ORIGINAL INVESTIGATION

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The cannabinoid receptor antagonist SR 141716 attenuates overfeeding induced by systemic or intracranial morphine

Abstract *Rationale:* Considerable interplay exists between the brain's opioid and cannabinoid systems. These systems are both involved in the control of appetite and research supports the notion that the opioid system modulates the role of the cannabinoid system on appetite. However, the ability of the cannabinoid system to modulate the opioid system's control over appetite has not been well studied. *Objectives:* The present study examined the role of cannabinoid CB_1 receptors in the control of opioid-induced feeding, and sought to identify specific brain regions underlying this role. *Methods:* After being habituated to the test environment and injection procedure, sated rats were injected with the cannabinoid CB_1 receptor antagonist SR 141716 (0.03–3.0 mg/kg, IP). Thirty minutes later, morphine or its vehicle were administered systemically (2.5 mg/kg SC, experiments 1 and 2) or intracranially into the nucleus accumbens (nAcc, experiment 3) or paraventricular nucleus of the hypothalamus (PVN, experiment 4). Food intake and locomotor activity was then recorded for 120 min. *Results:* A significant increase in food intake was observed following systemic and intracranial (10 nmol) application of morphine in all experiments. SR 141716 suppressed systemic and intra-PVN morphine induced feeding (experiments 2 and 4), but did not attenuate food intake induced by intra-nAcc application of morphine (experiment 3). *Conclusions:* Because SR 141716 had no effect on intra-nAcc morphine-stimulated feeding, it would appear that cannabinoid receptors do not modify opioid-mediated hedonic responses to food. Rather, we conclude that cannabinoid CB_1 receptor blockade may suppress opioid-induced feeding by stimulating the release of satiety-related peptides within the hypothalamus. Further, because SR 141716 did not block morphine induced locomotor activity, the observed effects on feeding do not appear to be due to a non-specific reduction in motivated behaviour.

Keywords Cannabinoids · Opioid · Eating · Rat · Nucleus accumbens · Hypothalamus

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Introduction

In the past few decades a tremendous increase in our understanding of ingestive behaviour has occurred. Animal studies have shown that the brain's opioid system is critically involved in the control of appetite. Opioid receptor agonists reliably stimulate the intake of food (Tepperman and Hirst 1982; Morley et al. 1984; Jackson and Sewell 1985; Bakshi and Kelley 1994), while opioid receptor antagonists such as naloxone reduce food intake (Kirkham and Blundell 1987; Cleary et al. 1996). A growing body of evidence has also firmly established that appetite is modulated by the brain's cannabinoid system. The administration of cannabis extracts such as $\triangle^9$-tetrahydrocannabinol (THC) stimulates appetite (e.g. Williams et al. 1998; Koch 2001), while the CB_1 cannabinoid receptor agonist SR 141716 reduces food intake (Arnone et al. 1997; Colombo et al. 1998; Simiand et al. 1998). Furthermore, more recent studies have shown that appetite is increased by the endogenous cannabinoid ligands 2-arachidonoylglycerol and anandamide (for reviews, see Kirkham and Williams 2001a; Berry and Mechoulam 2002).

Evidence suggests that the cannabinoid and opioid systems not only control appetite individually, but also interact with each other. For example, the orexigenic effects of THC on feeding induced by electric stimulation of the lateral hypothalamus can be attenuated by naloxone (Trojniar and Wise 1991). In addition, the increased consumption of food and an alcoholic beverage produced by THC or anandamide (Williams et al. 1998; Gallate et al. 1999; Williams and Kirkham 1999; Hao et al. 2000;

Koch 2001; Colombo et al. 2002) can be attenuated by naloxone and SR 141716, but not by the CB_2 cannabinoid receptor antagonist SR 144528 (Williams et al. 1998; Williams and Kirkham 1999). Naloxone and SR 141716 also synergistically depress food intake at doses that do not alter food intake on their own (Williams and Kirkham 2002). Several lines of evidence show that cannabinoid effects on appetite can be modulated by the opioid system. However, it is presently unclear whether opioid control of appetite can be mediated by the cannabinoid system. Hence, the first aim of this study was to determine whether the cannabinoid CB_1 receptor antagonist SR 141716 can modulate the well-known stimulatory effects of the opioid receptor agonist morphine on food intake.

Secondly, this study examined the neural substrates that mediate cannabinoid and opioid interactions in feeding behaviour. Two sites, the nucleus accumbens (nAcc) and the paraventricular nucleus of the hypothalamus (PVN), were of particular interest because of their involvement in modulating different aspects of food intake. The nAcc appears to be involved in controlling motivated behaviours such as the intake of food and water (for review, see Bardo 1998). Indeed, the nAcc is ideally positioned to receive and communicate relevant incentive-motivational information to limbic and motor areas (Zahm and Brog 1992; Groenewegen et al. 1996) and it has been argued that opioid neurons located in the ventral striatum mediate the hedonic (i.e. "pleasure") responses to food (reviewed by Kelley et al. 2002).

A high density of opioid and cannabinoid receptors are found in the nAcc (Goodman et al. 1980; Herkenham et al. 1991). There is evidence that the increase in dopamine levels induced by THC within the nAcc (Taylor et al. 1988; Chen et al. 1990; Tanda et al. 1997) can be attenuated by naloxone (Gardner and Lowinson 1991; Tanda et al. 1997). In addition, anandamide administered into the nAcc increases feeding (Williams and Kirkham 1999). It is therefore possible that the opioid and cannabinoid systems within the nAcc interact to influence a neural substrate involved in processing and translating information relating to the incentive-motivational properties of food.

The PVN regulates food intake by influencing metabolic, hormonal, and endocrine responses relating to the nutritional state of the organism (Williams et al. 2001). This structure is the target of converging orexigenic and anorexigenic pathways originating from various hypothalamic sites (Elmquist et al. 1998) and is therefore considered to be the chief site mediating hypothalamic regulation of energy homeostasis. The PVN is richly innervated by, and is particularly sensitive to, neurons that release neurochemicals known to alter food intake such as neuropeptide Y (NPY), galanine, serotonin, cocaine- and amphetamine-regulated transcript (CART), pro-opiomelanocortin (POMC), as well as opioid and cannabinoid peptides (Williams et al. 2001).

Opioid and cannabinoid receptors are densely co-localised in the PVN (Goodman et al. 1980; Herkenham

et al. 1991). The decrease in PVN neuronal activation produced by an opioid receptor agonist can be blocked by naloxone (Pittman et al. 1980). In addition, we have shown that administration of either an opioid or cannabinoid agonist leads to widespread Fos expression in many regions of the hypothalamus, with the PVN, VMH and dorsomedial hypothalamus showing the highest levels (Verty et al. 2002; Allen et al. 2003). The PVN receives projections from all the hypothalamic regions (for review, see Swanson and Sawchenko 1983), and the administration of anandamide into the ventromedial hypothalamus (VMH) stimulates appetite (Jamshidi and Taylor 2001). It is therefore possible that the hyperphagic effects of anandamide may be due to stimulation of CB_1 cannabinoid receptors within the VMH leading to trans-synaptic excitation of PVN cells.

The first experiment reported here examined the ability of morphine to stimulate consumption of laboratory chow in non-deprived rats. This was done to assess the suitability of the morphine dose and determine the time-course of the effects. In a second experiment, the ability of various doses of SR 141716 to attenuate the orexigenic effects of systemically administered morphine was examined. Two additional experiments examined the effects of systemically administered SR 141716 on feeding produced by the intracranial administration of morphine into either the nAcc or PVN. It was predicted that SR 141716 would dose-dependently attenuate opioid-induced feeding in all experiments. Locomotor activity was also measured in all experiments to ensure that the anorexigenic effects of SR 141716 could not be attributed to a non-specific reduction in motivated behaviour.

Materials and methods

Animals were treated in accordance with the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) and the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. This study was reviewed and approved by the University of New England Animal Ethics Committee.

Subjects

Experimentally naive male Wistar rats, approximately 8 weeks of age at the beginning of the experiment, were used. Rats were housed four to six per group in opaque polypropylene cages (640 mm×410 mm×250 mm high) with stainless steel wire lids. Cages were lined with dust-free wood chips and were housed in a climate-controlled room maintained on a 12 h:12 h reverse light-dark cycle (lights off at 0800 hours). Experimental testing commenced at 0830 hours, that is, 30 min following the onset of the dark cycle. Rats had ad libitum access to standard laboratory chow (Rat and mouse chow; Ridley AgriProducts, Australia) and tap water while in their home cages.

Surgery

Stereotaxic surgery was carried out under deep fluothane (Halothane; Veterinary Companies of Australia) anaesthesia. Once anaesthetised, rats were mounted in a stereotaxic frame with the

incisor bar set so that bregma and lambda were level. Custom-made stainless steel guide cannulae (0.64 mm in diameter) aimed 1 mm above the medial shell of the nucleus accumbens (nAcc, bilateral) or the paraventricular nucleus of the hypothalamus (PVN, unilateral) were implanted and secured to the skull with stainless steel screws and dental acrylic. Stereotaxic coordinates for the nAcc were 1.2 mm anterior to bregma, 1.0 mm lateral to the midline, and 7.6 mm ventral to the surface of the skull according to the atlas of Paxinos and Watson (1997). For the PVN, the insertion angle was offset 10° from vertical to avoid penetration of the sagittal sinus. A hole was drilled 1.8 mm posterior to bregma, and 1.8 mm lateral to the midline, and the cannula was inserted to a depth of 7.7 mm. Between injections, the guide cannulae were occluded with pins made from 0.305 mm diameter stainless steel wire (O-SWX-3012; Small Parts Inc., Miami Lakes, Fla., USA).

Lignocaine HCl (2% Lignomav; MAVLAB Australia) was administered SC in the area surrounding the incision, and the wound was closed with a tissue adhesive. Buprenorphine HCl (Temgesic 0.05 mg/kg SC; Reckitt & Colman Products) was administered during the surgery, and again 8–12 h later to manage post-operative pain. In addition, post-operative pain and inflammation were controlled by the daily administration of vedaprofen (Quadrisol 5, 0.1 ml/kg PO mixed in strawberry jam) for 3 post-operative days. To reduce flavour neophobia, the jam mixture was presented on 2 separate days prior to surgery. Animals were closely monitored and allowed to recover for at least 7 days prior to behavioural testing. At the completion of testing, rats were killed by brief CO_2 exposure and brains were extracted and post-fixed in formalin. The brains were then sectioned with a sliding microtome with freezing stage, stained with cresyl violet, and mounted onto microscope slides for confirmation of injection sites.

Drugs

SR 141716 [N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide, Sanofi-Synthelabo] was first mixed with a few drops of Tween 80 (polyoxtethylene sorbitan monooleate; ICN Biomedicals). Physiological saline was then added and the solution was stirred, then sonicated. The final vehicle solution contained 1 drop of Tween 80 per 2 ml saline. SR 141716 was administered intraperitoneally at doses of 0.03, 0.3, and 3.0 mg/kg in a volume of 1 ml/kg.

Morphine HCl (API/AMED, Australia) was dissolved in physiological saline. In experiments 1 and 2, morphine was administered SC at a dose of 2.5 mg/kg in a volume of 1 ml/kg. In experiment 3, morphine was administered at a dose of 10 nmol (3.218 μg) bilaterally into the medial shell of the nAcc. Because of the close proximity of the PVN with the midline, morphine (10 nmol) was administered unilaterally in experiment 4. For intracranial administration, the injection cannula consisted of a length of 30-gauge stainless steel tubing (O-HTX-30; Small Parts Inc.), which was 1.0 mm longer than the implanted guide cannulae. Tygon micro-bore tubing (O-TGY-010; Small Parts Inc.) was used to attach the injection cannula to a 10 μl Hamilton microsyringe (Hamilton Bonoduz AG, Switzerland), which was connected to an infusion pump (Stoelting, model 53200 V). Drug infusions occurred over a period of 30 s in a volume of 1 μl. The injection cannula was left in place for an additional 30 s to promote diffusion of the drug. Bilateral infusions were delivered simultaneously using two injection cannulae and two Hamilton syringes. Rats were held gently for the entire injection procedure.

Apparatus

The experiment was conducted in eight identical dimly lit (13 Lux) rectangular Perspex locomotor activity chambers (280 mm wide×230 mm deep×300 mm high). The walls were constructed of aluminium and Perspex, and the floors were made of galvanized wire mesh (1 cm^2).

The test food (Rat and mouse chow, Ridley AgriProducts, Australia; Analysis: acid detergent fibre=10.2%, neutral detergent fibre=29.4%, crude protein=22.4%, crude fat=3.8%, crude fibre=6.7%, digestible energy=16.6 MJ/kg dry matter, average carbohydrate=27.9%) was presented in cylindrical glass dishes (105 mm diameter x 35 mm high) placed in one corner of the test chambers. Dishes were washed daily at the end of testing with a detergent solution (Pyroneg; DiverseyLever Australia Pty Ltd). Each rat received the same dish for the entire duration of the experiment. Plastic drinking bottles containing tap water were always available in the test chambers.

A computer controlled passive infrared detector (Quantum passive infrared motion sensor, part no. 890-087-2; NESS Security Products, Australia) mounted to the ceiling of each box was used to quantify locomotor activity using custom designed software. A 10 μF capacitor located near LK2 of the sensor's printed circuit board was replaced with a 0.1 μF capacitor serving to alter the sensor alarm period from 5 s to approximately 50 ms. Locomotor activity was defined as time spent in motion in seconds and recorded in 1-min bins. Activity chambers were located inside sound attenuating boxes (800 mm×360 mm×650 mm high) and fitted with a fan, which provided ventilation and masking noise.

Experiment 1: effects of systemic morphine on feeding

Rats (n=12) received one test session every 48 h at approximately the same time each day. On the first day, rats were placed in the activity chambers with 100 g of laboratory chow for a single habituation session. During this session, rats were given 30 min of drug-free access to the test food (this portion of each session is hereafter called the "prefeed phase"). The remaining food was weighed and animals were injected SC with saline (1 ml/kg) and returned to the test chambers with the remaining food for 4 h. Food intake was determined by weight every hour.

Two subsequent sessions were conducted that were identical to the habituation session, with the exception that animals were injected SC with either saline (1 ml/kg) or morphine HCl (2.5 mg/kg) following the prefeed phase. Half the rats received morphine, and half received vehicle during the first session. In the following session the conditions were reversed such that each animal received the opposite treatment.

Locomotor activity (time in motion) was recorded during all sessions and grouped into four 1-h bins for statistical analysis.

Experiment 2: effects of SR 141716 on systemic morphine-induced feeding

Test sessions were conducted every 48 h at approximately the same time each day. First, rats (n=12) were given one habituation session identical to that used in experiment 1, with the exception that the vehicle for SR 141716 was administered IP just prior to the prefeed phase, and the duration of the drug test was reduced from 4 to 2 h. Next, all rats received additional sessions in which they were injected IP with vehicle or SR 141716 (0.03. 0.3, or 3.0 mg/kg) immediately prior to being placed in the test chamber for the 30-min prefeed phase. The remaining food was weighed and rats were then injected with morphine HCl (2.5 mg/kg, SC) and immediately returned to the test chamber for 2 h. The amount of food consumed was determined by weight 1 and 2 h later. Locomotor activity (time in motion) was recorded and grouped into two 1-h bins for statistical analysis. Drugs tests were conducted every 48 h and all rats received all treatments. The order of treatments was counterbalanced across animals. On the day between drug sessions, animals were placed in the apparatus and the session conducted as usual, but no data were collected.

Experiment 3: effect of SR 141716 on intra-nAcc morphine-induced feeding

The procedure was similar to experiment 2, except that morphine (10 nmol in 1 μl) or its vehicle was administered intracranially into the nAcc (n=16). The same doses of SR 141716 were used. All animals received all treatments in a counterbalanced order.

Experiment 4: effect of SR 141716 on intra-PVN morphine-induced feeding

The procedure was identical to experiment 2, except that morphine (10 nmol in 1 μl) or its vehicle was administered intracranially into the PVN (n=18). The same doses of SR 141716 were used. All animals received all treatments in a counterbalanced order.

Statistical analysis

The amount of food consumed (g) and time spent in motion (s) were used as dependent variables and analysed separately. Data for the prefeed phase were analysed using paired t-tests or repeated measures ANOVAs.

Data were collapsed into 1-h bins and bin was treated as a factor. Separate two-factor repeated measures (dose by bin) ANOVAs were conducted on each dependent variable. Where significant main effects were found, pairwise comparisons were conducted using Bonferroni adjustments for multiple comparisons. Mauchly's W was computed to check for violations of the sphericity assumption. When Mauchly's W test was significant, the Greenhouse-Geisser correction was applied.

All analyses were conducted using SPSS 10.0.8 for Macintosh (SPSS Inc., Chicago, Ill., USA) using a probability level of 0.05. Results from animals in experiments 3 and 4 with incorrect cannula placements were excluded from the statistical analyses.

Results

Experiment 1: effects of systemic morphine on feeding

The mean quantity of food consumed prior to drug administration during the 30-min prefeed phase in experiment 1 was relatively low (see Table 1) and did not differ significantly between treatments. In the drug test, morphine significantly increased the total food intake relative to vehicle (Fig. 1, top) [$F(1,11)=32.32, P<0.001$]. The main effect of bin and the treatment by bin interaction were significant [$F(1,11)=49.44, P<0.001$, and $F(1,11)=61.40, P<0.001$, respectively]. Food consumption was similar during each of the 4 h of testing following the vehicle treatment (Fig. 1, top). However, morphine produced a large increase in food consumption during the first 2 h of testing, and a large decrease in food consumption during the last 2 h of testing. Comparisons of food intake between treatments conducted at each hour were all significant.

There was no significant difference in locomotor activity between treatments during the prefeed phase (Table 1), but morphine significantly increased locomotor activity relative to vehicle during the drug test (Fig. 1, bottom) [$F(1,11)=52.92, P<0.001$]. The main effect of bin and the treatment by bin interaction for the locomotor drug data were also significant [$F(1,11)=8.63, P<0.001$, and $F(1,11)=8.76, P<0.001$, respectively]. Inspection of Fig. 1 (bottom) reveals that morphine produced modest increases in locomotor activity in each of the first 2 h of testing, but had very little effect in the final 2 h. Pairwise comparisons of treatments conducted at each hour of testing revealed that morphine significantly increased locomotor activity during the first 2 h of testing only.

Table 1 Food consumption and locomotor activity during the prefeed phase for all experiments. *SAL* saline; *MOR* morphine; *V* vehicle; *SR* SR 141716. Morphine or its vehicle was administered after the prefeed phase; therefore, data shown for experiments 2–4 represent the actions of SR 141716 or its vehicle only

Experiment	Route of administration	Treatment	Amount consumed (g±SEM)	Time in motion (s±SEM)
1	Systemic	SAL	1.17±0.16	660.84±29.06
		MOR	1.13±0.23	581.32±53.02
2	Systemic	V+MOR	1.57±0.26	685.03±39.10
		0.03 mg/kg SR+MOR	1.68±0.29	648.68±32.75
		0.3 mg/kg SR+MOR	0.88±0.17	632.23±45.35
		3.0 mg/kg SR+MOR	0.50±0.15*	668.39±48.82
3	Intra-nAcc	V+SAL	1.83±0.16	474.90±40.53
		V+MOR	1.67±0.14	481.98±40.28
		0.03 mg/kg SR+MOR	1.04±0.26	522.92±36.76
		0.3 mg/kg SR+MOR	0.57±0.18*	490.28±48.95
		3.0 mg/kg SR+MOR	0.42±0.16*	547.39±55.99
4	Intra-PVN	V+SAL	1.73±0.15	568.64±42.69
		V+MOR	1.56±0.14	518.44±48.95
		0.03 mg/kg SR+MOR	0.96±0.21**	568.79±28.42
		0.3 mg/kg SR+MOR	0.77±0.21*	527.61±38.13
		3.0 mg/kg SR+MOR	0.20±0.11*	647.18±38.50

* Significantly different from V+MOR, $P<0.05$
** Significantly different from 3.0 mg/kg SR+MOR, $P<0.05$
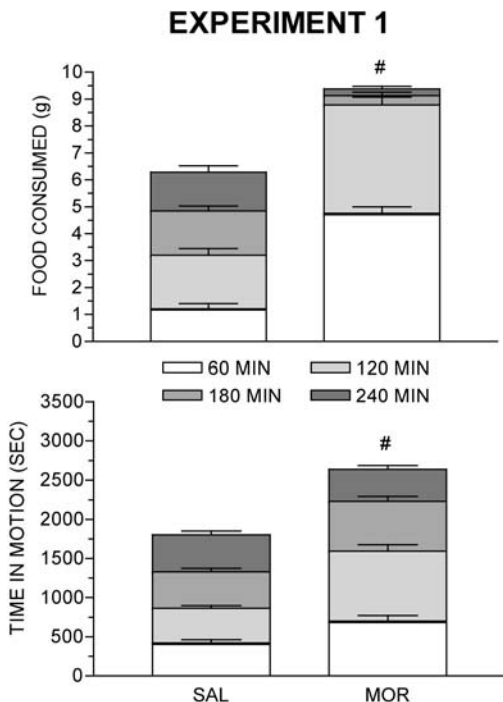
EXPERIMENT 1

Fig. 1 Laboratory chow consumed (*top*) and locomotor activity (*bottom*) in non-deprived rats following systemic administration of saline (*SAL*) or 2.5 mg/kg morphine (*MOR*). Data shown are means at each of four 60-min measurement intervals. Error bars represent the standard errors of the mean. $^{\#}P<0.05$, significantly different from saline

EXPERIMENT 2

Fig. 2 Laboratory chow consumed (*top*) and locomotor activity (*bottom*) in non-deprived rats following systemic administration of 2.5 mg/kg morphine combined with vehicle, 0.03, 0.3 or 3.0 mg/kg SR 141716. Data are means at two separate 60-min measurement intervals. Error bars represent the standard errors of the mean. *V* vehicle; *MOR* morphine; *SR* dose of SR 141716 in mg/kg; $^{\#}P<0.05$, significantly different from V+MOR

Experiment 2: effects of SR 141716 on systemic morphine-induced feeding

In experiment 2, administration of SR 141716 decreased food intake during the prefeed phase at the highest dose tested [$F_{(3,33)}=9.84$, $P<0.001$], without significantly affecting locomotor activity (Table 1). During the drug test, morphine-induced feeding was attenuated by pretreatment with SR 141716, shown by a significant main effect of dose (Fig. 2, top) [$F_{(3,33)}=13.41$, $P<0.001$]. Pairwise comparisons revealed that the 3.0 mg/kg dose differed significantly from all other doses tested. The main effect of bin ($P=0.055$) and the dose by bin interaction were not significant.

Analysis of the locomotor activity results for the drug test revealed that SR 141716 had no effect on morphine-stimulated locomotion (Fig. 2, bottom). The dose by bin interaction was not significant, but the main effect of bin was, showing that locomotor activity was generally higher in the second hour relative to the first hour (Fig. 2, bottom) [$F_{(1,11)}=33.10$, $P<0.001$].

Experiment 3: effect of SR 141716 on intra-nAcc morphine-induced feeding

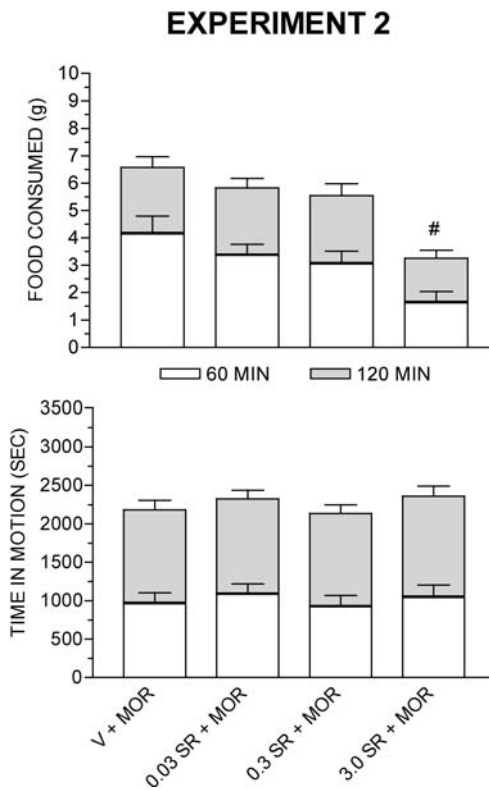Microscopic examination of stained tissue sections revealed that cannula tips were located bilaterally within the shell region of nAcc in 12 of the 16 animals implanted in experiment 3. The locations of the injector tips for all animals are shown in Fig. 3.

The amount of food consumed and locomotor activity during the prefeed phase did not differ significantly between the vehicle+saline and vehicle+morphine treatments (Table 1). However, administration of SR 141716 decreased food intake significantly during the prefeed phase at the 0.3 and 3.0 mg/kg doses [$F_{(3,33)}=11.50$, $P<0.001$], without significantly affecting locomotor activity (Table 1).

Comparison of the vehicle+saline and vehicle+morphine treatments in the drug phase (Fig. 4, top) revealed that food consumption was significantly increased by morphine administration [$F_{(1,11)}=66.52$, $P<0.001$]. Morphine also significantly increased locomotor activity relative to the vehicle+saline treatment (Fig. 4, bottom) [$F_{(1,11)}=85.42$, $P<0.001$]. However, the main effect of bin and the dose by bin interaction were not significant for either dependent measure. SR 141716 had no significant effect on morphine-stimulated food consumption and locomotor activity (Fig. 4) as evidenced by nonsignificant main effects of dose. A significant main effect of bin was, however, noted for food consumption
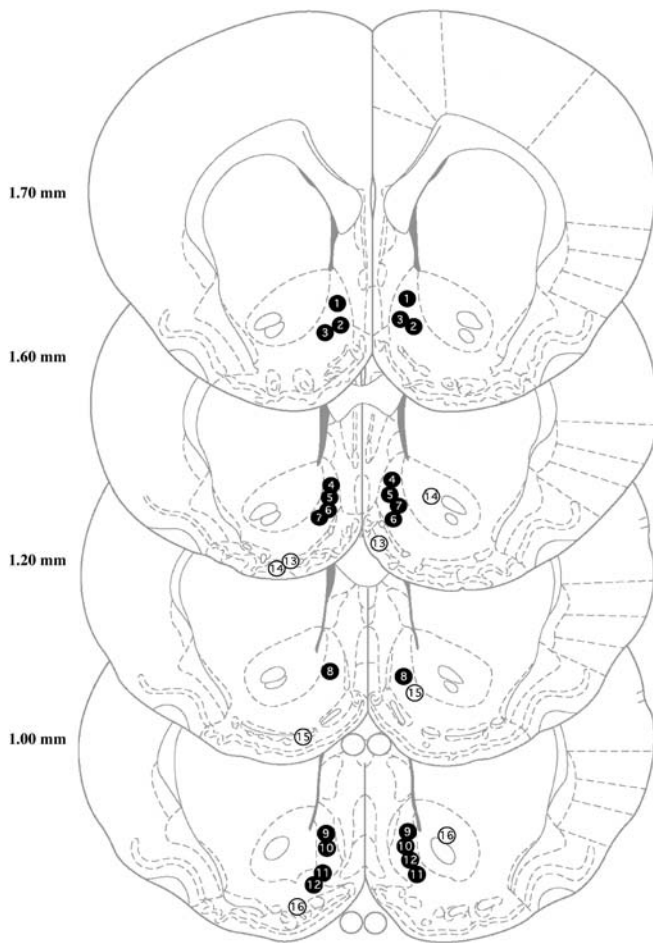
Fig. 3 Coronal sections showing locations of the injector tips for all rats in experiment 3 (intra-nAcc morphine administration). Illustrations are adapted from Paxinos and Watson (1997). Numbers to the left of each section represent the distance anterior to bregma. *Filled circles* represent placements within the target region (i.e. "hits"), and *open circles* represent placements outside the target structure (i.e. "misses"). *Matching numbers* represent left and right cannula placements in the same animal

indicating that more food was consumed in the first hour than in the second hour of testing [$F(1,11)=5.04$, $P<0.05$].

Experiment 4: effect of SR 141716
on intra-PVN morphine-induced feeding

Examination of tissue sections revealed that the location of the cannula tips were within the margins of the PVN in 14 of the 18 animals implanted in experiment 4 (Fig. 5).

During the prefeed phase, the amount of food consumed and locomotor activity did not differ significantly between the vehicle+saline and vehicle+morphine treatments (Table 1). An ANOVA comparing all doses of SR 141716 (including vehicle) to each other revealed that administration of SR 141716 decreased food intake significantly during the prefeed phase [$F(3,39)=14.44$, $P<0.001$]. Pairwise comparisons revealed that the 0.3 and
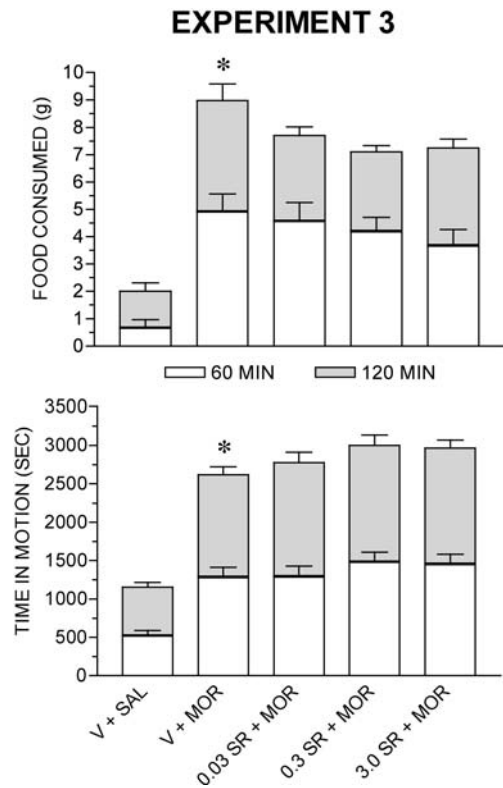

Fig. 4 Laboratory chow consumed (*top*) and locomotor activity (*bottom*) in non-deprived rats following intra-nAcc administration of saline or morphine (10 nmol) alone or in combination with vehicle, 0.03, 0.3 or 3.0 mg/kg SR 141716. Data shown are means at two separate 60-min measurement intervals. Error bars represent the standard errors of the mean. Abbreviations as in Fig. 2; [*]$P<0.05$, significantly different from V+SAL

3.0 mg/kg doses differed significantly from the vehicle control treatment, and the 0.03 mg/kg dose differed significantly from the 3.0 mg/kg dose. SR 141716 had no significant effect on locomotor activity during the prefeed phase (Table 1).

Comparing the vehicle+saline with the vehicle+morphine treatments in the drug phase revealed that morphine significantly increased both food consumption (Fig. 6, top) [$F(1,13)=76.43$, $P<0.001$], and locomotor activity (Fig. 6, bottom) [$F(1,13)=24.17$, $P<0.001$]. A significant dose by bin interaction was observed for locomotor activity [$F(1,13)=4.99$, $P<0.05$], but not for amount of food consumed. The main effect of bin was not significant for either of the dependent measures.

A separate ANOVA comparing all doses of SR 141716 (including vehicle) to each other was conducted for the food consumption and locomotor results. SR 141716 produced a significant dose-dependent reduction of morphine-stimulated food consumption (Fig. 6, top) [$F(3,39)=19.51$, $P<0.001$], but had no effect on locomotor activity (Fig. 6, bottom). Pairwise comparisons of the effect of different doses of SR 141716 on the amount of food consumed revealed that all SR 141716 treatments differed significantly from the vehicle+morphine treat-
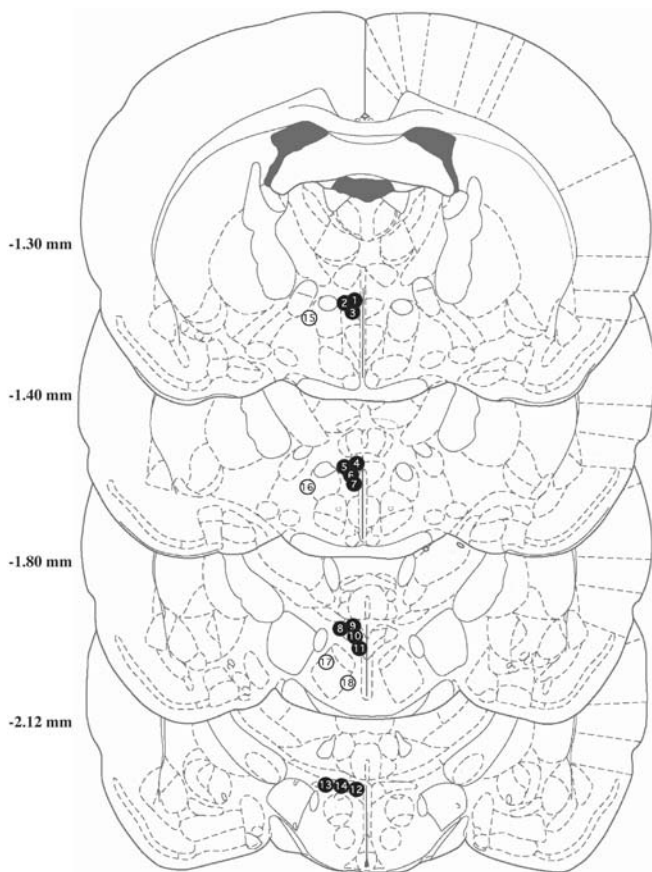
Fig. 5 Coronal sections showing locations of the injector tips for all rats in experiment 4 (intra-PVN morphine administration). Notations as in Fig. 3
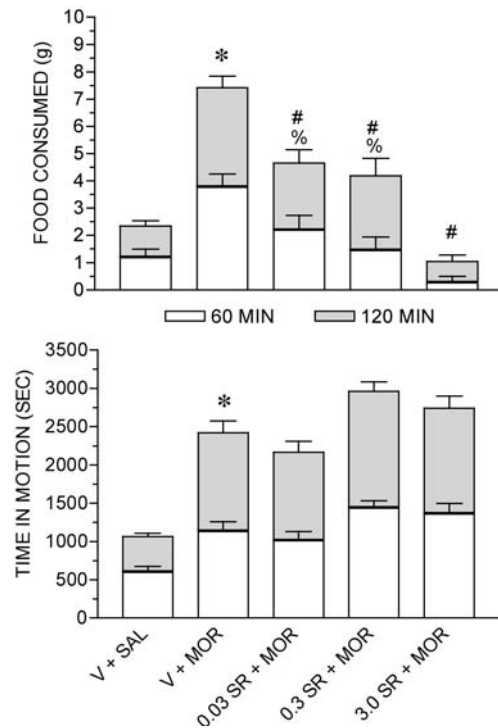

Fig. 6 Laboratory chow consumed (*top*) and locomotor activity (*bottom*) in non-deprived rats following intra-PVN administration of saline or morphine (10 nmol) alone or in combination with vehicle, 0.03, 0.3 or 3.0 mg/kg SR 141716. Data shown are means at two separate 60-min measurement intervals. Error bars represent the standard errors of the mean. Abbreviations as in Fig. 2; [*]$P<0.05$, significantly different from V+SAL; [#]$P<0.05$, significantly different from V+MOR; [%]$P<0.05$, significantly different from 3.0 SR+MOR

ment. Furthermore, the 0.03 and 0.3 mg/kg doses differed significantly from the 3.0 mg/kg dose. SR 141716 had no significant effect on morphine-stimulated locomotor activity ($P=0.072$, Fig. 6, bottom). The main effect of bin and the dose by bin interaction for the SR 141716 results were not significant for either of the dependent measures.

Discussion

The present study supports the notion that the cannabinoid system of the brain is involved in modulating opioid-induced food intake. Results showed that morphine increases food intake in non-deprived rats when administered either systemically, or intracranially into the medial shell region of the nucleus accumbens (nAcc) or the paraventricular nucleus of the hypothalamus (PVN). The CB_1 cannabinoid receptor antagonist SR 141716 attenuated the orexigenic effects of systemic or intra-PVN morphine administration, but had no effect on feeding induced by intra-nAcc morphine. Furthermore, SR 141716 had little effect on morphine-stimulated locomotor activity in any of the experiments suggesting that the effects on feeding were not due to a general reduction in motivated behaviour.

The finding that the systemic administration of morphine increased feeding (experiment 1) is in good agreement with previous reports (e.g. Sanger and McCarthy 1980; Morley et al. 1984; Gosnell and Krahn 1993) and further supports the notion that opioid receptors play an important role in the regulation of feeding. In addition, the observation that SR 141716 attenuated feeding produced by systemically administered morphine (experiment 2) complements a recent study by Williams and Kirkham (2002) showing that THC-induced hyperphagia can be attenuated by the opioid antagonist naloxone. Taken together, these results provide convincing evidence for a functional relationship between endogenous cannabinoid and opioid systems in the control of appetite. This hypothesis is further supported by recent demonstrations that cannabinoid and opioid receptor antagonists act synergistically to attenuate food intake (Kirkham and Williams 2001b; Rowland et al. 2001). Results from experiment 2 also complement a recent study showing that SR 141716 attenuates morphine-stimulated ethanol consumption in Sardinian alcohol-preferring rats (Vacca et al. 2002).

Experiments 3 and 4 were designed to examine specific brain regions that may underlie the opioid-cannabinoid interactions in feeding observed in experiment 2. To this end, morphine was administered intracranially into two regions selected for their preferential involvement in mediating the hedonic responses to food (nAcc) and feeding-related homeostasis (PVN). Results revealed that SR 141716 attenuated intra-PVN morphine-induced feeding, demonstrating that the influence on feeding mechanisms by opioid receptors located within the PVN is modulated by CB_1 cannabinoid receptors. Although the results of the present study alone are insufficient to elucidate the specific PVN-related mechanisms involved, other studies serve to suggest several possibilities. One such hypothesis is that cannabinoid and opioid interactions within the PVN may be regulated by leptin, a peptide hormone that has an inhibitory effect on food intake and body weight (Elmquist et al. 1999). Opioid receptor pathways modulate leptin responses in the hypothalamus (Dunbar and Lua 1999), and leptin exerts a negative control on hypothalamic endocannabinoid levels (Di Marzo et al. 2001). Thus, CB_1 receptors may alter hypothalamic leptin levels to attenuate opioid regulated feeding.

Other neuropeptides expressed within the hypothalamus that are involved in the regulation of feeding behaviour may also serve to modulate cannabinoid and opioid interactions in food intake. POMC (pro-opiomelanocortin) neurons are densely expressed and co-localized within hypothalamic regions (Kuhar and Dall Vechia 1999). POMC-derived neuropeptides such as α-MSH (melanocyte stimulating hormone) and ACTH (1–24) (adrenocorticotropin) seem to be involved in food intake and body weight regulation (Huzar et al. 1997; Krude et al. 1998; Cone 1999). Interactions between the melanocortin system with the opioid and cannabinoid systems have been demonstrated. For example, melanocortin peptides are considered to be antagonists of endogenous opioid peptides (de Wied and Jolles 1982) and melanocortin effects are reversed by morphine (Bertolini et al. 1986). In addition, cannabinoid and melanocortin systems act synergistically to reverse hemorrhagic shock in rats, suggesting a functional interaction between the cannabinoid and the melanocortin systems (Cainazzo et al. 2002). Therefore, inhibition of the cannabinoid system by SR 141716 may stimulate the release of melanocortin peptides serving to attenuate opioid-induced feeding.

SR 141716 failed to block food intake stimulated by intra-nAcc morphine administration in experiment 3. That is, despite the significant SR 141716-induced reduction in feeding during the prefeed phase, the intra-nAcc application of morphine resulted in a dramatic increase in feeding (Fig. 4). These results suggest that SR 141716 does not affect the hedonic responses to food related to opioid-related mechanisms within the nAcc (Kelley et al. 2002). In support of this possibility, it has been shown that SR 141716 failed to alter sham feeding at doses that were ten times higher than those required to block THC- or anandamide-stimulated feeding (Kirkham and Williams 2001b). It would therefore appear that the opioid systems within the accumbens are influenced by non-cannabinoid mechanisms (Znamensky et al. 2001). Further studies are needed to examine these possibilities.

The observation that administration of SR 141716 alone decreased intake of food during the prefeed phase of all experiments (Table 1) is consistent with previous reports of the anorexigenic effects of SR 141716 (e.g. Arnone et al. 1997; Colombo et al. 1998; Simiand et al. 1998; Freedland et al. 2000; Rowland et al. 2001) and provides further support for the role of endocannabinoids in feeding control (for review, see Berry and Mechoulam 2002). The effect of SR 141716 on food consumed during the prefeed phase needs to be considered here, as it admittedly complicates the interpretation of its interactive effects with morphine. Pre-existing differences in food consumption during the prefeed phase may affect subsequent morphine-induced feeding. That is, the attenuation of morphine-induced feeding produced by SR 141716 may be unrelated to an interaction with morphine, but may in fact result from increased hunger following a period of diminished food intake a short time earlier (i.e. during the prefeed phase). It is therefore important to note that results from the intra-nAcc experiment (experiment 3) clearly refute this possibility; there, morphine administration produced a clear increase in feeding that was independent of prefeed phase food consumption, which varied according to the dose of SR 141716 administered (Fig. 5).

In experiments 2, 3 and 4, the effects of SR 141716 alone were not examined beyond the prefeed phase. A robust suppression of feeding by SR 141716 has been independently documented by several laboratories (discussed above) and in our own laboratory (unpublished) using similar methods to those described here. Administration of SR 141716 alone would undoubtedly reduced feeding, with the highest dose producing a nearly complete block. It is admittedly clear in hindsight that the inclusion of an SR 141716 alone condition would have been informative in experiment 4, given that the combination of the highest dose of SR 141716 with morphine reduced feeding below that found with morphine alone (Fig. 6). Using only the results from experiment 4, one might argue that SR 141716 simply blocks all feeding by a downstream mechanism that is not necessarily related to the opioid system. The results from experiment 3 are therefore quite important in that they refute this possibility. That is, stimulation of the nAcc opioid system by morphine can overcome the suppression of feeding produced by even a large systemically administered dose of SR 141716, supporting the notion that this particular opioid feeding system is not critically dependent on cannabinoid receptors.

In conclusion, this study adds to a growing body of evidence demonstrating a functional interaction between the opioid and cannabinoid systems in mediating feeding behaviour. The results suggest that CB_1 cannabinoid receptors do not influence the hedonic properties of food by interacting with opioid-related mechanisms within the

nAcc; rather, we propose that CB_1 cannabinoid receptor blockade may serve to suppress opioid-induced feeding by stimulating the release of satiety-inducing peptides within hypothalamic circuitry.

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