Toxicology and Applied Pharmacology 269 (2013) 100–108

Contents lists available at SciVerse ScienceDirect

Toxicology and Applied Pharmacology

journal homepage: www.elsevier.com/locate/ytaap

Human metabolites of synthetic cannabinoids JWH-018 and JWH-073 bind with high affinity and act as potent agonists at cannabinoid type-2 receptors

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ARTICLE INFO

Article history:
Received 31 January 2013
Revised 14 March 2013
Accepted 15 March 2013
Available online 26 March 2013

Keywords:
Drug abuse
Drug metabolism
K2
Synthetic *cannabis*
Spice
Δ^9-Tetrahydrocannabinol

ABSTRACT

K2 or Spice is an emerging drug of abuse that contains synthetic cannabinoids, including JWH-018 and JWH-073. Recent reports indicate that monohydroxylated metabolites of JWH-018 and JWH-073 retain high affinity and activity at cannabinoid type-1 receptors (CB_1Rs), potentially contributing to the enhanced toxicity of K2 compared to marijuana. Since the parent compounds also bind to cannabinoid type-2 receptors (CB_2Rs), this study investigated the affinity and intrinsic activity of JWH-018, JWH-073 and several monohydroxylated metabolites at human CB_2Rs (hCB_2Rs). The affinity of cannabinoids for hCB_2Rs was determined by competition binding studies employing CHO-hCB_2 membranes. Intrinsic activity of compounds was assessed by G-protein activation and adenylyl cyclase (AC)-inhibition in CHO-hCB_2 cells. JWH-073, JWH-018 and several of their human metabolites exhibit nanomolar affinity and act as potent agonists at hCB_2Rs. Furthermore, a major omega hydroxyl metabolite of JWH-073 (JWH-073-M5) binds to CB_2Rs with 10-fold less affinity than the parent molecule, but unexpectedly, is equipotent in regulating AC-activity when compared to the parent molecule. Finally, when compared to CP-55,940 and Δ^9-tetrahydrocannabinol (Δ^9-THC), JWH-018, JWH-018-M5 and JWH-073-M5 require significantly less CB_2R occupancy to produce similar levels of AC-inhibition, indicating that these compounds may more efficiently couple CB_2Rs to AC than the well characterized cannabinoid agonists examined. These results indicate that JWH-018, JWH-073 and several major human metabolites of these compounds exhibit high affinity and demonstrate distinctive signaling properties at CB_2Rs. Therefore, future studies examining pharmacological and toxicological properties of synthetic cannabinoids present in K2 products should consider potential actions of these drugs at both CB_1 and CB_2Rs.

Introduction

K2 has emerged as a very popular drug of abuse that is heavily marketed to young teens and first-time drug users as "legal marijuana" (Seely et al., 2011; Vardakou et al., 2010). Various formulations of K2 products are sold at "head shops" and internet sites under the brand names of Spice, Spice Dream, or Yucatan Fire (Auwarter et al., 2009). Most K2 preparations consist of inert plant materials laced with a mixture of several synthetic cannabinoid compounds possessing psychoactive properties similar to those produced by Δ^9-tetrahydrocannabinol (Δ^9-THC) found in marijuana. Δ^9-THC produces psychotropic actions by activating CB_1 cannabinoid receptors (CB_1Rs) in the CNS (Fujiwara and Egashira, 2004). Although structurally distinct from Δ^9-THC, the synthetic compounds found in K2 products are derivatives of the well characterized aminoalkylindole (AAI) chemical class of ligands that also bind and activate CB_1Rs (Manera et al., 2008). Therefore, the abuse liability of both Δ^9-THC and K2-aminoalkylindoles (K2-AAIs) results from their ability to potently and efficaciously activate CB_1Rs. As many as 10 different K2-AAIs are reported to be present in various K2 preparations, but the two compounds that are commonly detected are JWH-018 [1-pentyl-3-(1-naphthoyl)indole] and JWH-073 [1-butyl-3-(1-naphthoyl)indole] (Vardakou et al., 2010).

Although controversial, marijuana has been used medicinally for centuries (Zuardi, 2006). In marked contrast, virtually no research has evaluated the safety or efficacy of any K2-AAI and several reports from Europe and the U.S. suggest that the clinical effects of K2 products are not only distinct from those caused by marijuana, but also pose significant health risks. For example, unlike effects typically observed with marijuana, some K2 smokers experience extreme paranoia, hallucinations, agitation, anxiety, seizures, elevated blood pressure and even death (Auwarter et al., 2009; Every-Palmer,

Abbreviations: AC, adenylyl cyclase; BSA, bovine serum albumin; CB_1R, cannabinoid type-1 receptor; CB_2R, cannabinoid type-2 receptor; CHO-hCB_2, CHO cells expressing human CB_2Rs; Δ^9-THC, delta-9-tetrahydrocannabinol; FRO, fractional receptor occupancy; GDP, guanosine diphosphate; GTPγS, guanosine 5′-O-[gamma-thio]triphosphate; GPCR, G-protein coupled receptor.

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http://dx.doi.org/10.1016/j.taap.2013.03.012

2010; Muller et al., 2010; Vardakou et al., 2010; Zimmermann et al., 2009). Therefore, understanding the basic pharmacological and toxicological properties of K2-AAIs is needed to provide insight into potential mechanisms underlying the unique adverse effect profile of these compounds. Such information should also aid in the development of efficacious pharmacotherapies to reduce the serious consequences following the use of these dangerous designer drugs of abuse.

Our laboratory has recently reported an important and unusual characteristic of K2-AAI metabolism that may contribute to the distinct clinical profile of the K2 products. Specifically, phase I metabolism of JWH-018 and JWH-073 produces several monohydroxylated metabolites that not only retain high nanomolar binding affinity for CB_1Rs, but also exhibit a range of intrinsic activity from neutral antagonism to full agonism (Brents et al., 2011, 2012). These observations are unexpected given that Δ^9-THC metabolism results in the production of only a single major active metabolite (11-OH-Δ^9-THC) with reduced CB_1R affinity (Kochanowski and Kala, 2005).

In addition to acting as agonists at CB_1Rs (Atwood et al., 2010, 2011), JWH-018 and JWH-073 are known to bind with high affinity to CB_2Rs, the second major cannabinoid receptor subtype (Aung et al., 2000; Chin et al., 1999). In contrast to CB_1Rs, CB_2Rs are expressed in highest density outside the CNS on immune cells (Klein et al., 2003) and regulate many important physiological processes ranging from inflammation to bone formation (Patel et al., 2010). Although also present in relatively low numbers in the brain and/or spinal cord, increasing evidence indicates that activation of CB_2Rs in the CNS modulates the addictive properties of several drugs of abuse, including cocaine (Xi et al., 2011), alcohol (Onaivi et al., 2008) and nicotine (Gamaleddin et al., 2012). Since metabolism of JWH-073 and JWH-018 unexpectedly produces metabolites retaining significant affinity and activity at CB_1Rs, it is important to determine if the parent compounds and/or metabolites of these drugs might be active at CB_2Rs as well.

Although JWH-018 and JWH-073 have been reported to act as agonists at CB_1Rs (Atwood et al., 2010, 2011; Brents et al., 2011, 2012), no studies to date have examined the pharmacological properties of these K2 synthetic cannabinoids or their metabolites at CB_2Rs. Therefore, the purpose of this study was to determine the affinity and intrinsic activity of JWH-018, JWH-073 and several of their major phase I hydroxylated metabolites at human CB_2Rs. We report that JWH-018, JWH-073 and several of their human metabolites exhibit high affinity and demonstrate distinctive signaling properties at CB_2Rs. These results indicate that future studies examining pharmacological and toxicological properties of synthetic cannabinoids present in K2 products should consider potential actions of these drugs at both CB_1 and CB_2Rs.

Methods

Cell culture

Chinese hamster ovary (CHO) cells stably expressing human CB_2Rs (CHO-hCB_2) or human mu-opioid receptors (CHO-hMOR), generated in our laboratory (Shoemaker et al., 2005a) were cultured in DMEM medium containing 10% fetal calf serum, 100 units/ml penicillin, 100 mg/ml streptomycin and 0.5 mg/ml geneticin (G418) in a humidified atmosphere of 5% CO_2, 95% air at 37 °C. Cells from passages 5–15 were used in all experiments.

Membrane preparation

CHO-hCB_2 cells were homogenized in ice-cold buffer (50 mM Hepes, pH 7.4, 3 mM MgCl2, and 1 mM EGTA) by 10 strokes employing a 40 ml Dounce glass homogenizer as detailed elsewhere (Shoemaker et al., 2005b). In brief, samples were centrifuged at 40,000 ×*g* for 10 min at 4 °C and homogenized similarly twice more. Samples were resuspended in Hepes buffer (50 mM, pH 7.4), aliquoted and stored at −80 °C. Protein concentration was determined by employing the BCA™ Protein Assay (Thermo Scientific, Rockford, IL).

Competition receptor binding assays

Competition receptor binding assays were conducted as previously described (Brents et al., 2012; Shoemaker et al., 2007). Briefly, the cannabinoid agonist [^{3}H]CP-55,940 (0.2 nM) and the competing non-radioactive ligands were allowed to equilibrate for 90 min at room temperature, in 1 ml of binding buffer (50 mM TRIS and 0.05% BSA), containing 25 μg of CHO-hCB_2 homogenates and 5 mM $MgCl_2$. Non-specific binding was defined by inclusion of 10 μM of the high affinity cannabinoid ligand WIN-55,212-2. After incubation, assay mixes were filtered through glass fiber filters and washed five times, and radioactivity was determined by liquid scintillation spectrophotometry.

[^{35}S]GTPγS binding assay

[^{35}S]GTPγS binding assays were performed as described previously (Shoemaker et al., 2007) in a buffer containing 20 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$, 20 units/l adenosine deaminase and 0.05% BSA at pH 7.4. Each binding reaction contained 25 μg of CHO-hCB_2 homogenates, cannabinoid ligands, 0.1 nM [^{35}S]GTPγS and 10 μM of GDP. Non-specific binding was defined by the addition of 10 μM of non-radioactive GTPγS. Samples were incubated for 30 min at 30 °C and rapidly filtered through glass fiber filters and radioactivity was quantified by liquid scintillation spectrophotometry.

Adenylyl cyclase (AC) assay

CHO-hCB_2 cells were plated in (17 mm) 24 well plates at a density of 4×10^6 cells per plate and cultured for 24 h prior to the assay as detailed elsewhere (Shoemaker et al., 2005b). On the day of the assay, cells were preincubated for 6 h at 37 °C in DMEM containing 0.9% NaCl, 500 μM 3-isobutyl-1-methyl xanthine and 2.5 μCi/ml of [^{3}H]adenine. The pre-incubation mix was removed and cannabinoid ligands were added for 15 min in a Krebs–Ringer–Hepes solution (10 mM Hepes, 110 mM NaCl, 25 mM Glucose, 55 mM Sucrose, 5 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, pH 7.4) containing 3-isobutyl-1-methyl xanthine and 30 μM forskolin. Reactions were terminated by adding 50 μl 2.2 N HCl. [^{3}H]cAMP was isolated by alumina column chromatography and radioactivity was quantified by liquid scintillation spectrophotometry.

Materials

Dulbecco's modified eagle's medium (DMEM), penicillin/streptomycin (10,000 IU/ml and 10,000 μg/ml) and geneticin (G418) were purchased from Fisher Scientific (Pittsburg, PA). Fetal calf serum was obtained from Gemini Bioproducts (West Sacramento, CA). GTPγS and GDP were purchased from EMD Chemical (Gibbstown, NJ), and Sigma Aldrich (St. Louis, MO), respectively. Cayman Chemical (Ann Arbor, MI) synthesized and verified the structures of JWH-018, JWH-073 and their respective metabolites (M1–M7, Fig. 1) through mass spectrometry and NMR. Δ^9-THC was supplied by the National Institute on Drug Abuse (NIDA, Bethesda, MD). CP-55,940, DAMGO and morphine were obtained from Tocris Biosciences (Ellisville, MO). [^{3}H]CP-55,940 (144 Ci/mmol) was purchased from PerkinElmer (Waltham, MA), [^{3}H]adenine (26 Ci/mmol) was procured from Vitrax (Placenia, CA) and [^{35}S]GTPγS (1250 Ci/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO).