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Entopeduncular nucleus endocannabinoid system modulates sleep–waking cycle and mood in rats

Mónica Méndez-Díaz [a,*], Seraid Caynas-Rojas [a], Vianney Arteaga Santacruz [a], Alejandra E. Ruiz-Contreras [b], Raúl Aguilar-Roblero [c], Oscar Prospéro-García [a]

[a] Grupo de Neurociencias, Laboratorio de Canabinoides, Depto. de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, Apdo. Postal 70-250, México, D. F. 04510, Mexico

[b] Grupo de Neurociencias, Laboratorio de Neurogenómica Cognitiva, Departamento de Psicofisiología, Facultad de Psicología, Universidad Nacional Autónoma de México, Apdo. Postal 70-250, México, D. F. 04510, Mexico

[c] División de Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Apdo. Postal 70-250, México, D. F. 04510, Mexico

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ABSTRACT

Since the pioneering work of Gadea-Ciria (Gadea-Ciria M, Stadler H, Lloyd KG, Bartholini G. Acetylcholine release within the cat striatum during the sleep–wakefulness cycle. Nature 1973; 243:518–519) indicating pointing to the involvement of acetylcholine and basal ganglia in sleep regulation; extensive literature has suggested that this brain complex participates in the control of the sleep–waking cycle (SWC). On the other hand, it has been demonstrated that the endocannabinoid system (eCBS) is prominently involved in the regulation of the SWC, mood and its related disorders. Since cannabinoid receptor 1 (CB1R) is highly expressed in basal ganglia, in particular in the entopeduncular nucleus (EP), we believe that it is important to know what the role of the EP CB1R is on SWC, depression, and anxiety.

To provide insight into the role of the EP CB1R in the regulation of wakefulness (W), non-rapid eye movement sleep (NREMs) and rapid eye movement sleep (REMs), rats were recorded for 24 h immediately after a single intra-EP administration of N-arachidonoylethanolamine (AEA) or 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-(1-piperidyl)pyrazole-3-carboxamide (AM251; CB1 inverse agonist). Likewise, the effect of these drugs on anxiety and depression was tested by means of the elevated plus maze (EPM) and forced swim test (FST), respectively.

Results demonstrate that AEA increases NREMs expression, while AM251 increases W and decreases both NREMs and REMs. In addition, administration of AM251 decreases the time rats spent in the open arms and increases immobility time in the FST. It seems that activation of the CB1R in the EP is important to induce sleep, while its blockade promotes W, as well as anxiety and depression, somewhat resembling insomnia in humans. These results suggest that the EP CB1R is modulating sleep and mood.

1. Introduction

The basal ganglia are involved in the regulation of numerous neurobiological processes, such as motor control (Ungerleider et al., 2002), cognitive functions (Shohamy et al., 2008), reward-related processes (Assadi et al., 2009) and emotions (Brown et al., 2012). Furthermore, several research groups have suggested that basal ganglia are important in the regulation of the sleep–waking cycle (SWC) (Gadea-Ciria et al., 1973; Qiu et al., 2010; Lazarus et al., in press). The basal ganglia include the striatum, the globus pallidus externus and internus (the latter is also named entopeduncular nucleus (EP) in non-human species, such as cats and rats), the subthalamic nuclei, and the substantia nigra. The role of several neurotransmitter systems on basal ganglia, for example, acetylcholine (Gadea-Ciria et al., 1973), GABA, glutamate, and dopamine (Rice et al., 2011) has also been well described. Likewise, the endocannabinoid system (eCBS) is highly present in basal ganglia, particularly in the EP (Giuffrida and Seillier, 2012; Svízenská et al., 2008).

Two of the most studied cannabinoids endogenous (eCB) are anandamide (AEA), and 2-arachydonyl-glycerol (2-AG); the enzymes involved in their biosynthesis and degradation, N-acyl phosphatidyl-ethanolamine phospholipase-D (NAPE-PLD) and fatty acid amide hydrolase (FAAH), are responsible for AEA metabolism; diacylglycerol lipase (DAGL) and monoacylglycerol lipase (MAGL) are responsible for 2-AG metabolism, and finally, the sites of action of this eCB are cannabinoid receptor 1 (CB1R) and cannabinoid receptor 2 (CB2R). CB1R is widely distributed in the central nervous system, and is mainly expressed in the buttons of GABAergic and Glutamatergic cells (Mechoulam and Parker, 2012). Within the basal ganglia, the globus pallidus is the nucleus with a higher expression of CB1R (Svízenská

* Corresponding author at: Laboratorio de Canabinoides, Grupo de Neurociencias, Depto. de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, Mexico. Tel.: +52 55 56 23 25 09; fax: +52 55 56 23 23 41.
E-mail address: mendezm@unam.mx (M. Méndez-Díaz).

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et al., 2008; Glass et al., 1997). On the other hand, several studies support the role of the eCBS in the SWC. Administration of AEA facilitates NREMs and REMs through CB1R activation (Rueda-Orozco et al., 2010; Murillo-Rodríguez et al., 2001). Mice lacking FAAH have increased sleep and reduced W (Huitron-Resendiz et al., 2004; Cravatt et al., 1996). In addition, systemic administration of SR141716A (a CB1 antagonist) increases W in rats (Santucci et al., 1996). In this context, we hypothesize that the eCBS in the EP has a role in the regulation of SWC.

In humans, there is a high degree of sleep and mood disorder (depression and anxiety) comorbidity (Atalay, 2011; Augner, 2011). A role of the eCBS in the modulation of anxiety and depression has also been suggested (Ashton and Moore, 2011; Viveros et al., 2005). For example, blocking FAAH, and MAGL activity produces anxiolytic effects in adult rats and mice, respectively (Busquets-Garcia et al., 2011; Gaetani et al., 2003). In the same way, it has been shown that N-palmitoylethanolamide elicits antidepressant-like effects in the FST and tail suspension test in mice (Yu et al., 2011). There are recent reports that CB1 deficient mice exhibit a phenotype characteristic of depression (Valverde and Torrens, 2012). Our objective is to analyze whether the EP eCBs has a role in SWC regulation and mood (depression and anxiety) in rats.

2. Material and methods

2.1. Subjects

A total of eighty male Wistar rats (250–350 g) were singly housed in standard plastic cages (42 × 25.5 × 20 cm) with wood shavings as bedding. They were maintained on a controlled light–dark cycle (12:12, 09:00 am lights off) at constant room temperature (22 ± 2 °C) and humidity (52%). Water and food (Rat Chow, Purina) were available ad libitum. All rats were subjected to stereotaxic surgery. To anesthetize the rats, we used a cocktail of ketamine hydrochloride, xylazine hydrochloride, and acepromazine maleate (70 mg/kg, 3 mg/kg, and 1.5 mg/kg, respectively) administered i.p. After surgery, rats were housed individually and monitored every day. One week was allowed for recovery. During this time, rats were manipulated for cleaning the surgical wound and their general health status was evaluated.

Animals were treated in accordance to the Official Mexican Regulation on "Technical specifications for the production, use and care of laboratory animals" (NOM-062-ZOO-1999). In addition, this study was approved by the Committee of Research and Ethics of the Facultad de Medicina, Universidad Nacional Autónoma de México.

2.2. Surgery and intra-EP administration of drugs

Twenty rats were stereotaxically implanted with a set of electrodes for conventional sleep recording. The electrodes used were: one bipolar electrode made of stainless steel Teflon-covered wire (0.013″, A-M Systems Inc. WA, USA) aimed at the hippocampus (P = 4.0, L = 2.5, V = 2.5, from the bregma) (Paxinos and Watson, 2007) to record the electroencephalogram (EEG), two electrodes made of stainless steel screws (0–80 × 1/16, Plastics One Inc. VA, USA) implanted into the frontal cortex (A = 3 from bregma) (Paxinos and Watson, 2007) used to ground the animal and two stainless steel Teflon-covered wire electrodes (0.013″, A-M Systems) inserted into the neck muscles to record the electromyogram (EMG). Additionally, during the same surgery time, rats were implanted with a couple of stainless steel guide cannula (23 GA × 1.3 cm, Plastics One) aimed at the EP (P = 2.3, L = 2.8, V = 7.4 from the bregma) (Paxinos and Watson, 2007). Electrodes were connected to a plastic head-cap. The entire electrode assembly was insulated and attached to the skull with dental acrylic.

After recovery from surgery and the handling period, rats were acclimated to the recording conditions for 24 h. Subsequently, they were randomly assigned to each experimental group to receive one of the following treatments: vehicle (dimethylsulfoxide [DMSO], 0.5 μl; control group, n = 8), AEA, 0.5 μg/0.5 μl (n = 6); and AM251 1 μg/0.5 μl (n = 6). Rats were injected with the aid of a KD Scientific pump at a rate of 0.1 μl/min through an injector inserted into the guide cannula. Immediately after the injection, rats were placed into the recording chambers to start the session.

2.3. Sleep recording

Based on the electrical activity recorded with these electrodes (EEG and EMG), we identified three sleep stages: W, as low-voltage and high frequency activity (theta rhythm, 5–8 Hz) or mixed activity associated to high EMG activity; NREMs, identified as high-voltage and low frequency waves (delta rhythm, 1.5–4 Hz), and low EMG activity; and REMs which was detected by its medium-voltage and high-frequency waves (theta rhythm, 5–8 Hz), and the absence of EMG activity (absence of postural tone). Polygraph signals were amplified and band pass filtered (1–30 and 30–100 Hz for EEG and EMG, respectively) by a Neurotop Mme 3116K polysomnograph (Nihon Kohden CA, USA). The signals were digitized and computer stored using Icelus® data collection software (Mark Opp, Ann Arbor, MI, USA) for subsequent off-line analysis. The length of the scoring segment was 12 s. The time spent in W, NREMs, and REMs during the 24 h recording was calculated in periods of 1, 4 and 12 h. Latency to REMs (time to first REMs epoch from first NREMs epoch) was evaluated. Frequency and mean duration of bouts (in min) during the dark and light part of the cycle were also calculated. Recordings started at the beginning of the dark period and lasted 24 h.

2.4. Elevated plus maze (EPM)

The EPM is a validated animal model of anxiety (Pellow et al., 1985). It consists of a 4-arm maze; each arm is 40 cm length × 12 cm width and 90 cm high. The 4 arms intersect (center section 12 cm × 12 cm) and form the shape of a plus sign (+); two of the arms are open and 30 cm high walls enclose the other two. An animal behavior video tracking system was used to record the animals during the test. Recordings were analyzed off-line (OMNIALVA®, Acolman, Estado de México, Mexico). The time spent in either the open or enclosed arms of the maze was estimated. The test was carried out at the beginning of dark period of the light–dark cycle, in a behavioral testing room with lights on.

Five minutes after receiving treatment (DMSO, 0.5 μl; AEA, 0.5 μg/0.5 μl or AM251 1 μg/0.5 μl, n = 10 per group) each rat was placed in the center of the maze facing an open arm and allowed to freely explore for 4 min. Rats were considered within an arm when their four paws were within the arm. After each rat exposure, the maze was cleaned with a 5% bleach solution.

2.5. Forced swim test (FST)

The FST is a test of behavioral despair used to evaluate the antidepressant activity of compounds (Porsolt et al., 1977). Rats were placed in a Plexiglas cylinder (Measurements: diameter 25 cm and height 45 cm), filled with water (23–25 °C) to a depth of 40 cm to prevent the animals from supporting themselves with their hind paws. The FST procedure consisted of a 15 min pre-test followed 24 h later by a 5 min test. After the session, rats were removed from the apparatus and immediately dried using paper towels. The test was recorded for off-line evaluation.

24 h after the pretest, rats received a treatment of either: DMSO, 0.5 μl; AEA, 0.5 μg/0.5 μl or AM251 1 μg/0.5 μl (n = 5 per group), and 5 min later were placed in the Plexiglas cylinder for 5 min. The animal behavior video tracking system (OMNIALVA® Acolman, Estado de México, Mexico) was also used to record the test and provide information for off-line analysis. The time spent in swimming (i.e. moving around in the cylinder), climbing (i.e. actively trying to climb the