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The great divide: Separation between in vitro and in vivo effects of PSNCBAM-based CB_1 receptor allosteric modulators

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ABSTRACT

While allosteric modulators of the cannabinoid type-1 receptor (CB_1) continue to be developed and characterized, the gap between the in vitro and in vivo data is widening, raising questions regarding translatability of their effects and biological relevance. Among the CB_1 allosteric modulators, PSNCBAM-1 has received little attention regarding its effects in vivo. Recently, pregnenolone was reported to act as an allosteric modulator of CB_1, blocking THC's effects in vitro and in vivo, highlighting the potential of CB_1 allosteric modulators for treatment of cannabis intoxication. We investigated the pharmacological effects of PSNCBAM-1 and two structural analogs, RTICBM-15 and -28, as well as pregnenolone, in both signaling and behavioral assays including [^{35}S]GTPγS binding, the cannabinoid tetrad and drug discrimination. While the CB_1 allosteric modulator PSNCBAM-1 attenuated THC-induced anti-nociception and its structural analog RTICBM-28 reduced THC's potency in drug discrimination, most cannabinoid effects in mice were unaffected. In contrast to the mouse studies, PSNCBAM-1 and analogs insurmountably antagonized CP55,940- and THC-stimulated [^{35}S]GTPγS binding and exhibited negative binding cooperativity with [^{3}H]SR141716 with similar apparent affinities. Notably, RTICBM-28, which contains a cyano substitution at the 4-chlorophenyl position of PSNCBAM-1, exhibited enhanced binding cooperativity with CP55,940. In contrast to previous findings, pregnenolone did not block THC's effects in drug discrimination or [^{35}S]GTPγS. These data further highlight the difficulty in translating pharmacological effects of CB_1 allosteric modulators in vivo but confirm the established pharmacology of PSNCBAM-1 and analogs in molecular assays of CB_1 receptor function.

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1. Introduction

The endogenous cannabinoid system's involvement in a wide range of physiological processes has led to the development of numerous potent and selective compounds with therapeutic promise in preclinical assays. Predominantly expressed in the central nervous system, the cannabinoid type-1 (CB_1) receptor has shown great potential as a target for the treatment of drug addiction, pain, mood disorders, obesity/metabolic syndrome, multiple sclerosis, and other diseases; this is evidenced by a large body of preclinical data demonstrating efficacy for drugs which bind the orthosteric site of the CB_1 receptor or inhibit metabolism of its endogenous ligands, anandamide (Devane et al., 1992) and 2-arachidonoylglycerol (Sugiura et al., 1995). Unfortunately, these drugs have had limited success due to their propensity to produce psychoactivity (agonists, e.g. dronabinol; Issa et al., 2014) or depression (antagonists, e.g. rimonabant; Christensen et al., 2007) or their lack of clinical efficacy (enzymatic inhibitors, e.g. PF-04457845; Huggins et al., 2012).

Fortunately, the determination that the CB_1 receptor (Matsuda et al., 1990) possesses a druggable allosteric site (Laprairie et al., 2016; Price et al., 2005; Shore et al., 2014) has provided a novel means through which receptor function can be studied and exploited for the development of better pharmacotherapeutics (Abood, 2016; Ross, 2007). Allosteric modulation allows for the fine-tuning of receptor pharmacology which may facilitate signaling bias towards pathways that are more therapeutically relevant while avoiding those involved in the untoward effects. There are now a handful of molecules which exhibit allosteric properties at the CB_1 receptor (for reviews see Morales et al., 2016; Nguyen et al., 2016). The majority of reported allosteric modulators differentially affect radioligand binding, exhibiting positive binding cooperativity with the CB_1/CB_2 agonist [^{3}H]CP55,940 and negative

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binding cooperativity with the selective CB_1 antagonist [^{3}H]SR141716. The allosteric antagonists exhibit insurmountable antagonism of receptor function while the positive allosteric modulators (PAMs) enhance agonist signaling.

It has now been over 10 years since the first reported CB_1 allosteric modulators (Price et al., 2005) and despite dozens of papers characterizing these and other CB_1 allosteric modulators in vitro, there is a dearth of articles reporting CB_1-mediated effects for these compounds in vivo. Results from the first systematic investigation into the in vivo effects of the prototypical CB_1 allosteric modulator, Org27569, were largely negative with only hypophagic effects reported in mice which occurred independently of the CB_1 receptor (Gamage et al., 2014). The second structural series reported two years after Org27569, PSNCBAM-1, was reported to reduce feeding in rats (Horswill et al., 2007); however, this effect has yet to be established as CB_1-mediated. While Org27569 was reported to have no effect in rats on CP55,940-induced catalepsy and antinociception, it did attenuate its hypothermic effects (Ding et al., 2014) and later was shown to reduce drug- and cue-induced reinstatement of cocaine and methamphetamine self-administration similar to SR141716 (Jing et al., 2014). In addition to the synthetic allosteric modulators, the hormone pregnenolone was reported to act as a CB_1 allosteric modulator both in vitro and in vivo, blocking the effects of THC and WIN55,212-2 (Vallee et al., 2014); however, recent attempts to observe effects in vitro have had limited success (Khajehali et al., 2015; Straiker et al., 2015), reporting only slight displacement of [^{3}H]SR141716 binding at micromolar concentrations but no observed effect in functional assays. The first positive allosteric modulator for the CB_1 receptor, ZCZ011, was recently reported to augment CB_1 agonist efficacy in both cellular and molecular assays as well as in rodent models including the cannabinoid tetrad and inflammatory pain models, the latter in which it exhibited efficacy on its own through hypothesized augmentation of endogenous cannabinoid tone (Ignatowska-Jankowska et al., 2015). Of note, the anti-nociceptive effects of ZCZ-011 were shown to be blocked by administration of SR141716, demonstrating CB_1 mediation.

While the majority of behavioral studies involving CB_1 allosteric antagonists have focused on Org27569 (Ding et al., 2014; Gamage et al., 2014; Jing et al., 2014), few have examined PSNCBAM-1 (Horswill et al., 2007) or any of its analogs. PSNCBAM-1 exhibits a very similar pharmacology to that of Org27569, exhibiting positive binding cooperativity with [^{3}H]CP55,940 (German et al., 2014; Horswill et al., 2007) and negative binding cooperativity with [^{3}H]SR141716 as well as insurmountable antagonism of CP55,940-stimulated [^{35}S]GTPγS binding (Horswill et al., 2007). PSNCBAM-1 also antagonizes the CB_1 receptor in other assays including depolarization-induced suppression of excitation (DSE; Straiker et al., 2015) and CP55940- and WIN55,212-induced beta-arrestin2 recruitment (Baillie et al., 2013). In the present study, PSNCBAM-1 and two of its structural analogs (Fig. 1), RTICBM-15 and -28 (compounds 11 and 29 respectively in German et al., 2014), were assessed for in vitro and in vivo activity as allosteric modulators. Furthermore, we evaluated pregnenolone for its purported allosteric effects at the CB_1 receptor. We hypothesized that PSNCBAM-1 and analogs would insurmountably antagonize receptor signaling in vitro (agonist-stimulated [^{35}S]GTPγS binding) and in vivo cannabimimetic activity (cannabinoid tetrad) and drug discrimination.

2. Materials and methods

2.1. Subjects

Adult male ICR mice (25–32 g; Harlan, Dublin, VA) and C57/Bl6J inbred mice (20–25 g; Jackson Laboratories, Bar Harbor, ME) were

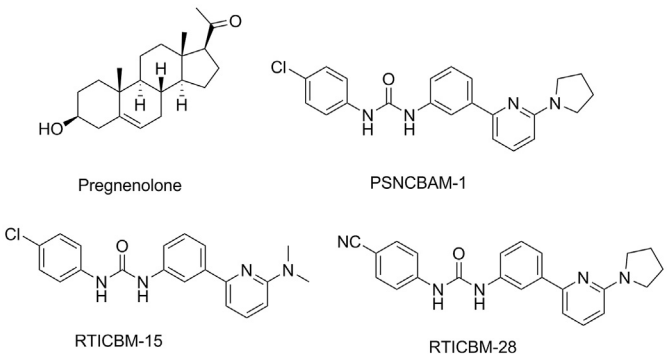

Fig. 1. Chemical structures of PSNCBAM-1, RTICBM-15, RTICBM-28 and pregnenolone.

housed singly in polycarbonate mouse cages. Each ICR mouse was tested with a single dose of compound in the tetrad battery. C57/Bl6J mice were used in the drug discrimination experiments. All animals were kept in a temperature-controlled (20–22 °C) environment with a 12-h light-dark cycle (lights on at 6 a.m.). ICR mice received food ad libitum when in their home cages. C57/Bl67 mice were maintained at 85–90% of free-feeding body weights by restricting daily ration of standard rodent chow. All mice received ad libitum water access when in their home cages. The in vivo studies reported in this manuscript were carried out in accordance with federal and state regulatory guidelines on the conduct of research in animals and were approved by our Institutional Care and Use Committee.

2.2. Apparatus

Measurement of spontaneous activity occurred in Plexiglas locomotor activity chambers (47 cm × 25.5 cm x 22 cm), with beam breaks (4 X 8 beam array) recorded by San Diego Instruments Photobeam Activity System software (San Diego, CA) on a computer located in the experimental room. Anti-nociception and rectal temperature were assessed with a standard tail flick device for rodents (Stoelting, Dale, IL) and a digital thermometer (Physitemp Instruments, Inc., Clifton, NJ), respectively. The ring immobility device consisted of an elevated metal ring (diameter = 5.5 cm, height = 28 cm) attached to a metal stand.

Mice in the drug discrimination experiment were trained and tested in mouse operant chambers (Coulbourn Instruments, Whitehall, PA), housed within light- and sound-attenuating cubicles. Each chamber contained two nose-poke apertures, with stimulus lights over each aperture, and a separate house light. A food dispenser delivered 20-mg food pellets (Bioserv Inc., Frenchtown, NJ) into a food cup (with a light) centered between the two aperture. Illumination of lights, delivery of food pellets, and recording of aperture responses were controlled by a computer-based system (Coulbourn Instruments, Graphic State Software, v 3.03, Whitehall, PA).

2.3. Chemicals

Δ^9-THC (NIDA Drug Supply Program, Bethesda, MD), SR141716/rimonabant (NIDA), PSNCBAM-1 and its analogs (synthesized in our laboratories), pregnenolone (Steraloids, Newport, RI), otenabant (Toronto Research Chemicals, Toronto, Canada), and the open ring degradant of the synthetic cannabinoid XLR-11 (1-[1-(5-fluoropentyl)-1H-indol-3-yl]-3,3,4-trimethyl-4-penten-1-one; Cayman Chemical, Ann Arbor, MI) were dissolved in a vehicle of 7.8% Polysorbate 80 N.F. (VWR, Marietta, GA) and 92.2% sterile saline USP (Butler Schein, Dublin, OH). For in vitro studies, Δ^9-THC,

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