Asians. Overall, the median body mass index (BMI) was significantly greater in the FAAH 385A/A genotype group compared to heterozygote and wild-type groups, with a higher frequency of the FAAH 385A/A genotype with increasing BMI: the strongest relationship was observed in the white cohort. The authors concluded that this mis-sense polymorphism could indicate an endocannabinoid risk factor in the development of overweight and obesity, specifically related to sensitivity to the rewarding effects of food.

The possibility of manipulating endocannabinoid systems in disease is already established – and not least in relation to appetite and body weight. With continuing advances, the number of therapeutic opportunities will also expand. We have already mentioned the use of THC to combat the reduction in appetite, metabolic dysfunction and wasting in AIDS and cancer (Plasse et al., 1991; Beal et al., 1995; Haney et al., 2005). However, the clinical application of THC in these conditions predates the current knowledge of endocannabinoids and their behavioural and metabolic functions. We anticipate that better understanding of these functions will produce enhanced treatments in future. Cannabinoids may also have application in the wasting and appetite loss associated with ageing and the dementias (Volker et al., 1997).

As the data discussed earlier imply, anorexia nervosa could be a potential target for the application of cannabinoids, despite its complex psychopathology. So far only a single study, in which THC was ineffective, has been reported; although more carefully designed studies may be more successful (Gross et al., 1983). There may also be useful interventions to be made in relation to the failure to thrive of infants. Recent work strongly suggests that endocannabinoid systems are vital to the ability of neonates to suckle (Fride et al., 2001). Thus when rimonabant is administered to newborn mice, milk ingestion and subsequent growth is completely inhibited, with fatal results. Similarly, CB1 knockout mice display deficient suckling at birth (Fride et al., 2005). Surprisingly, the initiation of the suckling response is further inhibited by rimonabant, suggesting the existence – and possible upregulation – of an additional non-CB1/non-CB2 receptor in the CB1–/– mice (Fride et al., 2003). Fride has proposed that at birth, brain 2-AG is sufficient to stimulate suckling, but that 2-AG present in maternal milk provides an essential additional stimulus to further feeding. By extrapolation, the oral-motor deficiency associated with the nursing and growth failure seen after CB1 receptor blockade in neonatal mice may indicate that a deficient endocannabinoid-CB1 receptor system may underlie the failure to thrive in human infants. Since 2-AG has also been detected in bovine and human milk (Di Marzo et al., 1998), and dietary supplementation with...
essential fatty acids has been shown to increase brain anandamide levels in young animals (Berger et al., 2001), deficiencies in maternal nutrition may lead to under-development of endocannabinoid systems in the fetus and neonate.

An obvious target for intervention in cannabinoid systems is the treatment of overweight and obesity. As we have seen, there is now a large body of evidence from animal studies to indicate the effectiveness of rimonabant and its analogues in reducing intake and, probably independently, to effect beneficial changes in the metabolic correlates of obesity. Moreover, recent Phase III clinical trials with rimonabant (Acomplia™) have indicated that the drug can effectively reduce weight and adiposity in obese people (for a critical evaluation of recently reported clinical data, see Vickers and Kennett, 2005). The first peer-reviewed report of a European trial (RIO-Europe) has recently been published (Van Gaal et al., 2005). In overweight and obese patients maintained on 20 mg/day for 1 year, rimonabant produced a mean weight reduction of 6.6 kg compared to only 1.8 kg after placebo (these data are for the intention to treat group; weight loss reached an average of 8.6 kg in completers). Significantly more of the rimonabant-treated group achieved greater than 10% weight loss, thus matching or exceeding the effects obtained with earlier classes of appetite suppressant. Additionally, the drug significantly reduced waist circumference, increased high-density lipoprotein (HDL)-cholesterol, lowered plasma free fatty acid levels, corrected hyperglycaemia, reduced plasma insulin levels and countered insulin resistance. Arguably, some of the reported effects exceed those that might be expected through weight loss alone, and may reflect the specifically peripheral actions of rimonabant discussed earlier; particularly in relation to the apparent ability of rimonabant to stimulate adiponectin. Although side-effects were reported to be minimal and transient, mood disorders were more prevalent in the 20 mg/day rimonabant group, which raises important questions as to the psychological consequences of disrupting a system that is potentially crucial to general reward processes.

Conclusion

The data reviewed here clearly indicate an important role of endocannabinoids in the processes that normally regulate appetite and feeding behaviour. The additional involvement of cannabinoid mechanisms in the peripheral regulation of adiposity and energy metabolism suggests that endocannabinoids represent a potentially unique target for the treatment of disorders of appetite and body weight regulation. The combined ability of rimonabant to regulate hormones linked to fat and glucose metabolism, promote weight loss, and to suppress food intake indicates that CB1 antagonists may be a powerful tool in the treatment of obesity and metabolic syndrome. Although publication in this area is increasing exponentially, further research into the behavioural actions of the endocannabinoids themselves (as opposed to CB1 antagonists) is an urgent priority. The outcome is likely to be a fuller understanding of motivational mechanisms in general, and a profitable target for clinically significant pharmaceutical developments; not only for suppressing appetite and body weight, but also to ameliorate appetite-loss and wasting in disease.

References


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