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Effect of chronic exposure to rimonabant and phytocannabinoids on anxiety-like behavior and saccharin palatability

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ABSTRACT

The acute effects of cannabinoid compounds have been investigated in animal models of anxiety-like behavior and palatability processing. However, the chronic effects of cannabinoids in such models are poorly understood. Experiment 1 compared the effects of both acute and chronic (14 days) exposure to the CB_1 receptor inverse agonist/antagonist, rimonabant, and the cannabis-derived CB_1 receptor neutral antagonist, tetrahydrocannabivarin (THCV), on: 1) time spent in the open, lit box in the Light–Dark (LD) immersion model of anxiety-like behavior and 2) saccharin hedonic reactions in the taste reactivity (TR) test of palatability processing. Experiment 2 compared the effects of chronic administration of cannabis-derived Δ^9-tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD) and cannabigerol (CBG) in these models. Tests were administered on Days 1, 7 and 14 of drug administration. In Experiment 1, rimonabant, but not THCV, produced an anxiogenic-like reaction in the LD immersion test and reduced saccharin palatability in the TR test; both of these effects occurred acutely and were not enhanced by chronic exposure. In Experiment 2, Δ^9-THC also produced an acute anxiogenic-like reaction in the LD immersion test, without enhancement by chronic exposure. However, Δ^9-THC enhanced saccharin palatability in the TR test on Day 1 of drug exposure only. CBD and CBG did not modify anxiety-like responding, but CBG produced a weak enhancement of saccharin palatability on Day 1 only. The results suggest that the anxiogenic-like reactions and the suppression of hedonic responding produced by rimonabant, are mediated by inverse agonism of the CB_1 receptor and these effects are not enhanced with chronic exposure.

1. Introduction

The endogenous cannabinoid system has been shown to mediate regulation of both feeding and emotional behavior (eg see Janero and Makriyannis, 2009; Lutz 2009). The CB_1 inverse agonist/antagonist, rimonabant, was developed and marketed as an anti-obesity drug before its withdrawal from the European markets due to the side effects of anxiety, depression and suicidal thoughts following long-term treatment (Janero and Makriyannis, 2009). Unfortunately, prior to the development of rimonabant, preclinical models of anxiety-like and depression-like responding yielded equivocal results; some reports indicated that rimonabant was anxiogenic (Arevalo et al., 2001; Navarro et al., 1997; Patel and Hillard, 2006), but others suggested that it was anti-depressant or anxiolytic (Griebel et al., 2005; Whitkin et al., 2005). However, all of these early studies evaluated acute effects of rimonabant. More recently, Beyer et al. (2010) reported that when chronically administered, rimonabant (10 mg/kg, but not 3 mg/kg) produced a depressant-like effect in the forced swim test and in a sucrose preference test following 21 days of administration.

Recent work with both the plant-derived tetrahydrocannabivarin (THCV) and synthetic THCV (0–4394) indicates that it acts as a CB_1 receptor antagonist in vitro (Thomas et al., 2005) and in-vivo at doses lower than 3 mg/kg, intraventricular (Pertwee, et al. 2007). In contrast to rimonabant, however, THCV (0-4394) does not produce inverse agonist activity in the [^{35}S] GTPγS bindng assay in mouse whole brain membrane and fails to produce stimulation of [^{35}S] GTPγS binding to such membranes (Thomas et al., 2005). Indeed, THCV shares the ability of the CB_1 inverse agonist/antagonist, AM251, to reduce the food intake and body weight of non-fasted and fasted mice but at lower doses (3 mg/kg) than AM251 (Riedel et al., 2009). If THCV is devoid of the inverse agonist properties of rimonabant, which are responsible for nausea (McLaughlin et al., 2005; Sink et al., 2008) and potentially anxiety and depression (Beyer et al., 2010; Sink et al., 2010a, 2010b), then THCV may be a potential candidate for therapeutic use. Indeed, in addition to reduced body weight (Riedel et al., 2009), THCV has been reported to suppress seizure activity (Hill et al., 2010), reduce inflammation and inflammatory pain (Bolognin et al., 2010), and even reduce Parkinson's disease symptoms, as well as disease progression (Garcia et al., 2011). However, the effect of chronic exposure to THCV has not been evaluated in animal models of anxiety.

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Not only has there been limited evaluation of the chronic effects of rimonabant and THCV in the preclinical models of anxiety and depression, but also there has been little study of the chronic effects of other phytocannabinoids in these models. Although there are over 60 compounds found in the cannabis plant, the only psychoactive compound found is Δ^9-tetrahydrocannabinol (Δ^9-THC), largely due to its agonism of the CB_1 receptor. When acutely administered, low doses of Δ^9-THC (<1 mg/kg) produce an anxiolytic effect, but higher doses (>1 mg/kg) are anxiogenic (Viveros et al., 2005; Patel and Hillard, 2006); this biphasic effect is characteristic of cannabinoids including the endogenous anandamide (Sulcova et al., 1988). When acutely administered the phytocannabinoid, cannabidiol (CBD), has also been reported to produce an anxiolytic effect at low doses (2.5–10 mg/kg), an effect that is mediated by the agonism of the 5-HT_{1A} receptors (Moreira et al., 2007; Campos and Guimaraes, 2008; Resstel et al., 2009; Zanelati et al., 2010; Gomes et al., 2011). Interestingly another compound found in cannabis, cannabigerol (CBG), has been reported to act as a 5-HT_{1A} antagonist in both in-vitro (Cascio et al., 2010) and in-vivo models (Rock et al., 2011). The actions of CBD and CBG on the 5-HT_{1A} receptor suggest that they may provide insights into the effects of cannabis on anxiety (e.g., Blier and de Montigny, 1994; De Vry, 1995). Most of the work on the effects of Δ^9-THC, CBD or CBG on emotional behavior has been conducted with the procedure of acute administration. In the single experiment that has evaluated chronic CBD in the rat models of anxiety-like behavior, ElBatsh et al. (2012) reported that daily administration of CBD (10 mg/kg) for 14 days resulted in enhanced conditioned freezing to a shock paired context. This finding contrasts with previous results demonstrating that an acute injection of 10 mg/kg of CBD reduced freezing time elicited by a shock paired context (Resstel et al., 2006). In contrast in mice, Long et al. (2010) demonstrated that daily administration of CBD for 21 days to male adult C57BL/6JArc mice produced an anxiolytic effect in the Light–dark immersion test of anxiety-like behavior at a low dose (1 mg/kg).

The Light–dark (LD) immersion test is a widely used rodent model of anxiogenic behavior and generalized anxiety that is relatively independent of locomotor activity (Bourin and Hascoët, 2003; File, 1985; Holmes, 2001) and is used to assess drug-induced changes in anxiety (Costall et al., 1989). This test relies upon the natural tendency of rodents to explore novel environments and may provide a less stressful baseline of responding relative to the more commonly used elevated plus maze test. Therefore, it may be more sensitive to both increases as well as decreases in anxiety (Bourin and Hascoët, 2003). High levels of time spent in the open area are interpreted as low-levels of anxiety-like behavior, whereas avoidance of the open field is associated with increased anxiety-like behavior (Holmes, 2001). Experiment 1 evaluated the effects of acute and chronic exposure to the CB_1 inverse agonist/antagonist, rimonabant, and the CB_1 neutral antagonist, THCV, in the LD immersion test of anxiety-like behavior. Experiment 2 evaluated the effects of acute and chronic exposure to Δ^9-THC, CBD and CBG in this test.

As well as modifying emotional responding, acute administration of CB_1 agonists and antagonists/inverse agonists modify palatability processing (eg. Higgs et al., 2003; Koch, 2001; Jarrett et al., 2005, 2007), which may serve as a model to evaluate hedonic/anhedonic effects of chronic exposure to these compounds. The taste reactivity (TR) test was designed to directly assess palatability in rats (Grill and Norgren 1978). During an intraoral infusion of sweet sucrose or saccharin solution, rats display the hedonic orofacial reactions of tongue protrusions and mouth movements. As well as sucrose consumption (Arnone et al., 1997; Koch, 2001; Higgs et al., 2003), these reactions are enhanced by pretreatment with Δ^9-THC (Jarrett et al., 2005) or by anandamide directly delivered to the shell of the nucleus accumbens (Mahler et al., 2007). These hedonic reactions are also suppressed by pretreatment with the CB_1 inverse agonist/antagonist AM251 (Jarrett et al., 2007; Limebeer et al., 2010). Interestingly, the CB_1 receptor neutral antagonists, AM6545 and AM6527, do not modify saccharin palatability in the TR test, unlike AM251. However, the effect of chronic exposure to cannabinoid compounds has not been evaluated in the TR test. Therefore, as well as evaluating the effect of chronic exposure to rimonabant and plant-derived cannabinoids on anxiety-like responding, the experiments below also evaluate their effect on saccharin palatability processing.

2. Materials and methods

2.1. Animals

All procedures were approved by the Animal Care Committee of the University of Guelph and adhered to the guidelines of the Canadian Council of Animal Care. The male Sprague Dawley rats weighing 225–265 g at the beginning of the procedure were maintained on ad libitum food (Highland Rat Chow [8640]) and water throughout the experimental procedure. They were individually housed in Plexiglas cages (48×26×20 cm) in the colony room at an ambient temperature of 21 °C with a 12/12 reverse light/dark schedule (lights off at 7 am). Rats were provided with two clean paper towels (replenished during weekly cage changes) and a soft plastic container that was 14 cm long and 12 cm in diameter that remained in the home cage. All experimental manipulations occurred during the cycle dark phase.

2.2. Drugs

All injections were intraperitoneal (ip). All drugs were prepared in the VEH of 1:1:18 formulation of EtOH, Cremophor, and saline; ethanol was evaporated under vacuum and the residue re-suspended in saline. The drugs evaluated included SR141716 (2.5 mg/kg; rimonabant; Sequoia Laboratories, UK), THCV (2.5 mg/kg; GW Pharmaceuticals), CBD (2.5 mg/kg, provided by R. Mechoulam) and CBG (2.5 mg/kg, provided by GW Pharmaceuticals). All drugs were administered in a volume of 1 ml/kg.

2.3. Intraoral cannulation surgery

As described by Limebeer et al. (2010), all rats were implanted with intraoral cannula for delivery of the 0.1% saccharin solution directly to the oral cavity in the TR test. On the day of surgery, the rats were injected with an antibiotic (Derapin: 100 mg/kg, sc; Ayerst) 30 min prior to being anesthetized with isoflurane (4–5% induction, 1.5% maintenance in 0_2). Once anesthetized a 2 cm^2 section of skin was shaved at the back of the neck at the level of the scapula. The skin was prepared by cleaning with soap (Bactistat; Ecolab, St. Paul, MN, USA) and wiping with 70% isopropyl alcohol followed by 7% Betadine solution (Purdue Products L.P., Stamford, CT, USA). The rat was then administered with a 5 mg/kg injection (i.p.) of the anti-inflammatory/analgesic drug carprofen (Rimadyl; Pfizer Canada Inc., Kirkland, QC, Canada). A thin-walled 15 G stainless steel needle was inserted at the shaved area on the neck, directed subcutaneously around the ear and brought out behind the first molar inside the mouth. A 10 cm length of Intra Medic PE90 tubing (Clay Adams Brand; Becton Dickinson and Co., Sparks, MD, USA) with an inner diameter (I.D.) of 0.86 mm and an outer diameter (O.D.) of 1.27 mm was then inserted through the needle after which the needle was removed. Betadine (10%) was applied to the puncture site and two elastic disks (2 cm^2) were placed over the exposed end of the tubing and drawn to the skin at the back of the neck for the purpose of stabilizing the cannula. The cannula was held secure in the oral cavity by a 6 mm disk of polypropylene mesh (297 μm; Small Parts Inc., Miramar, FL, USA) secured behind the heat flanged intraoral opening. Each cannula was constructed prior to surgery and kept in a cold sterilant solution (Germiphene Corporation, Brantfor, ON, Canada). Rats were returned to their home cage immediately following surgery and food pellets were loosely provided in the cage. For 3 days following surgery