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Δ^9 -Tetrahydrocannabinol acts as a partial agonist/antagonist in mice

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Abstract

Δ^9 -Tetrahydrocannabinol (THC) has been characterized as a partial agonist at cannabinoid CB1 receptors *in vitro*; however, it often produces the same maximum effects *in vivo* as other cannabinoid agonists. This study was carried out to determine whether THC would antagonize the hypothermic effects of another cannabinoid agonist, AM2389, in mice. Male mice were injected with 1–100 mg/kg THC, 0.01–0.1 mg/kg AM2389, or a combination of 30 mg/kg THC and 0.1–1.0 mg/kg AM2389, and rectal temperature was recorded for up to 12 h after injection. THC reduced the temperature by 5.6°C at a dose of 30 mg/kg; further increases in the dose did not produce larger effects, indicating a plateau in the THC dose–effect function. AM2389 reduced temperature by 9.0°C at a dose of 0.1 mg/kg. One hour pretreatment with 30 mg/kg THC attenuated the hypothermic effects of 0.1 mg/kg AM2389; a 10-fold higher dose, 1.0 mg/kg AM2389, was required to further decrease temperature, reflecting a five-fold rightward shift of the lower portion of the AM2389 dose–effect function following THC pretreatment. These results indicate that, in an assay of mouse hypothermia, THC exerts both agonist and antagonist effects following acute administration, and mark the first demonstration of partial agonist/antagonist effects of THC *in vivo*.

Keywords

AM2389; efficacy; mouse; partial agonist; Δ^9 -tetrahydrocannabinol

Introduction

Δ^9 -Tetrahydrocannabinol (THC) is the major psychoactive component of cannabis and is often the prototypic drug used in studies of cannabinoid pharmacology. THC binds with equal affinity to CB1 and CB2 cannabinoid receptors, with K_i values ranging from 11 to 300 nmol/l across different studies (Felder *et al.*, 1995; Tao and Abood, 1998; Govaerts *et al.*, 2004) and, on the basis of effects *in vitro*, has been characterized as a partial agonist at both cannabinoid receptor types. For example, in cerebellar membranes, THC produces only 20–50% of the effects of WIN55,212-2, CP55,940, and methanandamide for stimulating [³⁵S]GTP γ S binding or inhibiting adenylyl cyclase activity (Sim *et al.*, 1996; Breivogel and Childers, 2000; Govaerts *et al.*, 2004). Similarly, THC only partially inhibits glutamatergic

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Conflicts of interest

There are no conflicts of interest.

firing in cultured hippocampal neurons, compared with the effects observed with WIN55,212-2 (Shen and Thayer, 1999). Important to its designation as a partial agonist, THC also serves as a partial antagonist *in vitro*, able to attenuate the effects of WIN55,212-2, CP55,940, and the endocannabinoid 2-arachidonylglycerol (2-AG) on [³⁵S] GTPγS binding or synaptic transmission (Sim *et al.*, 1996; Petit *et al.*, 1998; Shen and Thayer, 1999; Kelley and Thayer, 2004). Notwithstanding the characterization of THC as a low-efficacy agonist *in vitro*, THC generally produces effects of the same magnitude as other CB1 agonists *in vivo* (e.g. see Fan *et al.*, 1994) and thus, the characterization of THC as a partial agonist has been difficult to corroborate in whole animals.

AM2389 is a recently developed cannabinoid agonist that is relatively selective for CB1 receptors. In HEK293 cells expressing CB1 receptors, AM2389 is a full agonist, inhibiting forskolin-stimulated cAMP accumulation with an EC₅₀ of 1.5 ± 0.3 nmol/l (Nikas *et al.*, 2010). In whole animals, AM2389 is a potent, long-lasting cannabinoid, capable of producing antinociception similar to morphine in rats and hypothermic effects of at least 10°C in mice (Nikas *et al.*, 2010; Järbe *et al.*, 2012). In contrast, high doses of THC typically decrease temperature in mice by 6°C or less (Wiley and Martin, 2003), suggesting that its agonist effects might be limited in this assay. The aim of the present study was to determine whether THC will antagonize the hypothermic effects of high doses of AM2389, thus providing evidence of partial agonist/partial antagonist effects of THC *in vivo*.

Methods

Male CD-1 mice, 25–30 g (Charles River Laboratories, Wilmington, Massachusetts, USA), were group-housed in a climate-controlled vivarium with free access to food and water. Mice were handled and acclimatized to experimental procedures before drug effect determinations. Experiments were conducted during the light portion of a 12-h light/dark cycle. Δ⁹-THC was obtained from the National Institute on Drug Abuse (NIDA, Rockville, Maryland, USA) and AM2389 was synthesized as described previously (Nikas *et al.*, 2010). Drugs were dissolved in 1 : 1 : 18 solutions of ethanol, alkamuls-620 (Rhone-Poulenc, Princeton, New Jersey, USA), and saline. Injections were administered subcutaneously in volumes of 1 ml/100 g body weight; drug doses are expressed in terms of the weight of the free base. Mice were maintained in accordance with the NIH Guide for Care and Use of Laboratory Animals (8th ed., 2011), and the research protocol was approved by the Institutional Animal Care and Use Committee.

Colonic temperature was measured by inserting thermal probes (YSI, No. 402; Yellow Springs Instruments Inc., Yellow Springs, Ohio, USA) rectally 2 cm for at least 30 s and until readings stabilized. Temperature was recorded before drug injection (baseline) and at specified times for 8–12 h after injection. Peak drug effects were taken as the maximum change in temperature at any time after injection and were used to construct dose–effect functions for THC and AM2389, from which equivalent doses were calculated by linear regression. Group means and SEMs were calculated for each treatment condition and statistical analysis was carried out using GraphPad Prism 5.03 (GraphPad Software, San Diego, California, USA). Peak drug effects were compared using one-way analysis of variance procedures with *P* set at 0.05, followed by Dunnett's or Tukey's multiple comparison tests.

Results

Both THC and AM2389 decreased temperature, although the time course and peak effects observed differed between drugs. THC, at doses of at least 10 mg/kg, significantly reduced temperature by 2.7–5.6°C. The greatest effects occurred following 30 mg/kg THC and,

across all doses, peak effects typically occurred within 1–2 h of injection (Fig. 1a). There was no difference between the peak effects of 30 and 100 mg/kg THC, indicative of a plateau in the dose–effect function at these doses (Fig. 1b). In contrast, AM2389 produced greater effects than THC, decreasing temperature by as much as 9°C when administered alone, and the peak effects emerged slowly, most often recorded 4 h after injection (Fig. 2a).

In antagonism studies, the dose of 30 mg/kg THC was administered 1 h before injections of 0.1 and 1.0 mg/kg AM2389. THC pretreatment decreased temperature by 3.6–4.2°C within 1 h and the addition of 0.1 mg/kg AM2389 did not further reduce temperature (Fig. 2b). The higher dose of 1.0 mg/kg AM2389 could surmount the effects of 30 mg/kg THC, decreasing temperatures by an additional 6.5°C. The antagonist effects of THC can be seen in the five-fold rightward shift in the bottom part of the AM2389 dose–effect function (Fig. 2b). The dose of AM2389 required to produce an 8°C decrease in temperature increased from 0.07 mg/kg (95% confidence interval: 0.06–0.09) under control conditions to 0.34 mg/kg (95% confidence interval: 0.27–0.43) following pretreatment with THC.

Discussion

These results show that THC antagonizes the hypothermic effects of a higher-efficacy cannabinoid agonist when both drugs are administered at sufficiently high doses. As reported previously, the maximum hypothermic effects of THC in mice were obtained at doses of 10–30 mg/kg and higher doses form a plateau in the dose–effect function (Wiley and Martin, 2003; McMahon and Koek, 2007). A plateau in a dose–effect function may indicate either that a physiological limit has been reached, such that greater effects are not possible, or that the drug has saturated the receptors and further increases in dose will not result in greater receptor occupancy (Ariëns *et al.*, 1960). Results with AM2389 confirm that further decreases in temperature can be achieved with other cannabinergic drugs; thus, the plateau in the THC dose–effect function indicates that the maximum hypothermic effects of THC are less than those produced by other drugs, providing in-vivo evidence for partial agonist actions of THC. Having identified saturating doses of THC, we next established that pretreatment with 30 mg/kg THC shifts the dose–effect function of a higher-efficacy agonist to the right. These results provide the first in-vivo evidence that THC can serve as an antagonist in the same assay (i.e. mouse hypothermia) in which it also acts as an agonist. Together, and in accordance with its characterization *in vitro*, our results show that THC can be considered a partial cannabinoid agonist *in vivo*.

Similar procedures have been used to characterize drugs that work in other receptor systems as partial agonists *in vivo*. For example, the opioids nalorphine and buprenorphine produce partial to full effects typical of μ -opioid agonists, and yet they also antagonize the effects of morphine, heroin, or other μ -opioid agonists (Holtzman, 1983; Walker *et al.*, 1995; Paronis and Bergman, 2011). Other studies have reported a plateau in the dose–effect function of the dopamine D1 partial agonist SKF83959 and shown that these saturating doses of SKF83959 will antagonize the effects of the full D1 agonist, R-(+)-6-Br-APB (Jutkiewicz and Bergman, 2004). Within the cannabinoid receptor system, our findings corroborate in-vitro data obtained with WIN55,212-2 and THC for stimulating [³⁵S]GTP γ S binding or inhibiting cell firing (Sim *et al.*, 1996; Shen and Thayer, 1999). Those studies also established that the dose–effect functions of THC plateau at levels below the maximum effects obtained with WIN55,212-2, and that THC antagonizes the effects of WIN55,212-2.

The designation of a drug as a full or a partial agonist is always related to the effects of other drugs in that pharmacological class on the variable being measured. Thus, although we find that THC is a partial agonist in producing hypothermia in mice, it must still be considered a full agonist under conditions in which it produces the maximum possible effect, including

antinociception, decreased locomotor activity, and THC discrimination (Compton *et al.*, 1992; Fan *et al.*, 1994; McMahon and Koek, 2007; Ginsburg *et al.*, 2012). Some studies have used the strategy of decreasing the number of available receptors to rank the relative efficacy of opioid drugs that have full agonist effects *in vivo* (Adams *et al.*, 1990; Paronis and Holtzman, 1992). A similar approach has been used to define THC as a partial agonist indirectly, insofar as it shows greater tolerance than other cannabinoid agonists *in vivo* (Hrubá *et al.*, 2012). Our results extend these findings by indicating that acute administration of THC has partial agonist and antagonist effects in otherwise drug-naïve animals. Insofar as the apparent partial or full agonist effects of drugs reflect their intrinsic properties, it seems likely that THC *in vivo* has lower efficacy than AM2389 and, as has been shown *in vitro*, other cannabinoid agonists.

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Fig. 1. Effects of THC on body temperature in mice, $n = 5-8$. (a) Effects of individual doses of THC at different times after injection. Vertical axis: colonic temperature (°C); horizontal axis: time since injection (h); points above BL represent baseline determinations obtained immediately before drug injection. (b) Peak effects of each dose of THC; point above SAL represent peak decreases in colonic temperature after saline. Vertical axis: change in colonic temperature from baseline (°C); horizontal axis: dose of THC (mg/kg body weight). *Doses of THC that had effects significantly different from saline; $P < 0.01$. BL, baseline; SAL, saline; THC, Δ^9 -tetrahydrocannabinol.

Fig. 2.

Effects of AM2389 on body temperature in mice, $n = 7-8$. (a) Effects of individual doses of AM2389 alone at different times after injection. (b) Peak effects of each dose of AM2389 administered either alone or after 30 mg/kg THC; points above SAL and THC represent decreases in colonic temperature at 1 h after saline or 30 mg/kg THC, respectively. * $P < 0.01$ relative to 0.1 mg/kg AM2389 alone; # $P < 0.01$ relative to 30 mg/kg THC alone. Other details as in Fig. 1. BL, baseline; SAL, saline; THC, Δ^9 -tetrahydrocannabinol.