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The CB1 inverse agonist AM251, but not the CB1 antagonist AM4113, enhances retention of contextual fear conditioning in rats

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ABSTRACT

The effects of CB1 antagonist/inverse agonists on the acquisition and consolidation of conditioned fear remain uncertain. Recent studies suggest that the CB1 antagonist/inverse agonist AM251 affects acquisition or consolidation of both contextual and discretely cued fear memories. AM251 is frequently referred to as a CB1 antagonist; however *in vitro* signal transduction assays indicate that this drug also elicits inverse agonist activity at CB1 receptors. The present studies were undertaken to compare the effects of AM251 on conditioned fear with those produced by AM4113, a novel CB1 antagonist with minimal inverse agonist activity. All drugs were administered prior to conditioning. In retention tests conducted two weeks after conditioning, both AM251 (4.0 mg/kg) and AM4113 (6.0 mg/kg)-treated animals exhibited reduced freezing during a conditioned tone cue played within a novel context. In contextual fear retention tests, animals previously treated with 4.0 or 8.0 mg/kg AM251 exhibited enhanced freezing. By contrast, no dose of AM4113 had any significant effect on contextual fear memory, which is consistent with the lower signal transduction activity of AM4113 at CB1 receptors compared to AM251. These results suggest that CB1 neutral antagonists may be less likely than CB1 inverse agonists to facilitate the acquisition or consolidation of contextual fear that may contribute to some clinical disorders.

1. Introduction

Drugs that interfere with cannabinoid CB1 transmission have been studied as potential treatments for obesity as well as other disorders. CB1 inverse agonists such as SR141716 (rimonabant) and AM251 have been shown to reduce food intake under a variety of conditions in animal models (Arnone et al. 1997; Colombo et al. 1998; Williams and Kirkham 1999; Wiley et al. 2005; McLaughlin et al. 2003, 2005; Gardner and Mallet 2006; Salamone et al. 2007). Clinical trials with the CB1 inverse agonists rimonabant and taranabant demonstrated that these drugs were effective at reducing body weight in humans (Curioni and Andre, 2006; Despres et al., 2005; Pi-Sunyer et al., 2006; Van Gaal et al., 2005; Addy et al., 2008). However, the high incidence of adverse emotional effects observed in clinical trials with these drugs has caused researchers to question their clinical usefulness (Le Foll et al. 2009). For example, CB1 inverse agonists have been shown to increase the incidence of nausea, anxiety and depression (Pi-Sunyer et al., 2006; Van Gaal et al., 2005; US Food and Drug Administration Advisory Committee, 2007; Addy et al., 2008). In view of these problems with CB1 inverse agonists, recent studies have begun to focus on the effects of CB1 receptor neutral antagonists such as AM4113, which is a pyrazole-3-carboxamide analog of rimonabant. (Salamone et al. 2007; Le Foll et al. 2009). In contrast to the inverse agonists AM251 and rimonabant, AM4113 demonstrated no significant inverse agonism at CB1 receptors as assessed with *in vitro* cAMP accumulation assays (Chambers et al. 2007; Sink et al. 2008a). Although AM4113 is able to suppress food intake and food-reinforced behavior, it does not induce nausea or malaise at comparable doses (Chambers et al. 2007; Sink et al. 2008a,b; Sink et al. 2009a). Furthermore, we recently found that, unlike AM251, AM4113 did not appear to evoke a pattern of anxiety-like behaviors in the elevated plus maze, a rodent anxiety test (Sink et al. 2010). Also, a study of c-Fos immunoreactivity showed that AM4113 induced less neural activation than AM251 in a number of brain structures, including the amygdala, a structure that is important for anxiety as well as fear conditioning (Sink et al. 2010).

In addition to studies of anxiety, it is also important to investigate how these compounds may affect the acquisition of conditioned fear. Classical fear conditioning in animals is thought to share many similarities with the acquisition and expression of memory-associated fears that characterize post-traumatic stress disorder (PTSD) and phobias in humans (Mineka and Oehlberg, 2008). Thus, a more thorough understanding of how CB1 antagonists and inverse agonists influence the acquisition of fear conditioning has important implications for understanding the clinical significance of these compounds.

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Numerous studies have implicated CB1 signaling in behaviors related to conditioned fear. Several brain areas important for fear conditioning (Barad et al. 2006; LeDoux, 2000; Rodrigues et al. 2009) including prefrontal cortex, hippocampus and basolateral amygdala, express a moderate to high density of CB1 receptor protein (Chhatwal et al. 2008; Herkenham et al. 1991; Katona et al. 2001; McDonald and Mascagni 2001). Both enhancement of CB1 transmission (Chhatwal et al. 2005; Mikics et al. 2006; Pamplona et al. 2008), and administration of CB1 antagonist/inverse agonists SR141716 (N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophe nyl)-4-methyl-1H-pyrazole-3-carboxamide; rimonabant) and AM251 (N-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophen yl)-4-methyl-1H-pyrazole-3-carboxamide; Arenos et al. 2006; Chhatwal et al. 2005; Finn et al. 2004; Marsicano et al. 2002; Mikics et al. 2006; Reich et al. 2008; Roche et al. 2007; Suzuki et al. 2004) can affect certain aspects of both cued and contextual classically conditioned fear. Particularly, modification of CB1 signaling consistently produces changes in fear extinction when given just prior to extinction training (Chhatwal et al. 2005; Chhatwal et al. 2008; Marsicano et al. 2002; Niyuhire et al. 2007; Pamplona et al. 2008). However, pre-conditioning administration of CB1 antagonist/inverse agonists has produced equivocal effects on conditioned fear. One study showed no effect of a CB1 antagonist/inverse agonist on fear conditioning in mice (Marsicano et al. 2002), but did report an effect of CB1 receptor knockout and injection of rimonabant on extinction of fear conditioning. Arenos et al. (2006) observed that CB1 antagonism impaired the expression of conditioned fear (Arenos et al. 2006). In contrast, Reich et al. (2008) showed that AM251 enhanced acquisition of freezing for both trace and delay forms of tone-cued fear conditioning.

In the present paper, we examined the effects of a CB1 antagonist (AM4113) and an inverse agonist (AM251) given prior to training on acquisition of classically conditioned fear, employing a two-week period between conditioning and testing. This interval, which is substantially longer than the typical one to four day period used in similar studies, was chosen because the longer delay between conditioning and test might make the procedure more sensitive to any differences in the strength of the associations, as stronger associations tend to take longer to forget (Annau and Kamin, 1961). In view of the differential effects of AM4113 and AM251 on anxiety-related behavior and neural activation (Sink et al. 2010), we hypothesized that the CB1 antagonist/inverse agonist, AM251, given prior to conditioning, would produce stronger effects on contextual or discretely cued fear memory than the neutral CB1 antagonist, AM4113. The same doses of AM251 and AM4113 that were used in the previous study of anxiety-related behavioral also were used in the present study.

2. Methods

2.1. Subjects

A total of 95 animals were used for these experiments. Adult male Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were pair-housed in a colony maintained at 23 °C, with a 12 h light/dark cycle (lights on 07:00). Food and water was available ad libitum in the home cages. Animal protocols were approved by the University of Connecticut Institutional Animal Care and Use Committee, and the studies were conducted according to NIH guidelines for animal care and use.

2.2. Drugs

AM251 and AM4113 (synthesized at the Center for Drug Discovery, Northeastern University) were dissolved in a vehicle of dimethylsulfoxide (DMSO; Fisher, Waltham, MA, USA), Tween-80 (Fisher), and 0.9% saline in a 1:1:8 ratio. This mixture also served as vehicle for these experiments. Doses and pretreatment times for AM251 and AM4113 were chosen based upon previous research demonstrating these doses to be efficacious for suppression of food intake (McLaughlin et al. 2003; Sink et al. 2008a,b, 2009a). Both drugs were administered IP in a volume of 1.0 mL/kg 30 min prior to conditioning.

2.3. Locomotor assessment

For assessment of locomotion, rats were placed in small activity chambers (28×28×28 cm) inside soundproof shells. These were different chambers than the fear conditioning boxes described below. The floor of each chamber consisted of two wire mesh panels (27×13 cm) connected through the center by a metal rod, which serves as a fulcrum for the floor panels. Locomotion by the subjects produced a slight deflection of one or more floor panels, which closed one or more of four microswitches mounted on the exterior of the chamber. Microswitch closure sent a signal to an external computer running a custom program, by means of an interface (Med Associates). Each microswitch closure was processed as a single activity count.

2.4. Fear conditioning procedures

Within the two weeks prior to conditioning, each animal received four adaptation sessions, which consisted of placing the rat in a novel chamber within a novel room for 5 min. The last of these adaptations took place in the locomotion assessment chambers described above during a 5 min baseline activity assessment session. Rats also received habituation injections of 0.3 mL 0.9% saline for four days prior to conditioning. On the day of conditioning, each rat was injected with drug and 30 min later carried by hand and placed in the shock chamber (28×21×21 cm, Med Associates, East Fairfield, VT) for a 95-s exploratory period. This period was followed by four tone-shock pairings. These pairings consisted of 35 second 70 dB tones co-terminating with 2 second 0.4 mA shocks generated by a scrambler (Lafayette Instruments, Lafayette, IN). The tone-shock pairings were interleaved with 95 second inter-tone intervals. The conditioning chamber was cleaned with dilute PineSol (The Clorox Co.; 1% solution, sprayed on the walls of the chamber with a spray bottle after which it was wiped off with a dry paper towel) between rats. The rats were given four more adaptation sessions as described above in the 14 days between conditioning and testing sessions. For context retention testing each animal was carried by hand into the room in which conditioning occurred and placed in the conditioning chamber for 95 s. The rat received neither conditioned tone nor shock during this test session. The next day, the rat was subject to an additional adaptation session as described above. The following day, each rat was carried into a novel test room inside a plastic mouse cage and placed in a novel chamber containing aspen shavings and smelling of a different odor (isopropyl alcohol; 30% solution of alcohol was sprayed on the walls of the chamber) than the conditioned context. After 95 s had elapsed, the conditioned tone sounded for 95 s. As with context testing, no shock was delivered at any time during the session. The novel chamber was cleaned with isopropyl alcohol and the shavings changed between rats. The same experimenter handled the rats during all conditioning and retention sessions. Conditioning and test sessions were observed from a video monitor in an adjacent room. The amount of time spent freezing, defined as the absence of movement, was determined by deducting the time in motion as detected using microwave activity monitors (RadioShack) and recorded on a PC running DOS (Oler and Markus, 1998).

2.5. Experiments

For each experiment, rats were randomly assigned to one of the drug treatment conditions, and between-subjects designs were used.