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Infusion of modafinil into anterior hypothalamus or pedunculopontine tegmental nucleus at different time-points enhances waking and blocks the expression of recovery sleep in rats after sleep deprivation

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ABSTRACT

Clinical studies have indicated that the primary pharmacological activity of modafinil (MOD) is inducing wakefulness; however, the brain targets that underlie its wake-promoting activity have not been described. In the present study, we show that MOD injected into sleep–wake related brain areas promoted alertness. If administered (10, 20, or 30 μg/1 μL) into either anterior hypothalamus (AH) or pedunculopontine tegmental nucleus (PPTg) at 08:00, 12:00 or 16:00 h, MOD enhanced wakefulness whereas diminished slow wave sleep as well as rapid eye movement sleep. In addition, microinjection of MOD (10, 20, or 30 μg/1 μL) either into AH or PPTg after total sleep deprivation prevented the sleep rebound. Taken together, these observations suggest that AH and PPTg play a key role in the wake-inducing effects of MOD and encourage further experimentation to draw a possible mechanism of action.

Introduction

Modafinil (2-[(diphenylmethyl) sulfinyl] acetamide, Provigil; MOD) is approved by the Federal Drug Administration in the United States of America for the treatment of hypersomnolence-associated narcolepsy (Bastuji and Jouvet, 1988; Boutrel and Koob, 2004; Littner et al., 2001; Losier and Semba, 1993). The mechanism of action of MOD on sleep is still unclear, even though several studies have indicated that wake-related neurotransmitters could be involved in the effects induced by MOD, such as serotoninergic (el Mansari et al., 1989; Ferraro et al., 1998), glutamatergic (Ferraro et al., 2002), histaminergic (Ishizuka et al., 2003), and dopaminergic (Minzenberg and Carter, 2008; Murillo-Rodríguez et al., 2008) system. In this regard, our group has demonstrated that a single intracerebroventricular (i.c.v.) injection of MOD (10 μg/5 μL) in rats was able to increase the extracellular levels of dopamine (DA; Murillo-Rodríguez et al., 2007). Similar results have been reported if administered (3.0–10 mg/kg, i.v.) to rhesus monkeys (Andersen et al., 2010).

It is interesting to note that the potential existence of a molecular pathway involved in MOD's effects on sleep has been also studied. In this regard, Petit et al. (2010) showed that expression of genes encoding glucose transporters is enhanced with MOD whereas *c*-Fos immunohistochemistry studies have demonstrated that wake-related brain areas respond to this drug (Lin et al., 1996; Scammell et al., 2000), including anterior hypothalamus (AH) and pedunculopontine tegmental nuclei (PPTg; Lin et al., 1996; Engber et al., 1998; Murillo-Rodríguez et al., 2008). Research evidence indicates that these brain nuclei, AH and PPTg, modulate alertness (el Mansari et al., 1989; Steriade et al., 1990; Szymusiak et al., 1998; Saper et al., 2001; Datta and Siwek, 2002; Kayama and Koyama, 2003; Szymusiak and McGinty, 2008; Murillo-Rodríguez et al., 2009). Thus, we hypothesized that MOD would induce wakefulness (W) if injected into AH or PPTg. The aim of the present study was to determine the effects of microinjections of this compound directly into either AH or PPTg at different time-points of the lights-on cycle (08:00, 12:00 or 16:00 h)

Abbreviations: AH, anterior hypothalamus; DA, dopamine; EEG, electroencephalogram; EMG, electromyogram; MOD, modafinil; PPTg, pedunculopontine tegmental nucleus; REMS, rapid eye movement sleep; SWS, slow wave sleep; TSD, total sleep deprivation; VEH, vehicle.

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and also that MOD would be able to counteract the sleep rebound after total sleep deprivation.

Materials and methods

Subjects

Experiments were performed following the guidelines on the Ethical Use of Animals from the Mexican Institutes of Health Research (DOF. NOM-062-Z00-1999) as well as the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 80-23, revised 1996) and approved by the Committee on the Ethics of Animal Experiments of the Universidad Anáhuac Mayab. All efforts were made to minimize animal stress and suffering. Sixty male Wistar rats (250–300 g) were housed at constant temperature (21 ± 1 °C) and under a controlled light–dark cycle (lights on: 07:00–19:00 h) in cages with sawdust bedding. Purina Rat Chow and tap water were available ad libitum.

Surgery

All surgical procedures were followed as previously reported (Murillo-Rodríguez et al., 2007, 2008). Briefly, using aseptic conditions and under deep anesthesia (cocktail of acepromazine [0.75 mg/kg], xylazine [2.5 mg/kg], and ketamine [22 mg/kg, i.p.]), animals were placed for surgeries into a stereotaxic frame (David Kopf Instruments; Tujunga, CA, USA). Microelectrodes (Plastics One; Roanoke, VA, USA) were implanted to record electroencephalographic activity (EEG) which consisted of 4 miniature screws with leads in contact with the dura. Two were placed on either side of the sagittal sinus over the occipital cortex through holes in the skull ($L = \pm 2.0$, $A = 6.0$). The remaining 2 electrodes were placed on either side of the sagittal sinus over the frontal cortex ($L = \pm 2.00$, $A = +3.00$). For the electromyogram (EMG) signal, 2 flexible wire electrodes were inserted bilaterally into the nuchal muscles of the rats. Additionally, animals were implanted with a unilateral 23-gauge guide cannula positioned into either AH ($A = -1.3$ mm; $L = +0.6$ mm; $H = -8.2$ mm) or PPTg ($A = -8.0$ mm; $L = +2.0$ mm; $H = -7.0$ mm). All stereotaxic coordinates were referred to Bregma (Paxinos and Watson, 2005). The electrodes and cannulae were anchored to the skull using dental cement and surgical site was swabbed with a lidocaine (5%; topical). At the end of the surgeries all rats received a pain relief (Buprenorphine [0.1 mg/kg, i.m.]) as well as amoxicillin (100 mg/kg, s.c.) to prevent infection. Right after surgical procedures, animals were placed in the sleep chambers for recovery and they were attached to the electroencephalographic recording leads for habituation to the handling associated with the microinjection procedure. This method consisted of wrapping the rat in a towel, placing it on the experimenter's lap for 5 min, replacing the stylet and inserting an injector into the guide cannula. After this, the stylet was replaced and rat was re-attached to sleep-recording cables inside the chamber. This procedure was done during 7 consecutive post-surgery days at the same time of the lights-on period. In previous studies, we noticed that this method reduces the amount of fecal droppings and urination, suggesting that this procedure reduces animal stress and, therefore, facilitates the manipulation of animals for administration of the treatments. Throughout experiments, rats had food and water available ad libitum.

Drugs

MOD (Cephalon, Inc. West Chester, PA, USA) was dissolved in vehicle (VEH: PEG/saline; 5:95 v/v). The doses of MOD tested are within the range of doses used in a plethora of functional studies (Murillo-Rodríguez et al., 2007, 2008) and which most likely result in a substantial increase in waking. All other compounds were obtained from Sigma Chemicals (St. Louis, MO, USA).

Pharmacological administrations

One week after surgeries, and 24 h before the experiment began, stylets were removed from guide-cannulae, and the injector was inserted and connected to a 10 or 50 μL Hamilton microsyringe driven by an automatic pump (CMA 400; flow rate: 1 μL/min) providing a total infusion volume of 1 μL.

Experiment 1 consisted of infusion of VEH ($n = 6$) or MOD (10, 20, or 30 μg/1 μL; $n = 6$ each dose group) into either AH or PPTg. All administrations were carried out at 08:00, 12:00 or 16:00 h. To determine whether composition of VEH would modify the sleep–wake cycle of the rats, an additional group was added that consisted of the withdrawal of the guide-cannula and placing the injector into the cannula without any administration (sham, $n = 6$). Immediately after the trials, animals were re-attached to the sleep-recording system.

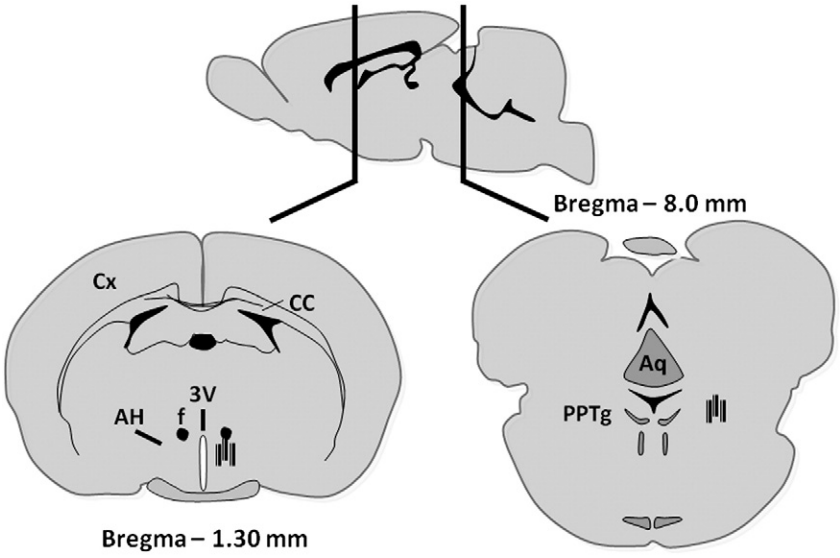

Fig. 1. Schematic representation of the cannulae localization into anterior hypothalamus or placed into the pedunculopontine tegmental nucleus (as indicated by the vertical black bars). Stereotaxic coordinates, drawings and abbreviations were taken from the Paxinos and Watson (2005) atlas. Abbreviations: 3V: third ventricle; AH: anterior hypothalamus; CC: corpus callosum; Cx: cerebral cortex; f: fornix; PPTg: pedunculopontine tegmental nucleus.