Effects of Anti-dementia Drugs on Morphine-Induced Somnolence

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The present study was undertaken to investigate the characteristics of morphine in rat sleep patterns and also the effects of donepezil and memantine on somnolence caused by morphine. Electrodes were chronically implanted into the cortex and dorsal neck muscle of rats for electroencephalogram (EEG) and electromyogram (EMG) recordings, respectively. EEG and EMG were recorded with an electroencephalograph. SleepSign ver.2.0 was used to analyse the sleep-wake state. Total times of wakefulness, non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep were measured from 10:00 to 16:00. Morphine at a high dose caused a significant decrease in sleep latency and total REM sleep time, although the drug at low doses caused significant increases in sleep latency and total awake time, and a significant decrease in NREM sleep time. Donepezil, memantine and methylphenidate antagonized the decrease in sleep latency caused by morphine. From these findings, it can be concluded that morphine caused somnolence, and donepezil and memantine are useful for somnolence caused by morphine, similar to methylphenidate.

Key words morphine; opioid; anti-dementia drug; methylphenidate; somnolence

It is well known that narcotics, such as morphine, fentanyl and oxycodone, have been used for patients with chronic pain. These narcotics cause several adverse effects; for example, somnolence, cognitive dysfunction, constipation and nausea. These adverse effects, especially somnolence, deteriorate the quality of life (QOL) of patients in the daytime.

Morphine has high affinity for μ , δ and κ receptors. On the other hand, it has been reported that opioid receptors were responsible for the sleep-wake cycle,^{1–4)} but there is little information about the effect of morphine on the sleep-wake pattern; therefore, we tried to clarify the role of opioid receptors in the sleep-wake cycle by studying the characteristics of morphine in the sleep-wake pattern.

Clinically, methylphenidate is used for the treatment of somnolence caused by opioid analgesics⁵⁾; however, drug dependence and tolerance were frequently observed during this drug therapy. On the other hand, it is recommended to use cholinesterase inhibitors for sedation or somnolence caused by narcotics in a clinical study⁶⁾; however, there is little evidence as to whether the above presumption is true or not. Donepezil is a centrally acting selective, competitive and reversible acetylcholinesterase inhibitor, widely used for the treatment of dementia. Moraes *et al.*⁷⁾ and we⁸⁾ reported that donepezil increased total times of wakefulness in human and animals, respectively. In addition, it has been reported that muscarinic and nicotinic acetylcholine receptors are responsible for the cognitive dysfunction caused by morphine in rats.^{9,10)} N-Methyl-D-aspartic acid (NMDA) receptor antagonist, memantine, is also used for the treatment of dementia, which protects against neuronal death. From these findings, we presumed that anti-dementia drugs may be effective in not only cognitive dysfunction but also somnolence caused by narcotics.

In the present study, therefore, we studied the characteristics of morphine in rat sleep patterns, and also the effects of donepezil and memantine on the somnolence caused by morphine in comparison with that of methylphenidate.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 240–320 g (Japan SLC, Shizuoka) were used. All animals were maintained in an air-conditioned room with controlled temperature ($24 \pm 2^\circ\text{C}$) and humidity ($55 \pm 15\%$). They were housed in aluminum cages with sawdust and kept under a light-dark cycle (lights on from 07:00 to 19:00). The animals were allowed free access to food and water, except during the experiments. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Surgery The animals were anesthetized with pentobarbital sodium (Nembutal[®], 35 mg/kg, i.p.; Abbott Laboratories, North Chicago, IL, U.S.A.), then fixed to a stereotaxic apparatus (SR-5N, Narishige, Tokyo). For electroencephalogram (EEG) recording, a stainless steel screw electrode (200 μm) was chronically implanted into the left frontal cortex (A: +1.0, L: –2.0) and the right occipital cortex (A: –4.0, L: +2.0) according to the atlas of Paxinos and Watson.¹¹⁾ To record the electromyogram (EMG), stainless steel wire electrodes (200 μm) were implanted into the dorsal neck muscle. The electrodes were connected to a miniature receptacle and the whole assembly was fixed to the skull with dental cement. At least 7 d were allowed for recovery from surgery.

EEG and EMG Recordings EEG and EMG were recorded with an electroencephalograph (Model EEG 4314, Nihon Kohden, Tokyo) for 6 h (10:00–16:00). The recording was carried out according to the method described previously.^{12,13)} The signals were amplified and filtered (EEG, 0.5–30 Hz; EMG, 16–60 Hz), digitized at a sampling rate of 128 Hz, and recorded using the data acquisition program SleepSign ver.2.0 (Kissei Comtec, Nagano). EEG and EMG of the rat were measured in a cylindrical plastic cage (diameter, 26 cm; height, 31 cm) with sawdust. The observation cage was placed in a soundproof and electrically shielded box (70×60×60 cm).

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Sleep-Wake State Analysis The sleep-wake states were automatically classified by 10-s epochs as wake and non-REM sleep by SleepSign ver.2.0, according to the criteria previously described.^{14,15} As a final step, defined sleep-wake stages were examined visually, and corrected, if necessary. Each state was characterized as follows: awake, low-amplitude EEG and high-voltage EMG activities; non-rapid eye movement (NREM) sleep, high-amplitude slow or spindle EEG and low-EMG activities; REM sleep, low-voltage EEG and EMG activities (Fig. 1). Sleep latency was defined as the time from the start of the experiment up to the first 12 consecutive 10-s epochs of sleep.

Drugs The following drugs were used: morphine hydrochloride (Takeda, Tokyo), donepezil hydrochloride (Ari-cept®; Eisai, Tokyo), memantine hydrochloride (Sigma, St. Louis, MO, U.S.A.) and methylphenidate (Ritalin®; Novartis Pharma K.K., Tokyo). Morphine was dissolved in distilled water and subcutaneously injected at 10:00, and EEG and EMG were measured for 6 h after drug administration. Donepezil, memantine and methylphenidate were suspended in 0.5% carboxymethylcellulose solution and administered orally at 9:30. Eight rats were used in each group and a counterbalanced design for drug dosage was used. Drugs were administered at intervals of 7 d when the same rats were used for repeated experiments.

Data Analysis and Statistics Values shown are the means $\pm$ S.E.M. Student's *t*-test was used for statistical analysis to estimate drug effects. A *p*-value <0.05 was considered significant.

RESULTS

Effect of Morphine on the Sleep-Wake Pattern Morphine at doses of 0.3 and 1 mg/kg caused a significant increase in sleep latency, and the drug at a dose of 1 mg/kg caused a significant increase in total awake time and a significant decrease in total NREM sleep time. On the other hand, morphine at a dose of 10 mg/kg caused a significant decrease in sleep latency and total REM sleep time (Fig. 2).

Effect of Morphine on Hourly Awake, NREM and REM Sleep Times Morphine at low doses caused a significant increase in hourly awake time and a significant decrease in hourly NREM sleep time (0.3 mg/kg, 0–1 and 1–2 h; 1 mg/kg, 1–2 and 2–3 h; 3 mg/kg, 2–3 and 3–4 h). On the other hand, morphine at a dose of 10 mg/kg caused a significant decrease in hourly awake time and a significant increase in hourly NREM sleep time at 0–1 and 1–2 h, but an opposite effect was observed at 3–4 and 4–5 h. In addition, morphine at doses of 0.3, 1, 3 and 10 mg/kg caused a significant decrease in hourly REM sleep time (Table 1).

Effects of Donepezil, Memantine and Methylphenidate on the Sleep-Wake Pattern Donepezil at a dose of 3 mg/kg, memantine at a dose of 10 mg/kg and methylphenidate at a dose of 10 mg/kg significantly increased sleep latency and total awake time, and decreased total NREM sleep time. In addition, memantine and methylphenidate significantly decreased total REM sleep time (Table 2).

Effects of Donepezil, Memantine and Methylphenidate on Somnolence Caused by Morphine Donepezil at a dose of 3 mg/kg, memantine at a dose of 10 mg/kg and methylphenidate at a dose of 10 mg/kg significantly antago-

Fig. 1. Representative EEG, EMG and EEG Power Value in Rats during Wakefulness, NREM Sleep and REM Sleep

Fig. 2. Effect of Morphine on the Sleep-Wake Pattern

Columns and vertical bars represent the mean $\pm$ S.E.M. ($n=8$). Morphine was subcutaneously injected. **, *Significantly different from the control group at $p<0.05$ and $p<0.01$, respectively.

nized the decrease in sleep latency caused by morphine at a dose of 10 mg/kg. In addition, donepezil, memantine and methylphenidate caused an increase in total awake time, and a decrease in total NREM sleep time. We measured hourly awake time to investigate the potency of the drugs in detail. Donepezil and methylphenidate significantly suppressed the

decrease in hourly awake and NREM sleep time at 0—1 and 1—2 h. In memantine, a significant suppressive effect was observed in hourly awake and NREM sleep time at 0—1 h (Table 3).

Fig. 3. Effects of Donepezil, Memantine and Methylphenidate on Somnolence Caused by Morphine

Columns and vertical bars represent the mean $\pm$ S.E.M. ($n=8$). Drugs were administered orally 30 min before morphine subcutaneous injection. *,** Significantly different from the morphine (10 mg/kg) administration group at $p<0.05$ and $p<0.01$, respectively.

DISCUSSION

Morphine showed a biphasic effect on the sleep-wake pattern. That is, morphine at low doses caused significant increases in sleep latency and total awake time, and a decrease in total NREM sleep time; however, at a high dose caused significant decreases in sleep latency and total REM sleep time. There are some reports that opioid receptors are involved in sleep-wake regulation; for instance, μ and κ receptor agonists caused an increase in NREM sleep time.^{1,2,16)} Morphine is a representative μ receptor agonist and also has agonistic effects on δ and κ opioid receptors. From these findings, it is reasonable to presume that morphine caused hypnotic effect *via* μ and κ opioid receptors. In addition, Li and van den Pol¹⁷⁾ reported that the sedative effect induced by narcotics is attributable to an inhibition of the orexin arousal system *via* μ receptors. On the other hand, Watson *et al.*¹⁸⁾ found that an increase in wakefulness time induced by a low dose of morphine is produced through the decreasing GABAergic transmission; therefore, the wakefulness-promoting effect induced by a low dose of morphine may be due to an inhibition of the GABAergic system. In addition, it is known that morphine *via* opioid receptors exerts anxiolytic effects, probably by interacting with the dopaminergic system by suppressing GABA inhibitory input to dopamine neurons, thereby augmenting dopamine release.^{19–22)} As shown in the results, morphine at a high dose caused a decrease in REM sleep time. It is well known that cholinergic system is involved in REM sleep attenuation. It has been reported that the amnesia induced by morphine was significantly antagonized by cholinesterase inhibitor physostigmine; therefore, it seems likely that anti-cholinergic effect of morphine is responsible for REM sleep decreasing effect.^{8,23)} Next, we studied the hourly awake, NREM and REM sleep times after the administration of narcotics to investigate the effect on the sleep-wake pattern in detail. As shown in the results, morphine at low doses (0.3, 1 mg/kg) caused an increase in the hourly awake time. On the other hand, morphine at a high

Table 1. Effect of Morphine on Hourly Awake, NREM and REM Sleep Times

Morphine (mg/kg, s.c.)	Time (min)					
	0—1 h	1—2 h	2—3 h	3—4 h	4—5 h	5—6 h
Wake						
Control	39.0 $\pm$ 2.3	29.6 $\pm$ 2.3	26.3 $\pm$ 0.7	14.2 $\pm$ 1.5	14.5 $\pm$ 1.5	15.6 $\pm$ 1.7
0.3	48.3 $\pm$ 2.9*	44.1 $\pm$ 3.1**	26.0 $\pm$ 2.2	14.8 $\pm$ 2.6	15.6 $\pm$ 2.5	19.3 $\pm$ 3.8
1	48.0 $\pm$ 2.9	50.5 $\pm$ 2.2**	33.2 $\pm$ 1.6**	18.4 $\pm$ 2.6	17.3 $\pm$ 3.6	16.1 $\pm$ 2.4
3	26.4 $\pm$ 5.9	37.3 $\pm$ 3.8	42.5 $\pm$ 2.8**	23.2 $\pm$ 1.6**	13.8 $\pm$ 2.1	17.1 $\pm$ 2.7
10	19.4 $\pm$ 1.6**	7.4 $\pm$ 1.7**	26.7 $\pm$ 2.6	47.6 $\pm$ 2.8**	34.4 $\pm$ 2.1**	20.3 $\pm$ 3.1
NREM sleep						
Control	18.8 $\pm$ 6.2	26.6 $\pm$ 2.0	28.5 $\pm$ 0.7	37.6 $\pm$ 1.1	36.4 $\pm$ 1.3	34.4 $\pm$ 1.1
0.3	10.1 $\pm$ 2.6*	14.3 $\pm$ 3.0**	30.4 $\pm$ 2.4	38.3 $\pm$ 2.4	37.0 $\pm$ 2.1	33.4 $\pm$ 3.0
1	10.4 $\pm$ 2.2*	8.6 $\pm$ 1.9**	23.3 $\pm$ 1.6*	35.8 $\pm$ 1.9	35.0 $\pm$ 2.6	34.6 $\pm$ 1.4
3	31.5 $\pm$ 5.5	20.7 $\pm$ 3.2	15.3 $\pm$ 2.4**	32.6 $\pm$ 1.5*	36.9 $\pm$ 0.8	34.4 $\pm$ 1.9
10	38.6 $\pm$ 1.6**	50.4 $\pm$ 2.0**	31.5 $\pm$ 2.7	11.8 $\pm$ 2.8**	23.1 $\pm$ 1.7**	33.5 $\pm$ 2.2
REM sleep						
Control	2.2 $\pm$ 0.6	3.8 $\pm$ 0.6	5.2 $\pm$ 0.5	8.2 $\pm$ 0.8	9.1 $\pm$ 0.8	10.0 $\pm$ 1.0
0.3	1.6 $\pm$ 0.5	1.6 $\pm$ 0.3**	3.6 $\pm$ 0.6	6.9 $\pm$ 1.1	7.4 $\pm$ 1.4	7.4 $\pm$ 1.3
1	1.5 $\pm$ 0.8	0.9 $\pm$ 0.3**	3.5 $\pm$ 1.0	5.8 $\pm$ 1.1	7.7 $\pm$ 1.4	9.3 $\pm$ 1.8
3	2.2 $\pm$ 0.5	2.1 $\pm$ 0.9	2.3 $\pm$ 0.9**	4.2 $\pm$ 0.5**	9.3 $\pm$ 1.5	8.6 $\pm$ 1.6
10	2.0 $\pm$ 0.6	2.2 $\pm$ 0.7	1.8 $\pm$ 0.4**	0.6 $\pm$ 0.2**	2.4 $\pm$ 0.6**	6.1 $\pm$ 1.3*

Each value represents the mean $\pm$ S.E.M. of 8 rats. Morphine was subcutaneously injected. ** Significantly different from the control group at $p<0.05$ and $p<0.01$, respectively (Student's *t*-test).

Table 2. Effects of Donepezil, Memantine and Methylphenidate on the Sleep-Wake Pattern

Sleep parameters	Time (min)			
	Control	Donepezil	Memantine	Methylphenidate
Sleep latency	60.9±13.1	139.8±19.8**	136.1±18.7**	130.9±10.9**
Awake	162.1±13.7	200.1±10.9**	207.5±12.1**	201.1±7.8**
NREM sleep	172.9±11.8	138.0±8.0**	139.6±10.1**	143.8±8.5**
REM sleep	25.0±3.9	21.9±4.7	12.9±3.2**	14.9±1.7**

Each value represents the mean±S.E.M. of 8 rats. Drugs were orally administered. ** Significantly different from the control group at $p<0.01$ (Student's *t*-test).

Table 3. Effects of Donepezil, Memantine and Methylphenidate on Hourly Awake and NREM Sleep Times Caused in Morphine-Treated Rats

	Time (min)	
	0—1 h	1—2 h
Wake		
Control	39.0±2.3	29.6±2.3
Morphine (10 mg/kg, s.c.)	13.5±1.6	2.6±1.0
Morphine+donepezil (3 mg/kg, <i>p.o.</i>)	22.5±3.0*	10.5±3.2*
Morphine+memantine (10 mg/kg, <i>p.o.</i>)	30.0±2.8**	4.8±1.1
Morphine+methylphenidate (10 mg/kg, <i>p.o.</i>)	30.7±3.1**	15.0±1.5**
NREM sleep		
Control	18.8±6.2	26.6±2.0
Morphine (10 mg/kg, s.c.)	43.8±1.5	55.8±1.3
Morphine+donepezil (3 mg/kg, <i>p.o.</i>)	34.8±3.1*	46.9±3.6*
Morphine+memantine (10 mg/kg, <i>p.o.</i>)	28.9±2.9**	53.8±1.3
Morphine+methylphenidate (10 mg/kg, <i>p.o.</i>)	26.7±2.7**	40.6±1.1**

Each value represents the mean±S.E.M. of 8 rats. *,** Significantly different from the morphine treated group at $p<0.05$ and $p<0.01$, respectively (Student's *t*-test).

dose (10 mg/kg) caused a decrease in the hourly awake time at 0—2 h, but an increase in the hourly awake time was observed at 4—6 h. As for NREM and REM sleep times, we sensitively detected the arousal and hypnotic effect of morphine. From these results, it is evident that morphine at low blood levels showed a wakefulness-promoting effect, whereas at a high blood level it caused somnolence.

As shown in the results, donepezil, memantine and methylphenidate suppressed the decrease in sleep latency caused by morphine. Almost the same results were observed in hourly awake time at 0—1 and 1—2 h. From these findings, it became apparent that donepezil and memantine are effective against the somnolence caused by morphine, similar to methylphenidate. It is considered that donepezil, memantine exerted wakefulness-promoting effect *via* cholinergic and dopaminergic system, respectively.⁸⁾ It has been reported that morphine at doses of 2.5 and 5 mg/kg impaired passive avoidance response, and both physostigmine and mecamylamine reversed morphine-induced impairment of passive avoidance response.^{9,10)} Therefore, we believed that the anti-dementia drugs used in the present study are effective against not only cognitive dysfunction but also somnolence caused

by morphine.

In conclusion, it was clarified that morphine caused somnolence. In addition, donepezil and memantine are useful against the somnolence caused by morphine, similar to methylphenidate.

REFERENCES

- Greco M. A., Fuller P. M., Zhou T. C., Martin-Schild S., Zadina J. E., Hu Z., Shiromani P., Lu J., *Brain Res.*, **1245**, 96—107 (2008).
- Alvira-Butero M. X., Garzon M., *Brain Res.*, **1123**, 101—111 (2006).
- Netz J., Medert H. A., Arndt J. O., *Psychopharmacology* (Berlin), **90**, 263—267 (1986).
- Walters A. S., *Sleep Med.*, **3**, 301—304 (2002).
- Bruera E., Fainsinger R., MacEachern T., Hanson J., *Pain*, **50**, 75—77 (1992).
- Slatkin N. E., Rhiner M., *J. Support Oncol.*, **1**, 53—63 (2003).
- Moraes W. S., Poares D. R., Guileminault C., Ramos L. R., Bertolucci P.H., Tufik S. D., *Sleep*, **29**, 199—205 (2006).
- Ishida T., Kamei C., *J. Pharmacol. Sci.*, **109**, 449—455 (2009).
- Darbandi N., Rezayof A., Zarrindast M. R., *Physiol. Behav.*, **94**, 604—610 (2008).
- Rezayof A., Darbandi N., Zarrindast M. R., *Neurobiol. Learn. Mem.*, **90**, 255—260 (2008).
- Paxinos G., Watson C., "The Rat Brain in Stereotaxic Coordinates," 6th ed., Academic Press, San Diego, 2007.
- Shinomiya K., Inoue T., Utsu Y., Tokunaga S., Masuoka T., Ohmori A., Kamei C., *Psychopharmacology*, **180**, 564—569 (2005).
- Takeda Y., Ishida T., Tsutsui R., Toide K., Tanimoto-Mori S., Watanabe S., Kanai Y., Kamei C., *J. Pharmacol. Sci.*, **107**, 246—250 (2008).
- Hirase M., Ishida T., Kamei C., *Eur. J. Pharmacol.*, **597**, 46—50 (2008).
- Tokunaga S., Tsutsui R., Obara Y., Ishida T., *Biol. Pharm. Bull.*, **32**, 51—54 (2009).
- McNamara P., *Palliat. Med.*, **16**, 425—434 (2002).
- Li Y., van den Pol A. N., *J. Neurosci.*, **28**, 2814—2819 (2008).
- Watson C. J., Lydic R., Baghdoyan H. A., *Neuroscience*, **144**, 375—386 (2007).
- Sasaki K., Fan L. W., Tien L. T., Ma T., Loh H. H., Ho I. K., *Brain Res. Bull.*, **57**, 689—694 (2002).
- Kabuto H., Yokoi I., Iwaya K., Mori A., *Life Sci.*, **56**, 1741—1748 (1995).
- Johnson S. W., North R. A., *J. Neurosci.*, **12**, 483—488 (1992).
- Rezayof A., Hosseini S. S., Zarrindast M. R., *Behav. Brain Res.*, **202**, 171—178 (2009).
- Zarrindast M. R., Farahmandfar M., Rostami P., Rezayof A., *J. Psychopharmacol.*, **20**, 59—66 (2006).