

Reversal of Δ^9 -THC hyperphagia by SR141716 and naloxone but not dexfenfluramine

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Abstract

Presatiated adult male Lister hooded rats received oral administration of the exogenous cannabinoid Delta-9-tetrahydrocannabinol (Δ^9 -THC; 1.0 mg/kg) in combination with subcutaneous injection of either the cannabinoid CB1 antagonist *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR141716; 0, 0.05, 0.1, 0.5 or 1.0 mg/kg), the CB2 antagonist *N*-[(1*S*)-endo-1,3,3-trimethyl bicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528; 0, 0.05, 0.1, 0.5 or 1.0 mg/kg), the general opioid antagonist naloxone (0.1, 0.5, 1.0 or 5.0 mg/kg) or the 5-HT agonist dexfenfluramine (0.05, 0.1, 0.5, 1.0 or 5.0 mg/kg). Food (chow) intake was measured over 2 h from the onset of the dark period. Δ^9 -THC induced significant hyperphagia, which was attenuated by subanorectic doses of SR141716 and naloxone. Neither SR144528 nor dexfenfluramine affected Δ^9 -THC-induced feeding. These data confirm mediation of Δ^9 -THC hyperphagia by central-type CB1 receptors, and support a functional relationship between cannabinoid and opioid systems in relation to appetite regulation. Stimulation of CB1 receptors may promote feeding by actions on food reward rather than by inhibition of serotonergic satiety mechanisms. © 2002 Elsevier Science Inc. All rights reserved.

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1. Introduction

The past decade has seen the discoveries of cannabinoid receptors within the central nervous system (Devane et al., 1988; Matsuda et al., 1990) and their endogenous ligands, the endocannabinoids arachidonoyl ethanolamide (anandamide; Devane et al., 1992), 2-arachidonoyl glycerol (2-AG; Mechoulam et al., 1995) and 2-arachidonoyl glyceryl ether (noladin; Hanus et al., 2001). Brain endocannabinoid systems have now been implicated in a number of physiological processes and, increasingly, the neural regulation of behaviour. Evidence so far indicates that endocannabinoids acting at the central type CB1 receptor may play an important role in the regulation of appetite for food. In animal models, the endogenous cannabinoids anandamide and 2-AG both increase feeding (Williams and Kirkham, 1999; Hao et al. 2000; Kirkham and Williams, 2001b; Williams and Kirk-

ham, 2001), while CB1 cannabinoid receptor blockade has been shown to suppress eating (Arnone et al., 1997; Colombo et al., 1998; Simiand et al., 1998).

Stimulation of appetite is one of the most commonly related effects of marijuana intoxication in humans (Abel, 1975), an effect that is ascribed to the effects of the exogenous cannabinoid Delta-9-tetrahydrocannabinol (Δ^9 -THC; Kirkham and Williams, 2001b). This hyperphagic action of Δ^9 -THC is presumed to involve stimulation of CB1 cannabinoid receptors, which are widely distributed throughout the brain (Herkenham et al., 1991), rather than CB2 receptors which are only expressed in peripheral tissues (Munro et al., 1993; Galiègue et al., 1995). Some of the psychological effects of Δ^9 -THC in humans have been shown to be mediated by the CB1 receptor, being blocked by administration of the selective antagonist, *N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR141716) (Huestis et al., 2001). However, to date, no thorough pharmacological characterisation of Δ^9 -THC-hyperphagia has been conducted. Given the renewed interest in the medicinal use of marijuana-based therapies and the clinical application of

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Δ^9 -THC to improve appetite in patients with cancer cachexia or HIV-induced wasting (Plassé et al., 1991; Beal et al., 1995), there is a clear need to explore the precise behavioural and motivational adjustments induced by the drug, together with their neurochemical underpinnings.

Various aspects of the behavioural pharmacology of Δ^9 -THC have been well-described previously (e.g., Fride and Mechoulam, 1993). However, the literature on Δ^9 -THC and feeding in animal models is relatively sparse, limited largely to rather inconclusive reports published in the 1970s (Kirkham and Williams, 2001b). We have reported that in satiated rats low doses of Δ^9 -THC will induce substantial, dose-dependent hyperphagia (Williams et al., 1998). Interestingly, this action of the drug appears to be enhanced when animals are fed palatable, high fat diets (Koch, 2001). Previously, Foltin et al. (1988) found that Δ^9 -THC administered in marijuana cigarettes preferentially increased the consumption of palatable food items like candy bars, cookies and cakes. Together with an apparently preferential suppression of preferred, palatable ingesta in animals by the CB1 antagonist SR141716 (Arnone et al., 1997; Simiand et al., 1998), such data have led to the hypothesis that cannabinoid receptor agonists may promote feeding by amplifying the incentive or reward value of foods. Indeed, cannabinoid involvement in general reward processes is supported by a number of different studies showing, for example, that SR141716 will reduce rats' sensitivity to rewarding electrical brain stimulation (Deroche-Gamonet et al., 2001), and prevent the acquisition of drug- or food-induced place preferences (Chaperon et al., 1998). Nevertheless, given the paucity of data, it is possible that cannabinoid hyperphagia may involve effects on other aspects of feeding regulation. For example, overconsumption may result from an inhibition of one or other of the chemical satiety signals that have been proposed to regulate appetite (Clapham et al., 2001).

The present experiments were designed to extend the pharmacological analysis of the hyperphagia produced by Δ^9 -THC. By comparing the effects of antagonists with selectivity for the predominantly central CB1 cannabinoid receptor (SR141716; Rinaldi-Carmona et al., 1994) or peripherally expressed CB2 receptors (*N*-[(1*S*)-endo-1,3,3-trimethyl bicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528); Rinaldi-Carmona et al., 1998), we sought to confirm specific cannabinoid receptor mediation of Δ^9 -THC hyperphagia.

In addition, we examined the potential involvement of reward systems in the feeding actions of Δ^9 -THC. A variety of experiments have suggested strong links between cannabinoids and endogenous opioid systems (Manzanares et al., 1999). Opioids are, in turn, associated with reward processes (Gardner and Vorel, 1998), including the pleasure associated with eating (Cooper and Kirkham, 1993). Previously, we have shown synergistic effects on feeding of combined blockade of CB1 and opioid receptors (Kirkham

and Williams, 2001a,b). Paired administration of low, sub-anorectic doses of SR141716 and the general opioid receptor antagonist naloxone produced marked anorexia, far exceeding the sum of the weak independent suppressive effects of either drug alone. Those data were interpreted as evidence for an important functional relationship between cannabinoids and opioids in relation to feeding. Here, we assess the effects of naloxone on Δ^9 -THC hyperphagia, to examine any opioid involvement in this action of the exogenous agonist.

As already noted, hyperphagia may also result from the inhibition of the normal process of satiation which limits meal size, or by some action on the state of intermeal satiety. If cannabinoid-induced hyperphagia involves such interactions, we might expect that Δ^9 -THC-feeding could be attenuated by manipulations which restore, or amplify, the normal functioning of satiety regulators. Among the various putative satiety signals proposed to regulate feeding, a vast body of data has accumulated to implicate serotonin (5-HT; Dourish, 1995). Just as cannabinoids have been linked to alterations in the functioning of endogenous opioids, activity at CB1 receptors has also been found to affect serotonergic neurotransmission (Nakazi et al., 2000), and anandamide may even exert some of its behavioural effects at brain 5-HT receptors (Kimura et al., 1998). To probe possible cannabinoid-serotonergic satiety interactions, we also examined the effect on Δ^9 -THC-induced eating of the potent anorectic compound dexfenfluramine, an indirect 5-HT agonist.

2. Methods

2.1. Animals

Forty male Lister hooded rats (Charles River), ~400 g, were housed individually under a reversed 12:12-h light–dark cycle (lights off at 10:00 h). Rats had free access to laboratory chow and tap water, except for 1-h food withdrawal on test days. All testing was conducted during the dark phase of the daily cycle. Animals were fully habituated to handling, feeding and drug administration procedures prior to the start of each experiment. The studies were conducted in accordance with the specifications of the United Kingdom Animals (Scientific Procedures) Act, 1986.

2.2. Drugs

In order to fully assess possible interactions between cannabinoid-mediated processes and opioid and serotonergic systems affecting feeding, a range of subanorectic doses of SR141716, SR144528, naloxone and fenfluramine were used (Kirkham and Williams, 2001a; Williams, 1999). A single dose of Δ^9 -THC (1 mg/kg) was chosen, having been shown to be optimal for promoting acute

hyperphagia in rats, while avoiding the appearance of sedative or motoric side effects (Williams et al., 1998; Koch, 2001). Oral administration was used as it has been shown to be an effective and reliable route for the induction of Δ^9 -THC hyperphagia (Brown et al., 1977; Williams et al., 1998).

Fresh solutions of drugs were prepared on each test day, 15 min before administration. Δ^9 -THC (in ethanol solution; Sigma) was dissolved in a palatable sesame seed oil vehicle and administered orally from a 1-ml syringe, in a volume of 1 ml/kg (Brown et al., 1977). SR141716A (Sanofi-Synthelabo, Montpellier, France) and SR144528 (Sanofi-Synthelabo, Montpellier, France) were suspended in 10% DMSO solution and administered subcutaneously, in a volume of 1 ml/kg. Naloxone hydrochloride (Sigma) and dexfenfluramine hydrochloride (Servier, Neuilly sur Seine, France) in 0.9% saline were administered subcutaneously in a volume of 1 ml/kg.

2.3. Statistics

Food intake data for each measurement interval were subjected to two-way ANOVA for repeated measures. Individual treatments were compared by Newman–Keuls test for multiple comparisons. All data were analysed using Statistica.

2.3.1. Experiment 1: effects of SR141716 on Δ^9 -THC hyperphagia

A prefeed design was adopted in order to induce low baseline food intake and allow the expression of cannabinoid hyperphagia (Williams et al., 1998). At dark onset (10:00), rats ($n = 10$) were presatiated by allowing 2 h access to 30 g of a palatable wet mash diet, consisting of 200 ml ground chow (Rat and Mouse Expanded Ground Diet; Special Diet Services, Witham, England) plus 250 ml tap water. At 12:00 h, all food was removed from cages and rats received oral doses of either sesame oil vehicle or Δ^9 -THC (1.0 mg/kg). Oral dosing was achieved by slowly dripping the test solution into the buccal cavity from a standard 1-ml syringe in a volume of 1 ml/kg, and allowing voluntary ingestion by each rat. Prior habituation to this technique, together with the palatability of the vehicle ensured that each rat consumed all of the specified doses.

At 12:30 h, rats were injected subcutaneously (1 ml/kg) with the specific CB1 receptor antagonist SR141716 (0, 0.05, 0.1, 0.5 or 1.0 mg/kg). Weighed food (chow) was made available from 13:00 h and food intake (adjusted for spillage) was subsequently measured after 1 and 2 h. This test length was chosen as our previous studies have shown Δ^9 -THC hyperphagia to be limited to 2 h under these conditions (Williams et al., 1998).

All animals received all treatments, counterbalanced according to a Latin square design, with at least 48 h separating successive treatments.

2.3.2. Experiment 2: effects of SR144528 on Δ^9 -THC hyperphagia

Using an identical procedure, a second group of 10 prefed rats were treated orally with Δ^9 -THC (1.0 mg/kg) or vehicle at 12:00 h. At 12:30 h, rats were injected with the selective CB2 receptor antagonist SR144528 (0, 0.05, 0.1, 0.5 or 1.0 mg/kg). Food was restored at 13:00 h and intake was measured after 1 and 2 h.

2.3.3. Experiment 3: effects of naloxone on Δ^9 -THC hyperphagia

Following the method outlined above, a third group of rats ($n = 10$) were prefed and subsequently treated with vehicle or Δ^9 -THC (1.0 mg/kg). At 12:30 h, rats were injected with either vehicle or naloxone (0.1, 0.5, 1.0 or 5.0 mg/kg). Preweighed food was presented at 13:00 h and subsequent food intake was measured after 1 and 2 h.

2.3.4. Experiment 4: effects of dexfenfluramine on Δ^9 -THC hyperphagia

Using the above procedure, a further group of prefed rats ($n = 10$) were treated orally with Δ^9 -THC (1.0 mg/kg) or

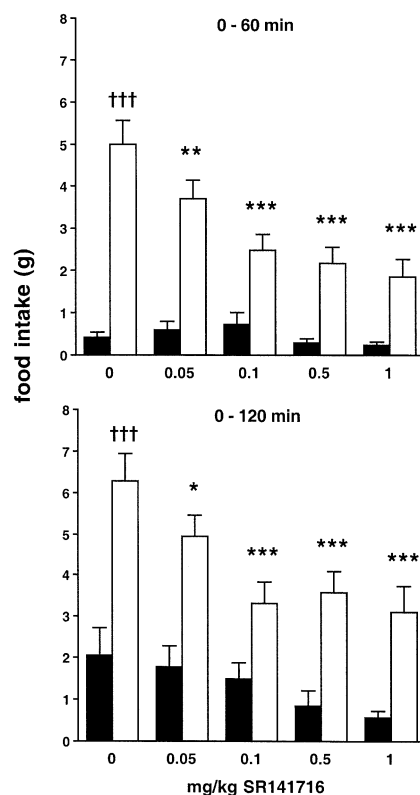


Fig. 1. Reversal of Δ^9 -THC hyperphagia in prefed rats by the selective CB1 antagonist SR141716. Solid bars represent effects of SR141716 alone; open bars display results of combined administration of Δ^9 -THC and SR141716. All values represent the mean ($\pm$ S.E.M.) food intake of 10 rats after 1 and 2 h of free access to chow. +++ Significant increase in intake compared to the vehicle–vehicle control ($P < .001$). * $P < .05$, ** $P < .01$, *** $P < .001$: significant attenuation of Δ^9 -THC feeding by SR141716.

vehicle at 12:00 h. At 12:30 h, rats were injected with either vehicle or dexfenfluramine (0.05, 0.1, 0.5, 1.0 or 5.0 mg/kg). At 13:00 h, preweighed chow was returned to the animals and food intake was subsequently measured after 1 and 2 h.

3. Results

Prefeeding, combined with the presentation of plain chow as the test diet, successfully produced low baseline test intakes, allowing the expression of the potent hyperphagic action of Δ^9 -THC. In each experiment, Δ^9 -THC produced at least a two-fold increase in total test intake, although actual hyperphagia was confined to the first hour of testing. Cannabinoid-induced overeating exhibited differential sensitivity to the coadministration of SR141716, SR144528, naloxone and dexfenfluramine.

In Experiment 1, Δ^9 -THC significantly increased food intake during the first hour of testing [$F(1,9)=133.16$, $P<.0001$]. This action was dose-dependently attenuated by the CB1 antagonist SR141716 [$F(4,36)=10.52$, $P<.0001$]: 0.05, 0.1, 0.5 and 1.0 mg/kg SR141716 suppressed agonist-induced intake by 26%, 50%, 57% and 63%, respectively

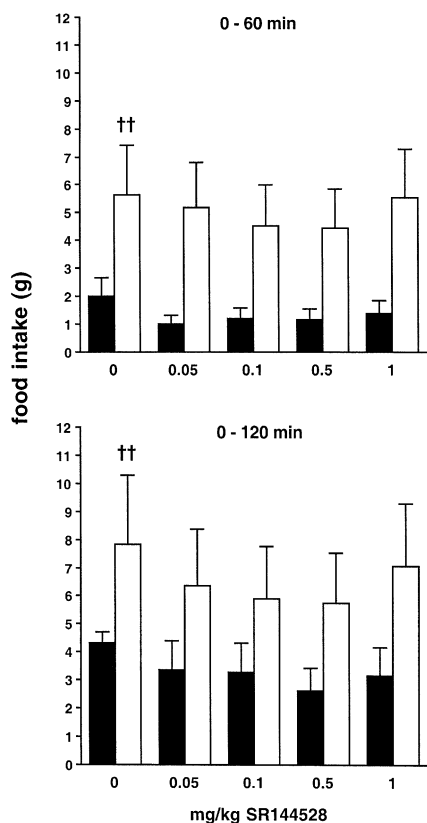


Fig. 2. Lack of effect of the CB2 antagonist SR144528 on Δ^9 -THC hyperphagia. Solid bars represent effects of SR144528 alone; open bars display results of combined administration of Δ^9 -THC and SR144528. †† Significant increase in intake compared to the vehicle–vehicle control ($P<.001$).

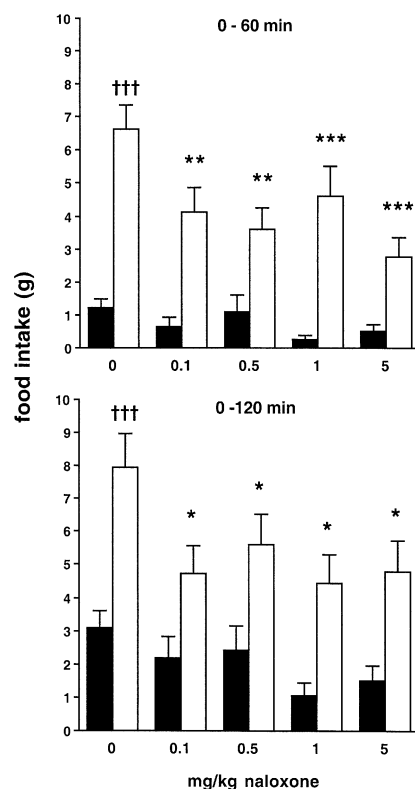


Fig. 3. Reversal of Δ^9 -THC hyperphagia in prefed rats by the general opioid antagonist naloxone. Solid bars represent effects of naloxone alone; open bars display results of combined administration of Δ^9 -THC and naloxone. ††† Significant increase in intake compared to the vehicle–vehicle control ($P<.001$). * $P<.05$, ** $P<.01$, *** $P<.001$: significant attenuation of Δ^9 -THC feeding by naloxone.

(Fig. 1). Administration of the antagonist alone had no reliable effect on intake during this period. During the second hour, low baseline intakes were unaffected by the agonist, and no effects of SR141716 alone were evident. Consequently, total 2-h intake measures retained both the significant elevation of intake by Δ^9 -THC [$F(1,9)=227.65$, $P<.0001$] and the dose-dependent attenuation of that hyperphagia by SR141716 [$F(4,36)=7.16$, $P<.001$]. Although there was a tendency for SR141716 to suppress intake across the whole test when administered alone, this effect was not significant. It is clear from the graph of total intake that the lowest antagonist doses, which alone had no suppressant action, reliably suppressed Δ^9 -THC hyperphagia. Moreover, for the first hour, a significant interaction between the effects of SR141716 alone or when combined with Δ^9 -THC [$F(4,36)=11.23$, $P<.001$] indicates that any hypophagic effects of the antagonist are insufficient to account for the observed attenuation of Δ^9 -THC hyperphagia.

In Experiment 2, Δ^9 -THC reliably increased total test intake [$F(1,9)=58.95$, $P<.0001$], again through a marked hyperphagic action during hour 1 [$F(1,9)=66.08$, $P<.0001$] (Fig. 2). In contrast to SR141716, the selective CB2 antagonist SR144528 had no effect on this agonist-induced

feeding, and exerted no obvious behavioural effects on its own [$F(4,36) = 1.46$, NS].

The results from Experiment 3 (Fig. 3) reveal that a significant increase in first hour feeding by Δ^9 -THC [$F(1,9) = 120.07$, $P < .0001$] was attenuated by the general opioid receptor antagonist naloxone [$F(4,36) = 6.98$, $P < .0001$]. This latter effect was evident at all naloxone doses, despite their failure to exhibit any reliable hypophagic effects when administered alone. Naloxone treatment reduced Δ^9 -THC-induced hyperphagia by 38%, 46%, 31% and 58% for 0.1, 0.5, 1.0 and 5.0 mg/kg, respectively. The lack of a marked naloxone dose–response is typical of the anorectic actions of this drug in rats feeding the relatively bland maintenance diet (Kirkham and Blundell, 1984).

Finally, in Experiment 4, the typical pattern of Δ^9 -THC-induced feeding was again observed (Fig. 4), with the drug inducing significant hyperphagia during the first hour [$F(1,9) = 221.77$, $P < .0001$]. Over this same period, dexfenfluramine displayed a tendency to reduce intake when administered alone, particularly at the highest dose [$F(5,45) = 3.17$, $P < .05$]. However, even 5 mg/kg dexfenfluramine failed to significantly attenuate Δ^9 -THC hyperphagia [$F(5,45) = 0.58$, NS]. A similar profile was maintained across

the whole test, with 5 mg/kg dexfenfluramine alone significantly suppressing total 2-h intake ($P < .01$), but not reliably affecting cannabinoid-induced eating ($P = .097$).

4. Discussion

These experiments have confirmed previous reports of the ability of peripherally administered Δ^9 -THC to promote overconsumption in rats (Williams et al., 1998; Koch, 2001). Although Δ^9 -THC is an agonist at both CB1 and CB2 receptors, the ability of SR141716, but not SR144528, to reverse these effects indicates that Δ^9 -THC hyperphagia is primarily mediated by the CB1 cannabinoid receptor. Although CB1 receptors are expressed in a number of peripheral tissues, they occur predominantly in the central nervous system (Breivogel and Childers, 1998). By contrast, CB2 receptors are not expressed in brain (Munro et al., 1993). So while not precluding some peripheral action of Δ^9 -THC, our data support a role for central, CB1-linked cannabinoid systems in the control of eating, and are in agreement with the widely accepted proposition that the behavioural effects of exogenous cannabinoids derive from actions on brain endocannabinoid systems (Ameri, 1999).

Previously, we have demonstrated that hyperphagia induced by administration of the endogenous cannabinoid anandamide is attenuated by SR141716 but not SR144528 (Williams and Kirkham, 1999). Taken together with reports that acute and chronic CB1 blockade by SR141716 (albeit at somewhat higher doses than those used here) will reliably suppress food intake (Arnone et al., 1997; Colombo et al., 1998; Simiand et al., 1998), our data suggest that blockade of CB1 receptors interrupts important feeding-related, endocannabinoid activity, and that Δ^9 -THC induces hyperphagia by mimicking or facilitating that activity.

It should be noted that SR141716 did not completely block Δ^9 -THC hyperphagia. This contrasts with the ability of the antagonist to abolish the feeding induced by exogenously administered anandamide (Williams and Kirkham, 1999). It is possible that Δ^9 -THC effects involve actions at targets other than CB1 receptors, or as yet unidentified subtypes of the CB1 receptor. Alternatively, the greater resistance of Δ^9 -THC to CB1 antagonism may reflect the persistent activity of the potent, centrally active metabolite 11-hydroxy- Δ^9 -THC (Abood and Martin, 1992).

Previously, we have hypothesized that the overconsumption produced by cannabinoids in satiated animals may be explicable in terms of either an increase in the palatability of the test food, or an inhibition of intermeal satiety mechanisms (Williams et al., 1998). These effects may, respectively, be associated with altered activity in opiodergic reward or serotonergic satiety systems.

Dexfenfluramine, acting as an indirect 5-HT agonist, reliably suppresses food intake by facilitating serotonergic processes involved in the satiation of eating and maintenance of postprandial satiety (Grignaschi and Samanin,

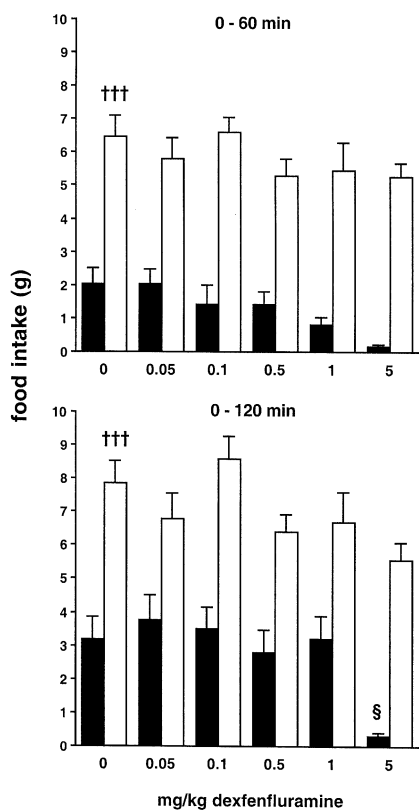


Fig. 4. Lack of effect of the 5-HT agonist dexfenfluramine on Δ^9 -THC hyperphagia. Solid bars represent effects of dexfenfluramine alone; open bars display results of combined administration of Δ^9 -THC and dexfenfluramine. ††† Significant increase in intake compared to the vehicle–vehicle control ($P < .001$). § Significant intake suppression by dexfenfluramine alone, compared to control ($P < .01$).

1992). The animals in our experiments were clearly satiated by overconsumption of food presented during the pre-feed stage: under control conditions, significant feeding was not apparent until the third hour following the removal of the prefeed (second hour of the intake test). Administration of dexfenfluramine might be expected to reinforce such meal-induced satiety. However, Δ^9 -THC produced equivalent levels of hyperphagia when given alone or when combined with even high, normally anorectic doses of dexfenfluramine. This implies that cannabinoid-induced overeating does not involve inhibition of serotonergic satiety processes. Of course, the lack of effect of dexfenfluramine on Δ^9 -THC-feeding does not preclude some interaction between endocannabinoid receptor stimulation and modulation of nonserotonergic satiety signals. Indeed, recent reports that elevated hypothalamic endocannabinoid levels in genetically obese rats and mice may be reduced by leptin administration (Di Marzo et al., 2001) suggest that endocannabinoids may be a crucial component of systems regulating long-term energy balance and food intake. Thus, interactions between the endocannabinoids and neurochemical systems involved in satiation may still be possible and deserve further investigation.

In contrast, the effects of the general opioid receptor antagonist naloxone on Δ^9 -THC feeding provide rather more straightforward support for some enhancement of food reward in cannabinoid hyperphagia. In this study, even low subanorectic naloxone doses were able to significantly attenuate Δ^9 -THC-induced feeding. Opioid involvement in food reward is now well established, with opioid receptor agonists and antagonists respectively increasing or reducing food intake in animal and human models. These effects are most marked when palatable ingesta are consumed (Cooper and Kirkham, 1993); in people, opioid receptor antagonists are reported to diminish the pleasantness of normally palatable foods (Drewnowski et al., 1995). The animals in the present tests were eating bland lab chow and, as our control data indicate, this food was relatively insensitive to the anorectic effects of naloxone. It is tempting to suggest that the enhanced anorectic potency of naloxone after Δ^9 -THC treatment reflects an action of the cannabinoid to increase the rewarding properties of the test food. Such an action may be mediated directly by endocannabinoid systems, or indirectly via alterations to opioid function. A number of studies have indicated that cannabinoids may increase the synthesis and/or release of opioids (Corchero et al., 1997, 1998, 1999; Manzanares et al., 1998, 1999). Possibly, Δ^9 -THC stimulation of cannabinoid receptors may promote activity in opioidergic reward pathways, thereby increasing food palatability and contributing to the elevated intake levels. Enhanced orosensory reward resulting from activation of opioid systems could then account for the greater suppressive effects of naloxone after Δ^9 -THC.

An alternative, if less attractive, account could be that the greater anorectic potency of naloxone in Δ^9 -THC treated rats is merely an artifact of higher baseline intake levels.

However, we have shown previously that these same low naloxone doses are ineffective in free-feeding, nonsatiated rats eating equivalent amounts to the Δ^9 -THC treated rats in Experiment 3 (Kirkham and Williams, 2001a).

There is in fact good evidence for significant convergent interactions between cannabinoid and opioid systems in relation to ingestive behaviour. For example, naloxone has been shown to reverse the facilitatory effects of the cannabinoid agonist CP-55,940 on the intake of palatable solutions such as beer and sucrose (Gallate et al., 1999), and of Δ^9 -THC on the feeding elicited by electrical stimulation of the lateral hypothalamus (Trojnar and Wise, 1991). Moreover, we have recently reported that combined administration of low, normally subanorectic doses of SR141716 and naloxone produces substantial, supra-additive intake suppression (Kirkham and Williams, 2001a,b).

Such findings are in line with a number of reports which indicate the existence of functional relationships between cannabinoid and opioid systems. For example, Ledent et al. (1999) reported that the reinforcing properties of opiates were reduced in CB1 receptor knockout mice. Similarly, Navarro et al. (2001) reported that SR141716 blocked heroin self-administration in rats, and morphine-induced place preference and morphine self-administration in mice. Functional opioid–cannabinoid associations have also been reported in relation to reward processes and the activity of mesolimbic dopaminergic pathways (Spanagel and Weiss, 1999). Endogenous opioids modulate the activity of this system by removing GABAergic inhibition of ventral tegmental dopamine neurons. Natural rewards, like food, cause dopamine release from terminals of these neurons within the nucleus accumbens, an effect which is mimicked by doses of Δ^9 -THC that can induce hyperphagia (Gardner, 1992; Tanda et al., 1997; Gardner and Vorel, 1998; Ameri, 1999). Importantly, cannabinoid stimulation of accumbens dopamine release is blocked by both the CB1 antagonist SR141716 and the opioid receptor antagonist naloxonazine (French, 1997; Tanda et al., 1997; Gessa et al., 1998).

To summarize, the exogenous cannabinoid Δ^9 -THC reliably produced hyperphagia in satiated rats. This effect appears to be largely mediated by central-type CB1 cannabinoid receptors since it was attenuated by the selective CB1 antagonist SR141716, but not by SR144528, an antagonist of peripheral CB2 receptors. Feeding induced by Δ^9 -THC was insensitive to high, anorectic doses of the 5-HT agonist dexfenfluramine, suggesting that cannabinoid hyperphagia does not depend on inhibition of serotonergic satiety mechanisms for its expression. By contrast, the opioid receptor antagonist naloxone effectively reversed Δ^9 -THC hyperphagia, even at doses, which exerted no intake suppression when administered alone. These data provide further evidence for functional relationships between endogenous cannabinoid and opioid systems in relation to motivational processes, and suggest the involvement of endocannabinoids in the hedonic evaluation of foods. Future research must therefore assess the susceptibility of feeding induced by the endogenous

cannabinoids to selective opioid receptor blockade, and investigate how these cannabinoid–opioid interactions are affected by modifications to food palatability. Overall, these studies have extended the pharmacological characterisation of the feeding effects of the exogenous cannabinoid Δ^9 -THC, and strengthen support for a role of endocannabinoid systems in the physiological regulation of appetite.

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References

- Abel EL. Cannabis: effects on hunger and thirst. *Behav Biol* 1975;15:255–81.
- Aboud ME, Martin BR. Neurobiology of marijuana abuse. *Trends Pharmacol Sci* 1992;13:201–6.
- Ameri A. The effects of cannabinoids on the brain. *Prog Neurobiol* 1999;58:315–48.
- Arnone M, Maruani J, Chaperon F, Thiebot MH, Poncelet M, Soubrié P, LeFur G. Selective inhibition of sucrose and ethanol intake by SR141716, an antagonist of central cannabinoid (CB1) receptors. *Psychopharmacology* 1997;132:104–6.
- Beal JE, Olson R, Laubenstein L, Morales JO, Bellman P, Yangco B, Lefkowitz L, Plasse TF, Shepard KV. Dronabinol as a treatment for anorexia associated with weight loss in patients with AIDS. *J Pain Symptom Manage* 1995;10:89–97.
- Breivogel CS, Childers SR. The functional neuroanatomy of brain cannabinoid receptors. *Neurobiol Dis* 1998;5:417–31.
- Brown JE, Kassoum M, Cross JK. Kinetic studies of food intake and sucrose solution preference by rats treated with low doses of delta-9-tetrahydrocannabinol. *Behav Biol* 1977;20:104–10.
- Chaperon F, Soubrié P, Puech AJ, Thiebot MH. Involvement of central cannabinoid (CB1) receptors in the establishment of place conditioning in rats. *Psychopharmacology* 1998;135:324–32.
- Clapham JC, Arch JRS, Tadyyon M. Anti-obesity drugs: a critical review of current therapies and future opportunities. *Pharmacol Ther* 2001;89:81–121.
- Colombo G, Agabio R, Diaz G, Lobina C, Reali R, Gessa GL. Appetite suppression and weight loss after the cannabinoid antagonist SR141716. *Life Sci* 1998;63:PL113–7.
- Cooper SJ, Kirkham TC. Opioid mechanisms in the control of food consumption and taste preferences. In: Herz A, Akil H, Simon EJ, editors. *Handbook of experimental pharmacology* vol. 104. Berlin: Springer-Verlag, 1993. pp. 239–63 (Part II).
- Corchero J, Fuentes JA, Manzanares J. Delta(9)-tetrahydrocannabinol increases proopiomelanocortin gene expression in the arcuate nucleus of the rat hypothalamus. *Eur J Pharmacol* 1997;323:193–5.
- Corchero J, Garcia-Gil L, Manzanares J, Fernandez-Ruiz JJ, Fuentes JA, Ramos JA. Perinatal delta(9)-tetrahydrocannabinol exposure reduces proenkephalin gene expression in the caudate–putamen of adult female rats. *Life Sci* 1998;63:843–50.
- Corchero J, Manzanares J, Fuentes JA. Repeated administration of delta(9)-tetrahydrocannabinol produces a differential time related responsiveness on proenkephalin, proopiomelanocortin and corticotropin releasing factor gene expression in the hypothalamus and pituitary gland of the rat. *Neuropharmacology* 1999;38:433–9.
- Deroche-Gamonet V, Le Moal M, Piazza PV, Soubrié P. SR141716, a CB1 receptor antagonist, decreases the sensitivity to the reinforcing effects of electrical brain stimulation in rats. *Psychopharmacology*. (online publication DOI 10.1007/s002130100804).
- Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC. Determination and characterisation of a cannabinoid receptor in rat brain. *Mol Pharmacol* 1988;34:605–13.
- Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R. Isolation of a brain constituent that binds to the cannabinoid receptor. *Science* 1992;258:1946–9.
- Di Marzo V, Goparaju SK, Wang L, Liu J, Batkai S, Jarai Z, Fezza F, Miura GI, Palmiter RD, Sugiura T, Kunos G. Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature* 2001;410:822–5.
- Dourish CT. Multiple serotonin receptors—opportunities for new treatments for obesity. *Obes Res* 1995;3:S449–62.
- Drewnowski A, Krahn DD, Demitrack MA, Naim K, Gosnell BA. Naloxone, an opiate blocker, reduces the consumption of sweet high-fat foods in obese and lean female binge eaters. *Am J Clin Nutr* 1995;61:1206–12.
- Foltin RW, Fischman MW, Byrne MF. Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. *Appetite* 1988;11:1–14.
- French ED. Delta(9)-Tetrahydrocannabinol-excites rat VTA dopamine neurons through activation of cannabinoid CB1 but not opioid receptors. *Neurosci Lett* 1997;246:159–62.
- Fride E, Mechoulam R. Pharmacological activity of the cannabinoid receptor agonist Δ^9 -tetrahydrocannabinol. *J Pharmacol Exp Ther* 1993;272:13–314.
- Galiègue S, Mary S, Marchand J, Dussossoy D, Carrière D, Carayon P, Bouaboula M, Shire D, Le Fur G, Casellas P. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur J Pharmacol* 1995;232:54–61.
- Gallate JE, Saharov T, Mallet PE, McGregor IS. Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. *Eur J Pharmacol* 1999;370:233–40.
- Gardner EL. Cannabinoid interaction with brain reward systems—the neurobiological basis of cannabinoid abuse. In: Murphy LL, Bartke A, editors. *Marijuana/cannabinoids: neurobiology and neurophysiology*. New York: CRC Press, 1992. pp. 275–335.
- Gardner EL, Vorel SR. Cannabinoid transmission and reward-related events. *Neurobiol Dis* 1998;5:502–33.
- Gessa GL, Melis M, Muntoni AL, Dianan M. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB1 receptors. *Eur J Pharmacol* 1998;341:39–44.
- Grignaschi G, Samanin R. Role of 5-HT receptors in the effect of *d*-fenfluramine on feeding patterns in the rat. *Eur J Pharmacol* 1992;212:287–9.
- Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE, Kustanovich I, Mechoulam R. 2-Arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci USA* 2001;98:3662–5.
- Hao S, Avraham Y, Mechoulam R, Berry EM. Low dose anandamide affects food intake, cognitive function, neurotransmitter and corticosterone levels in diet-restricted mice. *Eur J Pharmacol* 2000;392:147–56.
- Herkenham M, Lynn AB, Johnson MR, Melvin LS, De Costa BR, Rice KC. Characterisation and localisation of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci* 1991;11:563–83.
- Huestis MA, Gorelick DA, Heishman SJ, Preston KL, Nelson RA, Moolchan ET, Frank RA. Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. *Arch Gen Psychiatry* 2001;58:322–8.
- Kimura I, Ohta T, Watanabe K, Yoshimura H, Yamamoto I. Anandamide, an endogenous cannabinoid receptor ligand, also interacts with 5-hydroxytryptamine (5-HT) receptor. *Biol Pharm Bull* 1998;21:224–6.
- Kirkham TC, Blundell JE. Dual action of naloxone revealed by behavioural analysis: separate effects on initiation and termination of eating. *Appetite* 1984;5:45–52.

- Kirkham TC, Williams CM. Synergistic effects of opioid and cannabinoid antagonists on food intake. *Psychopharmacology* 2001a;153:267–70.
- Kirkham TC, Williams CM. Endogenous cannabinoids and appetite. *Nutr Res Rev* 2001b;14:65–86.
- Koch JE. Δ^9 -THC stimulates food intake in Lewis rats: effects on chow, high-fat and sweet high-fat diets. *Pharmacol Biochem Behav* 2001;68:539–43.
- Ledent C, Valverde O, Cossu G, Petite F, Aubert J-F, Baslet F, Bohme GA, Imperato A, Pedrazzini T, Roques BP, Vassart G, Fratta W, Parmentier M. Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* 1999;283:401–4040.
- Manzanares J, Corchero J, Romero J, Fernandez-Ruiz JJ, Ramos JA, Fuentes JA. Chronic administration of cannabinoids regulates proenkephalin mRNA levels in selected regions of the rat brain. *Mol Brain Res* 1998;55:126–32.
- Manzanares J, Corchero J, Romero J, Fernandez-Ruiz JJ, Ramos JA, Fuentes JA. Pharmacological and biochemical interactions between opioids and cannabinoids. *Trends Pharmacol Sci* 1999;20:287–94.
- Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* 1990;346:561–4.
- Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR, Pertwee RG, Griffin G, Bayewitch M, Barg J, Vogel Z. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem Pharmacol* 1995;50:83–90.
- Munro S, Thomas KL, Abu-Shaar M. Molecular characterisation of a peripheral receptor for cannabinoids. *Nature* 1993;365:61–5.
- Nakazi M, Bauer U, Nickel T, Kathmann M, Schlicker E. Inhibition of serotonin release in the mouse brain via presynaptic cannabinoid CB1 receptors. *N-S Arch Pharmacol* 2000;361:19–24.
- Navarro M, Carrera MRA, Fratta W, Valverde O, Cossu G, Fattore L, Chowen JA, Gómez R, del Arco I, Villanua MA, Maldonado R, Koob GF, Rodríguez de Fonseca F. Functional interaction between opioid and cannabinoid receptors in drug self-administration. *J Neurosci* 2001;21:5344–50.
- Plassé TF, Gorter RW, Krasnow SH, Lane M, Shepard KY, Wadleigh RG. Recent clinical experience with dronabinol. *Pharmacol Biochem Behav* 1991;40:695–700.
- Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D, Ferrara P, Soubrié P. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* 1994;350:240–4.
- Rinaldi-Carmona M, Barth F, Millan J, Derocq JM, Casellas P, Congy C, Oustric D, Sarran M, Bouaboula M, Calandra B, Portier M, Shire D, Breliere JC, Le Fur GL. SR144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J Pharmacol Exp Ther* 1998;284:644–50.
- Simiand J, Keane M, Keane PE, Soubrié P. SR141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. *Behav Pharmacol* 1998;9:179–81.
- Spanagel R, Weiss F. The dopamine hypothesis of reward: past and current status. *Trends Neurosci* 1999;22:521–7.
- Tanda G, Pontieri FE, DiChiara G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common μ_1 opioid receptor mechanism. *Science* 1997;276:2048–50.
- Trojniar W, Wise RA. Facilitory effect of Δ -9-tetrahydrocannabinol on hypothalamically induced feeding. *Psychopharmacology* 1991;103:172–6.
- Williams CM. Cannabinoid influences on appetite regulation. PhD Thesis, University of Reading, 1999.
- Williams CM, Kirkham TC. Anandamide induces overeating: mediation by central cannabinoid receptors. *Psychopharmacology* 1999;143:315–7.
- Williams CM, Kirkham TC. Hyperphagia induced by intra-accumbens injection of 2-arachidonoyl glycerol. Symposium on the cannabinoids. Burlington, VT: International Cannabinoid Research Society, 2001.
- Williams CM, Rogers PJ, Kirkham TC. Hyperphagia in pre-fed rats following oral Δ^9 -THC. *Physiol Behav* 1998;65:343–6.