Journal of Pharmacological Sciences xxx (2018) 1–6

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

journal homepage: www.elsevier.com/locate/jphs

Full paper

Modafinil alleviates levodopa-induced excessive nighttime sleepiness and restores monoaminergic systems in a nocturnal animal model of Parkinson's disease

Rina Ando [a], Mohammed Emamussalehin Choudhury [b, *], Yuki Yamanishi [a], Win Thiri Kyaw [a], Madoka Kubo [a], Mariko Kannou [a], Noriko Nishikawa [c], Junya Tanaka [b], Masahiro Nomoto [a], Masahiro Nagai [a]

[a] Department of Neurology and Clinical Pharmacology, Ehime University Graduate School of Medicine, 454 Shitsukawa, Toon, Ehime, 791-0295, Japan
[b] Department of Molecular and Cellular Physiology, Ehime University Graduate School of Medicine, 454 Shitsukawa, Toon, Ehime, 791-0295, Japan
[c] Department of Neurology, National Center Hospital of Neurology and Psychiatry, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo, 187-8551, Japan

ARTICLE INFO

Article history:
Received 10 January 2018
Received in revised form
1 March 2018
Accepted 20 March 2018
Available online xxx

Keywords:
Parkinson's disease
Excessive day time sleepiness
Monoamines
MPTP
Modafinil
Neurorestoration
Nocturnal

ABSTRACT

Treatment with dopaminergic agents result excessive daytime sleepiness (EDS) and some studies have shown the benefit of using modafinil for treating excessive daytime sleepiness of Parkinson's disease (PD) patient. We investigated whether modafinil have ameliorative properties against levodopa induced excessive nighttime sleepiness (ENS) in MPTP-treated murine nocturnal PD model. Our EEG analyses of whole day recordings revealed that modafinil reduce ENS of this nocturnal PD models with levodopa medications. Therefore, we investigated whether, modafinil post-treatment followed by MPTP shows any effect on monoamine contents of brain and found to robustly increased noradrenaline (NA) concentration of MPTP treated mice. Modafinil post-treatment, in neurorestorative context (5 days post-lesion) led to increased striatal dopamine (DA) concentrations of MPTP-treated mice. Here, we first confirmed that modafinil ameliorates levodopa induced excessive sleepiness and restores monoaminergic systems. The arousal and anti-parkinsonian effects displayed by modafinil indicate that in combination with dopaminergic agents, modafinil co-administration may be worthwhile in trying to suppress the excessive daytime sleepiness and progressive dopaminergic neuron loss in PD.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder where dopaminergic neurons are greatly affected. Symptoms of PD encompass mainly motor disorder such as rigidity, bradykinesia, resting tremor, and impaired cooperation. The main treatment for PD is currently the replenishment of dopamine by administrating levodopa or various types of dopaminergic agonists. Epidemiological studies reported that treatment with dopaminergic agent also results in sleep disorder like sleep attack and excessive daytime sleepiness (EDS).[1–5] Looking at sleep disorder in PD, accumulating evidences suggested that diminished neural structural and functional features in patients may have influence on EDS. More specifically, arousal systems are also compromised in PD brains, including noradrenergic neuron of locus coeruleus, cholinergic neuron of pedunculo-pontine nucleus and the basal forebrain, serotonergic neuron of median raphe, and orexinergic neuron of lateral hypothalamus.[6] PD patients are commonly experiencing sleep disturbances including insomnia at night time and hypersomnia at day time. EDS has a large impact on the quality of life of PD patients as well as their caregivers.

Modafinil is a psychostimulant drug used to treat EDS experienced in narcolepsy and obstructive sleep apnea and estimated to promote wakefulness via improvement in dopaminergic transmission.[7] A randomized trial reported that modafinil is effective for treating PD patients with EDS without deterioration in motor state in PD patients.[8] Several animal model studies have been conducted

* Corresponding author. Department of Molecular and Cellular Physiology, Graduate School of Medicine, Ehime University, Toon, Ehime, 791-0295, Japan.
E-mail address: mechoudh@m.ehime-u.ac.jp (M.E. Choudhury).
Peer review under responsibility of Japanese Pharmacological Society.

https://doi.org/10.1016/j.jphs.2018.03.005

with modafinil, to clarify the mechanism of action of modafinil as an anti-parkinsonian drug.[9–17] However, most of the mechanisms of action of modafinil thus far identified relate to neuroprotection of dopaminergic neurons, we appraised whether modafinil, could reverse ongoing dopaminergic degeneration in an animal model of Parkinson's disease. Besides, we first here, showed that modafinil are effective to reduce sleepiness in active period of day light cycle using electroencephalographic means.

2. Materials and methods

2.1. Animals

C57BL/6 male mice with 10 weeks of age were used in this study. The animals were housed in an insulated and soundproof recording room that was maintained at an ambient temperature 25 ± 1 °C, illumination intensity ~100 lux and humidity 50 ± 5% and that was on an automatically controlled in cycles of 12-h light/12-h dark where lights on was set at 7:00 o'clock (ZT0) and light off was set at 19:00 o'clock (ZT12). The animals had free access to food and water. The animal maintenance and experimental protocols were approved by the Animal Experimentation Ethics Committee, Ehime University, Japan.

2.2. Drugs

Methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride (MPTP), Modafinil, levodopa and benserazide hydrochloride (Sigma, St. Louis, MO, USA) were purchased and stored at room temperature. The preparation of MPTP injection was prepared at draft chamber.

2.3. Polygraphic recordings (EEG) studies

Mice were anaesthetized using isoflurane (Wako, Osaka, Japan) and placed onto a mouse stereotactic frame (Narishige, Tokyo, Japan), a pre-manufactured head mount (Part #8201, Pinnacle Technology, Inc., Lawrence, KS) was placed on skull and fixed with two stainless steel screws (Part #8209, Pinnacle Technology) which were positioned in the frontal region (A/P: +1.0 mm, M/L ±1.5 mm) and also another two screws, which were positioned over the posterior brain (A/P: −2.0 mm, M/L ±1.5 mm). The screws were served as EEG electrodes and the dual platinum-iridium electrodes attached to head mount were inserted bilaterally into the neck/back region (nuchal) muscles for monitoring EMG activity. All electrode leads were sealed with dental acrylic resin. After surgery, the mice were allowed for one week for complete recovery and then they were treated with drugs for recording. The mice were treated with MPTP at 25 mg/kg for five consecutive days followed by levodopa (50 mg/kg) and modafinil at 100 mg/kg as illustrated by Fig. 1A. A 48-h interval was maintained in each treatment regimen recording, just to avoid sleep pressure resulted by preceding wakefulness of previous recording as described.[18] Individual whole day (ZT12/7PM to ZT11/6PM) recording was performed for each treatment. A mouse pre-amplifier unit (Part #8202, Pinnacle Technology), was firmly attached to the head mount, enabled first stage amplification (100×) and initial high-pass filtering (1st-order 0.5 Hz for EEG and 10 Hz for EMG). The signals were then routed to an 8206 conditioning/acquisition system (Pinnacle Technology) via a swivel (Part #8204, Pinnacle Technology). The signals were then sampled at 400 Hz, digitized using a 14-bit A/D converter and routed to a PC based acquisition and analysis software package via USB. The wave form recognition scoring, signal analysis and scoring were accomplished using software Sleep Sign ver. 3 (Kissei Comtec, Nagano, Japan) as described by Kohtoh and his colleagues.[19]

2.4. Postmortem sampling

After the animal experimentation has been performed as described in Fig. 1B, the mice were euthanized with CO2 and brain were dissected out, quickly put on an ice-cold glass plate. Striatum and 1 mm ventral mid brain were collected for post mortem studies. The striatum and ventral midbrain tissues containing the SN were dissected out stored at −80 °C for further analyses.

2.5. High performance liquid chromatography

The monoamines content of the striatum were measured using high performance liquid chromatography with electrochemical detection (HPLC) as previously described by our previous work.[20] In brief, right striatum sample from was homogenized with an ultrasonic cell disruptor (Tomy Seiko, Tokyo, Japan) in 0.2 M perchloric acid containing 5 mM EDTA (Wako) and 3,4-dihydroxybenzamine (Wako), and were centrifuged. 10-μL aliquots of the filtered supernatant from the homogenized striatum were injected into a HPLC apparatus with a reversed-phase column. The mobile phase consisted of 15% (v/v) methanol containing 0.1 M sodium acetate (Wako), 0.1 M citric acid (Wako) adjusted to pH 3.5 (0.1 M sodium acetate−citric acid buffer, pH3.5), 180 mg/L sodium octydyl sulfate (Wako), and 10 mM EDTA, pumped through the column at a rate of 0.25 mL/min. The reversed-phase C18 column (150-mm length x 2.1-mm internal diameter; SC-5ODS, Eicom, Kyoto, Japan) were used.

2.6. Quantitative real-time RT-PCR

The ventral midbrain tissues containing the SN were processed for qPCR as described by our previous work.[20] In brief, total RNA was extracted from tissue using Qiagen RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality and quantity of obtained RNAs were examined by NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). cDNA was synthesized from 0.5 μg RNA using ReverTra Ace qPCR RT Master Mix with gDNA remover kit (Toyobo, Osaka, Japan), following the manufacturers' protocols. The cDNA samples were diluted by 3-fold, and qPCR analysis was performed in triplicate using an MJ mini instrument (BioRad, Hercules, CA) using Fast Start Universal SYBR Green Master (Roche Diagnostic, Tokyo, Japan). PCR conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. All gene-specific mRNA expression values were normalized against Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The primer sequences for each gene are listed in Supplementary Table 1.

2.7. Cell cultures

PC12 cells were cultured on poly-L-lysine (PLL) (Wako)-coated 75 cm^2 flasks (BD Falcon, NY) in RPMI 1640 medium (Wako) supplemented with 5% fetal calf serum (Gibco, Grand Island, NY) and 10% horse serum (Gibco). Cell cultures were incubated at 37 °C in a humidified atmosphere of 95% air and 5% CO_2. Confluent PC12 cells were seeded into 6-well cell culture plates (Nunc™ Thermo Scientific, Tokyo, Japan) and grown for 7 days, then exposed to drugs for 24 h. For drug study, control medium containing 1% fetal calf serum and 1% penicillin and streptomycin was used. The

Please cite this article in press as: Ando R, et al., Modafinil alleviates levodopa-induced excessive nighttime sleepiness and restores monoaminergic systems in a nocturnal animal model of Parkinson's disease, Journal of Pharmacological Sciences (2018), https://doi.org/10.1016/j.jphs.2018.03.005