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An NMDA antagonist (LY 235959) attenuates abstinence-induced withdrawal of planarians following acute exposure to a cannabinoid agonist (WIN 52212-2)

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Abstract

The mechanisms that facilitate the development and expression of cannabinoid physical dependence in humans and other mammals are poorly understood. The present experiments used a planarian model to provide evidence that pharmacological antagonism of NMDA receptors significantly attenuates the development of cannabinoid physical dependence. Abstinence-induced withdrawal from the cannabinoid agonist WIN 55212-2 (10 μM) was manifested as a significant ($P<0.05$) decrease in the rate of planarian spontaneous locomotor velocity (pLMV) when WIN 55212-2 (10 μM)-exposed planarians were placed into drug-free water. No change in pLMV occurred when WIN 55212-2 (10 μM)-exposed planarians were placed into water containing WIN 55212-2 (10 μM). WIN 55212-2 (10 μM)-exposed planarians placed into water containing LY 235959 (1 or 10 μM) did not display withdrawal (no significant difference, $P>0.05$, in pLMV). In addition, withdrawal was not observed (no significant difference, $P>0.05$, in pLMV) in planarians that were co-exposed to a solution containing WIN 55212-2 (10 μM) and LY 235959 (10 μM). The present results reveal that NMDA receptor activation mediates the development of cannabinoid physical dependence and the expression of cannabinoid withdrawal in planarians.

Keywords: WIN 55212-2; Cannabinoid; Planaria; Glutamate; NMDA; LY 235959; Dependence; Withdrawal

1. Introduction

Discontinuation of chronic marijuana consumption causes spontaneous withdrawal in humans (Crowley et al., 1998; Kouri et al., 1999). The cannabinoid withdrawal signs resulting from marijuana abstinence are milder compared to those signs caused by opioid withdrawal and are not prevalent in all individuals (Pertwee, 1999, 2006; Perkonigg et al., 1999). The situation in animals remains inconclusive (Lichtman and Martin, 2002; Gonzalez et al., 2005). A number of investigators demonstrate that the administration of a cannabinoid receptor antagonist, SR 141716A (rimonobant), to rats receiving chronic Δ^9-tetrahydrocannabinol (Δ^9-THC) precipitates a withdrawal syndrome (Aceto et al., 1995, 1996; Tsou et al., 1995; Beardsley and Martin, 2000; Rubino et al., 1998). However, spontaneous withdrawal from Δ^9-THC does not cause any withdrawal signs in rats. Yet, rats spontaneously withdrawn from a synthetic cannabinoid agonist with higher pharmacological potency or significantly different pharmacokinetics than Δ^9-THC do display withdrawal signs (Aceto et al., 2001).

It is clear that some cannabinoids can produce physical dependence in mammals, but the complexity of mammalian models and the pharmacokinetic properties (i.e., late onset, greater duration, slow metabolic clearance, etc.) of the cannabinoids themselves (Justinova et al., 2005; Pertwee, 1997; Gonzalez et al., 2005) is most likely responsible for the inconsistent results cited above. An attractive alternative to a mammalian model of drug withdrawal is a planarian model (Raffa and Valdez, 2001). Planarians are free-living, fresh-water flatworms that are considered to be the most primitive extant animals having bilateral symmetrical nerve processes consisting

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of cephalic ganglia and peripheral nerve cords (Sarnat and Netsky, 1985). Planarians provide a useful and convenient model for the study of nervous system function and drug-induced effects (Algeri et al., 1983; Agata, 2002; Agata and Watanabe, 1999; Carolei et al., 1975; Newmark et al., 2003; Newmark and Sanchez Alvarado, 2002; Palladini et al., 1996; Passarelli et al., 1999; Venturini et al., 1989). Our laboratory has developed and utilized a metric-change in spontaneous locomotor velocity that quantifies withdrawal behavior in planarians following exposure to cocaine, amphetamine, or opioids (Raffa and Desai, 2005, Raffa et al., 2001, 2000, 2003, Raffa and Martley, 2005, Raffa and Valdez, 2001, Umeda et al., 2005, 2004). The effect is not due to factors such as osmolarity or pH (Umeda et al., 2004). Most recently, we have demonstrated that cannabinoid-exposed, but not naïve, planarians undergo an abstinence-induced decrease in spontaneous locomotor velocity when placed into cannabinoid-free, but not cannabinoid-containing, water (Rawls et al., 2006a,b).

A series of molecular and neurochemical events accompany the withdrawal signs precipitated by rimonobant in rats exposed chronically to Δ^9-THC. These included decreased dopamine release, *c-fos* induction, changes in adenylate cyclase/cAMP signaling, and increased corticotrophin releasing factor (Hutcheson et al., 1998; Tzavara et al., 2000; Diana et al., 1998). Another neurotransmitter that may be involved is glutamate, the major excitatory transmitter in the mammalian brain. For example, it is known that blockade of glutamatergic transmission at NMDA receptors abolishes withdrawal signs precipitated by naloxone in rats exposed to chronic morphine (Tanganelli et al., 1991; Koyuncuoglu et al., 1990; Rasmussen et al., 1991; Tokuyama et al., 1996). In the case of opioids, these data indicate that NMDA receptor activation contributes to the development and/or expression of physical dependence in rats. The role of NMDA receptors in cannabinoid dependence is not yet known. Because planarians contain endogenous glutamate, express the genes for glutamate receptors and undergo abstinence-induced withdrawal from a cannabinoid agonist, they are a desirable model for investigating a role for NMDA receptors in cannabinoid physical dependence and withdrawal (Rawls et al., 2006a,b; Cebria et al., 2002).

The present study used planarians to test the hypothesis that NMDA receptor antagonism decreases: (1) development of physical dependence to a cannabinoid agonist and (2) expression of abstinence-induced withdrawal from a cannabinoid agonist. Actual experiments revealed that the NMDA receptor antagonist LY 235959 blocked the development of physical dependence to WIN 55212-2 and the expression of withdrawal following abstinence from WIN 55212-2. These results reveal that an increase in glutamatergic transmission at NMDA receptors mediates cannabinoid physical dependence and withdrawal in planarians.

2. Materials and methods

2.1. Animals and drugs

Planarians (*Dugesia dorotocephala*) were purchased from Carolina Biological Supply Company (Burlington, NC). Planarians were acclimated to temperature-controlled room temperature (21 °C) and tested within 72 h. Each planarian was used only once. WIN 55212-2 [4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenylcarbonyl)-6*H*-pyrrolo [3,2,1ij]quinolin-6-one] and LY 235959 [(−)-6-[phosphonomethyl-1,2,3,4,4a,5,6,7,8,8a-decahydro-isoquinoline-2-carboxylate] were purchased from Tocris-Cookson (St. Louis, MO). LY 235959 was dissolved in room-temperature (21 °C) tap water containing AmQuel® water conditioner. A stock solution of 1 mM WIN 55212-2 was prepared in 10/90% cremophor/water (Rawls et al., 2006a,b). Treatment solutions were diluted with tap water containing AmQuel® water conditioner.

2.2. Behavioral measurements

Planarian locomotor velocity (pLMV) was quantified by placing individual planarians into a clear plastic petri dish (Raffa et al., 2001) containing room-temperature (21 °C) tap water containing AmQuel® water conditioner. The dish was placed over paper with gridlines spaced 0.5 cm apart. pLMV was quantified as the number of gridlines crossed or re-crossed per min over a 5-min observation period. pLMV was expressed as the mean (± S.E.M.) of the cumulative number of gridlines crossed by each planarian per min. Prior to behavioral observations, each planarian was placed into individual 0.5 ml vials containing room-temperature vehicle or test drug(s) for 60 min. In the first set of experiments, planarians were exposed to either water or LY 235959 (10 μM) for 60 min and then tested individually for pLMV in either water or LY 235959 (10 μM). In a second set of experiments, planarians were exposed to either water or WIN 55212-2 (10 μM) and then tested individually for pLMV in one of the following: water, WIN 55212-2 (10 μM), LY 235959 (0.1 μM), LY 235959 (1 μM), or LY 235959 (10 μM). In a final experimental set, planarians were exposed for 60 min to water, WIN 55212-2 (10 μM), LY 235959 (1 μM), or WIN 55212-2 (10 μM) plus LY 235959 (1 μM) and then tested individually for pLMV in water.

2.3. Statistical analysis

Comparisons of the cumulative group means at 5 min were evaluated by a one-way ANOVA followed by a Tukey's post-hoc analysis. Values of $P<0.05$ were considered to be statistically significant.

3. Results

3.1. LY 235959 does not affect pLMV

The results with LY 235959 by itself are shown in Fig. 1. Drug-naïve planarians displayed a nearly constant pLMV of approximately 15–18 gridlines/min when tested in drug-free water (Raffa and Valdez, 2001; Raffa et al., 2001, 2003; Rawls et al., 2006a,b). Planarians that were tested for 60 min in LY 235959 (10 μM) and then tested in drug-free water or water containing the same concentration of LY 235959 displayed pLMV that was not significantly different ($P>0.05$) than LY