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RAPID COMMUNICATION

Claire M. Williams · Tim C. Kirkham

Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors

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Abstract *Rationale*: Central cannabinoid systems have been implicated in appetite regulation by the respective hyperphagic actions of exogenous cannabinoids, such as Δ9-THC, and hypophagic effects of selective cannabinoid receptor antagonists. *Objective*: This study examined whether an endogenous cannabinoid, anandamide, could induce overeating, via a specific action at central (CB1) cannabinoid receptors. *Methods*: Pre-satiated male rats (*n*=18), received subcutaneous injections of anandamide (0.5, 1.0, 5.0, 10.0 mg/kg) before 3-h, nocturnal food intake tests. In a second series of intake tests (*n*=8), anandamide injection (1.0 mg/kg) was preceded by injection of the specific CB1 receptor antagonist, SR141716 (0.1, 0.5, 1.0 mg/kg SC). *Results*: All doses of anandamide induced significant overeating, with 1.0 mg/kg being most potent. Additionally, hyperphagia induced by 1.0 mg/kg anandamide was dose-dependently attenuated by SR141716 pretreatment. *Conclusion*: This first demonstration of anandamide-induced, CB1-mediated, overeating provides important evidence for the involvement of a central cannabinoid system in the normal control of eating.

Key words SR141716 · Hyperphagia · Rat · Pre-feed · Eating · Appetite

Introduction

In the past decade, cannabinoid receptors and their putative endogenous ligands, such as anandamide, have been found to be widely distributed within the mammalian central nervous system (Devane et al. 1992; Herkenham 1995; Matsuda et al. 1990; Tsou et al. 1998). Although little is currently known about the involvement of these systems in the control of behaviour, there is historical support for a possible role in the regulation of appetite. For example, a commonly reported effect of marijuana intoxication is spontaneous, compulsive eating. This effect involves the principal psychoactive constituent of marijuana, Δ9-tetrahydrocannabinol (Δ9-THC), a cannabinoid which binds with high affinity to central-type CB1 cannabinoid receptors.

Several studies have confirmed that Δ9-THC can promote overconsumption in humans and laboratory animals (Brown et al. 1977; Mattes et al. 1994; Williams et al. 1998). By contrast, a selective CB1 antagonist, SR141716 (Rinaldi-Carmona et al. 1994), has been reported to suppress food intake in laboratory animals (Arnone et al. 1997; Colombo et al. 1998; Simiand et al. 1998). Such data suggest that an endogenous cannabinoid receptor agonist, mimicked by Δ9-THC and antagonised by SR141716, may normally be involved in the central regulation of appetite. Despite the theoretical implications of these findings, there has so far been no successful demonstration that hyperphagia can be induced by administration of an endogenous cannabinoid, and only a single report of a lack of effect of anandamide on food intake (Crawley et al. 1993).

In this study, we re-examined the effect on feeding of peripherally administered anandamide, alone or in combination with the antagonist SR141716. To facilitate the detection of overeating, we assessed anandamide's effects on nocturnal feeding in pre-satiated rats; a design which is particularly sensitive to the hyperphagic actions of drugs (Kirkham et al. 1995; Williams et al. 1998).

Materials and methods

Animals

Male Lister-Hooded rats (Harlan UK Ltd, UK), weighing 510 (±26) g at the start of the experiment, were maintained in a temperature-controlled room at 20–22°C, under a 12:12-h light-dark cycle (lights off at 1000 hours). Laboratory chow (PCD Mod C; Special Diet Services, Witham, UK) and tap water were freely available at all times. All tests were performed in home cages during the dark phase, under low intensity red light.

Drugs

Anandamide ([*N*-(2hydroxyethyl)-5,8,11,14-eicosatetraenamide]; Tocris Cookson Inc.), supplied in a 1:4 soya oil/water emulsion

C.M. Williams · T.C. Kirkham (✉)
Department of Psychology, University of Reading, Whiteknights, PO Box 238, Reading RG6 6AL, UK
e-mail: t.c.kirkham@reading.ac.uk, Fax: +44-1189-316-715

was dissolved in 0.9% saline solution. SR141716 ([*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide]; Sanofi Recherche, Montpellier, France) was solubilised in a 10% DMSO solution. Fresh drug solutions were prepared on each test day and administered subcutaneously at a volume of 1 ml/kg.

Procedure

Experiment 1: anandamide dose-response

Immediately after dark onset at 1000 hours, rats (*n*=18) were presented with 30 g of a palatable wet mash diet, consisting of 200 ml ground chow (Rat and Mouse Expanded Ground Diet, Special Diet Services, Witham, UK) mixed with 250 ml tap water. Any remaining food or spillage was removed after 2 h. At 1200 hours, rats received a subcutaneous injection of either vehicle or anandamide (0.5, 1.0, 5.0, 10.0 mg/ kg). Pre-weighed chow was available throughout the pre-feed period. Immediately following injection, and at hourly intervals thereafter, remaining chow was re-weighed (with adjustments for spillage) to determine food intake at 1, 2 and 3 h post-treatment.

Experiment 2: anandamide feeding and CB1 receptor blockade

Eight of the animals from experiment 1 were subsequently retested using a slightly modified procedure. After the pre-feed, rats received two subcutaneous injections: SR141716 (0.1, 0.5 or 1.0 mg/kg) or vehicle at 1130 hours, followed by either saline or 1.0 mg/kg anandamide at 1200 hours. Chow intake was measured at 1, 2 and 3 h after injection.

Drug administration began only after habituation of the animals to housing conditions (2 weeks) and test procedures (1 week), and pre-feed and chow intakes under test conditions had stabilized. In both experiments, each animal received all treatments according to a Latin Square design. At least 48 h separated successive treatments. All procedures were performed in compliance with the requirements of the United Kingdom Animals (Scientific Procedures) Act 1986.

Statistical analysis

For experiment 1, dose-related effects of anandamide on chow intake were analysed for each measurement interval using one-way ANOVA. Effects of single or combined administration of SR141716 and anandamide in experiment 2 were assessed using two-way ANOVA. The significance of differences between specific treatment means was determined by the Newman-Keuls test for multiple comparisons. All data were analysed using Statistica.

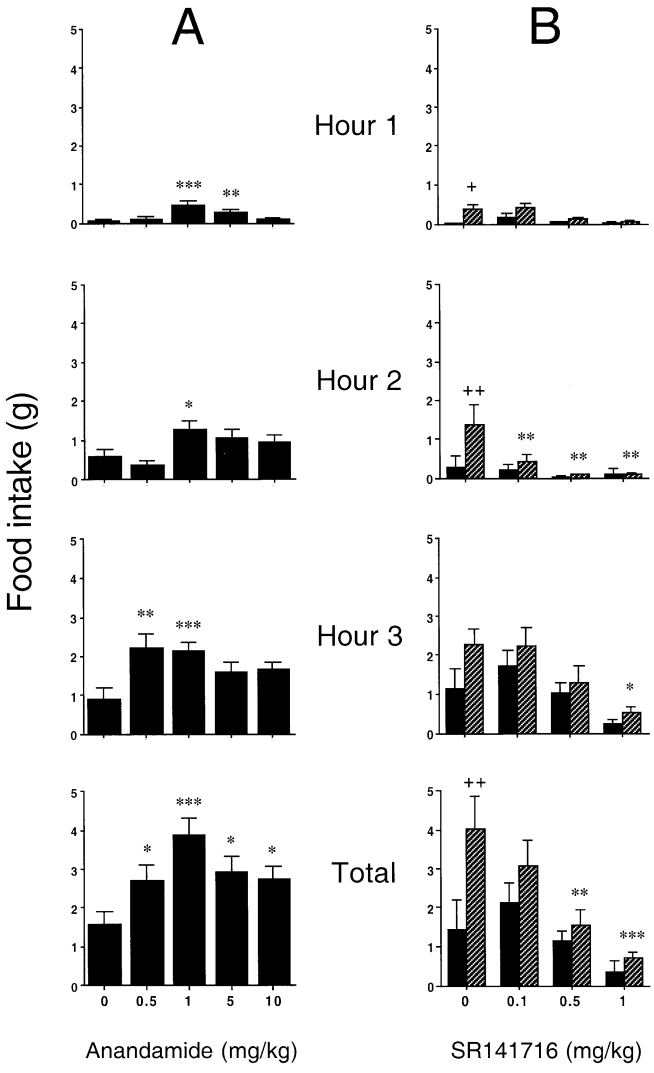

Fig. 1 **A** Effects of subcutaneous anandamide on nocturnal chow intake in pre-fed rats (*n*=18); **P*<0.05, ***P*<0.01, ****P*<0.001, significantly different from vehicle. **B** Attenuation of anandamide hyperphagia by the CB1 antagonist SR141716 (*n*=8). *Solid bars*: intake following SR141716 (0, 0.1, 0.5, 1.0 mg/kg, SC) plus anandamide vehicle. *Hatched bars*: intake following SR141716 plus anandamide (1.0 mg/kg, SC); **P*<0.05, ***P*<0.01, ****P*<0.001, relative to vehicle-anandamide condition. †*P*<0.05; ††*P*<0.01, compared to vehicle-vehicle control. All values are means (±SEM)

Results

Under control conditions, rats consumed the entire 30 g of mash within the first hour of the pre-feed stage. Baseline chow intakes during the subsequent 3-h intake tests were uniformly low (<2 g), thus permitting the easy detection of hyperphagia.

In experiment 1 (Fig. 1A), all doses of anandamide significantly increased total 3-h chow intake [$F(4,68)= 5.554$, $P<0.001$]. Overall, the 1.0 mg/kg dose was most potent, producing a greater than 2-fold increase in total intake ($P<0.001$). Examination of data for each hour of the test revealed this dose to have the most reliable effects, significantly increasing intake at each measurement interval. Other doses displayed different patterns of effects. The lowest (0.5 mg/kg) dose had a rather slow onset, exerting marked effects only during the third hour of testing ($P<0.01$). The 5 mg/kg dose produced a reliable increase during hour 1 ($P<0.01$), while later increases just failed to achieve significance. The highest, 10 mg/kg dose was less reliable, with non-significant increases through hours 2 and 3.

In experiment 2 (Fig. 1B), we attempted to block the hyperphagic effects of 1.0 mg/kg anandamide with the specific CB1 antagonist, SR141716. As before, anandamide produced a significant elevation of total intake in pre-fed rats [$F(1,14)=5.53$, $P<0.05$], with increases evident from the first hour ($P<0.05$). The most marked hyperphagia occurred during the second hour of testing, with a greater than 4-fold increase of chow intake ($P<0.001$).

Anandamide-induced eating was successfully blocked by SR141716 pretreatment. Over the whole test,

SR141716 produced a dose-dependent attenuation of anandamide hyperphagia [$F(3,42)=9.099$, $P<0.001$]. Considering hour 2, when the feeding effects of anandamide were most pronounced, hyperphagia was abolished by all doses of SR141716 [$F(3,42)=8.213$, $P<0.001$]. The antagonist had a tendency to reduce intake at the highest dose when administered alone. However, this effect was not significant at any point. Moreover, a significant second hour interaction between the effects of SR141716 alone, and when combined with anandamide [$F(3,42)=3.033$, $P<0.05$] indicates that any suppressive effects of the antagonist are insufficient to account for the observed abolition of anandamide hyperphagia.

Discussion

This study provides the first demonstration of hyperphagia induced by the administration of an endogenous cannabinoid receptor ligand. Anandamide provoked significant and reproducible overeating in satiated rats. Moreover, the dose-dependent attenuation of this effect by the selective CB1 receptor antagonist, SR141716, indicates that the anandamide hyperphagia is mediated by central cannabinoid receptors, not peripheral CB2 receptors.

Additionally, the data suggest that anandamide increased intake by modifying normal motivational processes, rather than by provoking some stereotyped, abnormal ingestive response. For example, after vehicle treatment, pre-satiation effectively delayed the appearance of substantial eating bouts until the third hour of testing. The largest absolute anandamide-induced increases in intake were obtained during this same period, with the two lowest doses being particularly potent. Thus, exogenous anandamide does not appear to have induced feeding directly but, instead, to have accentuated normally engendered, episodic eating motivation.

This pattern of anandamide effects on feeding contrasts with the effects of Δ9-THC obtained under similar test conditions (Williams et al. 1998). Generally, Δ9-THC was more potent, producing a much greater degree of overeating. However, Δ9-THC hyperphagia was restricted to the first hour of testing, and was followed by rebound, compensatory intake suppression lasting several hours. Possibly, these moderate effects of anandamide are due to its susceptibility to rapid metabolism. For example, in other behavioural assays, anandamide's effects have been accentuated through co-administration of the hydrolysis inhibitor phenylmethylsulphonyl fluoride (Mallet and Beninger 1998).

Alternatively, since anandamide can remain undegraded while in circulation and easily crosses the blood-brain barrier; Fride and Mechoulam 1993; Wenger et al. 1997), the milder, more sustained feeding effects of anandamide may reflect a subtler, more physiological adjustment to ongoing endogenous cannabinoid activity. Together with the apparent maintenance of normal eating patterns, our data suggest that the exogenous anandamide may act to facilitate, or augment, the actions of an endogenous anandamide system which normally contributes to the stimulation of appetite.

Overall, these observations provide strong support for a role of endogenous anandamide in the central regulation of feeding, and indicate the need for further research to define the precise aspects of eating motivation influenced by the anandamide system.

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