

# CB<sub>1</sub> cannabinoid receptor-mediated modulation of food intake in mice

\*<sup>1</sup>Jenny L. Wiley, <sup>1</sup>James J. Burston, <sup>1</sup>Darnica C. Leggett, <sup>2</sup>Olga O. Alekseeva, <sup>2</sup>Raj K. Razdan, <sup>2</sup>Anu Mahadevan & <sup>1</sup>Billy R. Martin

<sup>1</sup>Department of Pharmacology and Toxicology, Virginia Commonwealth University, Box 980613, Richmond, VA 23298-0613, U.S.A. and <sup>2</sup>Organix, Inc., Woburn, MA, U.S.A.

**1** Marijuana's appetite-increasing effects have long been known. Recent research suggests that the CB<sub>1</sub> cannabinoid receptor antagonist SR141716A may suppress appetite. This study represents a further, systematic investigation of the role of CB<sub>1</sub> cannabinoid receptors in the pharmacological effects of cannabinoids on food intake.

**2** Mice were food-restricted for 24 h and then allowed access to their regular rodent chow for 1 h. Whereas the CB<sub>1</sub> antagonist SR141716A dose-dependently decreased food consumption at doses that did not affect motor activity, Δ<sup>9</sup>-tetrahydrocannabinol (Δ<sup>9</sup>-THC) increased food consumption at doses that had no effect on motor activity. O-3259 and O-3257, structural analogs of SR141716A, produced effects similar to those of the parent compound.

**3** Amphetamine (a known anorectic) and diazepam (a benzodiazepine and CNS depressant) decreased food consumption, but only at doses that also increased or decreased motor activity, respectively. The CB<sub>2</sub> cannabinoid receptor antagonist SR144528 and the nonpsychoactive cannabinoid cannabidiol did not affect food intake nor activity.

**4** SR141716A decreased feeding in wild-type mice, but lacked pharmacological activity in CB<sub>1</sub> knockout mice; however, basal food intake was lower in CB<sub>1</sub> knockout mice. Amphetamine decreased feeding in both mouse genotypes.

**5** These results suggest that SR141716A may affect the actions of endogenous cannabinoids in regulating appetite or that it may have effects of its own aside from antagonism of cannabinoid effects (e.g., decreased feeding behavior and locomotor stimulation). In either case, these results strongly suggest that CB<sub>1</sub> receptors may play a role in regulation of feeding behavior.

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**Abbreviations:** 2-AG, 2-arachidonoyl glycerol; AM251, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide; Δ<sup>9</sup>-THC, Δ<sup>9</sup>-tetrahydrocannabinol

## Introduction

Obesity has been identified as one of the top 10 global health problems by the World Health Organization and may soon exceed smoking as the primary cause of preventable death in the U.S. (Mokdad *et al.*, 2004). One important contributor to obesity is increased caloric intake without concomitant increase in activity. In humans and other mammals, appetite regulation is a complex physiological process that involves interactions among many neuromodulatory systems (e.g., Chiesi *et al.*, 2001). Leptin, a key regulator of feeding behavior, is found mainly in white adipose tissue and is released into circulation on feeding. Upon reaching the hypothalamus, leptin binds to receptors on neurons that ultimately link to neuromodulatory pathways that release anorexigenic peptides, pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript, or orexigenic peptides, neuropeptide Y and agouti-related protein. Interestingly, in this regard, it has been reported that (a) levels of endocannabinoids (i.e., anandamide

and/or 2-arachidonoyl glycerol (2-AG)) are under the negative control of the hormone leptin and (b) obese mice or rats with congenitally disrupted leptin signaling show higher amounts of endocannabinoids in the hypothalamus (Di Marzo *et al.*, 2001). Further, endocannabinoid levels in the limbic forebrain and hypothalamus are increased in hungry rats and return to basal levels when rats are satiated (Kirkham *et al.*, 2002).

These findings and other recent evidence suggest that the endogenous cannabinoid system may also play a role in appetite regulation (for a review see Cota *et al.*, 2003a). Pharmacological manipulation of this system through exogenous administration of cannabinoid agonists, such as Δ<sup>9</sup>-tetrahydrocannabinol (Δ<sup>9</sup>-THC), the primary psychoactive constituent of the marijuana plant, revealed increased food intake in humans and rodents (Williams *et al.*, 1998; Koch, 2001; Hart *et al.*, 2002). In contrast, SR141716A, the prototypic antagonist of CB<sub>1</sub> cannabinoid receptors, decreased food intake (Rowland *et al.*, 2001; McLaughlin *et al.*, 2003) and is presently undergoing Phase III clinical trials as an appetite suppressant. The present study represents a further,

\*Author for correspondence; E-mail: jwiley@hsc.vcu.edu  
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systematic investigation of the role of CB<sub>1</sub> cannabinoid receptors in the pharmacological effects of cannabinoids on food intake and supports the hypothesis that the endogenous cannabinoid system is involved in appetite regulation.

## Methods

### Subjects

All compounds were tested in adult male ICR mice (25–32 g), obtained from Harlan (Dublin, VA, U.S.A.), that were housed in groups of five. SR141716A and amphetamine were also tested in male and female CB<sub>1</sub> knockout (CB<sub>1</sub><sup>-/-</sup>) and wild-type (CB<sub>1</sub><sup>+/+</sup>) mice, bred on a C57BL/6 background, as described previously (Zimmer *et al.*, 1999). These mice were derived from breeding pairs of heterozygotes (obtained from A. Zimmer, National Institute of Mental Health, Bethesda, MD, U.S.A.) and were born at Virginia Commonwealth University. All animals were kept in a temperature-controlled (20–22°C) environment with a 12-h light–dark cycle (lights on at 7 a.m.). For the feeding experiments, each ICR mouse was tested with each dose of a single drug, presented in randomized Latin square order. CB<sub>1</sub> knockout and wild-type mice were tested with all doses of SR141716A and then, with all doses of amphetamine, presented in randomized order. Separate mice were used for testing each dose of each compound in the locomotor activity studies. The studies reported in this manuscript were carried out in accordance with the guidelines published in the guide for the care and use of laboratory animals (National Research Council, 1996).

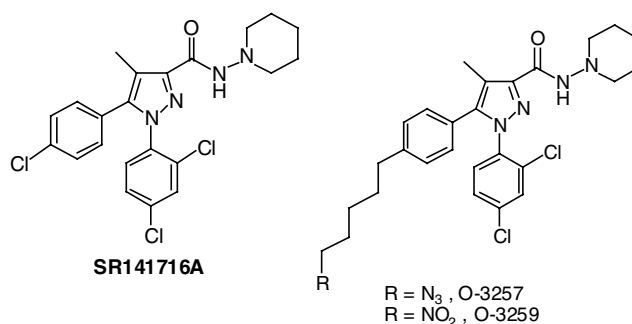
### Apparatus

The weight of food pellets was measured with a Mettler AT261 Delta Range scale (Toledo, OH, U.S.A.) at 0.01 mg accuracy. Assessment of spontaneous activity in mice occurred in standard activity chambers interfaced with a Digiscan Animal Activity Monitor (Omnitech Electronics, Inc., Columbus, OH, U.S.A.).

### Procedure

Feeding trials normally occurred on Tuesdays and Fridays between 12:00 and 14:00 h. At 24 h before the start of a feeding trial, all food was removed from the home cages of mice to be tested. The next day mice were transported to the laboratory at least 1 h before the beginning of the feeding trial. They were injected with the test compound at the specified pre-session injection interval. Subsequently, they were placed in a clear plastic cage with thick brown paper lining the bottom and allowed access to a pre-measured amount of their regular lab chow. At the end of 1 h, mice were removed from the test cage and placed back into their home cage. The amount of food left in the test cage, including crumbs, was measured, and amount consumed was calculated. Mice received no more than two feeding trials per week, separated by at least 72 h.

Locomotor trials occurred in a different laboratory. For these tests, mice were injected intraperitoneally (i.p.) at the same pre-session injection interval as for the feeding trial and were placed in individual activity chambers and spontaneous



**Figure 1** Chemical structures of SR141716A, O-3257, and O-3259.

activity was measured for 10 min. Activity was measured as the total number of interruptions of 16 photocell beams per chamber during the 10-min test and expressed as % inhibition of activity of the vehicle group.

### Drugs

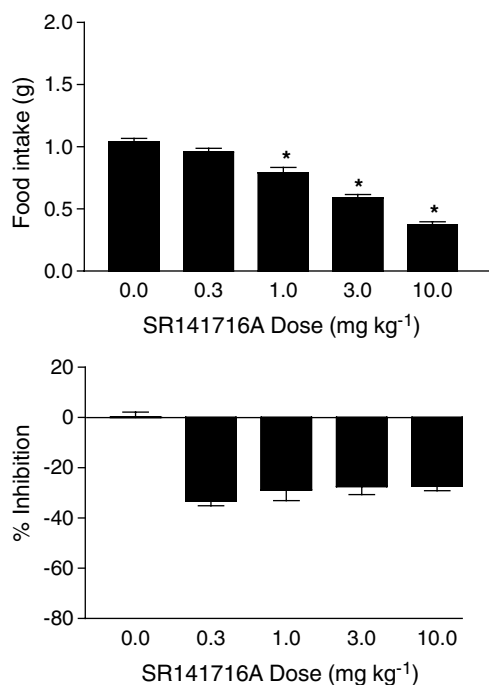
$\Delta^9$ -THC, SR141716A, SR144528, and cannabidiol were obtained from the National Institute on Drug Abuse (Rockville, MD, U.S.A.). They were suspended in a vehicle of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Inc., Princeton, NJ, U.S.A.), and saline in a ratio of 1 : 1 : 18. The two pyrazole analogs, O-3257 and O-3259 (Figure 1), were synthesized in our lab (Organix, Inc., Woburn, MA, U.S.A.) and were also prepared in 1 : 1 : 18 vehicle. Diazepam (Elkin-Sinn, Cherry Hill, NJ, U.S.A.) was purchased commercially in a concentration of 5 mg ml<sup>-1</sup>. A vehicle of ethanol : propylene glycol : distilled water in a 1 : 4 : 5 volume ratio was used to dilute this stock concentration to lower doses. D-Amphetamine (NIDA) was dissolved in physiological saline. All drugs were administered to the mice i.p. at a volume of 0.1 ml for every 10 g body weight. Pre-session injection times were based on our previous experience with these compounds, were identical for the feeding and locomotor studies, and were as follows: 60 min for SR141716A, O-3257, O-3259, and SR144528; 30 min for  $\Delta^9$ -THC and cannabidiol; and 15 min for amphetamine and diazepam.

### Statistical analysis

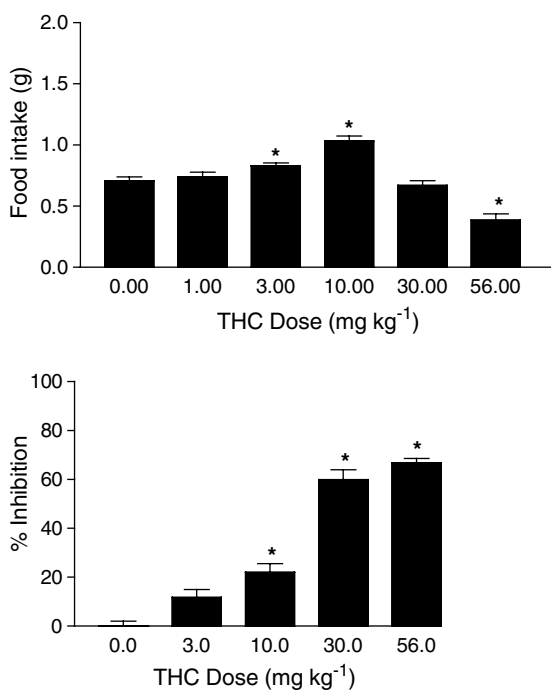
For each dose–effect curve in the feeding study, a repeated-measures analysis of variance (ANOVA) was used to analyze the food intake (in grams). For the locomotor activity experiments, separate independent sample ANOVAs were used to analyze the activity across doses for each compound. Tukey *post hoc* tests ( $\alpha = 0.05$ ) were used to specify differences revealed by significant ANOVAs.

## Results

Figure 2 shows the effects of SR141716A on food intake (top panel) and % inhibition of locomotor activity (bottom panel). SR141716A produced a significant dose-dependent decrease in food intake. Although some minimal stimulation of locomotor activity was observed following administration of SR141716A, this effect was not significant, nor was it dose-dependent. In contrast,  $\Delta^9$ -THC significantly and dose-dependently increased



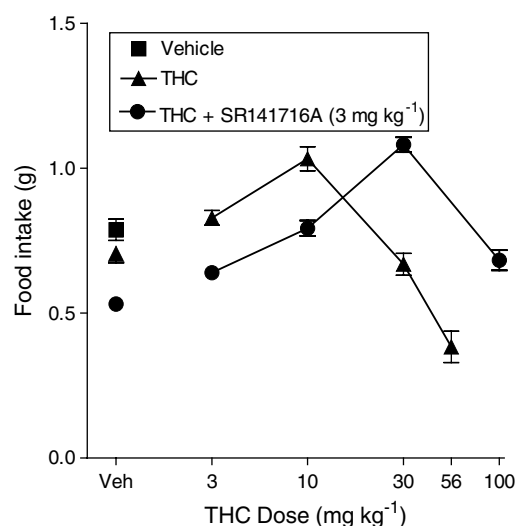
**Figure 2** Effects of SR141716A on food intake (top panel) and % inhibition of locomotor activity (bottom panel). Bars represent the mean ( $\pm$ s.e.m.) of data from the same 10 mice at each dose for food intake and data from five separate mice at each dose for assessment of locomotor activity. \* indicates significant difference from vehicle control ( $P < 0.05$ ). (Note: On all graphs that illustrate % inhibition, negative numbers represent stimulation of locomotor activity.)



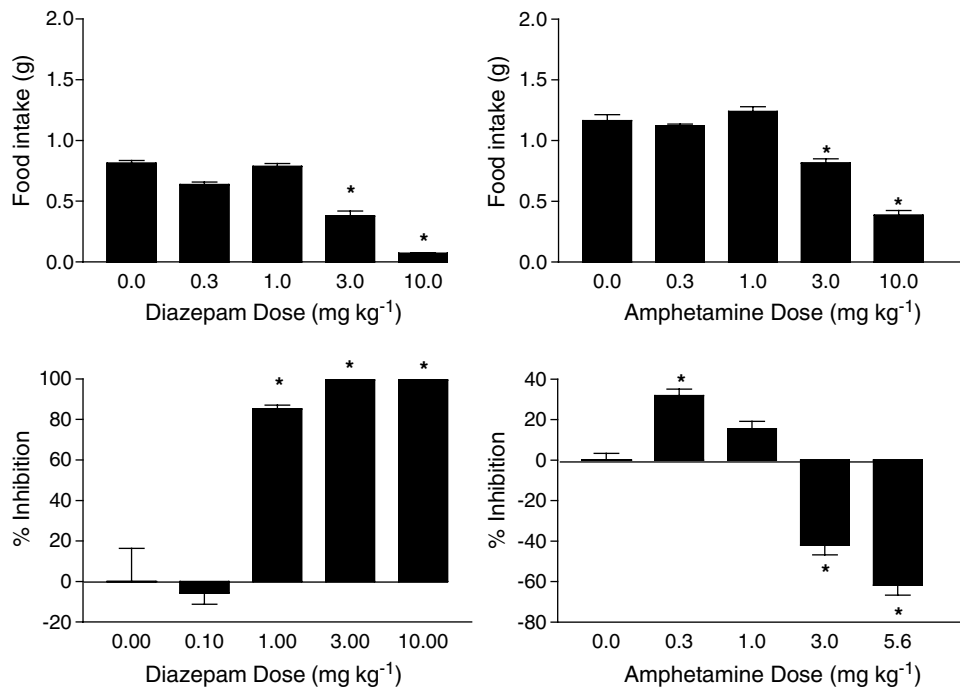
food intake at doses up to  $10 \text{ mg kg}^{-1}$  (Figure 3, top panel), but significantly decreased food intake at a higher dose of  $56 \text{ mg kg}^{-1}$ . At doses of  $10 \text{ mg kg}^{-1}$  and higher,  $\Delta^9$ -THC significantly decreased locomotor activity (Figure 3, bottom left panel). When administered in combination with  $\Delta^9$ -THC,  $3 \text{ mg kg}^{-1}$  SR141716A shifted the  $\Delta^9$ -THC dose-effect curve for food intake to the right (Figure 3, right panel), suggesting that SR141716A was acting as an antagonist in this model.

Figure 4 shows the results of tests with diazepam (left panels) and amphetamine (right panels). Although both drugs significantly decreased food intake at higher doses (top panels), these decreases were accompanied by significant changes in locomotor activity (bottom panels). Diazepam produced substantial inhibition of locomotor activity at a dose of  $1 \text{ mg kg}^{-1}$  and almost complete elimination of activity at doses of 3 and  $10 \text{ mg kg}^{-1}$ . In contrast, amphetamine significantly stimulated locomotor activity at higher doses.

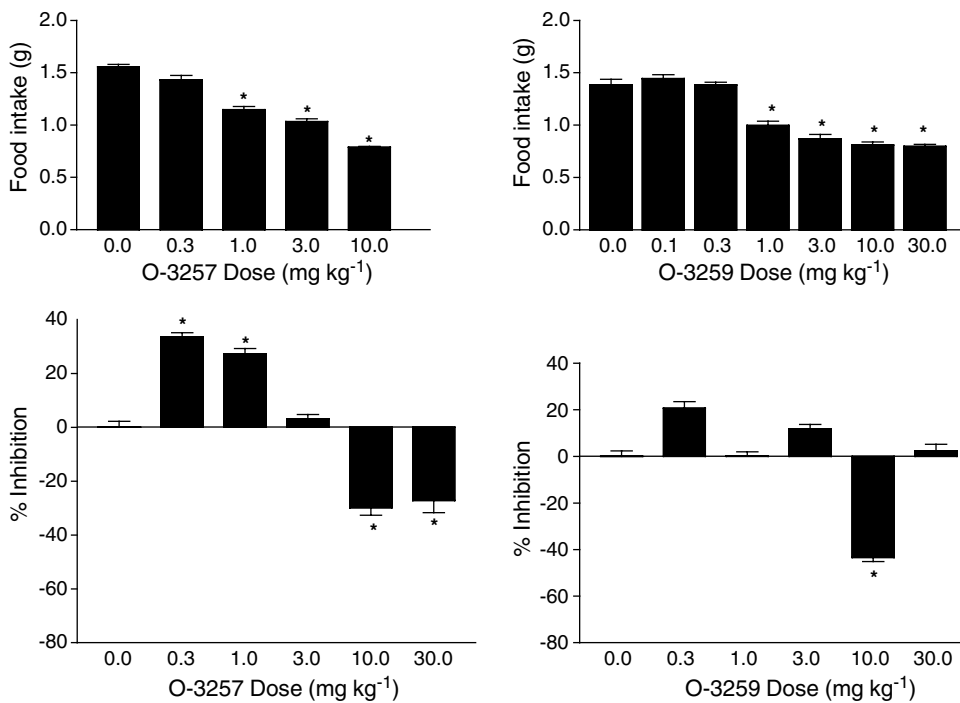
O-3257 and O-3259, two structural analogs of SR141716A, also significantly decreased food intake in this model (Figure 5, top panels); however, unlike diazepam and amphetamine, both compounds did so at one or more doses that did not significantly compromise locomotor activity. O-3257 had a biphasic effect on locomotor activity (Figure 5, bottom left panel), significantly decreasing the activity at lower doses and significantly increasing the activity at higher doses. Overall, O-3259 had little effect on locomotor activity (Figure 5, bottom right panel), except at a dose of  $10 \text{ mg kg}^{-1}$  where it significantly stimulated activity. In contrast with these results, tests with the CB<sub>2</sub> antagonist SR144528 and the nonpsycho-



**Figure 3** Effects of  $\Delta^9$ -THC on food intake (top panel) and % inhibition of locomotor activity (bottom left panel). Also shown are the effects of combination of  $3 \text{ mg kg}^{-1}$  SR141716A and different doses of  $\Delta^9$ -THC (bottom right panel). Bars represent the mean ( $\pm$ s.e.m.) of data from the same 9–10 mice across doses for food intake (but different mice for each  $\Delta^9$ -THC dose-effect curve) and data from six separate mice at each dose of each drug for assessment of locomotor activity. \* indicates significant difference from vehicle control ( $P < 0.05$ ).



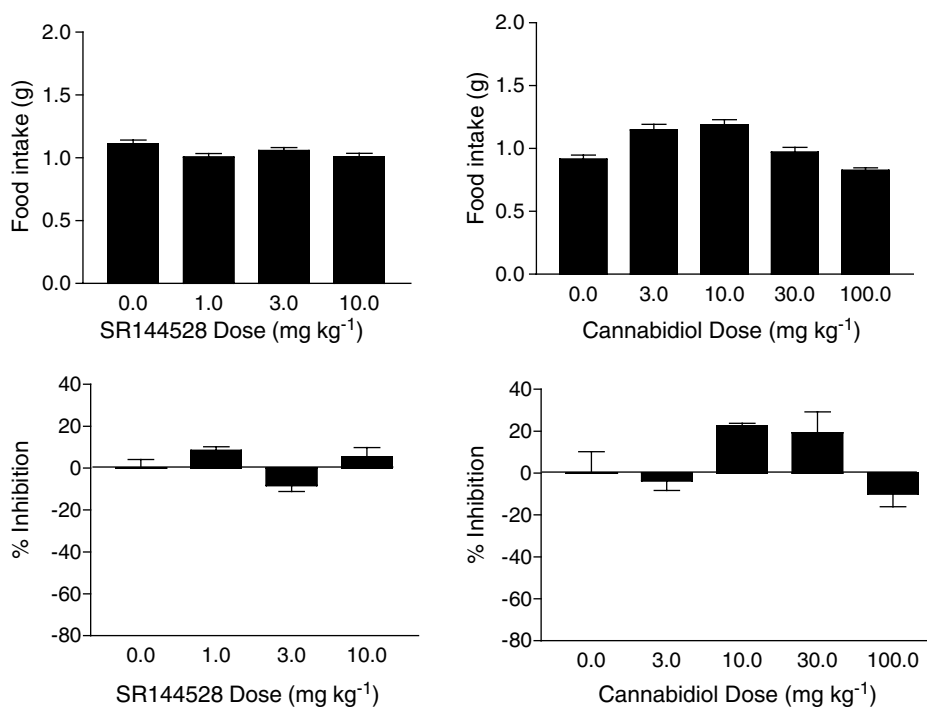
**Figure 4** Effects of diazepam (left panels) and amphetamine (right panels) on food intake (top panels) and % inhibition of locomotor activity (bottom panels). Bars represent the mean ( $\pm$ s.e.m.) of data from the same 10 mice across doses for food intake (but different mice for each drug) and data from 5–6 separate mice at each dose of each drug for assessment of locomotor activity. \* indicates significant difference from vehicle control ( $P < 0.05$ ).



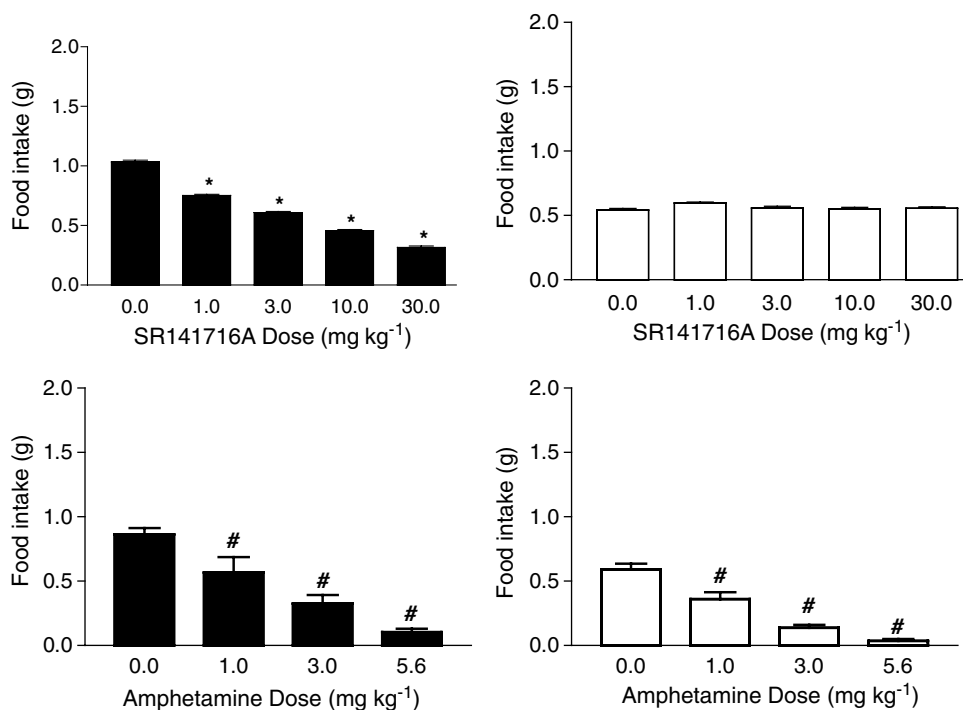
**Figure 5** Effects of two structural analogs of SR141716A, O-3257 (left panels) and O-3259 (right panels), on food intake (top panels) and % inhibition of locomotor activity (bottom panels). Bars represent the mean ( $\pm$ s.e.m.) of data from the same 10 mice across doses for food intake (but different mice for each analog) and data from 4–5 separate mice at each dose of each analog for assessment of locomotor activity. \* indicates significant difference from vehicle control ( $P < 0.05$ ).

active cannabinoid cannabidiol resulted in no significant effects on food intake (Figure 6, top panels) or on locomotor activity (Figure 6, bottom panel).

Figure 7 shows the effects of SR141716A (top panels) and amphetamine (bottom panels) in wild-type and CB<sub>1</sub> knockout mice (left and right panels, respectively). In C57BL/6 wild-type



**Figure 6** Effects of the CB<sub>2</sub> antagonist SR144528 (left panels) and cannabidiol (right panels) on food intake (top panels) and % inhibition of locomotor activity (bottom panels). Bars represent the mean ( $\pm$ s.e.m.) of data from the same 10 mice across doses for food intake (but different mice for each drug) and data from five separate mice at each dose of each drug for assessment of locomotor activity. \* indicates significant difference from vehicle control ( $P < 0.05$ ).



**Figure 7** Effects of SR141716A (top panels) and amphetamine (bottom panels) in wild-type (left panels) and CB<sub>1</sub> knockout (right panels) mice. Bars represent the mean ( $\pm$ s.e.m.) of data from the same six CB<sub>1</sub> knockout and six wild-type mice across all doses of both drugs. For each test drug, a main effect of genotype was found ( $P < 0.05$ ), indicating that basal food intake for CB<sub>1</sub> knockout mice was decreased compared to that of wild-type mice. \* indicates significant difference from vehicle control ( $P < 0.05$ ). # indicates significant difference from vehicle control (main effect of amphetamine dose) ( $P < 0.05$ ).

mice, SR141716A and amphetamine produced significant decreases in food intake similar to those produced by these drugs in ICR mice. Two major differences in these patterns

were observed with CB<sub>1</sub> knockout mice. The first difference was that baseline food intake under vehicle conditions was significantly decreased for CB<sub>1</sub> knockout mice as compared to

that of wild-type mice. The second difference was that SR141716A did not have any effect on food intake in CB<sub>1</sub> knockout mice. In contrast, amphetamine's effect on food intake was similar in both mouse strains.

## Discussion

The results of the present study show that SR141716A and two of its pyrazole analogs (O-3257 and O-3259) dose-dependently decreased food intake in moderately food-restricted mice over a dose range that had no significant effect on locomotor activity. The binding affinities of O-3257 and O-3259 for the CB<sub>1</sub> cannabinoid receptor are similar to that of SR141716A ( $K_i = 1.98 \pm 0.36$  (Rinaldi-Carmona *et al.*, 1994) as compared to  $K_i = 2.2 \pm 0.17$  for O-3257 and  $K_i = 1.2 \pm 0.05$  for O-3259 (B.R. Martin and J.L. Wiley, unpublished observations)), as is the minimal effective dose for reduced food intake for all three pyrazoles ( $1 \text{ mg kg}^{-1}$ ). Consistent with these results, a third pyrazole analog of SR141716A, *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251), has also been shown to decrease feeding in rats (McLaughlin *et al.*, 2003) and in two mouse models of obesity, but not in lean mice controls that were not food restricted (Zhou & Shearman, 2004). SR141716A itself has been found to decrease food intake in deprived and non-deprived rats (Rowland *et al.*, 2001; McLaughlin *et al.*, 2003; De Vry *et al.*, 2004; Verty *et al.*, 2004), in wild-type mice (Di Marzo *et al.*, 2001; Poncelet *et al.*, 2003), in mouse pups (Fride *et al.*, 2001; 2003), in lean and obese (*fal/fa*) Zucker rats (Vickers *et al.*, 2003), and in a diet-induced obesity model in mice (Ravinet-Trillou *et al.*, 2003). It also decreased the intake of other oral reinforcers such as sucrose and ethanol (Arnone *et al.*, 1997; Higgs *et al.*, 2003). Further, the anorectic effect was selective for the CB<sub>1</sub> cannabinoid receptor antagonist SR141716A and its analogs, as administration of the CB<sub>2</sub> cannabinoid receptor antagonist SR144528 did not change food intake. Together, these results strongly suggest that SR141716A and other pyrazole-based cannabinoids decrease feeding *via* their interaction with CB<sub>1</sub> cannabinoid receptors, either through antagonism of the effects of increased endocannabinoids in the brains of hungry rats or through production of opposing effects to those of endocannabinoids (i.e., inverse agonism). An action on peripheral CB<sub>1</sub> cannabinoid receptors may also be involved. Gomez *et al.* (2002) reported that anandamide levels were increased in the small intestine in food-deprived rats (as compared to basal levels during satiation). Further, they reported that systemic, but not central, administration of SR141716A reduced food intake in these rats. Destruction of the sensory neurons innervating the gut resulted in failure to observe SR141716A-induced decreases and cannabinoid agonist-induced increases in feeding. While these results seem to suggest that a primary peripheral effect of SR141716A on CB<sub>1</sub> cannabinoid receptors might be responsible for its hypophagic effect, SR141716A action on brain CB<sub>1</sub> cannabinoid receptors cannot be discounted. For example, Kirkham *et al.* (2002) found that site-selective, bilateral administration of 2-AG into the nucleus accumbens shell produced increased feeding that was reversed by SR141716A. Failure to observe an anorectic effect of centrally administered SR141716A in the Gomez *et al.* (2002) study may have resulted from lack of diffusion to relevant brain areas after *i.c.v.* injection.

The similarity of results with structurally distinct cannabinoid antagonists suggest that food intake may be regulated at least in part by antagonist effects on endocannabinoid modulation of appetite, a hypothesis that has received additional indirect support from studies that have reported leptin-induced regulation of anandamide levels in the hypothalamus (Di Marzo *et al.*, 2001), SR141716A-induced decreases in leptin (Ravinet-Trillou *et al.*, 2003), increased endocannabinoid levels in limbic forebrain and hypothalamus of hungry rats (Kirkham *et al.*, 2002; Hanus *et al.*, 2003), and SR141716A antagonism of increases in food intake produced by exogenously administered anandamide and 2-AG (Williams & Kirkham, 1999; Kirkham *et al.*, 2002). Our finding that the baseline food intake of CB<sub>1</sub> knockout mice was significantly reduced as compared with wild-type mice is also consistent with this hypothesis. Further reduction of food intake was observed when CB<sub>1</sub> knockout mice were injected with amphetamine, but not with SR141716A, suggesting that the response of these mice to a noncannabinoid anorectic drug resembles that of wild-type mice. These results are consistent with previous results in which Di Marzo *et al.* (2001) found that food-restricted CB<sub>1</sub> knockout mice showed decreased baseline feeding behavior when they were maintained on a reverse light–dark cycle, but showed no further decrease when SR141716A was administered. In another study, Cota *et al.* (2003b) reported that a reduction of caloric intake in non-restricted CB<sub>1</sub> knockout mice occurred across development. The decreased food intake in these mice was accompanied by alterations in neuropeptides in the hypothalamus that are involved in appetite regulation. Interestingly, CB<sub>1</sub> knockout mice were not sensitive to overeating induced by exogenous administration of neuropeptide Y, as were wild-type mice (Poncelet *et al.*, 2003).

In contrast with SR141716A and its analogs, the cannabinoid agonist  $\Delta^9$ -THC increased food intake. Anecdotal reports and historical accounts suggest that the stimulatory effects of marijuana on appetite in humans have long been known (e.g., Kalant, 2001). Indeed, oral formulations of  $\Delta^9$ -THC are currently used therapeutically to treat cachexia in cancer and AIDS patients (Beal *et al.*, 1995; Jatoi *et al.*, 2002). In rodents,  $\Delta^9$ -THC and  $\Delta^8$ -THC also increased food intake (present study; Williams *et al.*, 1998; Koch, 2001; Williams & Kirkham, 2002; Avraham *et al.*, 2004). Further, this effect was selective; that is, the dose range at which  $\Delta^9$ -THC produced this effect did not entirely overlap that at which it decreased locomotor activity. In contrast, cannabidiol, a nonpsychoactive cannabinoid with little affinity for the CB<sub>1</sub> cannabinoid receptor ( $K_i = 2283 \pm 453$ ; Thomas *et al.*, 1998), failed to alter either food intake or locomotor activity at doses that were up to 10-fold greater than those used with  $\Delta^9$ -THC, suggesting activation of CB<sub>1</sub> cannabinoid receptors is necessary to increase food intake and suppress locomotion. This hypothesis is strengthened by our observation that SR141716A produced a rightward shift in the  $\Delta^9$ -THC dose–effect function.

In summary, the results of the present study strongly suggest that cannabinoid regulation of appetite occurs *via* interaction with CB<sub>1</sub> cannabinoid receptors. Although not entirely conclusive, evidence available to date suggests that the appetite suppressive effects of SR141716A and other cannabinoid antagonists occurred as the result of antagonism of the action of endogenous cannabinoids at CB<sub>1</sub> cannabinoid receptors in

the brain and/or periphery rather than through inverse agonism at these receptors.

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on internship at VCU from the University of the West of England, Bristol, U.K. D.C.L. is a student at the Virginia Union University, Richmond, Virginia, and is enrolled in the Minority Access to Research Careers (MARC) Program, supported by NIGMS T34 grant GM-08503. We thank Ramona Winckler and Rhys Davies for technical assistance.

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