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Research report

Endocannabinoid/GABA interactions in the entopeduncular nucleus modulates alcohol intake in rats

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ABSTRACT

Alcohol use disorder is a compulsive behavior driven by motivational systems and by a poor control of consummatory behavior. The entopeduncular nucleus (EP) seems to be involved in the regulation of executive mechanisms, hence, in the expression of behavior. Endocannabinoids (eCB) are involved in alcohol intake mechanisms. The eCB receptor name cannabinoid receptor 1 (CB1R) is expressed in the EP in GABAergic terminals. The role of the eCB system (eCBs) of the EP in the modulation of alcohol seeking and intake behavior is unknown. Therefore, we decided to investigate the role of the eCBs and its interaction with GABA transmission in rat EP, in the regulation of alcohol intake behavior.

Rats were submitted to a 10-day period of moderate alcohol (10% in tap water) ingestion. No tap water was available. On day 11, either anandamide (AEA, CB1 receptor agonist), AM251 (CB1R inverse agonist), baclofen (BAC, GABAB receptor agonist), or CGP35348 (GABAB receptor antagonist) was administered into the EP. One bottle of water and one of alcohol (10% in water) were available ad libitum for the following 24 h, and consumption was quantified at the end of this period.

Results show that administration of AEA into the EP decreased alcohol consumption while AM251 and BAC administered independently increased alcohol consumption. AEA prevented the increase induced by AM251 or BAC. Likewise, CGP35348 prevented alcohol ingestion induced by AM251.

These data suggest that eCBs dysfunction in the EP may be playing a crucial role in the abuse and dependence of alcohol and other drugs.

1. Introduction

Alcohol use disorder (AUD) is a worldwide medical problem. Prevalence of AUD ranks between 0.2% (Afghanistan) to 18.87% (Russian Federation) (WHO, 2004). The reward system, basically formed by the ventral tegmental area (VTA) and the nucleus accumbens (NAcc) and the modulatory system, basically formed by the 3rd gyrus of the right prefrontal cortex, the subthalamic nucleus and the globus pallidus internus (GPi), also known as the entopeduncular nucleus (EP) in non-human species, such as cats and rats, drive the "wanting" behavior. It has been shown that electrical stimulation of the EP prevents haloperidol-induced vacuous chewing movements, and "compulsive" lever pressing behavior in rats (Klavir et al., 2011), suggesting that this nucleus down-regulates motor expression, thereby reducing impulsivity. Impulsivity, defined as the quick response to stimuli without considering the potential consequences of such a response, has been linked to alcohol and other drugs of abuse (Crews and Boettiger, 2009; Urcelay and Dalley, 2012; Peles et al., 2012). It has been described, that the Δ9-tetrahydrocannabinol (THC) increases impulsivity in humans (McDonald et al., 2003).

On the other hand, the eCBs has been strongly linked tomechanisms of drug abuse and dependence, such as the reinforcing effects of alcohol, opioids, nicotine, and cocaine among others (González et al., 2002; Shoaib, 2008; Orio et al., 2009; Luchicchi et al., 2010), and relapse (triggering and/or preventing reinstatement of drug seeking behaviors) (Li et al., 2008; Ward et al., 2009; Gamaleddin et al., 2011).

It has been demonstrated that endogenous cannabinoid levels (anandamide, AEA and 2-arachidonil-glicerol, 2-AG) increase in reward-related areas after chronic alcohol exposure (González et al., 2002). Volkow's group (Thanos et al., 2005) demonstrated that CB1R knockout mice, exhibited reduced voluntary alcohol consumption compared to wild type (WT) mice. WT mice treated with a

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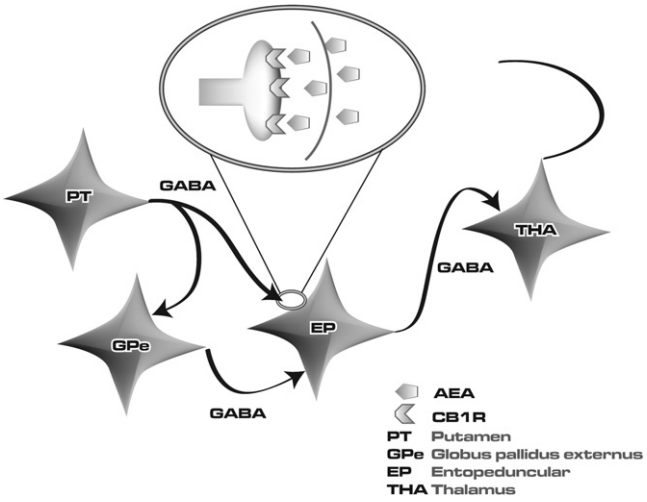

Fig. 1. Scheme describing interactions between CB1R and GABAergic system into EP, PT and thalamus.

CB1Rinverse agonist SR141716A, displayed a similar behavior. The same study showed that mice lacking the CB1 receptor are unable to develop conditioned place preference to alcohol (Thanos et al., 2005). Additional studies reported that systemic administration of the CB1Rinverse agonist, AM251, besides decreasing voluntary ethanol consumption, also prevents changes in gene expression caused by alcohol in regions involved in addiction (Femenía et al., 2010). Likewise, SR141716A microinjected into the NAcc or VTA prevents alcohol self-administration (Malinen and Hyytiä, 2008).

It is widely accepted that eCB activate inhibitory protein G coupled to CB1R in presynaptic axons, thereby reducing the probability of neurotransmitter release (Schlicker and Kathmann, 2001; Wilson and Nicoll, 2002). The CB1R is expressed principally in the buttons of GABAergic and Glutamatergic cells and is widely distributed throughout the central nervous system. One of the structures with a higher expression of CB1Ris theEP (Glass et al., 1997; Svízenská et al., 2008). Major EP (GABA) projections are directed to the thalamus, where they exert inhibitory action on thalamic activity, thereby interfering with the initiation or continuation of a motor response (Fig. 1) (Hauber, 1998).

Electrophysiological studies have shown that eCB inhibit presynaptic neurotransmitter release from the striatopallidal pathway and from the EP(Engler et al., 2006). eCB stimulate or inhibit GABA release depending on the dose (Gonzalez et al., 2009). This interaction of eCB and GABAergic cells may be crucial to regulate motor response.

Whether eCBs in the EP have a role in the modulation of alcohol intake behavior is unknown. Therefore, we decided to investigate the role of eCBs, and their interaction with GABA transmission in rat EP, in the regulation of alcohol intake behavior. In this context, we postulate that a dysfunction of the eCBs of the EP may play a crucial role in alcohol intake behavior.

2. Experimental procedures

2.1. Subjects

A total of 100 adult male Wistar rats weighing between 250 and 300 g were used. Rats were housed under a controlled light-dark cycle (12/12, 09:00 am lights off) at constant room temperature ($22 \pm 2\,^{\circ}C$) and humidity (52%). Water and food were available ad libitum.

2.2. Surgery

Ninety rats were implanted with bilateral guide cannulae (stainless steel cannula 23 GA × 1.3 cm, Plastics One) aimed at the EP (P = –2.3 mm, L = ±2.8 mm, V = –7.4 mm; Paxinos and Watson, 2007). Rats were deeply anesthetized with a cocktail of ketamine hydrochloride (250 mg), xylazine hydrochloride (10 mg), and acepromazine maleate (5 μg) in 6 ml of isotonic saline solution. A dose of 2.7 ml/1000 g of rat body weight was administered intraperitoneally (i.p.). After surgery, rats were housed individually and monitored every day. At least one week was allowed for recovery.

2.3. Alcohol intake

Toinducealcoholintake, rats were submitted to forced consumption of moderate alcohol (10% (v/v) in tap water) for ten days (no other liquid was available) and had ad libitum access to food (base line, BL). A freshly prepared 10% alcohol solution was provided daily. This concentration of alcohol (10%) has been used in protocols of forced consumption by others (Sarviharju et al., 2004; Van Waes et al., 2011). From the eighth to the tenth day, body weight, liquid (alcohol solution) and food intake were quantified (weighed on a digital scale, OHAUS, NJ, USA) to estimate the consumption of liquid and food every 24 hrs. The amount of liquid or food ingested has been expressed as an index calculated as follows (g liquid or food ingested/g body weight) × 100, indicating the amount of liquid or food ingested per g of rat's body weight.

2.3.1. Experimental day

On the 11th day, one of the following treatments was infused into the GPi: dimethylsulfoxide 100% (DMSO 0.5 μl/side), isotonic saline solution (SAL) (0.5 μl/side), AEA (0.5/0.5 μlside), AM251 (1.0 μg/0.5 μlside), BAC(0.1 μg/0.5 μlside), CGP35348 (0.1 μg/0.5 μl side), and as a cocktail AM251 (1 μg) + AEA (0.5 μg)/0.5 μl side, BAC (0.1 μg) + AEA (0.5 μg)/0.5 μl side, CGP35348 (0.1 μg) + AM251(1.0 μg)/0.5 μl side, each group had 10 rats. Drugs were injected with the aid of a KD Scientific pump at a rate of 0.1 μl/min through an injector (stainless steel cannula 30 GA × 1.3 cm, Plastics One) inserted into the guide cannula. We decided to use AM251 and AEA in the range of micrograms, based on our previous experience (Rueda-Orozco et al., 2008; Soria-Gómez et al., 2010a; Herrera-Solis et al., 2010) and of others (Bakkali-Kassemi et al., 2011; Rodriguez et al., 2011). DMSO 100% as vehicle has been used previously by our group (Díaz-Ruiz et al., 2001; Herrera-Solis et al., 2010; Méndez-Díaz et al., 2012) and we have not observed macroscopic differences in cresyl violet stained brain slices, or behavioral differences when compared to saline-treated groups.

Immediately after the rats received the corresponding treatment, they were provided with ad libitum access to two test bottles simultaneously: one containing 10% alcohol and other containing fresh water. Twenty-four hours later, alcohol and water consumption, as well as food intake, were quantified. All experiments were carried out in the middle (01:00 hours) of the dark phase of the photoperiod.

Additionally, to assess whether the amount of alcohol consumed by the subjects submitted to this protocol would produce behavioral signs of intoxication, an additional group of rats ($n = 10$) was exposed to the same conditions of alcohol consumption; on the eleventh day at 01:00 hours, we evaluated neurological signs including autonomic, sensory, and spontaneous motor functions, and their behavior in the Rota Rod test. Immediately before the evaluation, alcohol was removed and subjects transferred to a novel environment for behavioral assessment.