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ORIGINAL ARTICLE

Behavioral effects of the cannabinoid CB_1 receptor allosteric modulator ORG27569 in rats

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Abstract

The cannabinoid CB_1 receptor system is involved in feeding behaviors and the CB_1 receptor antagonist SR141716A is an effective antiobesity drug. However, SR141716A also has serious side effects, which prompted the exploration of alternative strategies to modulate this important drug target. Recently a CB_1 receptor allosteric modulating site has been discovered and the allosteric modulating activity of several modulators including ORG27569 has been characterized in vitro. Yet, little is known of the in vivo pharmacological effects of ORG27569. This study examined the behavioral pharmacology of ORG27569 in rats. ORG27569 (3.2–10 mg/kg, i.p.) selectively attenuated the hypothermic effects of CB_1 receptor agonists CP55940 (0.1–1 mg/kg) and anandamide (3.2–32 mg/kg). In contrast, SR141716A only attenuated the hypothermic effects of CP55940 but not anandamide. SR141716A but not ORG27569 blocked CP55940-induced catalepsy and antinociception. In addition, ORG27569 did not modify SR141716A-elicited grooming and scratching behaviors. In feeding studies, ORG27569 decreased palatable and plain food intake which was partially blocked by CP55940. The hypophagic effect of ORG27569 developed tolerance after 4 days of daily 5.6 mg/kg treatment; however, the effect on body weight gain outlasted the drug treatment for 10 days. These data suggest that ORG27569 may not function as a CB_1 receptor allosteric modulator in vivo, although its hypophagic activity still has potential therapeutic utility.

Abbreviations

Anandamide, (5*Z*,8*Z*,11*Z*,14*Z*)-*N*-(2-hydroxyethyl)icosa-5,8,11,14-tetraenamide; 2-BFI, (2-(2-benzofuranyl)-2-imidazoline); CP55940, 2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol; MK212, (6-chloro-2-(1-piperazinyl) pyrazine hydrochloride); ORG27569, 5-chloro-3-ethyl-1H-indole-2-carboxylic acid [2-(4-piperidin-1-yl-phenyl)ethyl]amide; SR141716A, 5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide.

Introduction

The cannabinoid CB_1 receptor system comprises the endocannabinoids, their receptors, and the key enzymes for synthesis and hydrolysis of endocannabinoids. The endocannabinoid system is an important regulator of many central and peripheral physiological and pathophysiological processes, including energy homeostasis, inflammation, emotion, memory, pain perception, and motivation for food and drug intake (Kirilly et al. 2012; Fine and Rosenfeld 2013; Silvestri and Di Marzo 2013; Morena and Campolongo 2014; Vlachou and Panagis 2014; Witkamp and Meijerink 2014). Therefore, great efforts have been made to pharmacologically modulate the endocannabinoid system for the treatment of various disorders, with some notable success. For example, Sativex®, a combination

of the cannabinoids tetrahydrocannabinol and cannabidiol, shows significant efficacy in the control of spasticity due to multiple sclerosis and is also in the development for the treatment of terminal cancer pain and neuropathic pain (Johnson et al. 2013; Serpell et al. 2013).

The first synthetic cannabinoid CB_1 receptor antagonist/inverse agonist SR141716A effectively attenuates the daily food intake and body weight gain both in normal rats and in obese Zucker rats (Colombo et al. 1998; Vickers et al. 2003), suggesting the modulation of CB_1 receptor blockade on energy balance and feeding behavior. Several large clinical trials confirmed these effects in overweight or obese patients (Pi-Sunyer et al. 2006; Despres et al. 2008; Van Gaal et al. 2008). These drug development efforts eventually moved SR141716A (Rimonabant®) into clinical practice for the management of obesity. However, it soon became clear that rimonabant induces severe adverse psychiatric events in some patients, including depression, anxiety, and suicidal ideation (Despres 2009; Kirilly et al. 2012). These adverse effects led to the withdrawal of rimonabant from the market (Traynor 2007). Although this withdrawal somewhat dampens the enthusiasm of targeting CB_1 receptors for body weight control, alternative strategies have been proposed. For example, it is possible to circumvent the central actions by designing peripheral CB_1 receptor antagonists, which may decrease the central adverse events related to CB_1 receptor blockade but preserve the antiobesity effects (Kirilly et al. 2012).

CB_1 allosteric negative modulators could be another possible strategy. Besides the orthosteric binding site, allosteric binding site(s) on CB_1 receptors has been reported although the exact location remains elusive (Price et al. 2005). Thus, theoretically drugs that act on this allosteric binding site can modulate the receptor activity through conformational changes in the receptor that are transmitted from the allosteric to the orthosteric site and/or to effector coupling sites (Christopoulos and Kenakin 2002). Several compounds demonstrate allosteric modulating activities on CB_1 receptors in in vitro assays (Price et al. 2005; Horswill et al. 2007; Navarro et al. 2009; Pamplona et al. 2012). For instance, ORG27569, ORG27759, and ORG29647 all increase the binding affinity of CB_1 receptor agonist CP55940 (positive allosteric modulation) and decrease the specific binding of CB_1 receptor antagonist SR141716A (negative allosteric modulation), indicative of allosteric binding cooperativity (Price et al. 2005). In contrast, other compounds such as lipoxin A4 and RTI-371 increase the affinity and efficacy of CB_1 receptor agonists and function as CB_1 receptor allosteric enhancers (Navarro et al. 2009; Pamplona et al. 2012). Importantly, some of these compounds (e.g., ORG27569 and ORG27759) behave as insurmountable antagonists in the mouse vas deferens bioassay via inhibition of G protein coupling, suggesting that they function as allosteric negative modulators (Price et al. 2005; Cawston et al. 2013). Although CB_1 receptor allosteric modulators could have important therapeutic potentials due to their functional antagonist activity on CB_1 receptors through distinct mechanism from orthosteric receptor antagonist such as SR141716A, the behavioral pharmacology of such compounds is largely unknown until recently (Gamage et al. 2014). This study examined the behavioral effects of one putative CB_1 receptor allosteric modulator, ORG27569, in several behavioral assays that are known to be related to CB_1 receptor activity in rats. The effects of ORG27569 on food intake to palatable food (Ensure®) and plain food on free-fed normal rats were also studied.

Materials and Methods

Subjects

Adult male Sprague–Dawley rats (Harlan, Indianapolis, IN) with initial weight of 250–300 g were housed individually on a 12/12 h light/dark cycle (behavioral tests were conducted during the light period) with free access to water and food except during test sessions. Animals were maintained and experiments were conducted in accordance with the 2011 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, Washington, DC). All studies were approved by the Institutional Animal Care and Use Committee, University at Buffalo, the State University of New York.

Behavioral procedures

Body temperature measurement

Body temperature was measured in a quiet procedure room maintained under identical environmental controls (temperature, humidity, and lighting) with the animal colony room. Rats (n = 5–8 per group) were habituated to the procedure room for at least 30 min before each test. Body temperature was measured by gently inserting a rectal probe (5.0 cm) and recording temperature from the digital thermometer (BAT7001H; Physitemp Instruments Inc., Clifton, NJ) (Li et al. 2009; Thorn et al. 2012). During a test session, a baseline body temperature measurement was immediately followed by the injection of a dose of a drug, and the follow-up measurements were conducted every 15 or 30 min until the effect of the drug dissipated. When a drug combination was studied, the first drug was administered 10 min before the first

measurement, which was immediately followed by the administration of a second drug. Rats were handled for at least 3 days before testing drugs in order to habituate rats to the procedure and were only used in one test.

Catalepsy

Drug-induced catalepsy was measured with a bar test. In the bar test, the two forelimbs of the rats (n = 7–8 per group) were placed on a horizontal, cylindrical metal bar (diameter, 1.0 cm; height, 10 cm; custom made) and the time until both forelimbs touched the table surface was recorded up to a maximum of 60 sec. Both hind limbs were placed on the same location for all the tests. The dose–effect relationships of the CB_1 receptor agonist CP55940-induced catalepsy were determined using a cumulative dosing procedure with 0.5 log unit increments and 30 min intertest intervals as described previously (Li et al. 2011, 2014). When a pretreatment drug was studied in combination with CP55940, the pretreatment time was 10 min.

Antinociception

Warm water tail flick test was used to detect the antinociceptive effects as described in detail elsewhere (Thorn et al. 2011; Sampson et al. 2012). Briefly, a water bath (model RS-PB-200; Revolutionary Science, Lindstrom, Minnesota) filled with tap water was maintained at 52°C and tail flick latencies were recorded with a hand-operated digital timer. During the test session, a baseline latency test and a postdrug test was separated by 30 min with a test drug being administered immediately after the baseline test. When a pretreatment drug was studied, the drug was administered 10 min prior to the baseline latency test. A 20-sec cutoff time was applied during the test to avoid tissue damage.

Grooming and scratching

The observation protocols of grooming and scratching behaviors were reported previously (Jarbe et al. 2002). Briefly, rats (n = 9–10 per group) were habituated to a quiet procedure room for at least 30 min before the test. A standard acrylic rat cage (26 × 47 × 22 cm) served as the observational arena and was cleaned between tests. Based on the literature (Jarbe et al. 2002) and our preliminary study, test began 15 min after SR141716A was administered and the duration of the observation was 10 min. For the tests, a rat was gently moved into the observation cage and two observers blind to the treatment monitored the frequency of grooming (the number of cleaning bouts) and scratching as defined according to the literature (Darmani and Pandya 2000) for 10 min. When ORG27569 was studied as a pretreatment, it was administered 10 min prior to SR141716A treatment. Rats were only used once.

Palatable food intake

Palatable food intake studies (n = 8) were conducted daily in a quiet procedure room adjacent to the animal colony room beginning at 12:00 noon. During initial training sessions, rats were removed from their home cages, weighed, and gently placed individually in acrylic standard rat cages equipped with a glass dish containing 40 mL of liquid food (milk chocolate-flavored Ensure® protein drink, 100 mL containing 148 kcal, including 4.6 g fat, 21.1 g carbohydrates, and 5.5 g protein; Abbott Laboratories, Lake Forest, IL). After 60 min, rats were returned to their home cages, and the amount of food consumed during the 60-min period was determined by subtracting the weight of the dish at the end of the session from its weight at the beginning of the session. Test began when daily food intake stabilized under these conditions (three consecutive days with ≤20% variability in consumption). For drug tests, ORG27569 or its vehicle was administered 30 min prior to test initiation. At least two baseline sessions were interspersed between drug test sessions.

Plain food intake

Rats that participated in the plain food intake experiment (n = 6–8 per group) were free fed throughout the study and the experiment occurred in their home cages. The daily food intake and body weight gain were monitored for 4 days before the experiment was initiated. Animals and their total food amount were weighed daily before the dark cycle started. On the first day of the experiment, drug or its vehicle was administered immediately before the start of the dark cycle and food intake was monitored by weighing the total residual food at 6, 12, and 24 h. For rats that participated in repeated dosing experiment, the test day was followed by repeated daily drug (8 days of daily 5.6 mg/kg ORG27569 followed by 4 days of daily 10 mg/kg ORG27569) or vehicle treatment (a total of 12 days). The ORG27569 dose was increased during the study because it was found that a significant tolerance to the hypophagic effect of 5.6 mg/kg ORG27569 was developed and thus a larger dose was used to confirm the presence of tolerance and recoup the effect. After that, daily treatment was terminated but the daily intake and body weight gain were continuously monitored for another 14 days. Thus, the entire repeated dosing experiment lasted for 30 days.

Data analyses

For the body temperature data, the body temperature changes (°C, mean ± SEM) were calculated by subtracting the baseline body temperature readings (first measurement of each test session) from all the subsequent measurements and plotted as a function of time. When single drug was studied, the data with 95% confidence limits not including 0 were considered significantly different from the baseline. When two drugs were studied, two-way repeated measures analysis of variance (ANOVA) (treatment × time) followed by Bonferroni's post hoc test was used to analyze the data. The area under curves (AUC) between times 0–135 min of individual doses of CP55940 alone and in combination with ORG27569 or SR141716 were also calculated and analyzed using two-way ANOVA (dose × treatment) followed by Bonferroni's post hoc test or Student's *t*-test. For the catalepsy, grooming and scratching experiments, the data were plotted as a function of drug dose and analyzed using two-way repeated measures ANOVA (dose × treatment) followed by Bonferroni's post hoc test. For the antinociception experiment, the tail flick data were analyzed using one-way ANOVA followed by Bonferroni's post hoc test. For the palatable food intake data, the total intake before drug test was used as control and the data after drug or vehicle treatment were normalized as % of control and were analyzed using one-way repeated measures ANOVA. For the plain food intake experiment, data from acute drug treatment were normalized as % of control and analyzed using one-way ANOVA, and data from repeated dosing study were analyzed using two-way repeated measures ANOVA (time × treatment) followed by Bonferroni's post hoc test. For all the statistical analyses, $P < 0.05$ was considered statistically significant.

Drugs

CP55940 (2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol), anandamide (5*Z*,8*Z*,11*Z*,14*Z*)-*N*-(2-hydroxyethyl)icosa-5,8,11,14-(tetraenamide), and SR141716A (5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide) were provided by Research Technology Branch (National Institute on Drug Abuse, Rockville, MD). Clonidine hydrochloride and MK212 (6-chloro-2-(1-piperazinyl) pyrazine hydrochloride) hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). ORG27569 and 2-BFI (2-(2-benzofuranyl)-2-imidazoline) hydrochloride were synthesized at Research Triangle Institute and verified by high-performance liquid chromatography (>95% pure), nuclear magnetic resonance, and elemental analysis. CP55940, anandamide, SR141716A, and ORG27569 were dissolved in a mixture of 1 part absolute ethanol, 1 part Emulphor-620 (Rhodia Inc., Cranbury, NJ), and 18 parts physiological saline. Clonidine, MK212, and 2-BFI were dissolved in 0.9% physiological saline. Doses were expressed as the weight of the forms listed above in milligrams per kilogram of body weight. All drugs were administered intraperitoneally. All drug/molecular target nomenclature used here conforms to *British Journal of Pharmacology's Guide to Receptors and Channels* (Alexander et al. 2011).

Results

The synthetic CB_1 receptor agonist CP55940, the endogenous cannabinoid anandamide, the $\alpha 2$ adrenoceptor agonist clonidine, and the imidazoline I2 receptor ligand 2-(2-benzofuranyl)-2-imidazoline (2-BFI) all dose dependently decreased the body temperature in rats. For CP55940, 0.32 and 0.56 mg/kg markedly decreased the body temperature with the nadirs reaching -2.9 ± 0.2 and -2.6 ± 0.3°C 60 min after drug administration. The duration of the hypothermic effect lasted between 2.5 and 3 h. A higher dose of CP55940 (1 mg/kg) did not further decrease the body temperature (nadir: -2.8 ± 0.2°C), but the effect lasted for at least 5 h (left panel, Fig. 1). For anandamide, a dose of 10 mg/kg decreased the body temperature up to -1.1 ± 0.4°C, but the effect only lasted for 30 min. A larger dose of anandamide (32 mg/kg) further decreased the body temperature to -2.2 ± 0.0°C and the effect lasted for 60 min (middle panel, Fig. 1). Clonidine at 1 mg/kg significantly decreased the body temperature and reached the nadir to -3.3 ± 0.2°C 3 h after the drug treatment. 2-BFI at 10 mg/kg significantly decreased the body temperature and reached the nadir to -1.7 ± 0.3°C 30 min after the drug treatment (right panel, Fig. 2). By contrast, ORG27569 did not significantly alter the body temperature within the dose range studied (3.2–10 mg/kg) (right panel, Fig. 1).

When studied as a combination, ORG27569 at doses of 3.2 and 10 mg/kg markedly attenuated the hypothermic effect of 0.32 mg/kg CP55940 (top left, Fig. 2). Two-way ANOVA revealed a significant main effect of time (3.2 mg/kg, $F_{13,52} = 17.22$, $P < 0.0001$; 10 mg/kg, $F_{9,36} = 21.13$, $P < 0.0001$) and a significant time × treatment interaction (10 mg/kg, $F_{9,36} = 3.74$, $P < 0.01$). Post hoc analyses indicated that the hypothermic effect of 0.32 mg/kg CP55940 was significantly attenuated between 60 and 120 min (3.2 mg/kg ORG27569) and between 45 and 135 min (10 mg/kg ORG27569). Similarly, 10 mg/kg ORG27569 also significantly attenuated the hypothermic effect induced by 32 mg/kg anandamide (top right, Fig. 2). Two-way ANOVA revealed significant main

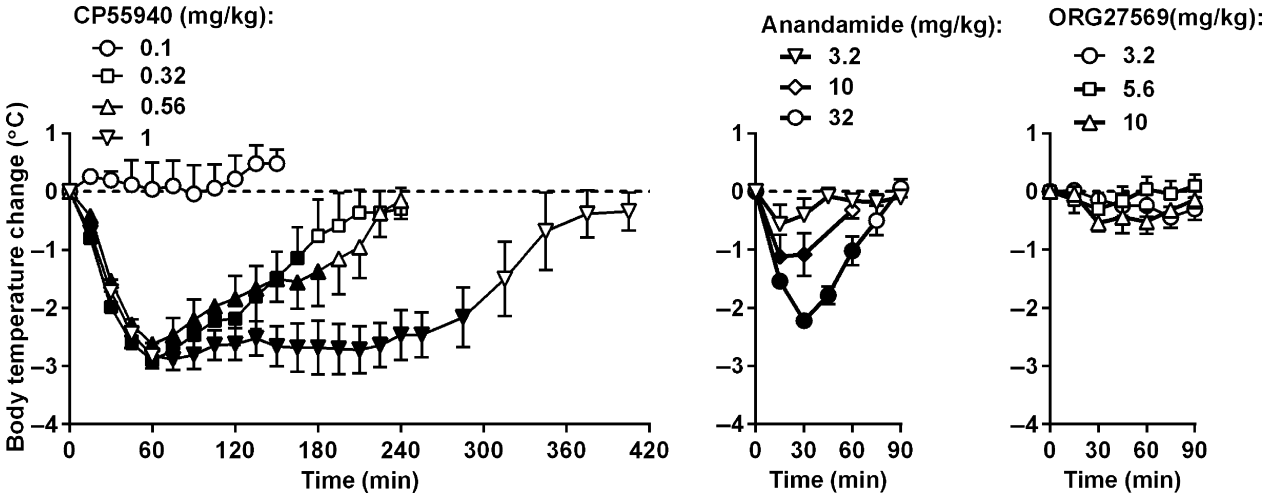

Figure 1. CP55940 (left) and anandamide (center) but not ORG27569 dose dependently decreased the rectal temperature in rats. Body temperature change (°C; ordinate) was plotted as a function of time (min; abscissa). Data are mean ± SEM of five rats/group (the 0.56 mg/kg CP55940 group included six rats). Filled symbols represent data that were significantly different from predrug control (95% confident limits not including 0).

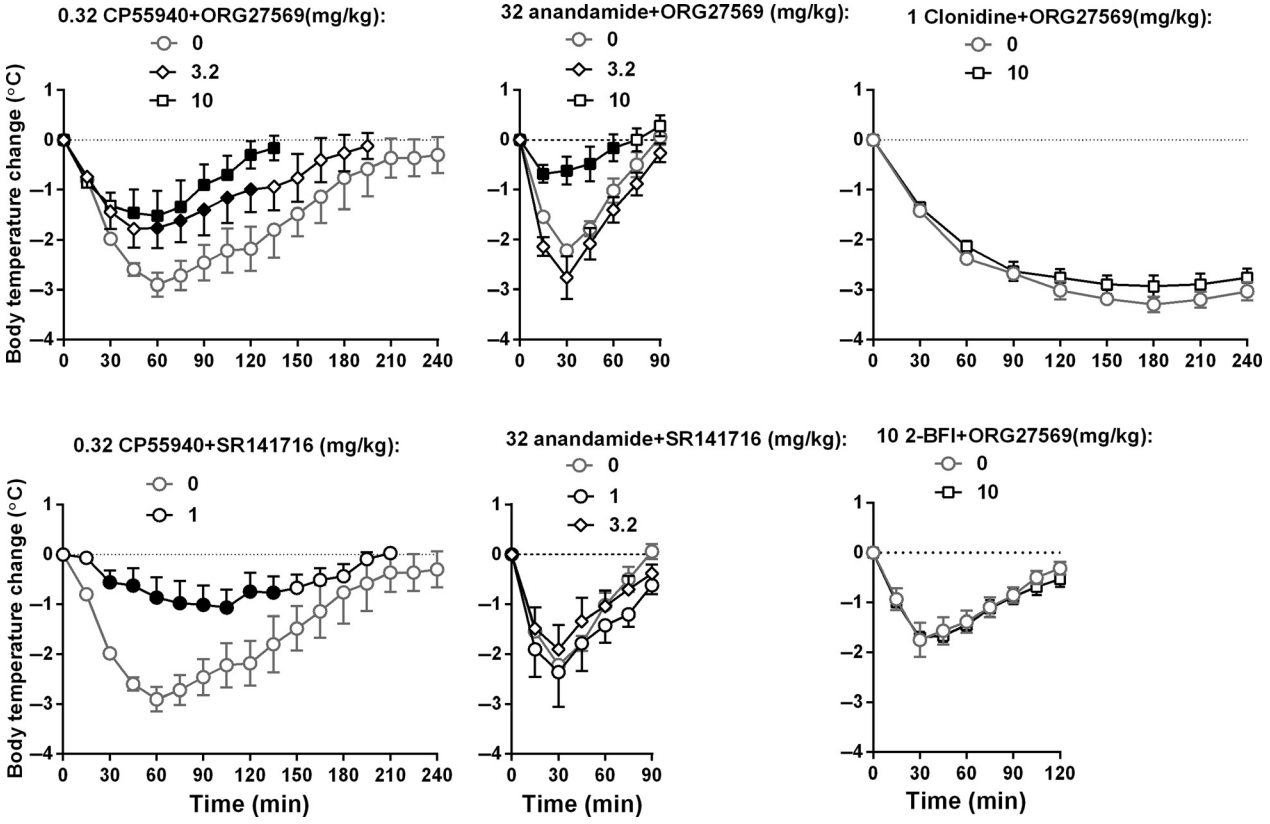

Figure 2. Effects of ORG27569 (top) or SR141716A (bottom, $n = 9$) on CP55940- (left, $n = 5$), anandamide- (middle, $n = 5$), clonidine- (right top, $n = 6$), or 2-BFI (right bottom, $n = 6$)-induced hypothermia in rats. Filled symbols represent data significantly different from control data (gray circles). See Figure 1 for other details.

effects of time ($F_{6,24}$ = 31.97, $P < 0.0001$), treatment ($F_{1,4}$ = 17.87, $P < 0.05$), and time × treatment interaction ($F_{6,24}$ = 10.82, $P < 0.0001$). Post hoc analyses indicated that the hypothermic effect of 32 mg/kg anandamide was significantly attenuated between 15 and 60 min. The CB_1 receptor antagonist SR141716A (1 mg/kg) also significantly attenuated the hypothermic effect of 0.32 mg/kg CP55940 (bottom left, Fig. 2). Two-way ANOVA revealed a significant main effect of time ($F_{14,56}$ = 10.97, $P < 0.0001$) and a significant time × treatment interaction ($F_{14,56}$ = 2.92, $P < 0.01$). Post hoc analyses indicated that the hypothermic effect of 0.32 mg/kg CP55940 was significantly attenuated between 30 and 150 min (3.2 mg/kg ORG27569). However, SR141716A (1–3.2 mg/kg) did not significantly alter 32 mg/kg anandamide-induced hypothermia (bottom middle, Fig. 2). ORG27569 at a dose of 10 mg/kg did not significantly change clonidine- and 2-BFI-induced hypothermia (bottle right, Fig. 2).

Two-way ANOVA analyses of the AUC data revealed that for the combination of 10 mg/kg ORG27569 with CP55940, there was a significant main effect of treatment ($F_{1,34}$ = 9.95, $P < 0.01$). Post hoc analysis indicated that the AUC of 0.32 and 0.56 mg/kg CP55940-induced hypothermia was significantly decreased by 10 mg/kg ORG27569. Student's *t*-test also revealed that 1 mg/kg SR141716A significantly decreased the AUC of 0.32 mg/kg CP55940-induced hypothermia (t_{12} = 3.31, $P < 0.01$) (Fig. 3).

CP55940 dose dependently and significantly increased the duration of catalepsy (top left, Fig. 4) (one-way ANOVA: $F_{4,24}$ = 9.56, $P < 0.0001$). Post hoc analyses indicated that 1 mg/kg CP55940 produced significant cataleptic effect in rats. Pretreatment with 3.2 or 10 mg/kg ORG27569 did not significantly alter the cataleptic effect of CP55940. However, 1.78 mg/kg SR141716A significantly decreased CP55940-induced catalepsy. Two-way ANOVA revealed a significant main effect of dose ($F_{2,12}$ = 10.52, $P < 0.01$) and significant dose × treatment interaction ($F_{2,12}$ = 7.72, $P < 0.01$). Post hoc analyses indicated that SR141716A significantly decreased the cataleptic effect induced by 0.1–1 mg/kg CP55940 ($P < 0.05$). CP55940 (0.32 mg/kg) produced significant antinociception, which was significantly attenuated by SR141716A but not ORG27569 ($F_{5,30}$ = 24.52; $P < 0.0001$) (bottom left, Fig. 4).

SR141716A dose dependently and significantly increased the frequency of grooming (top right, Fig. 4) (one-way ANOVA: $F_{3,34}$ = 10.37, $P < 0.0001$) and scratching (bottom right, Fig. 4) (one-way ANOVA: $F_{3,34}$ = 11.26, $P < 0.0001$). Post hoc analyses indicated that 1.78 and 5.6 mg/kg SR141716A significantly increased the frequency of grooming and 5.6 mg/kg SR141716A significantly increased the frequency of scratching. ORG27569 alone did not elicit increased grooming and scratching behaviors (symbols above "V") and also did not significantly modify the grooming and scratching behaviors induced by SR141716A. Two-way ANOVA revealed a significant main effect of SR141716A (grooming: $F_{2,80}$ = 22.99, $P < 0.0001$; scratching: $F_{2,80}$ = 34.42, $P < 0.0001$). Post hoc analyses found no significant difference.

Acute treatment with ORG27569 dose dependently decreased the palatable food intake ($F_{7,21}$ = 8.02, $P < 0.01$) (left panel, Fig. 5) with 5.6 and 10 mg/kg ORG27569 reaching statistical significance. Similarly, acute treatment with 5.6 mg/kg ORG27569, 0.32 mg/kg CP55940, and 3.2 mg/kg MK212 all significantly decreased food intake ($F_{5,42} \geq 6.77$, $P < 0.0001$; right panel, Fig. 5). In addition, a combination of 3.2 mg/kg MK212 with 5.6 mg/kg ORG27569 further significantly decreased the plain food intake ($P < 0.05$) while a combination of 0.32 mg/kg CP55940 with 5.6 mg/kg ORG27569 did not. In rats that continued to receive daily ORG27569 treatment, the drug continued to decrease the daily food intake in the rats (top panel, Fig. 6). Two-way repeated measures ANOVA revealed significant main effects of time ($F_{29,145}$ = 6.92, $P < 0.0001$) and time × ORG27569 treatment ($F_{29,145}$ = 4.67, $P < 0.0001$). Post hoc analyses indicated that for the first 4 days of daily 5.6 mg/kg ORG27569 treatment and for the first 2 days of daily 10 mg/kg ORG27569 treatment, food intake was significantly less compared with rats that received daily vehicle treatment (top panel, Fig. 6).

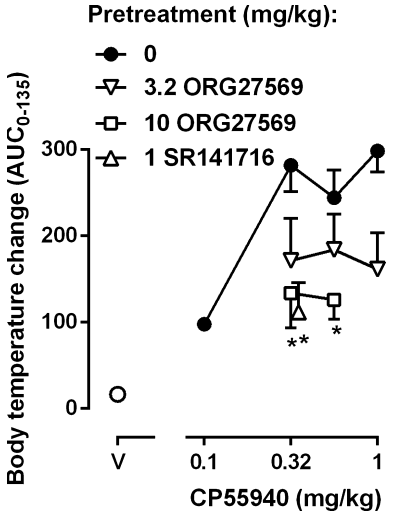

Figure 3. ORG27569 and SR141716A decreased the AUC of CP55940-induced hypothermia in rats. Ordinate: area under curve (AUC) between 0 and 135 min postdrug administration; abscissa, CP55940 dose (mg/kg). *$P < 0.05$ as compared to CP55940 alone.

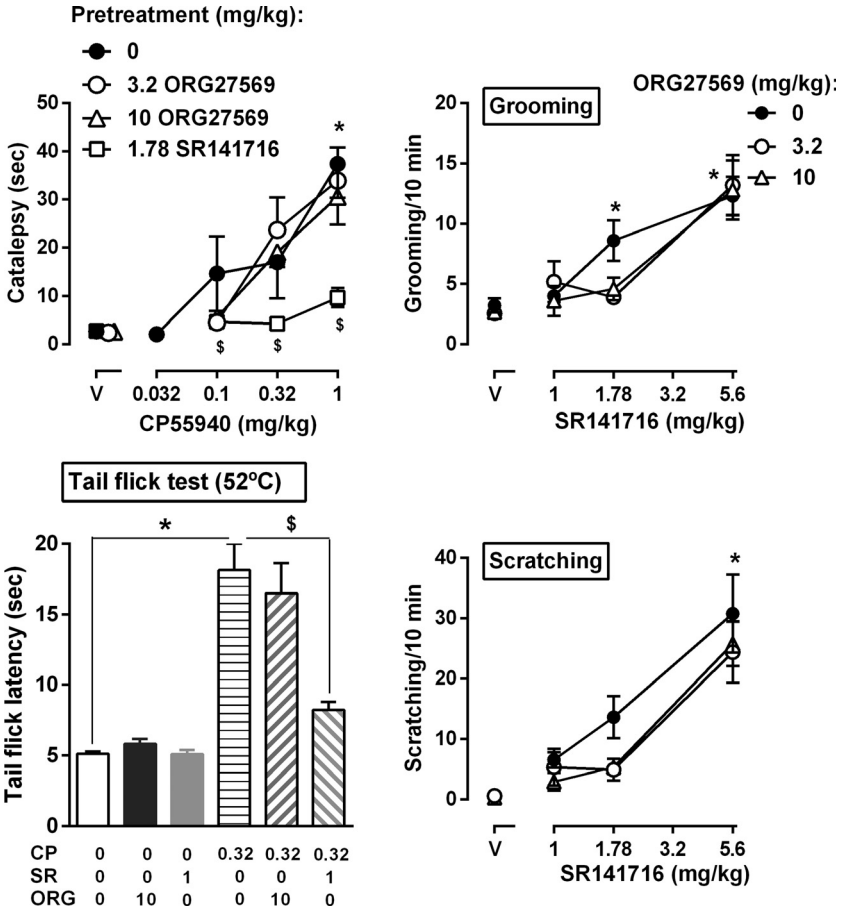

Figure 4. Effects of ORG27569 on CP55940-induced catalepsy (top left, n = 7–8), antinociception (top right, n = 6) and SR141716A-induced grooming (top right, n = 9–10) and scratching (bottom right, n = 9–10) behaviors in rats. Ordinate: top left, the duration of catalepsy (sec); bottom left, tail flick latency (sec); right, frequency of grooming (top) and scratching (bottom) behaviors as measured within a period of 10 min. Abscissa: dose of CP55940 (mg/kg, left) or dose of SR141716A (right). *$P < 0.05$ as compared to vehicle; $^{\$}P < 0.05$ as compared to CP55940 alone.

During the course of daily ORG27569 treatment, the body weight gain in rats that received ORG27569 was significantly less compared with those that received daily vehicle treatment (bottom panel, Fig. 6). Two-way repeated measures ANOVA revealed significant main effects of time ($F_{29,145} = 119.30$, $P < 0.0001$), ORG27569 treatment ($F_{1,5} = 13.77$, $P < 0.05$), and time × ORG27569 treatment interaction ($F_{29,145} = 6.42$, $P < 0.0001$). Post hoc analyses indicated that the body weight gain was significantly less between days 5 and 26 in rats that received daily ORG27569 treatment compared with rats that received daily vehicle treatment.

Discussion

This study reported the behavioral pharmacology of the putative allosteric CB_1 receptor modulator ORG27569 in rats. We found that ORG27569 selectively attenuated the hypothermic effects of two CB_1 receptor agonists, CP55940 and anandamide, but did not alter CP55940-induced catalepsy and antinociception. ORG27569 also did not alter the CB_1 receptor antagonist SR141716A induced grooming and scratching behaviors. Importantly, ORG27569 decreased the intake of both palatable food and plain food. Together, these results suggest that ORG27569 may not function as an allosteric negative modulator in vivo in rats, although its efficacy on feeding behavior may have potential therapeutic utility.

Cannabinoid receptor agonists produce various behavioral effects including hypothermia, antinociception, and catalepsy (Little et al. 1988). Both synthetic CB_1 receptor agonist CP55940 and the endogenous cannabinoid anandamide can produce hypothermia in rodents (De Vry et al. 2004; Singh et al. 2011), as shown in the current study. The hypothermic effects of CP55940 can be

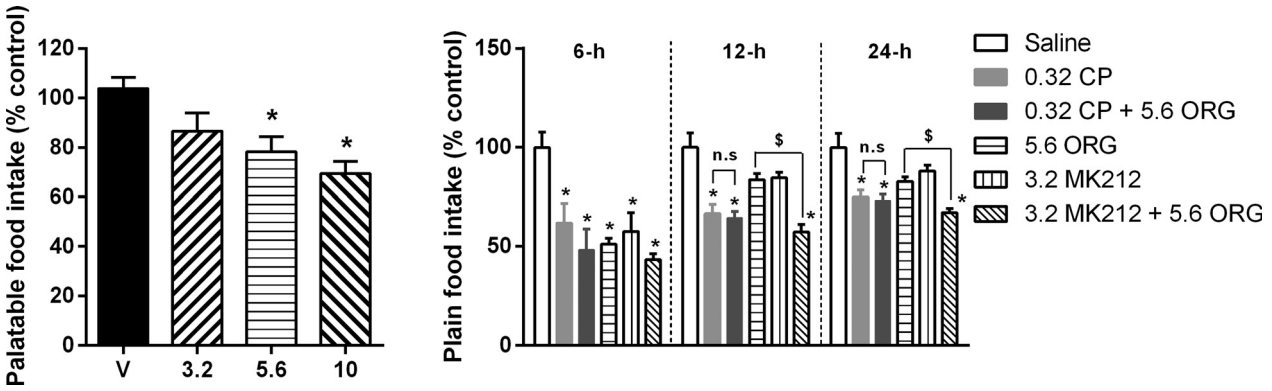

Figure 5. Effects of ORG27569 on palatable food (left, $n = 8$) and plain food intake (right, $n = 6$–8) in rats. Left: normalized total palatable food intake (no treatment condition set as 100%) within a period of 1 h. Right: normalized cumulative plain food intake after treatments with ORG27569, CP55940, and MK212 alone or in combination. $^{*}P < 0.05$ as compared to control condition. $^{\$}P < 0.05$ as compared to 5.6 mg/kg ORG27569 or 3.2 mg/kg MK212 alone. n.s., no statistical significance. CP: CP55940; ORG: ORG27569.

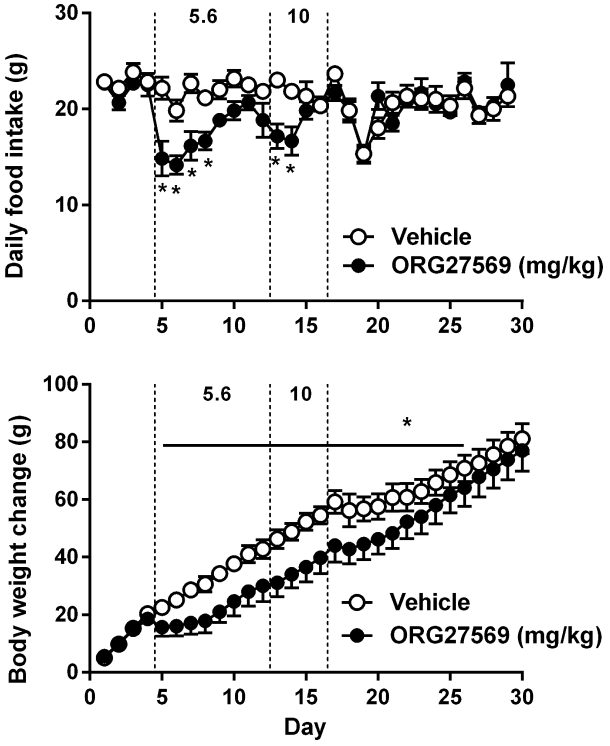

Figure 6. Effects of daily ORG27569 treatment on food intake (top) and body weight gain (bottom) in rats ($n = 6$). Vertical dashed lines indicate time frames that daily 5.6 or 10 mg/kg ORG27569 were administered. $^{*}P < 0.05$ as compared to vehicle-treated conditions.

blocked by CB_1 receptor antagonists (De Vry et al. 2004; Giuffrida and McMahon 2010), but the hypothermic effects of anandamide are usually not attenuated by CB_1 receptor antagonists such as SR141716A, suggesting the involvement of non-CB_1 receptor mechanism (Adams et al. 1998; Giuffrida and McMahon 2010; Singh et al. 2011). In the present study, ORG27569 attenuated the hypothermic effects induced by both CP55940 (CB_1-mediated effect) and anandamide (possibly non-CB_1-mediated effect), suggesting that ORG27569 may work through non-CB_1 mechanism. In addition, the antihypothermic effect of ORG27569 seems to be pharmacologically specific because ORG27569 did not alter the hypothermic effects induced by two pharmacologically unrelated drugs, although the potential underlying mechanism is unknown. The hypothermic effect of clonidine is mediated by α2 adrenoceptors and that of 2-BFI is mediated by imidazoline I_2 receptors (Thorn et al. 2012). This study also showed that ORG27569 did not alter CP55940-induced cataleptic and antinociceptive effects while SR141716A markedly blocked both effect in rats, which is consistent with a previous study in mice (Gamage et al. 2014). Combined, these results suggest that ORG27569 may not function as an active CB_1 allosteric negative modulator.

SR141716A induces various behavioral effects on its own including increased grooming and scratching behaviors and the effects are at least partially mediated through CB_1 receptors (Jarbe et al. 2002), although SR141716A also exerts non-CB_1-mediated effects (Erdozain et al. 2012). In contrast, ORG27569 did not elicit grooming and scratching behaviors at doses that markedly blocked CB_1 agonist-induced hypothermia. Because ORG27569 induces a CB_1 receptor state that decreases the receptor affinity of SR141716A (Ahn et al. 2012), we hypothesized that ORG27569 will attenuate the behavioral effects of SR141716A. No significant attenuation was observed. This is surprising and suggests that the in vitro results do not translate to in vivo conditions where behavioral changes are a result of integrated actions by exogenous and

endogenous ligands, brain region-specific receptor expression, and cross-talk among multiple systems (Herkenham et al. 1990). Nevertheless, ORG27569 also did not potentiate the effects of SR141716A for inducing grooming and scratching behaviors, which further suggests that ORG27569 does not function as an CB_1 receptor allosteric modulator.

Acute treatment with ORG27569 markedly decreased palatable food intake. The attenuation was transient as the 1-h palatable food intake 24 h later was not different from that without drug treatment (data not shown). It is well established that marijuana (*Cannabis sativa*) stimulates appetite, particularly for sweet and palatable foods, and that CB_1 receptor antagonism decreases the motivation for palatable foods both in laboratory animals and in humans. For instance, SR141716A has been shown to decrease palatable food self-administration, decrease the motivation to consume palatable food, and decrease plain food intake (Rowland et al. 2001; Ward and Dykstra 2005; Ward and Walker 2009). This property is the main drive that eventually led to the clinical development of SR141716A as an effective medication for overweight and obesity. ORG27569 reduces food intake in food-restricted mice (Gamage et al. 2014). In free-fed animals, ORG27569 also markedly decreased the intake of plain food, although the receptor mechanism of such effect is unclear. In drug combination studies, when two compounds with different pharmacological mechanisms produce similar hypophagic effects, it is expected that a combination of the two compounds should produce a larger effect than that of each compound alone (*enhancement*), as seen here with the serotonin $5\text{-}HT_{2C}$ receptor agonist MK212 and ORG27569. However, a combination of CP55940 and ORG27569 did not further reduce plain food intake, suggesting that the combined effect is less than expected (*attenuation*), an effect that is unlikely due to "floor effect" as much larger suppression is achievable (Varga et al. 2012). In animals, it is well studied that SR141716A-induced hypophagic effect develops tolerance over repeated drug treatment (Colombo et al. 1998; Gessa et al. 2006; Rigamonti et al. 2006), although the effect of SR141716A for delaying body weight gain usually lasts much longer (Colombo et al. 1998; Rigamonti et al. 2006). In the current study, ORG27569 showed a similar effect for decreasing plain food intake and delaying body weight gain. Tolerance developed within 5 days of daily 5.6 mg/kg ORG27569 treatment, and increasing the daily treatment dose to 10 mg/kg further decreased the food intake for two more days. The body weight gain slowly increased and 10 days later the difference between drug- and vehicle-treated groups became insignificant after ORG27569 treatment stopped. Despite the unclear receptor mechanism, the clear suppression of palatable and plain food intake and the lasting effect after the termination of drug treatment suggest that ORG27569 may be useful for the control of food intake and body weight.

ORG27569 is one of the first described CB_1 receptor allosteric modulators (Price et al. 2005). The in vitro modulating activity of ORG27569 on a novel CB_1 receptor allosteric modulating site has been extensively studied (Ahn et al. 2012, 2013; Cawston et al. 2013; Shore et al. 2014). This study described the behavioral pharmacological effects of ORG27569 in rats in a battery of behavioral assays, which largely suggests that ORG27569 does not modulate CB_1-receptor-mediated behavioral effects in rats and, as reported previously (Gamage et al. 2014), fails to function as a CB_1 receptor allosteric modulator. This lack of translation of ORG27569 from in vitro to in vivo does not support the potential usefulness of this compound as a research tool to understand CB_1 receptor allosteric sites. However, given the clear efficacy on feeding behaviors, ORG27569 may have potential for the control of overweight and obesity, although its underlying mechanism is unclear and may not be mediated via CB_1 receptors.

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Disclosures

None declared.

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