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Minireview

"Herbal incense": Designer drug blends as cannabimimetics and their assessment by drug discrimination and other in vivo bioassays

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ABSTRACT

Recently, synthetic cannabinoids originally designed for testing in the laboratory only have found use recreationally in designer herbal blends, originally called "Spice". The myriad of compounds found are for the most part potent full agonists of the cannabinoid receptor 1, producing effects similar to tetrahydrocannabinol (THC) and marijuana. Drug discrimination of these compounds offers a specific behavioral test that can help determine whether these new synthetic compounds share a similar "subjective high" with the effects of marijuana/THC. By utilization of drug discrimination and other behavioral techniques, a better understanding of these new "designer" cannabinoids may be reached to assist in treating both the acute and chronic effects of these drugs. The paper provides a brief exposé of modern cannabinoid research as a backdrop to the recreational use of designer herbal blend cannabimimetics.

Contents

Introduction

Amidst current controversies surrounding medical marijuana and even legalization of recreational use of cannabis products is the issue about so called designer cannabinoids, i.e., non-plant research chemicals affecting the endocannabinoid signaling system (ECS), which can produce a marijuana-like "high". Such research compounds represent diverse chemical templates although cannabimimetic indoles often have been used in the clandestine production of herbal blends, here collectively referred to as "Spice". This mini-review will provide a background to the current situation with a focus on in vivo behavioral procedures emphasizing the drug discrimination assay as highly relevant for understanding the psychopharmacology of marijuana/cannabinoids as well as the recent addition of designer cannabimimetics being presented as "legal highs".

Although cannabis has been known to mankind for millennia (Hanus, 2009), it was not until the mid 1960's that the main psychoactive constituent in marijuana/hashish was isolated and its exact structure elucidated (Gaoni and Mechoulam, 1964; Mechoulam et al., 1967). This phytocannabinoid is known as (−)-Δ^9-trans-tetrahydrocannabinol (THC), based on the formal chemical rules for numbering pyran compounds; according to a monoterpenoid system, this compound would be labeled Δ^1-THC (see Fig. 1). A minor isomer is Δ^8-THC (alternatively $\Delta^{1(6)}$- or Δ^6-THC) with an in vivo potency less than that of Δ^9-THC. However, Δ^8-THC is more chemically stable than Δ^9-THC, i.e., less prone to decomposing in the presence of light and oxygen. This presumably is the reason why a considerable amount of structure–activity-relationship

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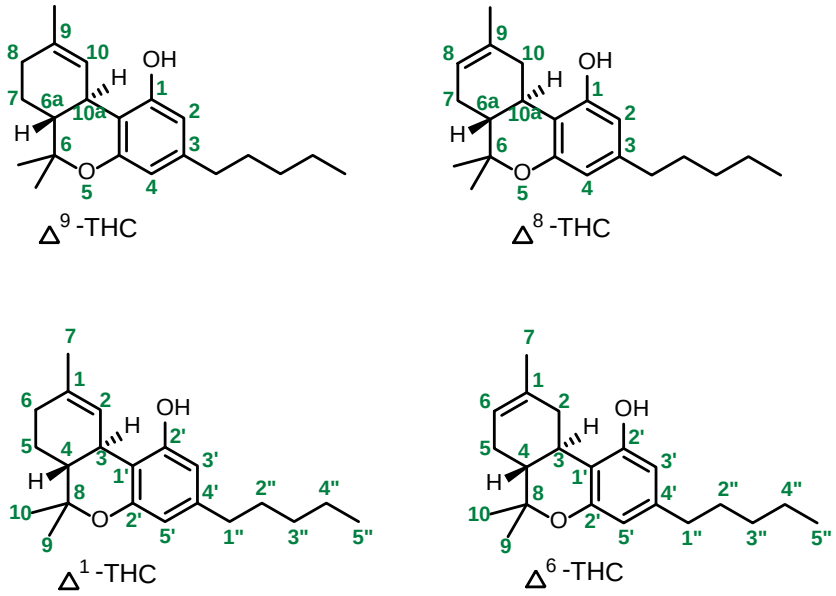

Fig. 1. Chemical structure of THC according to pyran numbering system (top row) or according to a monoterpenoid system (lower row).

(SAR) studies based on the THC template has been carried out with Δ^8-THC rather than Δ^9-THC. The pyran numbering system is the most commonly used descriptor for labeling the more than 60 C_{21} terpenophenolic cannabinoids identified in the cannabis plant to date.

Once the exact structure of THC had been elucidated, routes for its synthesis were explored in order to improve chemical efficiency and yield (Mechoulam and Gaoni, 1967; Petrzilka, 1971; Petrzilka and Sikemeier, 1967; Razdan, 1986). These efforts set the stage for the initial scientific efforts to better understand the pharmacology of cannabis, including pharmacokinetics and bio-distribution (Agurell et al., 1986; Pertwee, 2006). Metabolism is complex resulting in several intermediaries before excretion, occurring primarily through the kidneys/urine. A THC metabolite of major biological significance was identified as 11-hydroxy-THC, exhibiting a potency exceeding that of the parent compound (Balster and Prescott, 1992; Järbe, 2011). Early symposia surrounding scientific discourses about cannabinoids were held in 1971, Stockholm, Sweden (Agurell and Nilsson, 1971) and in 1972, London, UK (Paton and Crown, 1972). Both symposia covered a range of topics illustrating the proliferation of cannabinoid research but primarily focusing on THC as the agent responsible for the "subjective high" as other plant cannabinoids were essentially considered inactive. Although early SAR studies emphasized the chemistry of the plant "tricyclic" classical cannabinoids (Mechoulam and Edery, 1973), efforts were also directed at more novel chemical templates such as the "bicyclic" nonclassical cannabinoid agonist CP55,940 [2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl)-cyclohexyl]-5-(2-methyloctan-2yl)phenol] (Melvin and Johnson, 1987). Tritiated CP55,940 was instrumental in discovering the first cannabinoid receptor (CB_1R) (Devane et al., 1988). A few years later, the first endogenous ligand for this receptor was identified and named anandamide (AEA) (Devane et al., 1992b). Subsequently, other endogenous ligands for CB_1R were identified, of which 2-arachidonoylglycerol is best known (Mechoulam et al., 1995; Sugiura et al., 1995), as well as a 2nd binding site, the cannabinoid 2 receptor (CB_2R), being discovered (Munro et al., 1993). CB_2R is primarily linked to immune function(s); the relationship between these two cannabinoid receptors is not well understood. This is collectively referred to as the ECS.

Behavioral components of cannabimimetics

Along with the efforts in elucidating the exact chemical structure of THC was the search for methods to assess the effects of cannabinoids in vivo. Two early assays were the Gayer areflexia test in rabbits (Gayer, 1928) and the "sway-test" in dogs (Dixon, 1899), the former measuring abolition of the rabbit blinking reflex and the latter evaluating motor incoordination (ataxia) and other cannabis induced reactions in dogs. Along with these bioassays, Loewe (1946) also noted that muscle rigidity (catalepsy), particularly in mice, appeared as a characteristic phenomenon after administration of higher doses of THC. This immobility reaction was reintroduced in the early 1970's as the "ring-test" (Pertwee, 1972). Other early bioassay/in vivo measures, including analgesia, have been summarized by do Valle (do Valle, 1969).

Subsequently additional in vivo methods were introduced for probing the THC induced cannabimimetic effects in animals. One peculiar model made use of a subset of white New Zealand rabbits that exhibited nonlethal convulsions when given THC acutely and the intensity of the convulsions diminished after repeated THC administration, i.e., an indication of tolerance development; when THC was discontinued, the animals regained their sensitivity to convulsion proneness (Consroe et al., 1982; Martin and Consroe, 1976). Combining measures of spontaneous locomotor activity using an open-field arena, rectal temperature recordings, analgesia assessment and indices of immobility (catalepsy) constitutes the cannabinoid tetrad bioassay (Martin et al., 1991). This has been and still is one of the most widely used bioassays for assessing cannabimimetic activity in rodents. Each of these four endpoints in the tetrad by themselves is not specific for cannabimimetic activity and therefore they need to be evaluated as a cluster rather than separately to diminish the potential risk for detecting false positives (Wiley et al., 2006). This bioassay procedure has exhibited good correlation between cannabinoid receptor 1 (CB_1R) binding affinity and potency ($r = 0.85–0.91$) when evaluating classical cannabinoids such as THC and related derivatives but the correlation is less ($r = 0.46–0.76$) for the endogenous CB_1R ligand AEA and derivatives thereof as cited by Wiley et al. (Wiley et al., 2006; Adams et al., 1998; Compton et al., 1993), suggesting differences between the two classes of compounds. Although AEA activates CB_1R, there is evidence that this endocannabinoid also activates transient receptor potential cation channel vanilloid type 1 (TRPV1) signaling (Wiley et al., 2006). The TRPV1 protein is activated by excessive heat and pungent chemicals present in red hot chili peppers and mustard. For example, mice given the mixed AEA/TRPV1 derivative O-1839 (1,1-dimethylheptyl-arvanil), which has good efficacy for TRPV1, behaved in a "THC-like" manner in the tetrad test even though the affinity for CB_1R was low ($K_i > 200$ nM) (Di Marzo et al., 2001). Additional testing, using rats discriminating between THC and vehicle, suggested that O-1839 did not mimic the discriminative stimulus effects of THC or the

stimulus effects of a high-affinity CB_1R (k_i 3–5 nM) AEA derivative O-1812 [(*R*,5Z,8Z,11Z,14Z)-20-cyano-N-(1-hydroxypropan-2-yl)-16,16-dimethylicosa-5,8,11,14-tetraenamide]; THC and O-1812, on the other hand, exhibited cross-substitution and the effects were blocked by the selective CB_1R inverse agonist/antagonist rimonabant (Wiley et al., 2004). Other, more recent examples of false tetrad positives occurred with a subset of analogs having 3-substituent replacements of rimonabant's pyrazole core where such compounds exhibited a "THC-like" or cannabimimetic tetrad test profile in mice (Walentiny et al., 2013; Wiley et al., 2012). These analogs did not substitute for, nor did they antagonize the discriminative stimulus effects of THC in mice trained to discriminate between vehicle and THC. Likewise, THC did not substitute in mice discriminating between one of these analogs [O-6629 = 5-(bromomethyl)-2-(5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-4,5-dihydrooxazole; CB_1R K_i = 24 nM] and vehicle; rimonabant neither blocked, nor did it substitute for O-6629 (Walentiny et al., 2013). As the above examples show, although selective the tetrad bioassay protocol is not specific for CB_1R activation in the central nervous system, the presumed neural basis underlying the "subjective high", fueling the ingestion of cannabis preparations and other synthetic cannabimimetics. Rather, the pharmacological specificity issue in the above described studies was approached by the use of drug discrimination, an assay we believe is highly relevant for understanding the psychopharmacology of THC/marijuana as well as the recent addition of so called "designer cannabimimetics" in humans.

Designer cannabimimetics in herbal blends

The concept of designer drugs is not new but rather can be traced back to the early 20th century. What is new is the expansion of drug classes that is covered by this concept and their global availability made possible by the world-wide-web. Synthetic cannabinoids in herbal blends were first detected near the end of 2008. For a history of the emergence of "designer cannabimimetics", originally branded under product names such as "Spice" and "k2" (see e.g. Fattore and Fratta, 2011). "Spice" and related herbal incense products have been widely available for purchase in so called "head-shops" and over the internet during the last decade. Some product ingredients may be listed on the package but if laced with synthetic cannabimimetics, such additions and the amounts thereof are not publicly revealed — after all, the product is "not for human consumption". The situation is succinctly stated by the article title "Hijacking of basic research: the case of synthetic cannabinoids" (Wiley et al., 2011). Cannabimimetics present in the early wave of incense products have been banned (schedule 1 in the USA) by most governments around the globe but this does not ensure the end of designer cannabimimetics as there is a plethora of CB_1R agonists and their synthesis described in the scientific literature. This information is easily available, enabling the replacement of agents classified as illegal. It is of note that the banned cannabimimetics generally have high affinity for, and are fully efficacious in activating the CB_1R. Thus, many of these designer cannabimimetics are considered full CB_1R agonists in contrast to THC, which is characterized as a partial CB_1R agonist. In practical terms, this means that many of the banned substances will likely produce stronger effects than THC.

Given that the designer cannabimimetics were not primarily developed with human consumption in mind but rather as tools for enhancing the understanding of the mechanisms behind cannabinoid receptor activation, information about their toxicology and other pre-clinical assessments are limited. One of the first clandestine cannabimimetics to be identified in "Spice" products was naphthalen-1-yl(1-pentyl-1*H*-indol-3-yl)methanone, a member of the aminoalkylindole chemical family. This cannabimimetic is probably better known as JWH018 or AM678. Later studies found that this compound produced five oxidized metabolites in mice. One of these metabolites was also examined in vivo and disclosed marked effects on rectal temperature and locomotor activity in mice at magnitudes on par with the parent compound (Brents et al., 2011). A complex metabolism was also described for a related designer cannabimimetic JWH073 [naphthalen-1-yl(1-butylindol-3-yl)methanone] in mice, including a metabolite with putative CB_1R antagonist properties (Brents et al., 2012; see also Vasiljevik et al., 2013). Metabolism of these compounds appears similar in man (Chimalakonda et al., 2011; Moran et al., 2011), and the potpourri of potential active breakdown products may affect the overall pharmacological activity of these cannabimimetics (Seely et al., 2011, 2012), perhaps contributing to more adverse effects compared to marijuana/THC.

Drug discrimination and cannabinoids

As alluded to earlier in this paper, drug discrimination is a technique whereby an organism is trained to recognize the effects of a compound at a given dose and its absence (e.g., vehicle or another drug). When under the influence of the training or reference drug, one particular response is required to achieve reinforcement, may it be food for a food restricted organism or for the subject to be able to postpone an aversive event such as electric shock. A different response is required from the organism when trained under the alternate condition. Along with animal studies, drug discrimination protocols have also been developed for use in humans (Perkins, 2011; Rush et al., 2011). Examples involving humans discriminating between THC and a non-drug condition have been provided by Lile et al. (2009, 2010, 2011). Using laboratory animals, training and testing are mostly conducted using operant chambers equipped with two response manipulanda mounted left and right on a response panel (see Fig. 2). In the early days of drug discrimination, T-shaped maze procedures were commonly used where the animal had to turn left or right at the cross section of the maze depending on the prevailing drug training condition.

Another variation is to use conditioned drinking aversion where the drinking bout is preceded by administration of the training compound and subsequent to the drinking session, an emetic toxin such as lithium chloride (LiCl) is given ("danger" session). Drinking sessions performed under the influence of the alternate training condition is followed by saline, a non-toxic agent ("safe" session). If discrimination is successful, after repeated pairings one will find that fluid intake is low during "danger" sessions and intake is comparatively high during "safe" sessions. Such an approach was used to demonstrate that the effects of the CB_1R inverse agonist/antagonist rimonabant could be used to differentially control behavior (Järbe et al., 2004, 2008, 2011b), in spite of failed attempts to establish rimonabant as a discriminative cue using operant approaches (Mansbach et al., 1996; McMahon, 2006b; Pério et al., 1996). Nonetheless, the operant environment offers great versatility as well as good experimental control and has been by far the most widely used approach in drug discrimination research across drugs and species (Glennon and Young, 2011). Once trained to discriminate the presence/absence of the effects of the reference compound, test sessions are interspersed between the regular maintenance sessions. There are two basic issues that can be addressed in a test session: a) substitution; and b) blocking (antagonism). In substitution testing, we ask if the administration of a new compound is perceived by the subject as eliciting effects similar to the reference drug or not, and the answer is revealed by the organism's choice of responding, i.e., pressing the drug or the non-drug associated lever to achieve reinforcement. If a drug blocks the discriminative stimulus effects of the training drug, responding will occur on the "default" manipulandum that had been associated with reinforcement during discrimination sessions when the training/reference drug was absent.

Modeling cannabinoid withdrawal and discrimination

Mostly, "drug absence" refers to non-drug (vehicle) training. However, in order to model "drug withdrawal", animals have been given THC on a continuous basis and "withdrawal" precipitated by rimonabant, i.e., such discriminations presumably would be based on

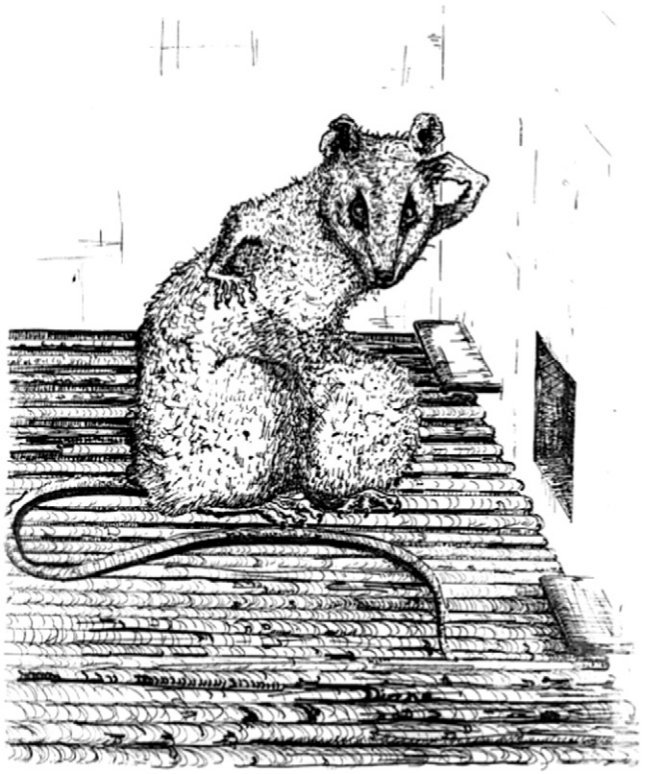

Fig. 2. Illustration of an operant two-lever choice drug discrimination set-up for rats. During training, reinforcement is contingent upon pressing the state-appropriate lever, e.g. when under the influence of the training drug, only presses on the left lever will produce the desired outcome (food, water, etc.) whereas during non-drug training sessions, only pressing the right lever will produce reinforcement. Once rats are reliably discriminating between the two training conditions, test sessions are interspersed between the regular training sessions. Tests examine drug effect similarity (generalization/substitution) or blocking (antagonism); see text for further details.
Drawing courtesy of Diane A. Mathis-Järbe.

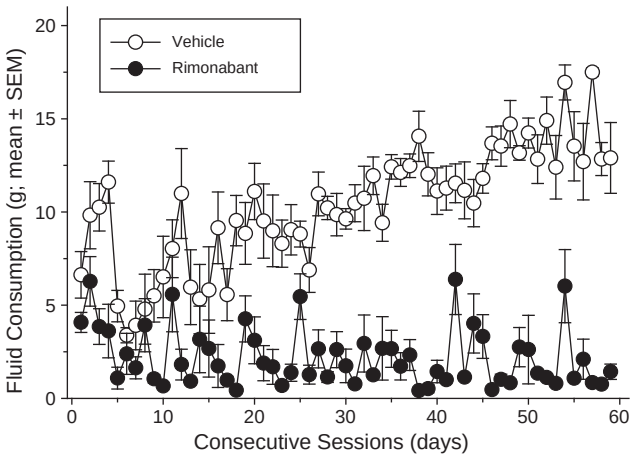

Fig. 3. Fluid consumption (0.1% saccharin flavored tap water) in male Sprague–Dawley rats chronically treated daily with i.p. injected 0.03 mg/kg AM2389. The 30 min drinking sessions were preceded by either vehicle or 3 mg/kg rimonabant given i.p. 20 min prior to initiating the drinking session. Immediately after the drinking session(s), the animals were treated with 120 mg/kg LiCl (10 ml/kg, i.p.) when the rats had been pre-treated with rimonabant and i.p. 10 ml/kg NaCl when the pre-treatment had been vehicle. Controls (not shown) were treated similarly except that NaCl replaced LiCl after rimonabant sessions (unpublished data).

the physiological consequences of displacing the agonist THC from CB_1R binding sites. "Withdrawal" discriminations using operant methodology in rhesus monkeys have been described by McMahon and colleagues (Ginsburg et al., 2012; McMahon, 2006a, b). An alternative approach is based on the previously mentioned drinking aversion protocol in which rats were treated on a daily basis with a potent and functionally long lasting CB_1R agonist (AM2389; [9β-hydroxy-3-(1-hexyl-cyclobut-1-yl)-hexahydrocannabinol]) (Järbe et al., 2012; Nikas et al., 2010) and 17 h later were challenged with rimonabant. The acquisition of this antagonist precipitated "withdrawal" discrimination (presence/absence of rimonabant) is illustrated in Fig. 3. Such presumptive "withdrawal" discriminations may be instrumental for a nuanced experimental analysis of the cannabinoid (THC) withdrawal syndrome as applied to designer cannabimimetics. It is noteworthy that within the drinking aversion approach, the pairing need not necessarily be between the feature drug and the unconditioned stimulus (e.g., LiCl) but rather, conditioning can be established using the alternate training condition (Mastropaolo et al., 1989). This may be useful if there are concerns about potential interactions between the training drug and the emetic agent (Parker et al., 2003).

Marijuana withdrawal in humans consists primarily of internal subjective events including irritability, anxiety, difficulty sleeping etc. with limited overt manifestation(s) unlike e.g., withdrawal from opioids such as heroin (Elkashef et al., 2008; Haney, 2005). Withdrawal symptoms associated with cessation of cannabis/marijuana ingestion in dependent subjects can be alleviated by THC (Marinol®) and the hexahydrocannabinol nabilone (Cesamet®) (Haney et al., 2004, 2013). Case reports of tolerance development, dependence, and withdrawal reactions have been described for "Spice" products in the scientific literature, although it is unclear exactly which synthetic cannabimimetic(s) had been ingested (Gunderson et al., 2012; Nacca et al., 2013; Vardakou et al., 2010). Factors conducive for developing drug tolerance and dependence in general involve fast onset of effect(s), short duration of action, and high efficacy agonism, i.e., full receptor activation. Compounds in Table 1 that seem to fit these general criteria are JWH018/AM678, JWH073 and AM2233 [(2-Iodophenyl)-[1-[(1-methyl-2-piperidinyl)-methyl]-1*H*-indol-3-yl]methanone], all three drugs being aminoalkylindoles with a shorter duration of action compared to THC in rhesus monkeys and rats (Ginsburg et al., 2012; Järbe et al., 2011a). This is indicated in Table 1 by the increasing ED_{50} values for tests with JWH018 and AM2233 at the 90-min as compared to the 30-min post-administration intervals. Ginsburg et al. (2012) examined the time-course of THC (0.1 mg/kg), JWH018 (0.032 mg/kg), and JWH073 (0.1 mg/kg) in rhesus monkeys as a function of elapsed time since the i.v. administration. Extrapolation of the original graph illustrating the decline of THC appropriate responding over time, resulted in the following functional half-life estimates expressed in min (±95% C.L.): THC 213 (207–219); JWH018 100 (86–118); and JWH073 65 (63–66). These estimates are based on non-linear regression using the software package Prism (v. 5; GraphPad Software, San Diego, CA; www.graphpad.com). Thus, the duration of action for the two cannabimimetic indoles were shorter than that of THC in rhesus monkeys. This agrees with the results for JWH018/AM678 in rats (Järbe et al., 1986, 2011a).

CB_1R mediation for these discriminative stimulus effects is indicated by surmountable reversal of the effects by rimonabant in both species. That is, increasing doses of the CB_1R agonists reversed the blockade of a fixed dose of the antagonist in a dose-dependent fashion. Such reversal is an indication that the agonist and the antagonist might exert their effects through a shared recognition site. Similarly, THC (2 mg/kg 24 h s.c.) dependent rhesus monkeys discriminating the effects of the presence/absence of i.v. 1 mg/kg rimonabant ("withdrawal") suggested that increasing doses of THC, JWH018 and JWH073 attenuated the discriminative stimulus effects of rimonabant (Ginsburg et al., 2012), again suggesting CB_1R mediation. Still, the question of whether or not tolerance and dependence would be more pronounced when established with a full efficacy agonist such as JWH018/AM678 remains to be ascertained as "withdrawal" studies to date in laboratory animals have relied on the partial CB_1R agonist THC as the reference compound in the drug discrimination model of cannabimimetic activity. This may be important in view of a case report indicating that withdrawal

Table 1
Drug discrimination of products found in synthetic "designer" marijuana.

Species	Training drug	Dose	Route/time	Training ED50 (±95% C.L.)	Test drug	route/time	Test ED50 (±95% C.L.)	Cited by
Rat	THC	3.2	IP/60	0.68 (0.38–1.2)	CP-47,497	IP/60	0.1 (0.06–0.15)	Weissman et al. (1982)
Rat	THC	3.0	IP/30	0.66 (nd)	HU-210	IP/90	0.017 (nd)	Järbe et al. (1989)
					HU-210	IP/270	0.01 (nd)	
					HU-210	IP/390	0.01 (nd)	
					THC	IP/90	1.78 (nd)	
Pigeon	THC	0.56	IM/90	0.16 (nd)	HU-210	IM/90	0.004 (nd)	
					HU-210	IM/270	0.002 (nd)	
					HU-210	IM/540	0.002 (nd)	
					THC	IM/30	0.53 (nd)	
					THC	IM/270	0.25 (nd)	
					THC	IM/540	1.21 (nd)	
Rat	CP-55,940	0.1	IP/30	0.02 (nd)	JWH-015	IP/30	15 (nd)	Wiley et al. (1998)
					JWH-007	IP/30	<1 (nd)	
Rat	THC	3.0	IP/30	0.6 (nd)	JWH-030	IP/30	4.7 (nd)	
Rat	BAY-38,7271	0.05	IP/30	0.018 (0.012–0.026)	HU-210	IP/120	0.003 (0.001–0.010)	De Vry and Jentzch (2002)
					THC	IP/30	0.34 (0.11–1.06)	
Rat	BAY-59,3074	0.5	PO/60	0.081 (0.047–0.142)	HU-210	IP/120	0.022 (0.015–0.034)	De Vry and Jentzch (2004)
					THC	IP/30	0.41 (0.26–0.66)	
Rat	mAEA	10	IP/20	2.907 (2.551–3.300)	JWH-018	IP/30	0.051 (0.045–0.056)	Järbe et al. (2010)
					JWH-018 + R (1.0)	IP/30	0.223 (0.177–0.282)	
Rat	THC	1.8	IP/20	0.497 (0.213–1.353)	JWH-018	IP/30	0.044 (0.037–0.050)	
					JWH-018 + R (1.0)	IP/30	0.570 (0.233–0.910)	
Rat	THC	3.0	IP/20 or 30	1.12 (0.96–1.31)	JWH-018	IP/30	0.14 (0.12–0.15)	Järbe et al. (2011a)
					JWH-018	IP/90	0.39 (0.17–0.93)	
					JWH-018 + R (1.0)	IP/30	1.05 (0.53–2.07)	
					JWH-018 + R (1.0)	IP/90	1.32 (0.66–2.64)	
					AM-2233	IP/30	0.49 (0.32–0.76)	
					AM-2233	IP/90	2.54 (1.40–4.58)	
Monkey	THC	0.1	IV/5	0.044 (0.032–0.061)	JWH-018	IV/5	0.013 (0.0091–0.019)	Ginsburg et al. (2012)
					JWH-073	IV/5	0.058 (0.036–0.094)	
					JWH-018 + R (0.32)	IV/5	0.07 (0.044–0.11)	
					JWH-018 + R (1.0)	IV/5	0.15 (0.11–0.2)	
					JWH-018 + R (3.2)	IV/5	0.44 (0.32–0.6)	
					JWH-073 + R (1)	IV/5	0.89 (0.57–1.4)	
					THC + R (0.32)	IV/5	0.24 (0.16–0.36)	
					THC + R (1.0)	IV/5	0.43 (0.31–0.59)	
					THC + R (3.2)	IV/5	1.3 (0.98–1.8)	

All doses are in mg/kg.
All times are in min.
mAEA = methanandamide.
R = rimonabant.
nd = not determined.
IP = intraperitoneal.
IM = intramuscular.
IV = intravenous.
PO = per os (intragastric).
JWH018 = AM678.

symptoms resulting from cessation of using a "Spice" blend were not alleviated by smoking marijuana (Nacca et al., 2013).

"Spice" compounds and discrimination

The bicyclic cannabimimetic CP47,497 [2-[(1S,3S)-3-hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol] was created by the drug company Pfizer as part of their efforts to develop new, non-opioid analgesics; Table 1 (Weissman et al., 1982). At the time, i.e., during the mid- to late 1970s, it was hoped that it would be possible to separate the analgesic from other cannabinoid induced effects. A study by Wilson et al. (1976) had suggested the potential for such a separation. However, this did not materialize and eventually Pfizer retreated from this early cannabinoid medication research program. The 7-fold potency difference in the discriminative stimulus effects of CP47,497 over THC in rats (see Table 1) is in reasonable accord with a recent study using mice discriminating between the presence/absence of 5.6 mg/kg THC; separate ED_{50} estimates for the drugs were not provided in the abstract. CP47,497 exhibited a similar potency in the tetrad test battery and these effects were blocked by 10 mg/kg rimonabant; CP47,497 was behaviorally ineffective in CB_1R knock-out mice, thus further implicating CB_1R mediation (Samano et al., 2013).

A compound found in early "Spice" products was HU-210, the 11-hydroxylated version of dimethylheptyl-Δ^8-THC. HU-210 is one of the most potent cannabimimetics discovered to date, exhibiting approximately 73 (rats) and 87 (pigeons) times the potency of THC (Järbe et al., 1989). This is congruent with the data obtained in rats discriminating between vehicle and the purported full, high-efficacy CB_1R agonist BAY 38-7271 [(−)-(R)-3-(2-hydroxymethylindanyl-4-oxy) phenyl-4,4,4-trifluorobutyl-1-sulfonate] (De Vry and Jentzsch, 2002). However, the potency difference between THC and HU-210 was only 20-fold in rats discriminating between vehicle and the partial CB_1R agonist BAY 59-3074 [3-[2-cyano-3-(trifluoromethyl)phenoxy]phenyl-4,4,4-trifluoro-1-butanesulfonic acid ester] (De Vry and Jentzsch, 2004); see Table 1. Note though that the routes of administration differed between these two cannabinergics in the latter study (De Vry and Jentzsch, 2004), making direct comparisons difficult. One important variable when evaluating HU-210 and related compounds is the time since administration until testing as these compounds have a very slow onset and a very long duration of action (Devane et al., 1992a;

Järbe et al., 1981, 1989, 2012). "Spice" preparations are mostly inhaled by smoking and HU-210 has not been examined in laboratory animals by that route of administration. However, if onset is relatively slow also after smoking, people may be fooled into thinking that they need to ingest more and thus could over-dose when the effects kick in.

Rats trained to discriminate the presence and absence of hashish-smoke will substitute for THC and vice versa (Järbe and Henriksson, 1974; Järbe et al., 1976). Similar findings have been obtained in mice (Vann and Walentiny, 2011). Mice have also been exposed to JWH018/AM678 by inhalation and levels of the drug measured in brain and other tissues after examining the mice in the tetrad test battery (Wiebelhaus et al., 2012). Thus, this cannabimimetic was biologically effective also by inhalation, the more common route of administration of herbal blends by human users.

As noted above, most drug discrimination studies have used the partial agonist THC as the reference. However, the designer cannabimimetics JWH007 = [(2-methyl-1-pentyl-1*H*-indol-3-yl)-1-naphthalenylmethanone] and JWH015 = [(2-methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenylmethanone] have been examined in rats discriminating between the presence and absence of the full CB_1R agonist CP55,940 and found to substitute, albeit with potencies lower than the training drug; Table 1 (Wiley et al., 1998).

Other more recently examined "herbal" cannabimimetics are JWH203 [1-pentyl-3-(2-chlorophenylacetyl)indole], JWH250 [1-pentyl-3-(2-methoxyphenylacetyl)indole] and AM2201 [N-(5-fluoropentyl)-3-(1-naphthoyl)indole] which were evaluated in rats discriminating between 3 mg/kg THC and vehicle. These three indoles "fully substituted for the discriminative stimulus effects of THC at doses that did not alter the rate of responding"; the abstract did not provide any potency estimates for the drugs (Gatch and Forster, 2013). So far all tested designer cannabimimetics have substituted for the cannabinergics used in training and thus add support for the validity of drug discrimination as a marker of the marijuana/THC-like "high".

Concluding remarks

This brief historical overview with an emphasis on in vivo bioassays useful for identifying drugs exhibiting a marijuana/THC-like "high" drug profile is intended as a background for the current situation where clandestine laboratories supply synthetic cannabinoids never tested in man for recreational use. Two currently commonly employed behavioral assays for identifying cannabimimetics are the tetrad and drug discrimination. Drug discrimination is more pharmacologically specific than the tetrad, but also more time consuming. However, employed in tandem, the two approaches ought to be mutually beneficial for characterizing cannabinoids. It is noteworthy that the drug companies Pfizer in the USA (Browne and Weissman, 1981; Weissman, 1978) and Janssen Pharmaceutica in Belgium (Colpaert, 2003, 2011) early on embraced drug discrimination as an important in vivo tool in the drug discovery process. Health concerns about these synthetic cannabinoids have only increased over the last years. Reports of kidney failure and seizures highlight the potential dangers of these compounds, especially as the exact contents of "Spice" blends are rarely known. While these "Spice" blends were originally created as a "legal" alternative that would not show up on drug tests for marijuana users, the original compounds found in "Spice" products were made illegal and analytical tests are being developed to detect additional synthetic cannabinergics in forthcoming herbal blends. A pressing issue is to determine in what way(s) the pharmacology of clandestine cannabimimetics may differ from that of marijuana/THC.

Conflict of interest statement

All authors declare that there is no actual or potential conflict of interest related to this manuscript.

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