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Modafinil enhances extracellular levels of dopamine in the nucleus accumbens and increases wakefulness in rats

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Abstract

Modafinil (MOD) is a wake-promoting drug that improves the alertness levels in narcolepsy; however, the molecular mechanism of action remains to be elucidated. We found that after a single icv injection of MOD (10\textmu g/5\mu l) the extracellular levels of dopamine (DA) and l-DOPA collected from the nucleus accumbens were increased and decreased, respectively. Separately, the icv administration of MOD (10\textmu g/5\mu l) to rats enhanced wakefulness (W) whereas diminished sleep during 4 h. Lastly, the alertness induced by MOD was partially antagonized by the sleep-inducing endocannabinoid anandamide (ANA). We conclude that MOD enhances the extracellular levels of DA, promotes W and its effects on sleep are partially blocked by ANA.

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Modafinil (MOD) is a wake-promoting medication for the treatment of excessive daytime sleepiness in narcoleptic patients [2,12]. It has been shown that MOD indeed induces and prolongs wakefulness (W) in several species [1,6,10,11,21].

But what might be the mechanism of action of MOD? Some studies have suggested the interaction between MOD and the dopaminergic system. For instance, Madras et al. have reported that MOD binds to dopamine (DA) transporters [13]. Additionally, it has been reported that alertness response to this drug is not observed in DA transporter-deficient mice [22]. Recently the same authors showed that the wake-inducing properties of MOD were blocked by the DA agonist quinpirole [23]. There is evidence suggesting that the wakefulness (W) caused by MOD might be a result of the enhancement in the release of DA [3,5]. Here we decided to analyze the effects of MOD on sleep. Finally, the molecular structure of MOD indicates that the acetamide moieties of this drug are linked by the hydrogen bonds as well as by contacts via the amide group [8]. Although there is no evidence of the interaction between MOD and the CB\textsubscript{1} cannabinoid receptor, we hypothesized that MOD could be binding with the cannabinoid receptor, since the endocannabinoid anandamide (ANA) does it via the amide bond [4]. For the last experiment, we tested whether the sleep-inducing endocannabinoid ANA [14,15,17] might block the waking effect caused by MOD.

Wistar male rats (n=46; 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). Food and water were provided ad libitum. For the microdialysis experiments, a group of rats (n=16) was anesthetized (acepromazine [0.75 mg/kg], xylazine [2.5 mg/kg], and ketamine [22 mg/kg], i.p.) and a guide-cannula (IC guide. BioAnalytical Systems [BAS], West Lafayette, IN, USA) was placed unilaterally and stereotaxically into the nucleus accumbens, core (AcbC; target coordinates: A = +1.2; L = 2.0; H = −7.0; [19]) as well as a cannula (23 gauge) was placed into one lateral ventricle (n=10; A = −0.8; L = −1.6; H = −3.6; [19]). The cannulaes were then fixed onto the skull with a thin layer of glue.
After the surgery, each animal was placed into the microdialysis bowl to habituate them to the experimental conditions. One week after the surgery and a day before the experiment, the microdialysis probe (1 mm of length. Polyacrylonitrile, MWCO = 30,000 Da; 340 µm o.d.; BAS) was inserted through the guide cannula into the target structure at 7:00 h and the tissue was allowed to stabilize for 24 h. During this period artificial cerebrospinal fluid (ACSF; composition: NaCl (147 mM), KCl (3 mM), CaCl₂ (1.2 mM), MgCl₂ (1.0 mM), pH 7.2) was continuously perfused through a FEP Teflon Tubing (0.65 mm o.d. × 0.12 mm i.d.) using a 2.5 ml gastight syringe. All procedure has been previously reported in Murillo-Rodríguez et al. (2003, 2006). The syringe Pump (CMA/100) controlled the perfusion speed of the ACSF (flow rate: 1 µl/min). For the experimental day, animals received at 07:00 h either vehicle (control group; n = 8) or MOD (10 µg/5 µl; n = 8). We collected every hour the dialysates during a total time of 4 h after injections across the lights-on phase. Immediately after sample collection from microdialysis study, all samples were injected into a HPLC (Gilson) for DA analysis as reported by our group previously [16]. A different group of rats (n = 10) were used for the sleep studies. Animals were anesthetized as described above and sleep electrodes were implanted as well as a icv cannulae. All electrodes and cannulae were placed and secured onto the skull using dental cement as previously reported by our group [15–17]. After the surgeries, animals were placed into the sleep-recording chambers for their post-surgery recovery (7 days). The rats received an injection of either vehicle (control group, n = 5) or MOD (10 µg/5 µl; n = 5). In the last experiment, an additional group of rats (n = 20) were implanted with electrodes for sleep studies as well as an icv cannulae as mentioned previously. Control group (n = 5) received vehicle. The following groups received either ANA (10 µg/5 µl; n = 5) or MOD (10 µg/5 µl; n = 5). The last group (n = 5) received ANA (10 µg/2.5 µl) and 15 min later MOD (10 µg/2.5 µl). MOD (Cephalon) and ANA (Sigma, USA) were dissolved in a vehicle (PEG/saline; 5:95 v/v). Sincere there is no available data about icv doses of MOD, we arbitrary decided to use the doses of 10 µg/5 µl. For the administration of ANA, the dose was chosen based in our previous reports [15,17]. All injections were given at 07:00 h and the administrations were done slowly over 1 µl/min with the injector left in the target for an additional 15 s to ensure extrusion from the tip and to minimize distribution of treatments upwards on the cannulae. After injections, the cannula was withdrawn and the stylet replaced. Right after the microinjections, the animals were attached to the sleep-recording system and we decided to collect and analyze the EEG/EMG data obtained from 4 h of recordings. All experiments were conducted with food and water ad libitum. The EEG/EMG data recordings were scored manually for epochs of W, SWS and rapid eye movement sleep (REMS) as previously described [15–17]. All studies were conducted in accordance with the principles and procedures described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The data are presented as means and standard errors. Student t-test was used to compare control and MOD groups in the microdialysis and sleep studies and a p-value < 0.05 was considered statistically significant. In the last experiment, statistical analysis was carried out by one-way analysis of variance (ANOVA), followed by the Sheffé test as a post-hoc test (STATVIEW, p < 0.05).

In experiment 1, we measured the levels of DA and t-DOPA after MOD injection over 4 h. Results showed that MOD-treated rats (10 µg/5 µl; icv) compared with the control group, displayed a significant enhancement in the levels of DA (d.f. = 14; t = 2.646; p = 0.005) whereas a diminution in extracellular levels of t-DOPA (d.f. = 14; t = 0.740; p = 0.470) was also found (Fig. 1A). Hourly analysis of the effects of MOD on extracellular levels of DA showed a significant enhancement across 4 h post-injection (Fig. 1B; 1 h: d.f. = 14; t = −15.09; p = 0.001; 2 h: d.f. = 14; t = −4.983; p = 0.002; 3 h: d.f. = 14; t = −9.299; p = 0.001; 4 h: d.f. = 14; t = −7.427; p = 0.001). A decrease profile in levels of t-DOPA hourly was found after MOD injection during the same time-points (Fig. 1C; 1 h: d.f. = 14; t = 2.07;
Fig. 2. (A) Effects on total time (4 h of sleep recordings) on wakefulness (W), slow wave sleep (SWS) and rapid eye movement sleep (REMS) after icv administrations of either vehicle or modafinil (MOD 10 μg/5 μl) during the lights-on period. The effects of MOD on sleep–wake cycle are shown hour by hour for W (B), SWS (C) and REMS (D). Each point represents the mean ± S.E.M. (* vs. Control, p < 0.05).

In the following experiment, we injected icv MOD (10 μg/5 μl) or vehicle during the rat’s normal sleeping period (07:00 h, lights-on phase). MOD markedly increased the total W time (d.f. = 8; t = −9.740; p < 0.0001) and induced a significant diminution of total SWS time (d.f. = 8; t = 8.188; p < 0.0001) as well as REMS (d.f. = 8; t = 5.223; p < 0.0008) over the next 4 h (Fig. 2A). As shown in Fig. 2B, MOD enhanced W about 1 h (d.f. = 8; t = −8.591; p < 0.0001) after the administration and this effect remained over the 4 h of sleep-recordings (2 h: d.f. = 8; t = −5.637; p < 0.0005; 3 h: d.f. = 8; t = −7.748; p < 0.0002; 4 h: d.f. = 14; t = −3.11; p < 0.01). We found that MOD decreased SWS hourly (Fig. 2C) right after the first h post-injection (1 h: d.f. = 8; t = 5.977; p < 0.003) and remained inhibited during the following time points (2 h: d.f. = 8; t = 3.865; p < 0.004; 3 h: d.f. = 8; t = 6.347; p < 0.0002; 4 h: d.f. = 8; t = 2.046; p = 0.07). The hourly analysis of REMS (Fig. 2D) after MOD injection showed that this sleep stage remained diminished over 3 h post-injection (1 h: d.f. = 8; t = 2.444; p < 0.04; 2 h: d.f. = 8; t = 4.919; p < 0.001; 3 h: d.f. = 8; t = 3.248; p < 0.01; 4 h: d.f. = 8; t = 0.858; p = 0.4).

In the last experiment (Fig. 3) after 4h of sleep recordings, we found once again that MOD enhanced the total time of W (d.f. = 16; F = 31.598; p < 0.0001) and decreased total sleep time (TST; d.f. = 16; F = 70.723; p < 0.001). As found in previous experiments [14,15,17], injection of ANA increased TST and decreased waking (p < 0.0001). Interestingly, administration of ANA 15 min before MOD partially prevented the waking effect caused by the last compound (p < 0.0001) as well as the decrease...
in TST (p < 0.0001). Therefore, ANA partially prevented the increase in W and the diminution in TST, both effects caused by MOD. The hourly analysis of the effects of ANA + MOD on waking and on TST are shown in Fig. 3B and C, respectively (1 h: d.f. = 16; F = 67.565; p = 0.00010.04; 2 h: d.f. = 16; F = 24.724; p < 0.0001; 3 h: d.f. = 16; F = 25.693; p < 0.0001; 4 h: d.f. = 16; F = 2.627; p < 0.05).

Our study shows that at the dose tested of MOD (10 µg/5 µl, icv), induced an enhancement in DA levels and a diminution in l-DOPA levels collected from AcbC (Fig. 1A). The effects of MOD on contents of DA and l-DOPA were present across 4 h of sample collection (Fig. 1B and C, respectively). The findings in the present study are supported by previous studies showing that MOD increases the release of DA [3]. However, this is the very first time that effects of MOD after icv injections have been reported on DA and l-DOPA levels collected from AcbC in freely moving rats. We believe that the possible mechanism in the wake-promoting effects of MOD involves the DA system. It is known that lesions of DA cell groups induce an arousal response reduction in rats [9] as well as in Parkinson’s disease (PD) patients [18,20,21]. PD is a progressive neurological disorder characterized by the degeneration of DA neurons in the substantia nigra leading to a deficiency in the DA neurotransmission. Among the motor symptoms, PD patients show postural instability, bradykinesia, tremor and rigidity. These and other motor signs improve with l-DOPA, the precursor of DA; therefore, l-DOPA plays a crucial element in the modulation of the symptoms in PD. It seems that the enzymatic process involved in the formation of catecholamines might be under the influence of MOD. Complementary experiments testing the role of MOD on the biosynthesis of catecholamines would provide us a better understanding of the phenomena.

On the other hand, despite we tested only one dose of MOD (10 µg/5 µl), we found that the administration of this drug during the lights-on period of the rats increased the total time of W while decreased SWS and REMS (Fig. 2A). The effect on W, SWS and REMS was observed after the very first hour post-injection (Fig. 2B–D, respectively). Those results are consistent with previous pharmacological systemic studies [7,10,21] suggesting the effective role of MOD inducing W. Finally, in the last experiment (Fig. 3), we tested if the administration of ANA might be able to prevent the effects of MOD on sleep. This hypothesis was derived from the data that shows that the molecular structure of MOD possess an amide moiety [8] similar to the hypothalamus after modafinil administration: a microdialysis study in rats. Neuroreport 2001;16:3533–7.

We conclude that icv administrations of MOD to rats produced an enhancement of DA levels as well as an increase of W. Additionally, the alertness induced by MOD was partially antagonized by the sleep-inducing endocannabinoid ANA. However, we cannot rule out that the sleep-promoting effect of ANA could offset the waking-enhancing actions of MOD mediated by increased DA levels.

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