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Cannabinergic aminoalkylindoles, including AM678=JWH018 found in ‘Spice’, examined using drug (Δ^9 -THC) discrimination for rats

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Abstract

We examined four different cannabinergic aminoalkylindole ligands, including one drug (AM678=JWH018) found in herbal ‘Spice’ concoctions, for their ability to substitute for Δ^9 -tetrahydrocannabinol (THC), and the ability of the cannabinoid receptor 1 (CB₁R) selective antagonist/inverse agonist rimonabant to block the substitution 30 and 90 min after i.p. injection. Rats trained to discriminate the effects of vehicle from those produced by 3 mg/kg THC were used. The order of potency was: AM5983 $\geq$ AM678>AM2233>WIN55,212-2 at both test intervals. AM5983 and AM678 appeared 8 times more potent than THC, followed by AM2233 (about twice as potent as THC), and WIN55,212-2 $\approx$ THC at the 30 min-test interval. The aminoalkylindoles showed reduced potency (i.e., an increased ED₅₀ value) at the longer injection-to-test interval of 90 min compared to testing at 30 min. The rightward shifts by co-administration of rimonabant were approximately 8 to 12-fold for AM5983 and AM678, compared to an approximately 3-fold rightward shift for the WIN55,212-2 curve. AM2233 (1.8 mg/kg) substitution was also blocked by 1 mg/kg rimonabant. In conclusion, AM5983 and AM678=JWH018 are potent cannabimimetics derived from an aminoalkylindole template. WIN55,212-2 seemed to interact differently with rimonabant, compared to either AM5983 or AM678, indicating potential differences in the mechanism(s) of action between cannabinergic aminoalkylindoles.

Keywords

THC; cannabinergic aminoalkylindoles; WIN55,212-2; AM678=JWH018; AM5983; AM2233; rimonabant; drug discrimination-rat

Introduction

The endocannabinoid system (ECS) has emerged as a regulatory system of major significance for both normal physiology as well as pathophysiology. The system is comprised of two cloned G-protein-coupled seven-transmembrane receptors (GPCRs), named cannabinoid receptor 1 (CB₁R) and 2 (CB₂R), and endogenous ligands. CB₁R/CB₂R binds the phytocannabinoid Δ^9 -tetrahydrocannabinol (THC), the main psychoactive

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constituent in preparations derived from *Cannabis sativa* L. Activation of CB₁R seems primarily responsible for the “subjective high”. Thus, ECS can be affected by both endogenous and exogenous ligands. Apart from issues related to drug abuse and dependence, ECS has also been implicated in various other pathophysiological states such as e.g., chronic pain and inflammation; for overview, see (Pertwee 2010).

The first cannabinergic indoles to be discovered were aminoalkylindoles of which WIN55,212-2 was the most potent *in vivo* (Compton et al. 1992), and this ligand has subsequently been widely used as a tool in cannabinoid research. Given the emphasis on CB₂R activation/inactivation as therapeutics (Poso and Huffman 2008) and the status of WIN55,212-2 as a relatively readily available “prototypical” CB₁R agonist, pharmacological information on other cannabimimetic indoles is scant and typically limited to binding assays regarding cannabinoid receptor affinity and subtype selectivity. Depending on the training drug used for discrimination (THC or methanandamide, a stable analog of the endogenous ligand anandamide), we previously observed different magnitudes of right-ward shifts of the dose-effect curves for the aminoalkylindoles WIN55,212-2 and AM678 in the presence of the selective CB₁R antagonist/inverse agonist rimonabant, suggesting potential differences in the mode of action between the two indoles (Järbe et al. 2010). Although the underlying mechanism for this differential effect is unknown, there are several instances where divergent effects on signaling and cellular/physiological responses between WIN55,212-2 and THC (or CP55,940) have been reported (Bosier et al. 2010).

Direct CB₁R activation can produce pronounced psychotropic effects and therefore other approaches have been pursued for developing therapeutics potentially affecting the ECS. Yet, it has become increasingly clear that a clandestine market has developed surrounding synthetic alternatives for achieving marijuana-like effects. It is also from this perspective that more *in vivo* information about cannabimimetic designer drugs is relevant. One of the currently examined compounds (AM678) is/was commonly used as an adulterant in herbal preparations such as ‘Spice’, initially offered primarily in Europe and presented as a legal alternative to marijuana (Hudson et al. 2010; Vardakou et al. 2010). This aminoalkylindole is more commonly known in the scientific literature as JWH018 (Huffman et al. 1994). Initial *in vivo* studies showed the compound to be effective in the so called “tetrad” battery of tests in mice (Wiley et al. 1998), and also in drug discrimination for rats, differentiating between vehicle and either THC or methanandamide (Järbe et al. 2010). In both studies, indications of differences between agonists were obtained even though the ligands exhibited a general cannabimimetic profile. Atwood and colleagues examined signaling characteristics of the drug at CB₁R and concluded that “JWH018 is a potent and efficacious cannabinoid CB₁ receptor agonist” (Atwood et al. 2010).

The current studies examined four cannabinergic aminoalkylindoles, including WIN55,212-2, for their ability to substitute for THC, and their interaction with rimonabant, in rats discriminating a higher dose of THC compared to the earlier report, since training dose can be an important determinant in drug discrimination (Järbe 1989), both in terms of efficacy and mechanism of action (Bergman et al. 2000; Hodge et al. 2006). Information on the duration of effect for indole-derived cannabinergics is very limited. To gauge duration of action, the currently examined aminoalkylindoles were tested at both 30 and 90 min post-administration to ensure that our protocol included an injection-to-test interval where maximum *in vivo* activity would be sampled (see below, Methods). THC discrimination is a pharmacologically selective animal model involving CB₁R activation (Järbe, 2011). Thus, the “perceived” effects of THC define the endpoint by which new compounds are evaluated.

Methods

Subjects

Two separate groups of 8 and 12 male Sprague-Dawley rats (Taconic Farms, Germantown, NY) were used. Animals were individually housed in colony rooms with an average temperature of 20°C and a 12-hour light/dark cycle (rats were trained and tested during the light phase). Animals ($\approx$ 90 days old at purchase) were experimentally naïve at the time of shaping the lever pressing response (see below). Post-session supplemental feeding with Harlan Rat Chow[®] (# 2018) was restricted to approximately 12 to 14 g/day. All procedures were approved by the Animal Care and Use Committee of Hahnemann University/AHERF (now Drexel University; n=8) and that of Temple University (n=12), Philadelphia, PA, USA. The “Principles of Animal Laboratory Care” (National Institutes of Health, 1996) were followed.

Apparatus

Training and testing occurred in 8 chambers (ENV-001, Med. Associates, St Albans, VT) equipped with two non-retractable response levers, house-and lever lights, and a grid floor. Each chamber was enclosed within a sound- and light-attenuating box with an exhaust fan and interfaced with a DOS/Windows compatible computer. Response contingencies were programmed using Med-PC software (v. 1.16; Med. Associates).

Training procedure

Rats were trained to eat food pellets (45 mg, BioServe[®]) from a food receptacle located midway between the two response levers, and shaped to lever press for food until responding 10 times for each reinforcer (fixed-ratio 10 schedule of reinforcement; FR-10). When the house light was off, and the stimulus lights above the response levers were lit, completion of 10 presses on the state-appropriate lever resulted in the delivery of two 45 mg food pellets, followed by a 10-sec (n=8) or a 6-sec (n=12) time-out period with only the house light on. At the end of the time-out period, the stimulus lights above the levers were lit, the house light was turned off, and the FR-10 schedule of reinforcement contingency reinstated. Sessions ended by all lights in the box being turned off.

Once daily, beginning 30 (n=8) or 20 (n=12) min after i.p. injection, the rats were trained in this two-choice task to respond on drug- (3 mg/kg THC) or vehicle-associated levers. The change from a 30 to a 20 min post-injection training interval was for logistical reasons (Järbe et al. 2001). The position of drug-appropriate levers was randomly assigned among rats so that it was to the right of the food cup for half the subjects and left for the other half. Throughout the session, the aforementioned FR-10 reinforcement schedule was in effect. Presses on the incorrect lever were recorded, but had no programmed consequences. The order of drug or vehicle administrations was nonsystematic, with no more than two consecutive drug or vehicle sessions. Approximately an equal number of drug and vehicle training sessions occurred throughout the study. To avoid the potential influence of odor cues left in a chamber by a preceding subject, the order in which drug/vehicle training sessions were conducted for rats trained in the same chamber was randomized (Extance and Goudie 1981). Training occurred Monday through Friday, and lasted 20 min. Training continued until and beyond the point at which rats reached the acquisition criterion of selecting the lever appropriate for the training condition on at least 8 out of 10 consecutive sessions. Correct selection was defined as total presses before the first reinforcement (FRF) being equal to, or less than 14 (i.e., the incorrect lever not pressed more than 4 times before completing 10 presses on the lever appropriate for the prevailing training condition; FRF $\leq$ 14).

Testing procedure

Once stable THC (3 mg/kg) discriminations were achieved (see Results), test (T) sessions were conducted on average 3 times every two weeks; on interim days, regular drug (D) or vehicle (V) training sessions of 20 min duration took place. Approximately two weeks before initial testing, animals began receiving two i.p. injections (2 ml/kg each) before the training sessions (i.e., D and V, or V and V) to accustom the animals to a double injection procedure such as that used for antagonism testing. Typically, the order of sessions was: D, V, T, V, D (week 1); V, T, V, D, T (week 2); V, D, T, D, V (week 3); and D, T, D, V, T (week 4). Tests were conducted only if responding during the training sessions immediately preceding the test had been correct ($FRF \leq 14$) during the initial six FR-10 cycles of the session. If incorrect, animals were retrained for at least three sessions where $FRF \leq 14$ before additional testing took place. In test sessions, food pellets were delivered for 10 presses on either lever for 6 reinforcement cycles or until 20 min had elapsed, whichever occurred first. There were one (THC) or two (aminoalkylindoles) sessions per test day. In the case of two test sessions, animals finished the first test at 30 min post-injection as described above, then were put back in their home cages and left undisturbed until being put back into the chambers for the 2nd test probe occurring 90 min post-injection, which was executed as described above. These intervals were based on time-course data for WIN55,212-2 (Pério et al. 1996) as well as our previous study regarding WIN55,212-2 and AM678 (Järbe et al. 2010). Doses and drugs 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 drug (THC)-associated lever to the total number of lever presses in a test session (excluding responding during the time-out periods). Only data for animals receiving at least one reinforcer during the test session were considered for this measure, i.e., animals must have made a minimum of 10 presses on one of the two levers. Response rate (responses per second) across all subjects was also calculated. This measure was based on the performance of all rats, including non-responders.

Data analysis

Response rate was averaged ($\pm$ SEM) among rats and plotted as a function of dose. The effects of a drug on response rate were considered significant when the mean rate of responding was not within the 95% confidence limits ($\pm$ 95% C.L.) of the mean control response rate, expressed as a percentage [rate (% control)]. This was defined in individual rats as the mean response rate pertaining to the initial 6 reinforcement cycles, calculated from vehicle training sessions in which the criteria for testing were met. The dotted horizontal lines in the graphs presented in “Results” represent the $\pm$ 95% C.L. of the % vehicle rate immediately preceding the tests in question. Data points outside these dotted lines are considered significant. Non-linear regression analyses of the average generalization and antagonism data after log-dose transformation were performed using Prism software (v. 5, GraphPad Software, San Diego, CA) to provide ED_{50} estimates and their $\pm$ 95% C.L. (regression model: log dose vs. response – variable slope with the top and bottom of the curves constrained to 100 and 0). Using the F-test, the Prism program estimates if slopes are parallel or not, and if parallel, evaluates whether the intercepts are equal or not (a measure of potency).

Drugs

The levo isomer of Δ^9 -THC (6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6H-benzo[c]chromen-1-ol), dissolved in ethanol (200 mg/ml), was kindly provided by the National Institute on Drug Abuse (NIDA; Bethesda, Maryland, USA) and stored at -20°C until used. To prepare suspensions, appropriate amounts of ethanol/THC were withdrawn, the ethanol evaporated under a stream of nitrogen, and the residue dissolved (w/v) in a solution of propylene glycol (PG) and Tween-80 (T-80), before the solute was diluted with

normal (0.9%) saline after sonication for 20 min. Rimonabant, as base (*N*-(piperidin-1-yl)-5-(4-chloro-phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) was also provided by NIDA and stored refrigerated at 4°C before being dissolved in the PG/T-80 (v/v) mixture and diluted with saline. For the most part, the final suspension consisted of 5% PG/3% T-80 and 92% saline, the exceptions being the three highest doses of WIN55,212-2 (5.6, 10 and 18 mg/kg) and the highest doses of AM678 (3 mg/kg) and AM5983 (3 mg/kg) where 5% T-80 were used, i.e., 5, 5 and 90%, respectively. (R)-(+)-WIN55,212-2 mesylate [(R)-5-methyl-3-(morpholinomethyl)-2,3-dihydro-[1,4]oxazino[2,3,4-*hi*]indol-6-yl)(naphthalene-1-yl)methanone; K_i (CB₁) = 1.9 nM; K_i (CB₂) = 0.3 nM; purity ≥98%] was purchased from Biomol (Plymouth Meeting, PA); k_i values for WIN were obtained from published literature (Thakur et al. 2005). AM678 [naphthalene-1-yl(1-pentyl-1*H*-indol-3-yl)methanone; K_i (CB₁) = 4.5 nM; K_i (CB₂) = 33.6 nM; MW = 341], (±) AM5983 [(1-((1-methylpiperidin-2-yl)methyl)-1*H*-indol-3-yl)(naphthalen-1-yl)methanone; K_i (CB₁) = 2.1 nM; K_i (CB₂) = 2.3 nM; MW = 383] and (±) AM2233 [(2-iodophenyl)(1-((1-methylpiperidin-2-yl)methyl)-1*H*-indol-3-yl)methanone; K_i (CB₁) = 2.9 nM; K_i (CB₂) = 3.3 nM; MW = 458] are crystalline compounds that were handled the same way as rimonabant and WIN55,212-2. The ligands AM678, AM5983 and AM2233 were synthesized in the Center for Drug Discovery, University of Connecticut at Storrs; for structures, see Fig. 1. Doses were generally administered i.p. in a volume of 2 ml/kg, the exceptions being the two highest doses of WIN55,212-2 (10 and 18 mg/kg) where 3 ml/kg were used. Suspensions were prepared fresh daily just prior to administration. Doses are expressed as the forms indicated. The smaller size THC group (n=8) examined the effects of AM678 and AM5983 alone in addition to generating the THC generalization gradient at 30 min post-injection. The other group (n=12) was used for all other tests.

Results

The 12 rats used to generate most of the current data were trained to discriminate between THC and vehicle for 37 sessions on the terminal FR-10 schedule of reinforcement before testing commenced; the other 8 rats were trained the discrimination for 41 sessions before being subjected to testing.

THC

Fig. 2 illustrates the generalization gradients (dose-response curves) for THC itself in animals discriminating between 3 mg/kg THC and vehicle, administered i.p. either 30 min (circles; n=8), or 20 min (hexagons; n=12) prior to session onset. The ED₅₀ estimates and the ± 95% C.L. are indicated in the upper graph. Neither the ED₅₀ values, nor the Hill slopes were significantly different between the two data sets [$F(2, 6) = 3.50$; NS]. When combined, the average ED₅₀ value (± 95% C.L.) was 1.12 (0.96 – 1.31) mg/kg (Table 1) and the corresponding values for Hill slope were: 2.95 (1.75 – 4.16). The rate of responding during these tests is indicated in the lower panel. Most of the latter data points were within or in close proximity to the ±95% C.L. of the combined vehicle base-line data for the two groups of rats.

Aminoalkylindoles

Figs. 3 and 4 illustrate the generalization gradients (dose-response curves) for WIN55,212-2, AM678, AM5983 and AM2233 alone (open symbols), and when tested together with 1 mg/kg rimonabant (filled symbols) at 30 min (Fig. 3) and 90 min (Fig. 4) post-injection in rats discriminating between 3 mg/kg THC and vehicle. The ED₅₀ estimates and the ± 95% C.L. are indicated in Table 1 (the data point in the left panel of Fig. 3 reflecting 2 responding rats out of 12 rats tested at 5.6 mg/kg WIN55,212-2 alone at 30 min post administration, is not included in the regression analysis). The lower panels of each figure indicate the associated

rates of responding, expressed as a percentage of the control rate obtained from the vehicle training sessions immediately preceding these tests.

WIN55,212-2

As indicated in the upper left panels of Figs. 3 and 4 and detailed in Table 1, the ED_{50} estimates for WIN55,212-2 alone differed significantly between the tests occurring 30 compared to 90 min post-administration (no overlapping values within the $\pm 95\%$ C.L.), as did the Hill slopes [$F(1, 9) = 7.02$; $p < 0.05$]. Co-administration of 1 mg/kg rimonabant shifted the generalization gradients of WIN55,212-2 significantly to the right as there were no overlaps in the ED_{50} ($\pm 95\%$ C.L.) values for the two test intervals, i.e., 30 (Fig. 3) and 90 (Fig. 4) min post administration. Like WIN55,212-2 alone, there was also a tendency for reduced THC-like responding for the longer compared to the shorter test interval in the drug combination tests (upper left panels of Figs. 3 and 4). Response rates for these tests are shown in the two lower left panels of Figs. 3 and 4. Most of these data points were within or in close proximity to the $\pm 95\%$ C.L. of the vehicle base-line data, exceptions being the effects resulting from the higher test doses of WIN55,212-2 alone and the rimonabant plus WIN55,212-2 combination at both injection-test intervals.

AM678

The next left panels of Figs. 3 and 4 illustrate the generalization gradients for AM678 alone (open symbols), and when tested together with 1 mg/kg rimonabant (filled symbols). AM678 alone potently substituted for THC at the 30 min post-injection test (Fig. 3); the THC-like cue effect was reduced at the 90 min test (Fig. 4). Co-administration of 1 mg/kg rimonabant shifted the generalization gradient of AM678 to the right at both test intervals (Table 1). The response rates during these tests are indicated in the two lower panels (AM678 alone, open symbols and rimonabant plus AM678, filled symbols). Most of these data points were within or in close proximity to the $\pm 95\%$ C.L. of the vehicle base-line data, the exception being the highest test dose of AM678 (3 mg/kg) in combination with rimonabant (lower section of Fig. 4).

AM5983

The next third set of panels of Figs. 3 and 4 illustrate the generalization gradients for AM5983 alone (open symbols), and when examined together with 1 mg/kg rimonabant (filled symbols). AM5983 alone potently substituted for THC at both the 30 min (Fig. 3) and the 90 min (Fig. 4) post-injection tests. Co-administration of 1 mg/kg rimonabant resulted in at least 10-fold parallel shifts to the right of the AM5983 generalization gradients (Table 1). Response rates for these tests are shown in the two lower sections of Figs. 3 and 4). With the exception of 1 mg/kg AM5983 examined alone, most data points were within or in close proximity to the $\pm 95\%$ C.L. of the vehicle base-line data.

AM2233

The furthestmost upper right panels of Figs. 3 and 4 illustrate the generalization test results for AM2233 alone (open symbols), and when 1.8 mg/kg AM2233 was tested together with 1 mg/kg rimonabant (filled symbols). The ED_{50} estimates for AM2233 differed significantly between the tests occurring 30 min compared to 90 min post administration (no overlapping values within the $\pm 95\%$ C.L.), with associated non-significant Hill slopes [$F(1, 8) = 0.82$; NS], suggesting that the drug effects are waning (Table 1). Not surprisingly, excluding the dose of 1.8 mg/kg of AM2233 from the analysis resulted in a significant difference in the slopes between the two time points [$F(2, 6) = 35.45$; $p < 0.001$]. Response rates were suppressed in tests with the two highest doses at 30 min and remained so for the 1.8 mg/kg dose also at 90 min post-injection. Although 1 mg/kg rimonabant attenuated the degree of

substitution at 30 min (Fig. 3, upper right panel), the rate-decreasing effects of 1.8 mg/kg AM2233 was not blocked (Fig. 3, lower right panel). However, the rate decrease was blocked at the 90 min test (Fig. 4), i.e., the rate being within the $\pm 95\%$ C.L. of the vehicle base-line data. All animals ($n=12$) obtained at least one reinforcement during both tests.

Discussion

To summarize, co-administration of rimonabant (1 mg/kg) resulted in wider right-ward shifts of the AM678 and AM5983 generalization curves compared to that of WIN55,212-2. All four aminoalkylindoles exhibited less THC-like cue effects 90 min compared to the results from the 30 min post-administration interval. Irrespective of the time interval studied, the following rank order of potency emerged: $AM5983 \geq AM678 > AM2233 > WIN55,212-2$. Based on data from the shorter injection-to-test interval, AM5983 and AM678 appeared 8 times more potent than THC, followed by AM2233, which was about twice as potent as THC, and $THC \geq WIN55,212-2$. All of these ligands showed reduced potency (i.e., an increased ED_{50} value) at the longer injection-to-test interval of 90 min compared to the initial tests conducted at 30 min. Such a decline over time in the ability to substitute for THC was also evident from tests involving antagonism of these ligands by rimonabant. The right-ward shifts caused by rimonabant were more pronounced for AM5983 and AM678 compared to WIN55,212-2, and of similar magnitude to that described earlier for THC/rimonabant combinations, i.e., approximately 8 to 12-fold based on data from the shorter injection-to-test interval of 30 min (Järbe et al. 2001). In contrast, there was only an approximately 3-fold right-ward shift of the WIN55,212-2 curve, consistent with previous data (Järbe et al. 2010) employing a lower training dose of THC (1.8 mg/kg), and hence extending the generality of this outcome by a systematic replication approach (Sidman 1960).

WIN55,212-2 has been characterized *in vitro* as a full CB_1R agonist and the ligand generally is considered more potent than the partial CB_1R agonist THC (Pertwee 2008). This contention was supported also by some early drug discrimination studies (Compton et al. 1992; Péroio et al. 1996) as well as later investigations (McMahon et al. 2008; Solinas et al. 2007). However, other studies, like this one, have not necessarily found that to be the case; see also (De Vry and Jentsch 2002; 2004; Järbe et al. 2010; Mauler et al. 2002; McMahon 2006a; b; Wiley et al. 1995). Although the reason for this discrepancy in relative potency across studies is not known, it is noteworthy that WIN appeared less effective than either of the CB_1R agonists THC or CP55,940 in attenuating or reversing the discriminative stimulus effects of rimonabant in monkeys maintained on twice/daily THC administration (Stewart and McMahon 2010). Thus, such an outcome from this presumed withdrawal discrimination seems to support the contention that there are differences in the way that CB_1R agonists interact with rimonabant and hence suggests differences in the mode(s) of action [see also (McMahon 2006b)].

The decline in the percentage of drug responding between the 30 and 90 min test intervals suggest that the THC-like effects of WIN55,212-2 have a relatively fast offset. Although not re-examined here, previous data (Järbe et al. 1986) suggested that for THC itself, the difference between these two intervals was less pronounced. Using the same regression model as that applied here resulted in ED_{50} ($\pm 95\%$ C.L.) estimates of 0.85 (± 0.63 –1.16) mg/kg and 1.06 (0.82–1.37) mg/kg of THC for these two injection-to-test intervals, respectively (Järbe et al. 1986). Also, applying the current non-linear regression model to the time-course data generated by rats discriminating WIN55,212-2 (0.3 mg/kg) from vehicle produced an *in vivo* functional half-life estimate of ($\pm 95\%$ C.L.) 129.9 (± 120.1 – 140.4) min, again suggesting a relatively short duration of action (Péroio et al. 1996). As for

THC, the discriminative stimulus effects of this CB₁R agonist are clearly reduced 4 hrs after i.p. administration in rats (Järbe et al. 1981, 1986).

At both of the test intervals examined, co-administration of rimonabant (1 mg/kg) and WIN55,212-2 shifted the CB₁R agonist generalization/dose gradient about 3-fold to the right, indicative of surmountable antagonism. That increasing doses of WIN55,212-2 can overcome the rimonabant-induced CB₁R blockade is consistent with previous findings, although the magnitude of the shift has varied (Järbe et al. 2010; McMahon 2006a; Wiley et al. 1995). The current magnitude of the right-fold shift of the WIN55,212-2 curve is similar to the 2.5-fold shift we observed in rats trained to discriminate between vehicle and 1.8 mg/kg THC (Järbe et al. 2010), and furthermore contrasts with the larger rightward displacement that occurred with rimonabant/AM678 combinations as reported previously (Järbe et al. 2010), as well as in the current study. The latter magnitude of the displacement of the dose-response curve was evident also for AM5983/rimonabant combinations. Both AM678 and AM5983 were about 8 times more potent than THC, and by extension also WIN55,212-2. Although we are cognizant that many factors determine *in vivo* potency, receptor affinity could explain the relative potency difference between THC [K_i (CB₁) = 46 nM] and AM678 [K_i (CB₁) = 4.5 nM] as well as AM5983 [K_i (CB₁) = 2.1 nM], but does not account for the diminished relative potency of WIN55,212-2 [K_i (CB₁) = 1.9 nM] as a substitute for THC. It has been argued that the mode of CB₁R activation is different for aminoalkylindoles compared to that of more “classical” cannabinoids such as THC and the potent analog CP55,940 (Huffman and Padgett 2005; Manera et al. 2008). Regardless, the current studies reaffirmed the previously reported activity of AM678, both when examined alone as well as when combined with rimonabant, by extending the finding to a situation involving a higher training dose and presumably therefore a more salient THC cue. An *in vivo* activity profile similar to AM678 was evident also for AM5983 in the current study.

The aminoalkylindole AM2233 was developed as a potent CB₁R (racemic K_i = 2.8 nM) agonist and as a probe for biochemical and imaging studies (Deng et al. 2005). Radioiodinated [¹³¹I]AM2233 followed a brain distribution pattern consistent with anatomical areas known to display an abundance of CB₁R expression and it was more potent than WIN55,212-2 (Deng et al. 2005). Therefore, it is not surprising that AM2233 substituted for the discriminative stimulus effects of THC. In that regard, the compound was twice as potent as THC (as well as WIN55,212-2) at the 30 min injection-to-test interval. Very limited THC-like responding occurred when testing the animals 90 min after injection, suggesting a comparatively short duration of action for the THC-like effects of AM2233. Likewise, a short duration of action of the primary active isomer *R*-[¹³¹I]AM2233 was evident also in brain binding studies in mice where the highest activity was registered 5 min i.v. post-injection with very little activity remaining 60 min later (Dhawan et al. 2006). In agreement with above reports, the substitution for THC by AM2233 was rimonabant-sensitive. Thus, co-administration of rimonabant together with a high dose of AM2233 (1.8 mg/kg) markedly attenuated the degree of substitution at both post-injection intervals, suggesting CB₁R mediation for the THC-like effects of AM2233.

Two of the four aminoalkylindoles examined here are racemic mixtures. For AM2233, most of the CB₁R activity resides with the (*R*)-isomer with little, if any contribution from the (*S*)-isomer (Deng et al. 2005; Dhawan et al. 2006). Regarding AM5983, both isomers are active in rats, i.e., substituting for the discriminative stimulus effects produced by the racemic mixture (AM5983) used for drug discrimination training, but similarly to AM2233, most activity appears attributable to the (*R*)-isomer (personal observation).

Current data also have heuristic value in demonstrating that AM678 (a common ingredient in ‘Spice’ herbal concoctions, but now banned in many countries including the USA)

substitutes for THC and that the substitution is blocked by rimonabant as previously found (Järbe et al. 2010). Coupled with other data (see Introduction), it is clear that this aminoalkylindole is bioactive and could produce marijuana-like effects though it may not be the only synthetic cannabimimetic ingredient in any given ‘Spice’ formulation (Atwood et al. 2011; Hudson et al. 2010; Vardakou et al. 2010). The binding affinity of JWH018/AM678 for CB₁R was previously reported to be 9 nM, i.e., a 4-to 5-fold difference in comparison to THC (Aung et al. 2000), and its *in vivo* potency *viz-a-viz* THC in mice depended upon which sub-test of the “tetrad” assay was being used for comparison (Wiley et al. 1998). Our binding affinity estimate of 4.5 nM, coupled with current and previous *in vivo* drug discrimination data (Järbe et al. 2010), would suggest that the potency difference could be as high as 8-fold. Regarding time-course, the decline in activity at the 90 min post-injection tests suggested that the THC-like effects were waning [see also (Uchiyama et al. 2011)]. Thus, a protracted time-course characteristic of dimethyl, heptyl THC analogs such as HU210 or a similarly derived hexahydrocannabinol (HU243, i.e., [(6aR,10aR)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,8,9,10,10a-hexahydro-6H-benzo[c]chromen-1-ol]) was not evident for any of the current aminoalkylindoles (Devane et al. 1992; Järbe et al. 1989; Järbe et al. 1981; Little et al. 1989).

In summary, we replicated and extended the previous observation that AM678 is a more potent cannabinergic “THC-like” ligand than WIN55,212-2 also when employing a higher training dose of THC. Additionally, the WIN55,212-2 induced behavioral disruption at higher doses in the 3 mg/kg THC group was not evident in the 1.8 mg/kg THC group (Järbe et al. 2010), suggesting that different training doses can be useful also in delineating mechanistic differences. The order of magnitude of the rightward shifts of the dose-response curves was larger for AM678 compared to WIN55,212-2 and this finding was extended to AM5983. That the shift to the right was less for WIN55,212-2, compared to the shifts for AM678 and AM5983 when these CB₁R agonists were co-administered with rimonabant, is somewhat surprising in light of recent findings where WIN55,212-2 binding to non-CB₁R binding sites in the mouse brain was shown to be also antagonized by rimonabant (Nguyen et al. 2010). Currently it is unknown if these novel non-CB₁R binding sites are present also in the brain of other mammals such as the rat and if so, would they be differentially affected by cannabinergic ligands? Although the binding affinity for cannabinoid receptors by AM2233 is similar to that of WIN55,212-2, the potency of AM2233 in eliciting “THC-like” effects was two-fold higher than that of WIN55,212-2. AM2233 also appeared to have the shortest duration of action among the currently examined aminoalkylindoles.

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References

- Atwood BK, Huffman J, Straiker A, Mackie K. JWH018, a common constituent of ‘Spice’ herbal blends, is a potent and efficacious cannabinoid CB receptor agonist. *Br J Pharmacol*. 2010; 160:585–93. [PubMed: 20100276]

- Atwood BK, Lee D, Straiker A, Widlanski TS, Mackie K. CP47,497-C8 and JWH073, commonly found in 'Spice' herbal blends, are potent and efficacious CB(1) cannabinoid receptor agonists. *Eur J Pharmacol.* 2011
- Aung MM, Griffin G, Huffman JW, Wu M, Keel C, Yang B, Showalter VM, Abood ME, Martin BR. Influence of the N-1 alkyl chain length of cannabimimetic indoles upon CB(1) and CB(2) receptor binding. *Drug Alcohol Depend.* 2000; 60:133–40. [PubMed: 10940540]
- Bergman J, France CP, Holtzman SG, Katz JL, Koek W, Stephens DN. Agonist efficacy, drug dependence, and medications development: Preclinical evaluation of opioid, dopaminergic, and GABAA-ergic ligands. *Psychopharmacology (Berl).* 2000; 153:67–84. [PubMed: 11255930]
- Bosier B, Muccioli GG, Hermans E, Lambert DM. Functionally selective cannabinoid receptor signalling: therapeutic implications and opportunities. *Biochem Pharmacol.* 2010; 80:1–12. [PubMed: 20206137]
- Compton DR, Gold LH, Ward SJ, Balster RL, Martin BR. Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from delta-9-tetrahydrocannabinol. *J Pharmacol Exp Ther.* 1992; 263:1118–26. [PubMed: 1335057]
- De Vry J, Jentzsch KR. Discriminative stimulus effects of BAY 38-7271, a novel cannabinoid receptor agonist. *Eur J Pharmacol.* 2002; 457:147–52. [PubMed: 12464360]
- De Vry J, Jentzsch KR. Discriminative stimulus effects of the structurally novel cannabinoid CB₁/CB₂ receptor partial agonist BAY 59-3074 in the rat. *Eur J Pharmacol.* 2004; 505:127–33. [PubMed: 15556145]
- Deng H, Gifford AN, Cui G, Li X, Fan P, Deschamps JR, Flippen-Anderson JL, Gatley SJ, Makriyannis A. Potent cannabinergeric indole analogs as radioiodinatable brain imaging agents for the CB1 cannabinoid receptor. *J Med Chem.* 2005; 48:6386–92. [PubMed: 16190764]
- Devane WA, Breuer A, Sheskin T, Järbe TUC, Eisen MS, Mechoulam R. A novel probe for the cannabinoid receptor. *J Med Chem.* 1992; 35:2065–9. [PubMed: 1317925]
- Dhawan J, Deng H, Gatley SJ, Makriyannis A, Akinfeleye T, Bruneus M, Dimaio AA, Gifford AN. Evaluation of the in vivo receptor occupancy for the behavioral effects of cannabinoids using a radiolabeled cannabinoid receptor agonist, R-[125/131I]AM2233. *Synapse.* 2006; 60:93–101. [PubMed: 16715483]
- Extance K, Goudie AJ. Inter-animal olfactory cues in operant drug discrimination procedures in rats. *Psychopharmacology (Berl).* 1981; 73:363–71. [PubMed: 6789359]
- Hodge CW, Grant KA, Becker HC, Besheer J, Crissman AM, Platt DM, Shannon EE, Shelton KL. Understanding how the brain perceives alcohol: neurobiological basis of ethanol discrimination. *Alcohol Clin Exp Res.* 2006; 30:203–13. [PubMed: 16441269]
- Hudson S, Ramsey J, King L, Timbers S, Maynard S, Dargan PI, Wood DM. Use of high-resolution accurate mass spectrometry to detect reported and previously unreported cannabinomimetics in "herbal high" products. *J Anal Toxicol.* 2010; 34:252–60. [PubMed: 20529459]
- Huffman JW, Dai D, Martin BR, Compton DR. Design, synthesis and pharmacology of cannabimimetic indoles. *Bioorg Med Chem Lett.* 1994; 4:563–6.
- Huffman JW, Padgett LW. Recent developments in the medicinal chemistry of cannabimimetic indoles, pyrroles and indenenes. *Curr Med Chem.* 2005; 12:1395–411. [PubMed: 15974991]
- Järbe, TUC. Discrimination learning with drug stimuli: Methods and applications. In: Boulton, AA.; Baker, GB.; Greenshaw, AJ., editors. *Neuromethods: Vol. 13. Psychopharmacology.* Humana Press; Clifton, NJ: 1989. p. 513-63.
- Järbe, TUC. Perceptual drug discriminative aspects of the endocannabinoid signaling system in animals and man. In: Glennon, RA.; Young, R., editors. *Drug discrimination: Applications to medicinal chemistry and drug studies.* Wiley Publ. Co; Hoboken, NJ: 2011. p. 241-85.
- Järbe TUC, Swedberg MD, Mechoulam R. A repeated test procedure to assess onset and duration of the cue properties of (–) Δ^9 -THC, (–) Δ^8 -THC-DMH and (+) Δ^8 -THC. *Psychopharmacology (Berl).* 1981; 75:152–7. [PubMed: 6275439]
- Järbe TUC, Hiltunen AJ, Lander N, Mechoulam R. Cannabimimetic activity (delta-1-THC cue) of cannabidiol monomethyl ether and two stereoisomeric hexahydrocannabinols in rats and pigeons. *Pharmacol Biochem Behav.* 1986; 25:393–9. [PubMed: 3020594]

- Järbe TUC, Hiltunen AJ, Mechoulam R. Stereospecificity of the discriminative stimulus functions of the dimethylheptyl homologs of 11-hydroxy- Δ^8 -tetrahydrocannabinol in rats and pigeons. *J Pharmacol Exp Ther.* 1989; 250:1000–5. [PubMed: 2550611]
- Järbe TUC, Lamb RJ, Lin S, Makriyannis A. (R)-methanandamide and Δ^9 -THC as discriminative stimuli in rats: tests with the cannabinoid antagonist SR-141716 and the endogenous ligand anandamide. *Psychopharmacology (Berl).* 2001; 156:369–80. [PubMed: 11498713]
- Järbe TUC, Li C, Vadivel SK, Makriyannis A. Discriminative stimulus functions of methanandamide and Δ^9 -THC in rats: Tests with aminoalkylindoles (WIN55,212-2 and AM678) and ethanol. *Psychopharmacology (Berl).* 2010; 208:87–98. [PubMed: 19902182]
- Little PJ, Compton DR, Mechoulam R, Martin BR. Stereochemical effects of 11-OH-delta-8-THC-dimethylheptyl in mice and dogs. *Pharmacol Biochem Behav.* 1989; 32:661–6. [PubMed: 2544901]
- Manera C, Tuccinardi T, Martinelli A. Indoles and related compounds as cannabinoid ligands. *Mini Rev Med Chem.* 2008; 8:370–87. [PubMed: 18473928]
- Mauler F, Mittendorf J, Horvath E, De Vry J. Characterization of the diarylether sulfonylester (–)-(R)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluoro-1-sulfonate (BAY 38-7271) as a potent cannabinoid receptor agonist with neuroprotective properties. *J Pharmacol Exp Ther.* 2002; 302:359–68. [PubMed: 12065738]
- McMahon LR. Characterization of cannabinoid agonists and apparent pA2 analysis of cannabinoid antagonists in rhesus monkeys discriminating Δ^9 -tetrahydrocannabinol. *J Pharmacol Exp Ther.* 2006a; 319:1211–8. [PubMed: 16943255]
- McMahon LR. Discriminative stimulus effects of the cannabinoid CB1 antagonist SR 141716A in rhesus monkeys pretreated with Δ^9 -tetrahydrocannabinol. *Psychopharmacology (Berl).* 2006b; 188:306–14. [PubMed: 16953389]
- McMahon LR, Ginsburg BC, Lamb RJ. Cannabinoid agonists differentially substitute for the discriminative stimulus effects of Δ^9 -tetrahydrocannabinol in C57BL/6J mice. *Psychopharmacology (Berl).* 2008; 198:487–95. [PubMed: 17673980]
- Nguyen PT, Selley DE, Sim-Selley LJ. Statistical Parametric Mapping reveals ligand and region-specific activation of G-proteins by CB1 receptors and non-CB1 sites in the 3D reconstructed mouse brain. *Neuroimage.* 2010; 52:1243–51. [PubMed: 20451624]
- Pério A, Rinaldi-Carmona M, Maruani J, Barth F, Le Fur G, Soubrié P. Central mediation of the cannabinoid cue: activity of a selective CB1 antagonist, SR 141716A. *Behav Pharmacol.* 1996; 7:65–71. [PubMed: 11224395]
- Pertwee RG. Ligands that target cannabinoid receptors in the brain: from THC to anandamide and beyond. *Addict Biol.* 2008; 13:147–59. [PubMed: 18482430]
- Pertwee RG. Receptors and channels targeted by synthetic cannabinoid receptor agonists and antagonists. *Curr Med Chem.* 2010; 17:1360–81. [PubMed: 20166927]
- Poso A, Huffman JW. Targeting the cannabinoid CB2 receptor: modelling and structural determinants of CB2 selective ligands. *Br J Pharmacol.* 2008; 153:335–46. [PubMed: 17982473]
- Sidman M. *Tactics of Scientific Research – Evaluating Experimental Data in Psychology.* Basic Books, New York. 1960
- Solinas M, Tanda G, Justinova Z, Wertheim CE, Yasar S, Piomelli D, Vadivel SK, Makriyannis A, Goldberg SR. The endogenous cannabinoid anandamide produces delta-9-tetrahydrocannabinol-like discriminative and neurochemical effects that are enhanced by inhibition of fatty acid amide hydrolase but not by inhibition of anandamide transport. *J Pharmacol Exp Ther.* 2007; 321:370–80. [PubMed: 17210800]
- Stewart JL, McMahon LR. Rimonabant-induced Δ^9 -tetrahydrocannabinol withdrawal in rhesus monkeys: discriminative stimulus effects and other withdrawal signs. *J Pharmacol Exp Ther.* 2010; 334:347–56. [PubMed: 20375197]
- Thakur GA, Nikas SP, Li C, Makriyannis A. Structural requirements for cannabinoid receptor probes. *Hand Exp Pharm.* 2005; 168:209–246.
- Uchiyama N, Kikura-Hanajiri R, Matsumoto N, Huang ZL, Goda Y, Urade Y. Effects of synthetic cannabinoids on electroencephalogram power spectra in rats. *Forensic Sci Int.* 2011

- Vardakou I, Pistos C, Spiliopoulou C. Spice drugs as a new trend: mode of action, identification and legislation. *Toxicol Lett.* 2010; 197:157–62. [PubMed: 20566335]
- Wiley JL, Barrett RL, Lowe J, Balster RL, Martin BR. Discriminative stimulus effects of CP 55,940 and structurally dissimilar cannabinoids in rats. *Neuropharmacology.* 1995; 34:669–76. [PubMed: 7566504]
- Wiley JL, Compton DR, Dai D, Lainton JA, Phillips M, Huffman JW, Martin BR. Structure-activity relationships of indole- and pyrrole-derived cannabinoids. *J Pharmacol Exp Ther.* 1998; 285:995–1004. [PubMed: 9618400]

WIN55,212-2

(R)-[5-methyl-3-[(morpholinomethyl)-2,3-dihydro-[1,4]oxazino[2,3,4-hj]indol-6-yl]]naphthalen-1-ylmethanone

AM2233

(±)-[1-[(2-iodophenyl)[1-[(1-methylpiperidin-2-yl)methyl]-1H-indol-3-yl]methanone

AM5983

(±)-[1-[(1-methylpiperidin-2-yl)methyl]-1H-indol-3-yl]naphthalen-1-ylmethanone

AM678

naphthalen-1-yl[1-pentyl-1H-indol-3-yl]methanone

Figure 1.
Chemical structures of cannabinergic agonists (aminoalkylindoles) used in study.

Figure 2. Generalization test results (upper panel) and corresponding response rate data (lower panel) for THC in THC- (3 mg/kg) versus vehicle-trained rats at 30 min (30'; n=8), and 20 min (20'; n=12). The generalization results represent the mean ($\pm$ SEM) percentage of lever presses on the THC appropriate lever out of the total number of lever presses emitted during a test session (vertical axis); doses examined in mg/kg (horizontal axis). Rate refers to the mean ($\pm$ SEM) number of lever presses per second emitted during a test session, expressed as a percentage of control, i.e., vehicle-training sessions immediately preceding above test sessions (vertical axis); doses in mg/kg (horizontal axis). Dotted lines represent the $\pm$ 95% confidence limits of vehicle control response rate determined from the initial 6 reinforcement cycles of the one to two vehicle training sessions immediately preceding these tests; symbols outside the confidence limits are considered significantly different from

control. Dose determinations are based on one to two observations for each rat and were obtained on separate test days. Test results are based on sessions of a maximum of 6 reinforcements (12 food pellets) or 20 min, whichever occurred first.

Figure 3.

Generalization test results (upper panels) and the corresponding response rate data (lower panels) for the test drug alone (open triangles; WIN55,212-2 $n=12$; AM678 $n=6-7$; AM5983 $n=7$; AM2233 $n=10-12$), and when combined with 1 mg/kg rimonabant (filled triangles; WIN55,212-2 $n=11-12$; AM678 $n=11-12$; AM5983 $n=11-12$; AM2233 $n=12$), 30 min post i.p. administration. Drug-lever responding results (upper panels) represent the mean ($\pm$ SEM) percentage of lever presses on the THC appropriate lever out of the total number of lever presses emitted during a test session (vertical axis); doses examined in mg/kg (horizontal axis). Rate refers to the mean ($\pm$ SEM) number of lever presses per second emitted during a test session (vertical axis); doses in mg/kg (horizontal axis). Numbers within brackets indicate the number of rats responding (i.e., accumulating at least 10 responses on either lever and thus obtaining at least one reinforcement) out of the total number used for the test. Other details are as described in the legend for figure 2.

Figure 4.

Generalization test results (top) for the test drug alone (open triangles) and when combined with 1 mg/kg rimonabant (filled triangles) and the corresponding response rate data (bottom), 90 min post i.p. administration. The 90 min test occurred subsequent to the 30 min test after a single injection and hence both intervals evaluated during the same test day. Drug-lever responding results represent the mean ($\pm$ SEM) percentage of lever presses on the THC appropriate lever out of the total number of lever presses emitted during a test session (vertical axis); doses examined in mg/kg (horizontal axis). Rate refers to the mean ($\pm$ SEM) number of lever presses per second emitted during a test session (vertical axis); doses in mg/kg (horizontal axis). The 90 min test occurred subsequent to the 30 min test after a single injection and hence both intervals evaluated during the same test day. Other details are as described in the legend for figures 2 and 3.

Table 1

Summary of generalization test data for THC (3 mg/kg) trained rats.

30 min tests	
Test Drug	ED₅₀ ± 95% C.L. (mg/kg)
WIN	1.30 (± 0.85–1.98)
WIN+R	4.11 (± 2.45–6.89)
AM678	0.14 (± 0.12–0.15)
AM678+R	1.05 (± 0.53–2.07)
AM5983	0.13 (± 0.10–0.17)
AM5983+R	1.43 (± 1.07–1.91)
AM2233	0.49 (± 0.32–0.76)
90 min tests	
Test Drug	ED₅₀ ± 95% C.L. (mg/kg)
WIN	3.66 (± 2.81–4.76)
WIN+R	11.01 (± 6.13–19.78)
AM678	0.39 (± 0.17–0.93)
AM678+R	1.32 (± 0.66–2.64)
AM5983	0.14 (± 0.08–0.27)
AM5983+R	2.23 (± 1.84–2.70)
AM2233	2.54 (± 1.40–4.58)
THC	1.12 (± 0.96–1.31) ^{*)}

^{*)} aggregate value (see Fig. 2)

R = Rimonabant (1 mg/kg)