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Exposure to cannabinoid agonist WIN 55,212-2 during early adolescence increases alcohol preference and anxiety in CD1 mice

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ABSTRACT

The endocannabinoid (eCB) system is involved in the modulation of the reward system and participates in the reinforcing effects of different drugs of abuse, including alcohol. The most abundant receptor of the eCB system in the central nervous system is the CB1 receptor (CB1R), which is predominantly expressed in areas involved in drug addiction, such as the nucleus accumbens, the ventral tegmental area, the substantia nigra and the raphe nucleus. CB1R is expressed in early stages during development, and reaches maximum levels during early adolescence. In addition, cannabinoid receptor 2 has been found expressed also in the central nervous system at postsynaptic level. In order to analyze the participation of the eCB system on ethanol (EtOH) preference, mice were exposed to cannabinoid agonist WIN 55,212-2 (WIN) for 5 consecutive days during early adolescence. Anxiety tests were performed the day after WIN treatment withdrawal, and EtOH preference was measured throughout adolescence. Mice exposed to WIN during early adolescence exhibited a significant increase in EtOH intake and preference after treatment. Moreover, WIN exposure during early adolescence induced an anxiogenic effect. Morphometric analysis revealed higher dendritic ramifications and fewer dendritic spines in neurons of the substantia nigra pars compacta in WIN-treated mice. On the other hand, immunohistochemical analysis revealed an increase in the number of tryptophan hydroxylase-expressing neurons in the dorsal raphe nucleus but no differences were found in the ventral tegmental area or substantia nigra pars compacta for tyrosine hydroxylase-expressing neurons. These results demonstrate that exposure to WIN in early adolescence can affect neural development and induce alcohol preference and anxiety-like behavior during late adolescence.

1. Introduction

Cannabis consumption is highly prevalent during adolescence, and most of the people start its use in the early adolescence, coinciding with the major neuronal changes in the central nervous system (Chadwick et al., 2013). The cannabis contains psychoactive components, notably Δ(9)-tetrahydrocannabinol (THC), which acts on the endogenous endocannabinoid (eCB) system. On the other hand, alcohol is one of the drugs of abuse and psychoactive substances most widely used by men worldwide (Robinson and Adinoff, 2016). Like other drugs of abuse, alcohol activates the reward system, by increasing the firing rate of dopaminergic neurons from the ventral tegmental area (VTA) (Brodie et al., 1999; Hauser et al., 2011), leading to an increase in the release of dopamine in the nucleus accumbens (Wise, 2009). Several studies have supported the idea that the eCB system has a modulatory role in the reward system, and that it participates in vulnerability to consumption and in the development of tolerance and dependence on different drugs of abuse (Gonzalez et al., 2004; Maldonado et al., 2006; Parsons and Hurd, 2015; Pradier et al., 2015).

The eCB system is mainly made up of two endogenous ligands,

Abbreviations: WIN, WIN 55,212-2; CB1R, Cannabinoid receptor 1; CB2R, Cannabinoid receptor 2; eCB, endocannabinoid; TH, Tyrosine hydroxylase; TrpOH, Tryptophan hydroxylase; VEH, vehicule.

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anandamide (AEA) and 2-arachidonyl-glycerol (2-AG). However, other endogenous molecules have been found to bind cannabinoid receptors (Lu and Mackie, 2016; Riebe and Wotjak, 2011). eCBs act mainly through cannabinoid receptors CB1 (CB1R) and CB2 (CB2R), which belong to the family of G protein-coupled receptors. CB1R is the most abundant receptor in the central nervous system (CNS), is located at the presynaptic level and leads to the inhibition of neurotransmitter release (Ohno-Shosaku et al., 2012). On the other hand, although predominantly located in cells from the immune system, CB2R has been found expressed also in the CNS at the postsynaptic level (Brusco et al., 2008; Riebe and Wotjak, 2011) and has also been implicated in reward and addiction (Kleczkowska et al., 2016; Parsons and Hurd, 2015; Pradier et al., 2015).

Besides being widely distributed in the CNS, CB1R is highly expressed in reward-related areas, such as the nucleus accumbens, VTA and prefrontal cortex (El Khoury et al., 2012; Mackie, 2005; Tsou et al., 1998), and it also participates in the addictive properties of different drugs of abuse (El Khoury et al., 2012; Maldonado et al., 2006; Parsons and Hurd, 2015). On the other hand, CB1R has been found expressed in the nucleus raphe as well as other areas involved in anxiety behavior (Haring et al., 2007). While the effects of alcohol on the eCB system have been widely studied, less is known about the modulation of the eCB system in alcohol vulnerability and consumption. Evidence suggests that pharmacological (Colombo et al., 2004, 2005; Gessa et al., 2005) or genetic (Houchi et al., 2005; Thanos et al., 2005; Vinod et al., 2008) blockade of CB1R suppresses ethanol (EtOH) consumption and preference, as well as the motivational and reinforcing effects of alcohol (Henderson-Redmond et al., 2016). On the contrary, the administration of cannabinoid receptor agonists induces voluntary EtOH consumption (Colombo et al., 2005; El Khoury et al., 2012; Linsenbardt and Boehm, 2009).

In addition to the relationship between the reward and the eCB systems, there is evidence of interactions between the eCB and the serotonergic systems (Haj-Dahmane and Shen, 2011; Haring et al., 2013). Both eCBs and serotonin (5-HT) influence several physiological functions and control a wide range of behaviors and emotions, including anxiety (Haring et al., 2015; Martin et al., 2002), although evidence suggests a contradictory role of cannabinoids. It has been reported that cannabinoids can induce both anxiogenic- and anxiolytic-like respones, depending on the doses and the familiarity of the environment (Hajizadeh Moghaddam et al., 2013; Haller et al., 2004; Patel and Hillard, 2006; Rubino and Parolaro, 2016; Ruehle et al., 2012), and that anxiogenic effects can be accompanied by a decrease in locomotor activity (Rutkowska et al., 2006).

Adolescence is a critical stage in neural development, characterized by neurobehavioral plasticity and maturation processes. During adolescent neural development, important dopaminergic reorganization takes place (Money and Stanwood, 2013; Spear, 2000), along with functional maturation of the eCB system (Crews et al., 2007; Ellgren et al., 2008; Gaffuri et al., 2012). In humans, cannabinoid consumption during early adolescent development can induce long-lasting neural alterations which affect behavior and develop psychiatric pathologies, such as depression, anxiety disorders and schizophrenia, and cognitive impairments in adulthood (Chadwick et al., 2013). Therefore, this extended development provides a wide window of critical periods in which potential disruptors could influence diverse effects, including a tendency and a vulnerability to drug abuse (Spear, 2000).

In particular, exposure to CB1R and CB2R cannabinoid agonist WIN 55,212-2 (WIN) during adolescence has been demonstrated to induce long-lasting behavioral consequences in adult rodents, affecting cocaine withdrawal (Aguilar et al., 2017), anxiety (Wegener and Koch, 2009), and inducing memory impairment in the Morris water maze and in fear conditioning (Tomas-Roig et al., 2017).

In this regard, to study how the consumption of cannabinoids in the adolescence can affect the neural development and the alcohol preference, we analyzed the effects of WIN exposure during early adolescence in mice. Given recent evidence, we hypothesized that exposure to WIN in early adolescence induces alterations in neural development that affect alcohol preference and anxiety-like behavior during late adolescence. Our findings provide new evidence of the sensitivity to pharmacological stimulation of eCB receptor activity during neural development, which affects alcohol preference and anxiety-like behaviors.

2. Materials and methods

2.1. Animals

Thirty-nine male CD1 mice, 21 days-old at the start of the study, were used throughout. Mice were housed at the animal facilities of *Instituto de Biología Celular y Neurociencias* (IBCN), in groups of three per cage, at controlled temperature conditions (20 ± 2 °C) and 12-h dark light cycle (7am-7pm light cycle). Standard rodent chow and tap water were available *ad libitum*, except during the period of forced EtOH consumption and the EtOH preference assay, in which alcohol was presented alone or in a two-bottle choice access, one with EtOH 6% v/v and the other with water, respectively. Each cage was randomly assigned to the vehicle (control group, VEH, 6 cages) or the WIN-treated group (treated group, WIN, 7 cages). All the procedures for the use and care of experimental animals were approved by the *Comité Institucional de Cuidado y Uso de Animales de Experimentación* (CICUAL, School of Medicine, University of Buenos Aires, Res CD 2935/2016).

2.2. Experimental protocol

The experimental protocol is summarized in Fig. 1A. Mice were subjected to forced EtOH consumption (see 2.2.1) to avoid rejection in the following EtOH preference assay. After the forced EtOH consumption period, mice received a daily administration of WIN or vehicle (VEH) from P30 to P35, and had available 2 bottles, one with water and the other with EtOH (6% v/v), in order to analyze the consumption of EtOH (EtOH preference) during WIN and VEH treatment (see 2.2.2). After the last WIN or VEH injection, mice continued the EtOH preference assay (see 2.2.4) for another 40 days to evaluate the long-lasting effects of WIN on alcohol preference.

2.2.1. Forced EtOH consumption

Mice received increasing concentrations of alcohol solutions as the only beverage from P21 to P30 (Fig. 1A). During 4 days (P21-P24), animals received EtOH 3% (v/v); then EtOH 6% (v/v) for 3 days (P25-P27); and finally EtOH 8% (v/v) for 2 days (P28-P29). Every bottle was filled with 200 ml of EtOH at the corresponding concentration. The bottles were changed twice a week during the morning with an EtOH solution prepared the same day, and alcohol consumption and body weight were registered.

2.2.2. Cannabinoid agonist WIN administration

As from P30, animals received a daily subcutaneous administration of WIN (3 mg/kg) or VEH, for 5 consecutive days. Twenty-one mice were assigned to the WIN-treated group, while 18 mice were assigned to the control VEH group. WIN was dissolved in tween 80 (0.3% v/v) in sterile saline solution, while and tween 80 (0.3% v/v) in saline solution was used as VEH.