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⁹-Tetrahydrocannabinol discriminative stimulus effects of AM2201 and related aminoalkylindole analogues in rats

Torbjörn U.C. Järbe, Roger S. Gifford, Alexander Zvonok, and Alexandros Makriyannis

Center for Drug Discovery, Department of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115, USA

Abstract

The recent recreational use of synthetic cannabinoid ligands, collectively referred to as ‘Spice’, has raised concerns about their safety and possible differences in their biological effect(s) from marijuana / ⁹-THC. AM2201, a highly efficacious, potent CB₁ receptor (CB₁R) agonist, is a recently detected compound in ‘Spice’ preparations. Furthermore, structural analogues of AM2201 are now being found in ‘Spice’. The present studies were conducted to investigate their ⁹-THC-like effects using drug (⁹-THC) discrimination in rats. Results show that the tested compounds were potent cannabinergics that generalized to the response to ⁹-THC, with AM2201 being most potent, exhibiting a 14-fold potency difference over ⁹-THC. The other analogues were between 2.5 and 4-fold more potent than THC. Surmountable antagonism of AM2201 with the selective CB₁R antagonist / inverse agonist rimonabant also established that the discrimination is CB₁R dependent. Time course data reveal that AM2201 likely peaks rapidly with an *in vivo* functional half-life of only 60 minutes. The present data confirm and extend previous observations regarding ⁹-THC-like effects of ‘Spice’ components.

Keywords

synthetic cannabinoids; ‘Spice/K2’; AM2201; analogues; drug discrimination; rat

Introduction

Ligands for the endocannabinoid signaling system (ECS) have been investigated for their use in commercial enterprises where synthetic cannabinoids are offered as alternatives to ⁹-tetrahydrocannabinol (THC), the main psychotropic component in cannabis preparations (marijuana, hashish etc). Structurally dissimilar to THC, most of these compounds have been based on an indole template created to uncover the structural requirements for preferentially activating the two main cloned ECS recognition sites, namely cannabinoid receptor 1 (CB₁R) and 2 (CB₂R). Clandestine production has focused on high-affinity CB₁R

Correspondence to: T.U.C. Järbe, Northeastern University, Center for Drug Discovery (CDD), 116 Mugar Hall, Department of Pharmaceutical Sciences, 360 Huntington Ave, Boston, MA 02115, Phone: 617-373-2273 (main office), Fax: 617-373-7493 (main office), t.jarbe@neu.edu.

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ligands, as the desired endpoint of recreational use (“high, stoned etc.”) is CB₁R receptor-mediated. Although these novel compounds were not initially intended for human consumption and their *in vivo* effects were not examined, the plethora of side effects reported in the recent literature has changed this perspective, e.g., clinical/emergency reports hint at effects not usually associated with cannabis consumption (Monte et al. 2014). Thus, further *in vivo* studies of these compounds are warranted.

Initially, the most commonly detected synthetic cannabinoid was JWH018/AM678 but, recently, AM2201, a fluorinated analogue of JWH018, has become more prevalent (Wohlfarth et al. 2015). This pattern of slightly modifying existing drugs to evade drug control legislation is commonly observed (Banister et al. 2015). Thus, as AM2201 has recently been made illegal, novel synthetic cannabinoids are expected to enter the market, likely including side chain analogues of AM2201, few of which have been researched in the laboratory. This paucity of data needs to be addressed to determine whether these novel compounds exhibit pharmacological/physiological differences from the previously detected “Spice” compounds. The current study used drug (THC) discrimination to examine the ability of side chain analogues of AM2201 to produce THC-like discriminative stimulus, i.e., “subjective”-like effects (Järbe 2011).

Methods

Subjects

Adult male Sprague–Dawley rats (N=12; Taconic Farms, Germantown, NY, USA) were individually housed in a colony room with an average temperature of 22°C and a 12-hour light/dark cycle (lights on at 07.00 h; rats were trained and tested during the light phase). Purina Rat Chow was restricted to approximately 12 g/day, maintaining body weights between 300 and 430 g.

Apparatus

Drug discrimination training and testing were conducted in eight operant chambers (ENV-001), constructed of Plexiglas and aluminum, each equipped with two response levers, house- and lever-lights, and a grid floor. Each chamber was enclosed within a sound- and light-attenuating enclosure equipped with an exhaust fan. Chambers were interfaced (model SG-502) with an IBM-compatible PC. Behavioral sessions and data collection were conducted using the Med-PC software program (v. 1.16). All equipment purchased from Med. Associates, St Albans, VT, USA.

Procedure

The drug discrimination procedure has been described in detail elsewhere (Järbe et al. 2010). Briefly, food restricted rats were trained to obtain one 45 mg chocolate flavored pellet (BioServe®), according to a fixed-ratio 10 schedule of reinforcement (FR-10). The rats were trained in this two-choice task to respond on drug (THC) - or vehicle-appropriate levers once daily. The position of drug-appropriate levers was randomly assigned among subjects so that it was to the right of the food cup for half the subjects. Animals were trained to discriminate between THC (3 mg/kg) and vehicle, administered i.p. 20 min before session

onset. Presses on the incorrect lever were recorded but had no programmed consequences. The schedule of drug or vehicle administrations was non-systematic, with no more than two consecutive drug or vehicle trials. To minimize the potential influence of odor cues left in a chamber by a preceding subject (Extance and Goudie 1981), the order of drug and vehicle training sessions for animals trained in the same chamber was varied. Twenty-min sessions were conducted Monday through Friday. The acquisition criterion was selecting the correct lever on at least 8 out of 10 consecutive training days. Correct selection was defined as total presses before the first reinforcement being equal to or less than 14 (i.e., an animal did not press the wrong lever more than 4 times before pressing 10 times on the injection-appropriate lever).

Once criterion was met, tests occurred on average three times every two weeks; on interim days, training sessions were conducted. There was one session per test day. Drug training sessions preceded half the test sessions; the other half were preceded by vehicle training sessions. Tests were conducted only if responding during both the preceding drug and vehicle training sessions met criterion. If not, animals were retrained for at least three sessions before additional testing. During testing, animals were reinforced for 10 presses on either lever, until 20 min had elapsed or six reinforcers had been delivered, whichever occurred first. Doses were examined in a mixed order. For each dose tested, the percentage of responding on the drug-appropriate lever was calculated from the ratio of the number of presses on the THC-associated lever to the total number of lever presses in a test session. Animals receiving at least one reinforcer during the test session were used for this measure. ED₅₀ estimates were obtained with non-linear regression after log dose transformation (Prism v. 5). Additionally, response rate was calculated and subjected to one-way ANOVA.

Drugs

THC and rimonabant were provided by The Research Technology Branch, National Institute of Drug Abuse, Rockville, MD. The other compounds were synthesized at the Center for Drug Discovery (CDD), Northeastern University, Boston, MA, USA, according to synthetic schemes outlined elsewhere (Makriyannis and Deng 2001). The cannabinoids were initially dissolved in absolute ethanol and the stock solutions kept in a freezer at -20°C. At the time of usage, appropriate amounts were withdrawn, the ethanol evaporated under a stream of nitrogen, the residue then dissolved (w/v) in an aqueous solution of dimethyl sulfoxide (2%), propylene glycol (4%) and Tween-80 (4%) prior to a final suspension with saline slowly added just prior to dosing. Suspensions of rimonabant were prepared the same way, except the compound was not initially dissolved in ethanol. All drugs were administered i.p. in a volume of 2 ml/kg 20 min before session onset. CB₁R/CB₂R binding affinity (K_i) estimates for the four test indoles and the generic structural chemotype are shown in Table 1; for methods/procedures on estimating binding affinity, see (Sharma et al. 2013).

Results and Discussion

All of the tested compounds fully substituted in the THC discrimination at 20 min post administration, with a rank order of potency of fluoro (AM2201) > alkene > cyano = bromo

> THC. Table 1 displays median dose-effect estimates (ED_{50}) and the corresponding 95% confidence intervals ($\pm 95\%$ C.L.) for the cannabinergic drug discrimination data.

The *in vivo* potency of AM2201 is similar to previously published work (Gatch and Forster 2014). Tetrad data on both the alkene and bromo analogues have been previously reported, and their potencies were comparable to the present bio-assay (Vigolo et al. 2015; Wiley et al. 1998). The prediction that these analogues might enter the clandestine market was recently confirmed as the bromo, chloro and alkene substitution versions have all been detected in batches of ‘Spice’ in the past year (Simoes et al. 2014; Vigolo et al. 2015). These analogues are readily synthesized employing the same precursors as those used in creating AM2201 or JWH-018/AM678. Besides being detected in some batches of ‘Spice’, the alkene analogue is also important as it has been identified, along with JWH-018/AM678, as a combustion product of AM2201. This means that people smoking AM2201 may also be inhaling the alkene analogue, which appears to be only slightly less potent than AM2201 (Donohue and Steiner 2012).

Combinations of AM2201 with a fixed dose of the CB_1R antagonist/inverse agonist rimonabant produced surmountable antagonism with a 7.87 right-fold difference in potency, supporting the notion of a CB_1R mechanism for the discrimination. A right-ward shift of this magnitude is consistent with previous antagonism studies involving rimonabant and JWH018/AM678 as well as THC in drug discrimination (Järbe et al. 2011; Järbe et al. 2001).

The time-course of AM2201 shows a behavioral/functional half-life of ~60min, which is in accord with previous data (Gatch and Forster 2014). This is also similar to most other reported ‘Spice’ indole compounds, in that they are shorter acting than THC, e.g. (Järbe et al. 2011; Rodriguez and McMahon 2014).

Overall, the present drug discrimination data add to the growing number of THC-like compounds identified by this mechanistic *in vivo* receptor assay. These new analogues of AM2201 all appear to be potent CB_1R agonists that mimic the discriminative stimulus effects of THC. Both the short duration of action and highly potent nature of AM2201 are common hallmarks of drugs of abuse and, as suggested by recent case-report documentation, highlight the dangers posed by these synthetic cannabinoids (Rodgman et al. 2014). While this study clearly demonstrates that these compounds produce similar discriminative-stimulus effects to THC/marijuana, their high efficacy at CB_1R compared to THC, and the scant information regarding potential side effects calls for further investigation of their potentially harmful effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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1. All procedures were approved by the Animal Care and Use Committee of the Northeastern University, Boston, MA, USA. Report dedicated to the memory of the late Dr. S.R. Goldberg for his many scientific contributions, especially in drug abuse research and behavioral pharmacology.

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Figure 1.

Results of testing the aminoalkylindole compounds and THC separately as well as AM2201 in combination with rimonabant (Rim.) in rats discriminating i.p.-administered THC (3 mg/kg) from vehicle. Panel A shows the average ($\pm$ SEM) dose response curves generated from testing varying doses of the drugs at 20 min post administration. Panel B shows the average response rate ($\pm$ SEM) of the animals during the same sessions. Panel C shows the average ($\pm$ SEM) drug discrimination results of testing AM2201 at various time points after its administration and each time point was obtained on a separate test day. Panel D shows the average ($\pm$ SEM) response rate of the animals at the corresponding time-point tests. There are 8 to 12 observations per data points, determined on separate test occasions. The grey circles are the average ($\pm$ SEM) results from training sessions immediately prior to testing, above V (vehicle) and D (drug = 3 mg/kg THC).

Table 1

Median dose-effect estimates (ED₅₀) and corresponding 95% confidence intervals ($\pm$ 95% C.L.), R = side-chain substituent (functional group), receptor binding affinity (K_i in nM).

Base Structure	R	CB ₁ R K _i	CB ₂ R K _i	ED ₅₀ ($\pm$ 95% C.L.) mg/kg
-CH ₂ F	1	20-30	0.0547 (0.0471 - 0.0634)
	=CH ₂	10-20	90-110	0.1968 (0.0968 - 0.4000)
	-CH ₂ CN	1-10	30-40	0.3005 (0.2807 - 0.3217)
	-CH ₂ Br	1-10	40	0.2843 (0.1718 - 0.4703)
	⁹ -THC	39.5 ^{a)}	40 ^{a)}	0.7611 (0.4533 - 1.278)
	-CH ₂ F + Rim. 1 mg/kg			0.3838 (0.3733 - 0.3946)

Rim. = Rimonabant;

^{a)} data from (Thakur et al. 2005)