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*Behav Pharmacol*. Author manuscript; available in PMC 2014 June 03.

Published in final edited form as:
*Behav Pharmacol*. 2014 April ; 25(2): 182–185. doi:10.1097/FBP.0000000000000027.

# *In-vivo* pharmacological evaluation of the $CB_1$-receptor allosteric modulator Org-27569

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## Abstract

Several allosteric modulators (AMs) of the $CB_1$ receptor have been characterized *in vitro*, including Org27569, which enhances $CB_1$-specific binding of [$^3$H]CP55,940, but behaves as an insurmountable $CB_1$-receptor antagonist in several biochemical assays. Although a growing body of research has investigated the molecular actions of this unusual AM, it is unknown whether these actions translate to the whole animal. The purpose of the present study was to determine whether Org27569 would produce effects in well-established mouse behavioral assays sensitive to $CB_1$ orthosteric agonists and antagonists. Similar to the orthosteric $CB_1$ antagonist/inverse agonist rimonabant, Org27569 reduced food intake; however, this anorectic effect occurred independently of the $CB_1$ receptor. Org27569 did not elicit $CB_1$-mediated effects alone and lacked efficacy in altering antinociceptive, cataleptic, and hypothermic actions of the orthosteric agonists anandamide, CP55,940, and $\Delta^9$-tetrahydrocannabinol. Moreover, it did not alter the discriminative stimulus effects of anandamide in FAAH-deficient mice or $\Delta^9$-tetrahydrocannabinol in wild-type mice in the drug discrimination paradigm. These findings question the utility of Org27569 as a 'gold standard' $CB_1$ AM and underscore the need for the development of $CB_1$ AMs with pharmacology that translates from the molecular level to the whole animal.

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**Conflicts of interest**
There are no conflicts of interest.

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**Keywords**

allosteric modulator; anandamide; cannabinoid; $CB_1$; CP55; 940; $\Delta^9$-tetrahydrocannabinol; mouse; rimonabant

## Introduction

The discovery of molecules acting as $CB_1$-receptor allosteric modulators (AMs) has fueled interest in developing novel therapeutic agents that elicit minimal cannabimimetic side effects associated with orthosteric $CB_1$ agonists and antagonists (Price *et al.*, 2005; Horswill *et al.*, 2007; Navarro *et al.*, 2009; Bauer *et al.*, 2012; Pamplona *et al.*, 2012). Org27569, the most widely investigated $CB_1$ AM, possesses an unusual pharmacological profile. It increases the binding affinity of CP55,940, a potent and efficacious orthosteric $CB_1$ agonist, but abates receptor responses in functional assays, including CP55,940-stimulated GTPγS binding, contractions of mouse vas deferens, agonist-induced inhibition of cAMP production, and β-arrestin recruitment assays (Price *et al.*, 2005; Ahn *et al.*, 2012; Baillie *et al.*, 2013). Other recent work has revealed that β-arrestin plays a role in $CB_1$-biased signaling produced by Org27569 (Ahn *et al.*, 2013). These paradoxical effects of Org27569 (i.e. enhanced orthosteric agonist binding, but antagonism of signaling events) have been proposed as being mediated by increased rates of $CB_1$ desensitization that concomitantly cause cAMP levels and hyperpolarization states to return to baseline more rapidly than in the absence of the modulator (Cawston *et al.*, 2013).

Although Org27569 represents the most extensively studied $CB_1$ AM reported in the scientific literature, this work is based solely on in-vitro studies. If $CB_1$ AMs are to be pursued as potential therapeutic agents, it will be important to ensure that prototypical compounds under investigation are active in the whole animal (Ross, 2007). Thus, the primary objective of the present study was to determine whether Org27569 functions as a $CB_1$ AM in mice. On the basis of its dampening effect on $CB_1$-receptor signaling, we initially assessed whether it would reduce food consumption, a hallmark of $CB_1$ blockade (Di Marzo and Matias, 2005). Previously, PSNCBAM-1, which is structurally and pharmacologically similar to Org27569, was shown to reduce food consumption in rats, although $CB_1$ involvement was not assessed (Horswill *et al.*, 2007). Thus, in the present study $CB_1$ (+/+) and (−/−) mice were used to infer whether any observed anorectic effects were $CB_1$ dependent. In addition, we tested Org27569 in well-established animal models sensitive to the cannabimimetic effects of $CB_1$ orthosteric agonists, alone and in combination with CP55,940 or anandamide (AEA), which have been used in its in-vitro characterization, as well as with $\Delta^9$-tetrahydrocannabinol (THC).

## Methods

### Subjects

Male and female $CB_1$ (+/+) and (−/−) mice were used in feeding studies, and male C57BL/6J mice (Jackson Laboratory, Bar Harbor, Maine, USA) or FAAH (−/−) mice were used in drug discrimination experiments. Male ICR mice (Harlan, Indianapolis, Indiana,

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USA) and C57BL/6 J mice were used in other experiments. All animal protocols were approved by the VCU IACUC and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (Resources IoLA, 2011).

### Effects of Org27569 on feeding

Following a 1-week acclimation period, $CB_1$ (+/+) and (−/−) mice were food-deprived, given an intraperitoneal injection of Org27569 (30mg/kg), rimonabant (10mg/kg; positive control), or vehicle at 23 h, and placed in a plastic cage with access to water. A premeasured amount (2.3–2.6 g) of sweet cereal (Froot Loops; Kellogg NA Co., Battle Creek, Michigan, USA) or standard chow (Teklad, Madison, Wisconsin, USA) was placed in the test cage from 24 to 26h. All mice received each treatment condition in a counterbalanced design, with at least 96 h between test days.

### Cannabimimetic pharmacological effects of Org27569 in combination with orthosteric agonists

Mice were tested for (i) catalepsy in the bar test (60 s); (ii) antinociception in the warm-water (52°C) tail withdrawal test (10 s cutoff); and (iii) hypothermia by measuring rectal temperature (Falenski *et al.*, 2010). Antinociception was calculated as the percentage of maximum possible effect (MPE):

$$\left(\%\,\mathrm{MPE}=\left[\frac{(\mathrm{test}-\mathrm{control\ latency})}{(10-\mathrm{control})}\right]\times 100\right).$$

Cumulative dose–response relationships were assessed for AEA, CP55,940, and THC.

### Drug discrimination

FAAH (−/−) and C57BL/6J mice were respectively trained to discriminate AEA (6.0mg/kg) and THC (5.6mg/kg) from vehicle using a fixed ratio 10 (FR10) schedule in nose-poke operant conditioning chambers (MED Associates, St Albans, Vermont, USA), as previously described (Ignatowska-Jankowska *et al.*, 2013). Bioserv 14mg Dustless Precision Pellets (product # F05684; Bioserv, Frenchtown, New Jersey, USA) served as the reinforcer. During test sessions, responses in either aperture delivered reinforcement under an FR10 schedule.

### Org27569 quantification in blood and brain

Org27569 was administered either intraperitoneally (30mg/kg) or intracerebroventricularly (100 μg) and levels of the drug were quantified, respectively, in the blood and brain, or brain, according to established methods (Kinsey *et al.*, 2009), using the HPLC/MS/MS method with an Applied Bio systems (Carlsbad, CA, USA) 3200 Q trap with a turbo V source for TurboIonSpray attached to a Shimadzu SCL HPLC system (Kyoto, Japan). Chromatographic separation was performed using a Discovery HS $C_{18}$, 2.1×150mm, 3 μm column (Supelco, St. Louis, MO, USA).

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### Drugs

Org27569 (University of Aberdeen, Scotland) and CP55,940, THC, AEA, PF-3845 (FAAH inhibitor that prevents AEA degradation), and rimonabant ($CB_1$ antagonist/inverse agonist; NIDA, Bethesda, Maryland, USA) were dissolved in ethanol (Pharmco Products Inc., Brookfield, Connecticut, USA), Emulphor-620 (Rhodia, Cranbury, New Jersey, USA), and saline, in a ratio of 1 : 1 : 18. Org27569 was administered systemically in a volume of 20μl/g, and for intracerebroventricular injection it was dissolved in 100 % dimethyl sulfoxide and administered in a volume of 5μl. The injection volume for all other systemically administered compounds was 10 μl/g.

### Data analysis

All data are reported as mean±SEM and were analyzed using analysis of variance. Dunnett's test was used for the dose–response experiments and the Bonferroni–Dunn test was used for other post-hoc comparisons. Analyses were considered significant at *P* values less than 0.05.

## Results

Org27569 (30mg/kg) significantly reduced consumption of standard chow (data not shown) and sweet cereal in both $CB_1$ (−/−) and (+/+) mice [genotype by drug treatment interaction: $F(2,60)=11.1$, $P<0.001$; Fig. 1a]. In contrast, rimonabant (positive control) reduced intake in $CB_1$ (+ /+) mice only. Org27569 did not alter the discriminative stimulus effects of or substitute for AEA (Fig. 1b and c) or THC (5.6mg/kg intraperitoneally; data not shown) in FAAH (−/−) mice and C57BL/6J mice, respectively. Moreover, Org27569 alone did not produce antinociception, catalepsy, or hypothermia. Further, Org27569 did not reduce the cataleptic, antinociceptive, or hypothermic effects produced by AEA in PF-3845-treated mice or FAAH (−/−) mice (data not shown). In fact, in FAAH (−/−) mice, Org27569 produced small but significant increases in the potency of AEA (potency ratio with 95 % confidence interval) to elicit catalepsy [1.3 (1.1–1.7)] and antinociception [1.9 (1.3–2.8)], but not hypothermia [1.1 (0.9–1.4)], compared with vehicle-pretreated FAAH (−/−) mice (data not shown). Org27569 (30mg/kg intraperitoneally) was detected in the brain (4.09±0.39 μg/mg) and blood (3.71±1.77mg/ml) 1 h after intraperitoneal administration. In an effort to assess the impact of further increasing brain levels of this compound, Org27569 (100 μg) was administered intracerebroventricularly. Despite achieving increased brain levels of Org27569 (i.e. 13.4±2.5 and 10.56±1.8 μg/mg at 0.75 and 1.75 h after injection, respectively), this compound did not alter the antinociceptive, cataleptic, or hypothermic dose–response relationships of intraperitoneally administered CP55,940 (0.1, 0.3, and 1mg/kg; Fig. 2). Finally, Org27569 (30 mg/kg, intraperitoneal) did not affect these pharmacological endpoints produced by intraperitoneally administered THC (3, 10, 30, and 100 mg/kg; data not shown).

## Discussion

This study represents the first systematic evaluation of the $CB_1$ AM Org27569 in well-established in-vivo assays sensitive to $CB_1$ orthosteric agonists and antagonists. Org27569 produced hypophagic effects in both $CB_1$ (−/−) and (+/+) mice. This finding is in contrast to

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that for rimonabant, which reduced food consumption only in $CB_1$ (+/+) mice. These results indicate that the $CB_1$ receptor is dispensable for Org27569-induced hypophagia. Org27569 did not elicit relevant behavioral or cannabimimetic effects when administered alone or in combination with three distinct $CB_1$ orthosteric agonists. Specifically, it did not substitute for either AEA or THC and did not modify the discriminative stimulus effects of either agonist in the drug discrimination paradigm. Moreover, Org27569 did not alter the cataleptic, antinociceptive, or hypothermic effects of THC in wild-type mice or AEA in PF-3845-treated FAAH (+/+) mice, although it slightly enhanced AEA-induced antinociception and catalepsy in FAAH (−/−) mice. Although pharmacokinetic issues represent a potential hurdle when studying drugs in the whole animal, it is noteworthy that 1 h after intraperitoneal administration, Org27569 was detected at equivalent concentrations in the brain and blood. Moreover, direct administration of Org27569 to the brain yielded a 2–3-fold increase in its levels in the brain; however, it still did not alter the cataleptic, antinociceptive, or hypothermic effects of systemically administered CP55,940.

The findings that Org27569 did not elicit $CB_1$-mediated effects alone and lacked efficacy in altering the actions of orthosteric agonists in established murine behavioral assays sensitive to cannabimimetic activity indicate a lack of translation between its interesting in-vitro actions at the $CB_1$ receptor and its effects in the whole animal. These results question the utility of Org27569 as a tool to elucidate $CB_1$ allosteric site(s), if these compounds are to be pursued as potential pharmacotherapies. The development of new $CB_1$ AMs with a pharmacology that extends from the molecular level to the whole animal is needed.

## Acknowledgments

This research has been supported by the National Institute on Drug Abuse grants DA009789, DA017259, DA027113, DA026449, and 2T32DA007027. The authors thank Scott O'Neil for his assistance with these studies.

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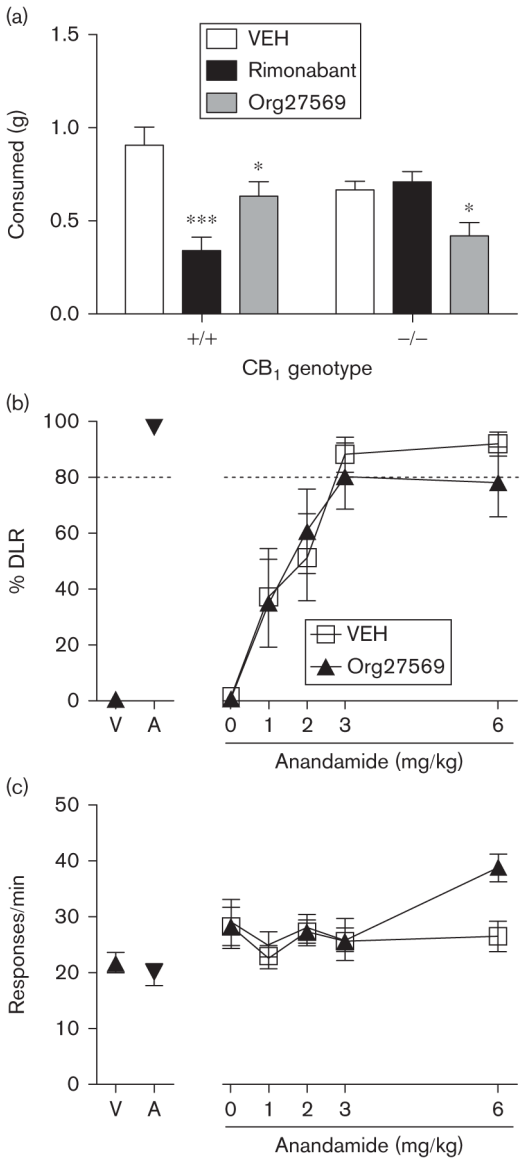


**Fig. 1.**

Org27569 produced $CB_1$-independent hypophagic effects and did not affect the discriminative stimulus effects of anandamide (AEA). (a) Amount of food consumed by male and female $CB_1$ (+/+) and (−/−) mice pretreated (1 h) with either vehicle (VEH; white bars), 30 mg/kg Org27569 (gray bars), or 3 mg/kg rimonabant (black bars), administered during 2-h access to sweet cereal. (b) AEA-like responding in mice trained to discriminate AEA (6 mg/kg) from vehicle, and (c) rates of responding. $n$=8–9 per group.*$P$<0.05, **$P$<0.01, ***$P$<0.001; significant differences from vehicle treatment; Bonferroni post-hoc test. (a) $n$=11 per group, (b, c) $n$ =8–9 per group. All data are reported as mean ± SEM. A, AEA training dose (6 mg/kg) control test; %DLR, percent drug-like responding; V, vehicle control test.

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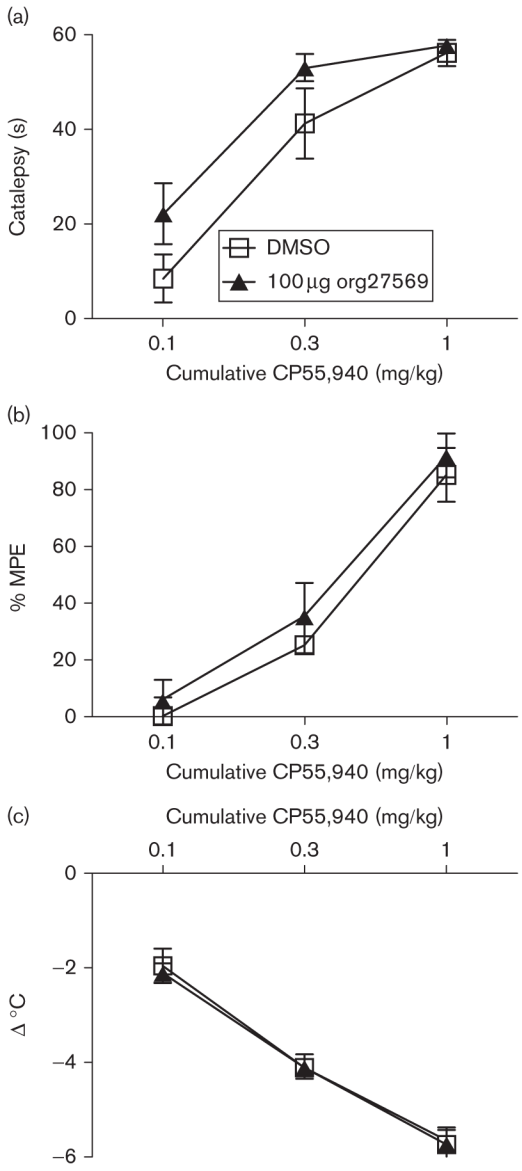


**Fig. 2.**

Org27569 (100 µg intracerebroventricularly; filled triangles) did not affect the pharmacologic effects of systemically administered CP55,940 compared with vehicle [dimethyl sulfoxide (DMSO); open squares]. (a) Catalepsy, (b) antinociception, and (c) hypothermia. *n*=7 per group. All data are reported as mean ± SEM. MPE, maximum possible effect.