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Evidence for an Interaction between CB_1 Cannabinoid and Melanocortin MCR-4 Receptors in Regulating Food Intake

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Melanocortin receptor 4 (MCR4) and CB_1 cannabinoid receptors independently modulate food intake. Although an interaction between the cannabinoid and melanocortin systems has been found in recovery from hemorrhagic shock, the interaction between these systems in modulating food intake has not yet been examined. The present study had two primary purposes: 1) to examine whether the cannabinoid and melanocortin systems act independently or synergistically in suppressing food intake; and 2) to determine the relative position of the CB_1 receptors in the chain of control of food intake in relation to the melanocortin system. Rats were habituated to the test environment and injection procedure and then received intracerebroventicular injections of various combinations of the MCR4 receptor antagonist JKC-363, the CB_1 receptor agonist Δ^9-tetrahydrocannabinol, the MCR4 receptor agonist α-MSH, or the cannabinoid CB_1 receptor antagonist SR 141716. Food intake and locomotor activity were then recorded for 120 min. When administrated alone, SR 141716 and α-MSH dose-dependently attenuated baseline feeding, whereas sub-anorectic doses of SR 141716 and α-MSH synergistically attenuated baseline feeding when combined. Δ^9-Tetrahydrocannabinol-induced feeding was not blocked by α-MSH, whereas SR 141716 dose-dependently attenuated JKC-363-induced feeding. Locomotor activity was not significantly affected by any drug treatment, suggesting that the observed effects on feeding were not due to a nonspecific reduction in motivated behavior. These findings revealed a synergistic interaction between the cannabinoid and melanocortin systems in feeding behavior. These results further suggested that CB_1 receptors are located downstream from melanocortin receptors and CB_1 receptor signaling is necessary to prevent the melanocortin system from altering food intake. (*Endocrinology* 145: 3224–3231, 2004)

IN THE PAST FEW decades, a tremendous increase in our understanding of ingestive behavior has occurred. Animal studies have shown that the hypothalamic neurotransmitter and neuropeptide systems play a key role in maintaining food intake and energy balance. Among these, the melanocortin (MC) and cannabinoid systems are considered to be important mediators of feeding behavior (for review, see Refs. 1 and 2).

MCs, such as α-MSH, a 13-amino acid peptide derived from the larger proopiomelanocortin (POMC) polypeptide precursor molecule, are potent catabolic agents (3, 4). In the brain, POMC mRNA is localized in neurons within the arcuate nucleus (5). These neurons project to hypothalamic nuclei such as the paraventricular nucleus (PVN) and dorsomedial and ventromedial nuclei (6), which are involved in mediating energy homeostasis. MC receptor-4 (MCR4) receptors are located in these regions and act as an important mediator of feeding behavior (for review, see Ref. 2). Indeed, the deletion of the MCR4 gene in mice leads to hyperphagia and profound obesity (7). In addition, hypothalamic MCR4 receptors are selectively down-regulated in diet-induced obese rats and up-regulated in food-restricted rats (8). Furthermore, intracerebroventicular (ICV) administration of α-MSH or its synthetic homolog MT-II suppresses food intake in rats (9, 10) via MCR4 receptor activation (10). Administration of HS014 or JKC-363, both pharmacological antagonists at MCR4 receptors, dose-dependently stimulates food intake (3, 11, 12). Taken together, these studies suggest an important role for the MCR4 receptors in mediating feeding behavior.

A growing body of evidence has also established that appetite is modulated by the cannabinoid system of the brain. The administration of the cannabis constituent Δ^9-tetrahydrocannabinol (THC) stimulates appetite (13–15), whereas the cannabinoid receptor antagonist SR 141716 reduces food intake (16–19). Furthermore, recent studies have indicated that appetite is increased by the endogenous cannabinoid ligands 2-arachidonoylglycerol and anandamide (for review, see Refs. 1 and 20). SR 141716 fails to reduce food intake in transgenic mice lacking CB_1 cannabinoid receptors, and these mice eat 50% less than their wild-type littermates (21). This provides additional evidence that the cannabinoid system is a positive modulator of food intake.

Indirect evidence suggests a possible interaction between the cannabinoid and MC systems in feeding behavior. Both cannabinoid and MC receptors are localized within the hypothalamus (22, 23), and administration of THC, SR 141716, and MT-II leads to *c-fos* expression, a marker of neural activation, in similar regions of the hypothalamus (24–26).

Abbreviations: ICV, Intracerebroventicular; LV, lateral ventricle; MC, melanocortin; MCR4, MC receptor-4; NPY, neuropeptide Y; POMC, proopiomelanocortin; PVN, paraventricular nucleus.

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Furthermore, high concentrations of α-MSH are found in the PVN and dorsomedial and ventromedial nuclei (6). Perhaps the strongest support for an interaction between the cannabinoid and MC systems comes from the observation that THC regulates β-endorphin, a POMC-related peptide, levels in the hypothalamus (27, 28) and that a subactive dose of SR 141716 enhances the hemorrhagic shock-reversing effects of MC (29).

To further investigate the interaction between the cannabinoid and MC systems in feeding behavior, a series of experiments were conducted with two primary purposes: 1) to determine whether a MC agonist and a CB_1 receptor antagonist synergistically block food intake; and 2) to determine the relative position of the CB_1 receptors in the chain of control of food intake in relation to the MC system.

Materials and Methods

Drugs

SR 141716 [*N*-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide; Sanofi-Synthelabo (Montpellier, France)] was first mixed with a few drops of Tween 80 (polyoxtethylene sorbitan monooleate; ICN Biomedicals, Seven Hills, New South Wales, Australia). Physiological saline was then added, and the solution was stirred and then sonicated. The final vehicle solution contained 15 μl Tween 80/2 ml saline. SR 141716 was administered ICV unilaterally into the lateral ventricle (LV) at doses of 0.03, 0.3, or 3.0 μg in a volume of 1 μl.

THC (AGAL, Pymble, Australia), available as a 2.0-mg THC/ml ethanol solution, was first mixed with a few drops of Tween 80 (ICN Biomedicals). The suspension was stirred continuously under a steady stream of nitrogen gas until all ethanol was evaporated. Physiological saline was then added, and the solution was stirred until the Tween 80/THC suspension was well dispersed. The final vehicle solution contained 15 μl Tween 80/2 ml saline. THC was administered ICV unilaterally into the LV at a dose of 5 μg in a volume of 1 μl.

α-MSH and JKC-363 (Sigma-Aldrich, Sydney, New South Wales, Australia) were dissolved in physiological saline and administered ICV unilaterally into the LV at doses of 0.1, 1.0, and 10 μg (α-MSH) and 1 nmol (1.5068 μg; JKC-363).

For intracranial administration, the injection cannula consisted of a length of 30-gauge stainless steel tubing (O-HTX-30; Small Parts Inc., Miami Lakes, FL), 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, Bonaduz, Switzerland), which was connected to an infusion pump (model 53200V; Stoelting, Wood Dale, IL). Drug infusions occurred over a period of 30 sec. The injection cannula was left in place for an additional 30 sec to promote diffusion of the drug. Rats were held gently for the entire injection procedure.

Experimental animals

Male Wistar rats (n = 28), approximately 8–10 wk of age at the beginning of the experiment, were used. Ten experimentally naïve rats were used in each of the first two experiments. These rats were combined and used again in experiment 3. An additional eight experimentally naïve rats were used in experiment 4 and subsequently reused in experiment 5; however, one rat lost its cannula mount, leaving seven rats for this experiment.

Rats were housed four to six per group in opaque polypropylene cages (640 × 410 × 250 mm high) with stainless steel wire lids. Cages were lined with dust-free wood chips, and rats were housed in a climate-controlled room maintained on a 12-h reverse light/dark cycle (lights off at 0800 h). Experimental testing commenced at 0830 h (*i.e.* 30 min after the onset of the dark cycle). Rats had *ad libitum* access to standard laboratory chow (rat and mouse chow; Ridley AgriProducts, Sydney, New South Wales, Australia) and tap water while in their home cages.

Animals were treated in accordance with the *Principles of Laboratory Animal Care* (National Institutes of Health 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.

Surgery

Rats had permanent custom-made stainless steel guide cannulae (0.64 mm in diameter) implanted stereotaxically above the LV and secured to the skull with stainless steel screws and dental acrylic (30). Unilateral stereotaxic coordinates for the LV were 1.0 mm posterior to bregma, 1.0 mm lateral to the midline, and 3.0 mm ventral to the surface of the skull according to the atlas of Paxinos and Watson (31).

Verification of cannula placements was done before the start of experimental testing by the administration of angiotensin II (100 ng/rat; Sigma-Aldrich). Angiotensin II reliably induces water drinking in nondeprived rats when administered into the ventricles (32). Only data from rats drinking more than 10 ml in a 30-min period were included in the analysis. Sixteen rats were implanted with guide cannulae for each of the first two experiments. Cannula placements were deemed acceptable for 10 rats from each of these experiments. In experiment 4, cannula placements in eight of 11 implanted rats were deemed acceptable.

Apparatus

The experiment was conducted in eight identical chambers located within sound-attenuating chambers fitted with a fan, which provided ventilation and noise masking (30). The test food (rat and mouse chow; Ridley AgriProducts) (analysis: acid detergent fiber, 10.2%; neutral detergent fiber, 29.4%; crude protein, 22.4%; crude fat, 3.8%; crude fiber, 6.7%; digestible energy, 16.6 MJ/kg dry matter; average carbohydrate, 27.9%) was presented in cylindrical glass dishes (105 mm diameter × 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., Sydney, New South Wales, Australia). 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 mounted on the ceiling of each box was used to quantify locomotor activity using custom designed software (30). Locomotor activity was defined as time spent in motion in seconds and recorded in 1-min bins.

Experiment 1: effects of SR 141716

Rats received one session every 24 h at approximately the same time each day. On the first day, all animals received a single habituation session. During this session, rats were given injections of the vehicle for SR 141716 (1 μl) and placed in the apparatus for 120 min with 100 g of laboratory chow, and food intake was measured by weight every hour. Spilled food was recovered before weighing. During the next session, all rats received additional sessions in which they were given injections of vehicle or SR 141716 (0.03, 0.3, or 3.0 μg) and immediately placed in the test chamber for 2 h. The amount of food consumed was determined by weight every hour. Locomotor activity (time in motion) was recorded and grouped into two 60-min bins for statistical analysis.

In this and all subsequent experiments, drug treatments were tested using a repeated measures design whereby each rat in a given experiment received each drug treatment. Drug tests were conducted every 48 h, and all rats received all treatments in a counterbalanced order. On the day between drug sessions, animals were placed in the apparatus and the session was conducted as usual, except that the rats received sham injections. No data were collected during this time.

Experiment 2: effects of α-MSH

The procedure was similar to experiment 1, except that rats received injections of saline or α-MSH (0.1, 1.0, and 10 μg). The order of drug treatments was counterbalanced across animals.

Experiment 3: effects of combined administration of SR 141716 and α-MSH

The procedure was similar to experiment 1, except that rats received SR 141716 or its vehicle, followed 5 min later by α-MSH or its vehicle

at doses that, on their own, did not affect food intake in experiments 1 and 2. The amount of food consumed was determined by weight every hour. The order of drug treatments was counterbalanced across animals.

Experiment 4: effects of α-MSH on feeding induced by THC

The procedure was similar to experiment 1, except that rats were first given 30 min of drug-free access to the test food (this portion of each session is hereafter called the "prefeed phase"). A prefeed phase was used to facilitate the observation of drug-induced food intake by ensuring low baseline food intake during the testing period. The remaining food was weighed, and in the habituation session, animals were given injections of saline, followed 25 min later by vehicle for THC. Rats were then immediately returned to the testing chamber for an additional 120 min. Next, all rats received additional sessions similar to the habituation session, except that after the prefeed phase, rats were given injections of saline or α-MSH (0.1, 1.0, or 10.0 μg), followed 25 min later by vehicle or THC (5 μg).

Experiment 5: effects of SR 141716 on feeding induced by JKC-363

The procedure was identical to experiment 4, with the exception that in the habituation session, the vehicle for SR 141716 was administered, followed 25 min later by saline. During the additional sessions, rats received vehicle or SR 141716 (0.03, 0.3, or 3.0 μg), followed by JKC-363 (1 nmol). The dose of JKC-363 was chosen based on a previous study demonstrating the hyperphagic effect of this compound (12). All animals received all treatments in a counterbalanced order.

Statistical analysis

The amount of food consumed (grams) and time spent in motion (seconds) were used as dependent variables and analyzed separately. Data for the prefeed phases in experiments 4 and 5 were analyzed using repeated measures ANOVAs. For the drug phase data in experiments 1–5, food intake and locomotor activity data were collapsed into two 60-min 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 tests. Mauchly's W test 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 version 11.0 for Macintosh (SPSS Inc., Chicago, IL) using a probability level of 0.05.

Results

Experiment 1: effects of SR 141716

Pretreatment with SR 141716 attenuated food intake relative to vehicle in a dose-dependent manner, as shown by a significant main effect of dose (Fig. 1, *top*) ($F_{(3,27)} = 22.07$; $P < 0.001$). Pairwise comparison revealed that the 0.3- and 3.0-μg doses were significantly different from vehicle. The 0.3-μg dose was significantly different from the 0.03-μg dose. Furthermore, the 3.0-μg dose was significantly different from the 0.03- and 0.3-μg doses. A significant main effect of bin was observed, indicating that more food was consumed in the first hour relative to the second hour of testing ($F_{(1,9)} = 158.65$; $P < 0.001$). The dose by bin interaction was not significant. Analysis of the locomotor activity results for the drug test revealed that SR 141716 did not affect locomotor activity (Fig. 1, *bottom*). The dose by bin interaction was not significant, but the main effect of bin was ($F_{(1,9)} = 6.41$; $P < 0.05$), showing that locomotor activity was generally higher in the first hour relative to the second hour (Fig. 1, *bottom*).

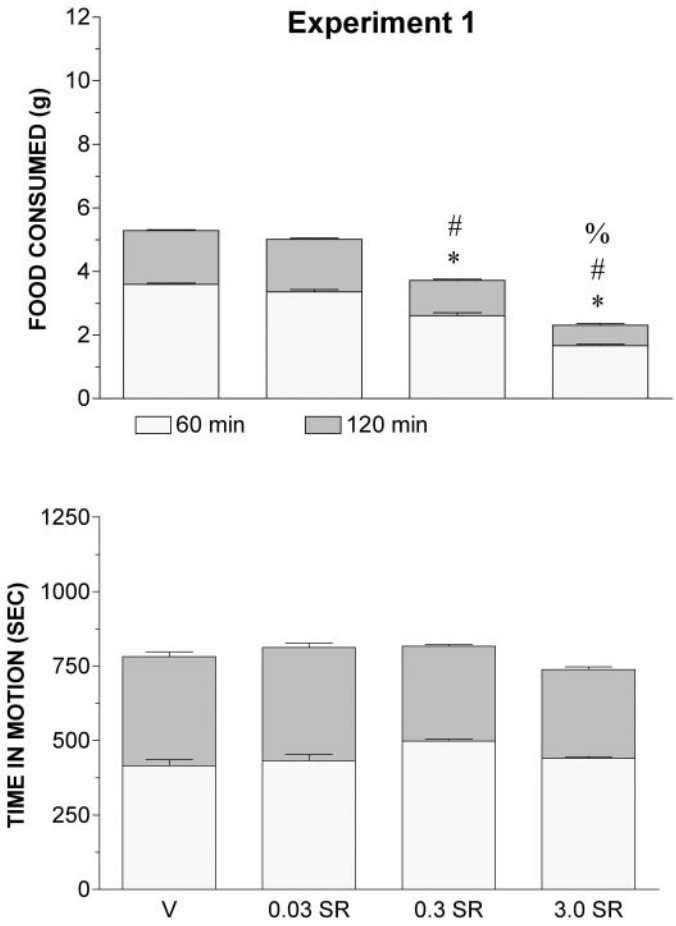

FIG. 1. Laboratory chow consumed (*top*) and locomotor activity (*bottom*) in nondeprived rats after ICV administration of vehicle or 0.03, 0.3, or 3.0 μg SR 141716. Data represent means (+SEM) at two consecutive 60-min measurement intervals. V, Vehicle; SR, dose of SR 141716 in micrograms; *, $P < 0.05$, significantly different from V; #, $P < 0.05$, significantly different from 0.03 SR; %, $P < 0.05$, significantly different from 0.3 SR.

Experiment 2: effects of α-MSH

Pretreatment with α-MSH attenuated food intake relative to vehicle in a dose-dependent manner, as shown by a significant main effect of dose (Fig. 2, *top*) ($F_{(3,27)} = 71.02$; $P < 0.001$). Pairwise comparison revealed that the 1.0- and 10.0-μg doses were significantly different from saline and the 0.1-μg dose. Furthermore, the 10.0-μg dose was significantly different from the 1.0-μg dose. A significant main effect of bin was observed, indicating that more food was consumed in the first hour relative to the second hour of testing ($F_{(1,9)} = 138.41$; $P < 0.001$). The dose by bin interaction was also significant ($F_{(3,27)} = 14.15$; $P < 0.001$). The main effects of dose and bin and the dose by bin interactions were not significant for locomotor activity (Fig. 2, *bottom*).

Experiment 3: effects of combined administration of SR 141716 and α-MSH

Combined administration of sub-anorectic doses of SR 141716 (0.03 μg) and α-MSH (0.1 μg) significantly attenuated food intake relative to vehicle, as shown by a significant main

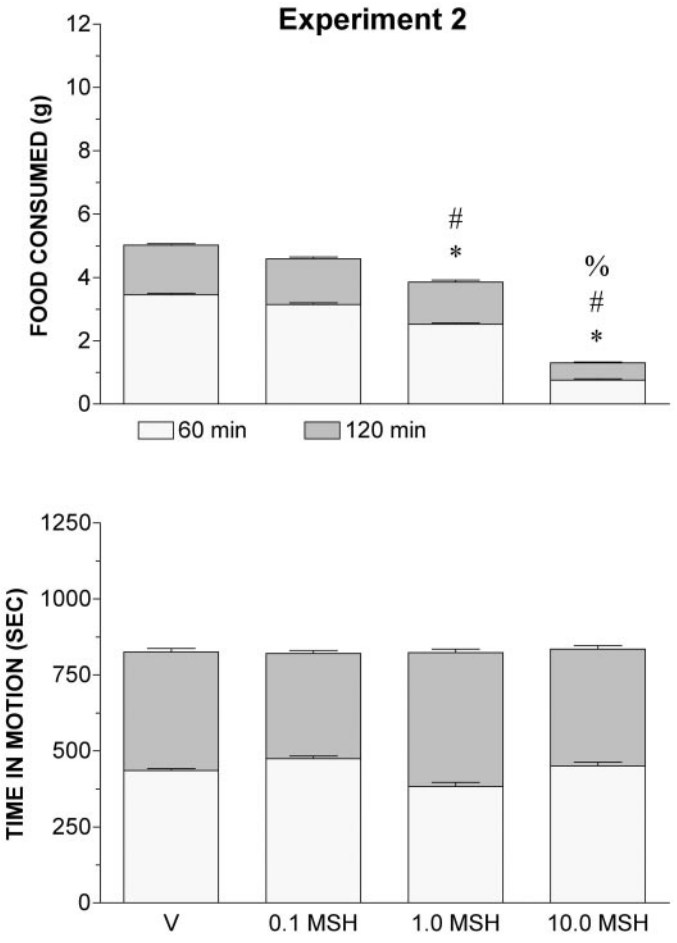

FIG. 2. Laboratory chow consumed (*top*) and locomotor activity (*bottom*) in nondeprived rats after ICV administration of saline or 0.1, 1.0, or 10.0 μg α-MSH. Data represent means (+SEM) at two consecutive 60-min measurement intervals. V, Vehicle; MSH, dose of α-MSH in micrograms; *, $P < 0.05$, significantly different from saline; #, $P < 0.05$, significantly different from 0.1 MSH; %, $P < 0.05$, significantly different from 1.0 MSH.

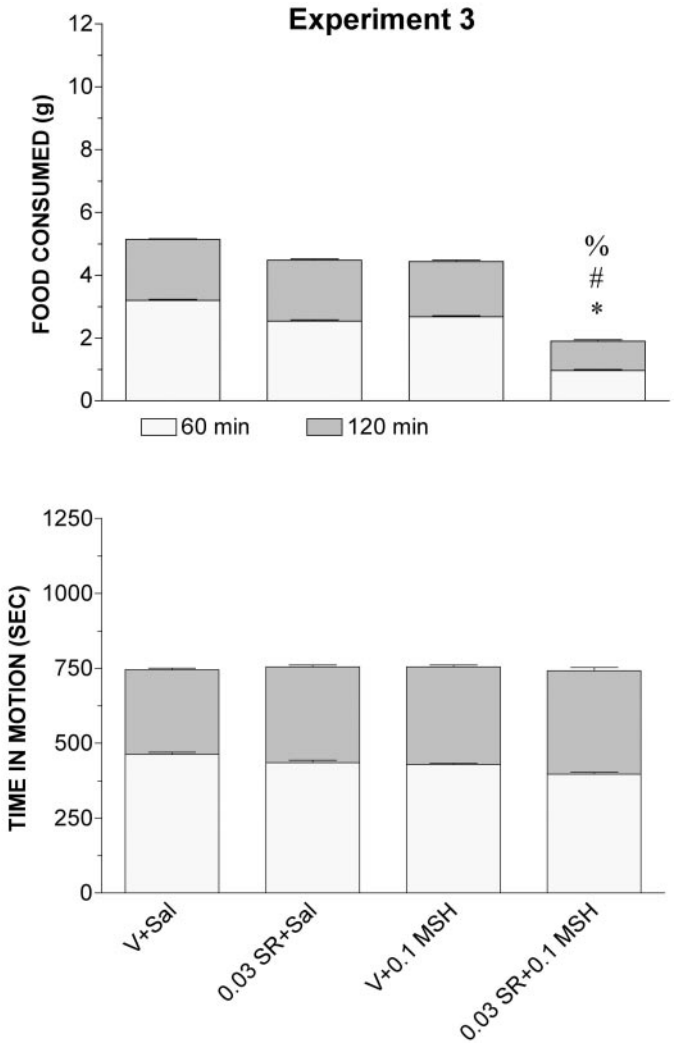

FIG. 3. Laboratory chow consumed (*top*) and locomotor activity (*bottom*) in nondeprived rats after ICV administration of vehicle+saline, 0.03 SR+Sal, vehicle+0.1 MSH, or 0.03 SR+0.1 MSH. Data represent means (+SEM) at two consecutive 60-min measurement intervals. V, Vehicle; Sal, saline; SR, dose of SR 141716 in micrograms; MSH, dose of α-MSH in micrograms; *, $P < 0.05$, significantly different from V+Sal; #, $P < 0.05$, significantly different from 0.03 SR+Sal; %, $P < 0.05$, significantly different from V+0.1 MSH.

effect of dose (Fig. 3, *top*) ($F_{(3,57)} = 79.01$; $P < 0.001$). Pairwise comparison revealed that 0.03 μg SR 141716 and 0.1 μg α-MSH alone did not significantly affect food consumption compared with vehicle. Furthermore, combined administration 0.03 μg SR 141716 and 0.1 μg α-MSH was significantly different from vehicle, 0.03 μg SR 141716, and 0.1 μg α-MSH. A significant main effect of bin was observed, indicating that more food was consumed in the first hour relative to the second hour of testing ($F_{(1,19)} = 50.47$; $P < 0.001$). The dose by bin interaction was also significant ($F_{(3,27)} = 5.58$; $P < 0.01$). Analysis of the locomotor activity results for the drug test revealed that SR 141716 and α-MSH alone or in combination did not affect locomotor activity compared with vehicle (Fig. 3, *bottom*). The dose by bin interaction was not significant, but the main effect of bin was ($F_{(3,57)} = 5.58$; $P < 0.001$), showing that locomotor activity was generally higher in the first hour relative to the second hour (Fig. 3, *bottom*).

Experiment 4: effects of α-MSH on feeding induced by THC

The mean quantity of food consumed (mean, 2.49 g; range, 2.39–2.58 g) and locomotor activity (mean, 480.90 sec; range, 468.41–496.45 sec) before drug administration during the 30-min prefeed phase in experiment 4 was relatively low and did not differ significantly between treatments. A repeated measures ANOVA comparing all treatments revealed a significant main effect of dose ($F_{(4,28)} = 16.09$; $P < 0.001$) and a significant main effect of bin ($F_{(1,7)} = 46.08$; $P < 0.001$) for food consumption (Fig 4, *top*). Pairwise comparison revealed that food intake with saline+THC, 0.1 M α-MSH+THC, 1.0 M SH+THC, and 10.0 M SH+THC was significantly higher than with saline+vehicle treatment, suggesting that THC-induced feeding was not blocked by the administration of α-MSH. A significant main effect of bin was observed for locomotor activity ($F_{(1,7)} = 316.85$; $P < 0.001$), again reflecting higher locomotor activity in the first hour relative to the second hour of testing. The dose by bin interaction was not significant for either of the dependent variables (Fig 4).

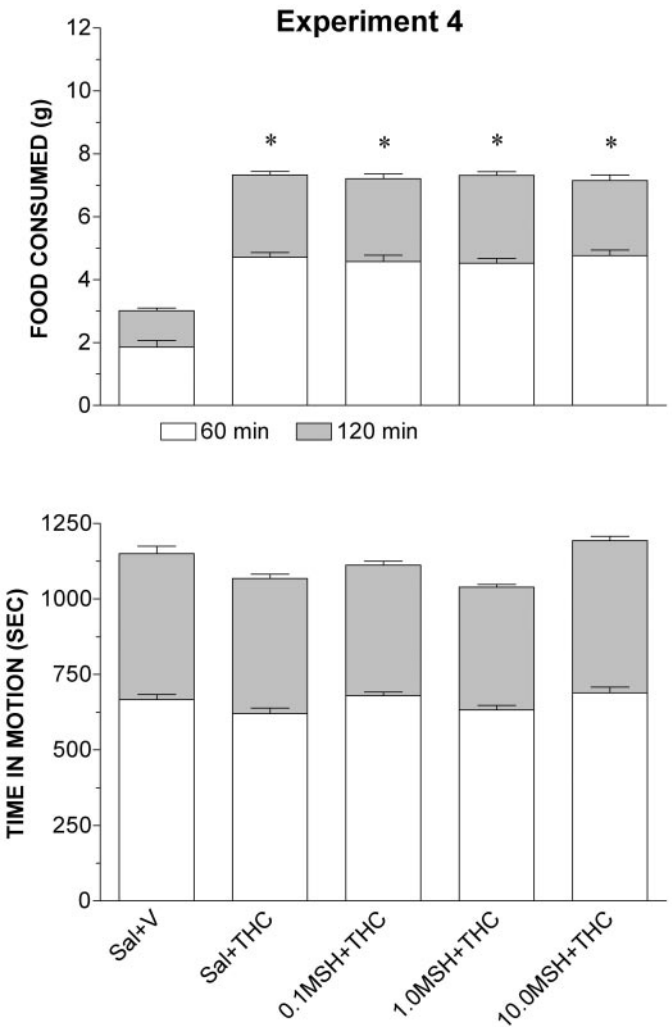

FIG. 4. Laboratory chow consumed (*top*) and locomotor activity (*bottom*) in nondeprived rats after ICV administration of vehicle or THC (5 μg) alone or in combination with saline or 0.1, 1.0, or 10.0 μg α-MSH. Data represent means (+SEM) at two consecutive 60-min measurement intervals. Sal, Saline; V, vehicle; THC, dose of THC in micrograms; MSH, dose of α-MSH in micrograms; *, $P < 0.05$, significantly different from Sal+V.

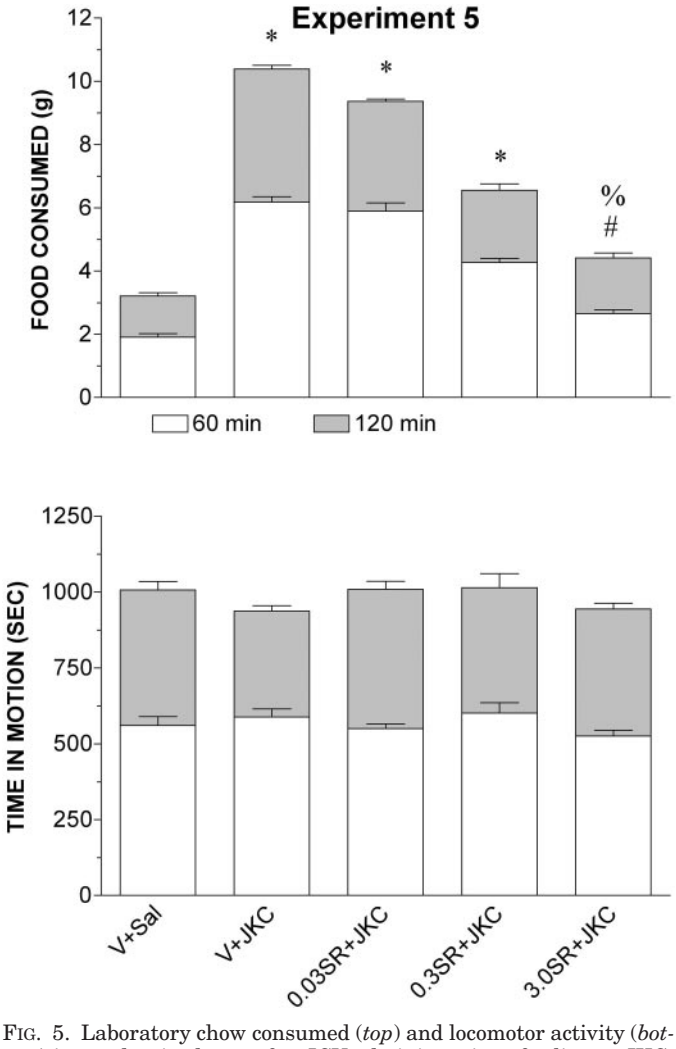

FIG. 5. Laboratory chow consumed (*top*) and locomotor activity (*bottom*) in nondeprived rats after ICV administration of saline or JKC-363 (1 nmol) alone or in combination with vehicle or 0.03, 0.3, or 3.0 μg SR 141716. Data represent means (+SEM) at two consecutive 60-min measurement intervals. V, Vehicle; SR, dose of SR 141716 in micrograms; Sal, saline; JKC, dose of JKC-363 in micrograms; *, $P < 0.05$, significantly different from Sal+V; #, $P < 0.05$, significantly different from JKC+V; %, $P < 0.05$, significantly different from 0.03 SR+JKC.

Experiment 5: effects of SR 141716 on feeding induced by JKC-363

The mean quantity of food consumed (mean, 2.34 g; range, 2.08–2.44 g) and locomotor activity (mean, 462.996 sec; range, 453.01–476.19 sec) before drug administration during the 30-min prefeed phase in experiment 5 was relatively low and did not differ significantly between treatments. A repeated measures ANOVA comparing all treatments was conducted for the food consumption and locomotor activity results. SR 141716 produced a significant dose-dependent reduction of JKC-363-stimulated food consumption (Fig. 5, *top*) ($F_{(4,24)} = 36.96$; $P < 0.001$) but had no effect on locomotor activity (Fig. 5, *bottom*). Pairwise comparison of the effects of different doses of SR 141716 on JKC-363-induced food consumption revealed that the 0.3 μg SR+JKC-363 and 3.0 μg SR+JKC-363 doses differed significantly from the vehicle+JKC-363 treatment. Furthermore, the 0.03 μg SR+JKC-363 treatment differed significantly from the 3.0 μg SR+JKC-363 treatment. There was a significant main effect of bin ($F_{(1,6)} = 37.35$; $P < 0.001$) showing that less food was consumed during the second hour compared with the first hour. There was also a significant main effect of bin for locomotor activity ($F_{(1,6)} = 37.35$; $P < 0.001$), suggesting that locomotion was greater during the first hour relative to the second hour of testing. The dose by bin interaction was not significant for food consumption or locomotor activity.

Discussion

The major findings of the present study can be summarized as: 1) when administered alone, SR 141716 and α-MSH dose-dependently attenuated baseline feeding; 2) administration of sub-anorectic doses of SR 141716 and α-MSH synergistically attenuated baseline food intake; 3) THC-induced

feeding was not blocked by α-MSH; 4) SR 141716 dose-dependently attenuated JKC-363-induced feeding behavior; and 5) locomotor activity was not significantly affected by any drug treatments, suggesting that effects on feeding were not due to a nonspecific reduction in motivated behavior.

The results from experiment 1 demonstrate that SR 141716 attenuates feeding, complementing previous findings with this drug (17–19, 33, 34) and offering additional support for the role of the endogenous cannabinoid system in the control of feeding (for review, see Ref. 1). The finding that α-MSH suppresses food intake (experiment 2) is also in good agreement with previous reports (3, 4) and further supports the notion that MC receptors play an important role in the regulation of feeding.

Experiment 3 examined whether α-MSH and SR 141716 act independently or synergistically to block food intake. Results revealed that the combined administration of doses of SR 141716 and α-MSH that did not alter food intake on their own produced a synergistic reduction of food intake. This finding complements a previous study demonstrating that CB_1 receptor blockade by SR 141716 enhances the ability of MCs to promote recovery from hemorrhagic shock (29). It is notable that only a single dose of α-MSH and SR 141716 were combined in experiment 3; additional doses should be studied to fully characterize the interactive effects of these drugs. Although the specific mechanisms underlying the synergistic action of α-MSH and SR 141716 are poorly understood, it is possible that SR 141716 and MCs may interact at the signal transduction mechanism level by increasing cAMP synthesis via an effect on G_i proteins. Both cannabinoids and MCs belong to the G protein-coupled receptor superfamily (for review, see Refs. 35 and 36), and blockade of CB_1 receptors with SR 141716 and stimulation of MC receptors increases cAMP production (37, 38). It is worth noting that the effect of SR 141716 on cAMP may be due to the inverse agonist action of SR 141716 combined with a reduction of the endogenous cannabinoid tone. cAMP has been proposed to be a biochemical signal mediating satiety (39). Thus, when given alone, sub-anorectic doses of SR 141716 or α-MSH may not be sufficient to stimulate cAMP production. However, when given together, they may stimulate the production of sufficient cAMP to reduce feeding, although the effects of combined administration of sub-anorectic doses of SR 141716 and α-MSH on cAMP production needs to be elucidated. The proposed mechanism underlying the synergistic effect of SR 141716 and α-MSH may, therefore, represent a biochemical, rather than a physiological, correlate.

The synergistic interaction between the cannabinoid and MC systems observed in this study may involve the opioid system. Thus, MCs are considered to be endogenous antagonists for the opioid system (40), morphine-induced depression of evoked potentials (41) and the addictive properties of opiates (42) are blocked by MCs, and MC effects on experimental hemorrhagic shock can be reversed by morphine treatment (43). Furthermore, opioids stimulate endogenous cannabinoid release (44), and SR 141716 reduces morphine-induced feeding behavior (30). It is, therefore, possible that the synergistic effect between the cannabinoid and MC systems may be modulated by opioid signaling.

Experiments 4 and 5 were designed to determine the relative position of CB_1 receptors in the chain of control of food intake in relation to the MC system. Results revealed that THC and JKC-363 significantly increased food intake, consistent with previous studies (12, 45). Interestingly, in experiment 4, α-MSH failed to block THC-induced feeding at doses that significantly reduced feeding on their own (experiment 2), whereas in experiment 5, SR 141716 reduced feeding induced by JKC-363. The results of experiments 4 and 5 suggest that CB_1 receptors may be located downstream from MCR4 receptors and that THC-induced feeding is not critically dependent on the MC system.

Although the results of the present study do not elucidate the specific brain regions and mechanisms involved, other studies serve to suggest several possibilities. One such hypothesis is that the cannabinoid and MC interaction observed in the present study may be mediated via the PVN (9, 10). The PVN regulates food intake by influencing the metabolic, hormonal, and endocrine responses relating to the nutritional state of the organism (2). This structure is the target of converging orexigenic and anorexigenic pathways originating from various hypothalamic sites (46) 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, as well as MC peptides and cannabinoids (2).

MC and cannabinoid receptors are densely located within the PVN (23, 47). Maximal suppression of food intake by α-MSH was observed with intra-PVN injections (48), and the synthetic MCR4 ligand MT-II produces widespread Fos expression in the PVN. In addition, we have shown that systemic administration of THC and SR 141716 produces Fos expression in many regions of the hypothalamus, with the PVN showing the highest levels (24, 25). We have recently demonstrated that administration of THC into the PVN produces a profound stimulation of feeding (our unpublished data). It is, therefore, possible that the MC and cannabinoid systems may interact within the PVN to regulate food intake. It should be noted that most synaptic transmission and intracellular signaling within the hypothalamus is mediated via axonal release of the neurotransmitter glutamate (for review, see Ref. 49). Evidence suggests that cannabinoid (50) and POMC (51) neurons use glutamate, suggesting that the interaction observed in the present study could be due to the activation or suppression of the excitatory synaptic drive to PVN anorexigenic signals. Indeed, a recent study has demonstrated cannabinoid-induced suppression of excitatory glutamatergic inputs to PVN neurons (52).

The results of the present study and other published reports provide evidence that CB_1 receptor activation is necessary to prevent MC-induced inhibition of feeding and CB_1 receptors are located downstream from MC receptors. For example, the NPY-Y1 receptor antagonist 1229U91 significantly reduces food intake stimulated by the MR4R receptor antagonist HS014 (53). MC4R receptor knockout mice still respond to the orexegenic effects of NPY (54), whereas NPY fails to stimulate feeding in CB_1 receptor-deleted mice (55). Fos expression induced by leptin, an adipocyte-derived hor-

mone, is blocked by the MC3R/MC4R receptor antagonist SHU9119 (56). HS014 reduces feeding inhibition and body weight loss induced by leptin in rats (57), and leptin-deficient C57BL/6JLep^{ob} mice respond to the anorectic effect of the MC agonist melanotan-II (3). These data clearly suggest that leptin exerts control over the melanocortinergic neurons, whereas the cannabinoid and NPY systems are modulated by the MC system. Thus, MC receptor signaling seems to be located downstream from leptin receptors and upstream from endogenous cannabinoid-, NPY-, and opioid-producing neurons. Taken together, it is hypothesized that melanocortinergic neurons would control endogenous cannabinoid activity. This conclusion is supported by the results of experiments 4 and 5, in which it was demonstrated that THC-induced feeding was not reduced by α-MSH, whereas SR 141716 reduced JKC-363-induced feeding. These results suggest a signaling cascade involving leptin→α-MSH→NPY→CB_1.

The present study also found a synergistic interaction between the cannabinoid and MC systems, suggesting that MCs may directly modulate endogenous cannabinoid activity bypassing NPY. It would be interesting to determine whether MCs affect food intake in CB_1 receptor-deleted mice. Based on the results of the present study and previous published reports, we hypothesize that CB_1 receptor-deleted mice should not respond to the anorexigenic effect of α-MSH or the orexigenic effect of JKC-363. Such a study is eagerly awaited.

The finding that the doses of SR 141716 used in the present study are effective in decreasing food intake when administered alone (16) admittedly complicated the interpretation of an interaction between the cannabinoid and MC systems. That is, attenuation of JKC-363-induced feeding by SR 141716 may be unrelated to an interaction with the MC system but may, in fact, result from suppression of all feeding by a downstream mechanism. It is, therefore, important to note the results of a previous study from our group (30). In this study, SR 141716 attenuated feeding produced by intra-PVN but not intra-accumbens application of morphine. These results clearly show that the anorectic effects of a systemically administered high dose of SR 141716 (*i.e.* 3.0 mg/kg) can be completely overridden by pharmacological treatment.

In conclusion, this study demonstrates for the first time a synergistic interaction between the MC and cannabinoid systems in regulating food intake. Results further suggest that the cannabinoid receptors are located downstream from the MC system and that CB_1 receptor activation may be necessary for MC antagonist-induced stimulation of food intake.

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