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RAPID REPORT

ADENOSINE AND THE HOMEOSTATIC CONTROL OF SLEEP: EFFECTS OF A1 RECEPTOR BLOCKADE IN THE PERIFORNICAL LATERAL HYPOTHALAMUS ON SLEEP–WAKEFULNESS

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Abstract—The orexinergic neurons of the lateral hypothalamus (LH) are critical for wakefulness [McCarley RW (2007) Neurobiology of REM and NREM sleep. Sleep Med 8:302–330]. Recent evidence suggests that adenosine (AD), a homeostatic sleep factor, may act via A1 receptor (A1R) to control orexinergic activity and regulate sleep–wakefulness [Thakkar MM, Winston S, McCarley RW (2002) Orexin neurons of the hypothalamus express adenosine A1 receptors. Brain Res 944:190–194; Liu ZW, Gao XB (2006) Adenosine inhibits activity of hypocretin/orexin neurons via A1 receptor in the lateral hypothalamus: a possible sleep-promoting effect. J Neurophysiol]. To evaluate the role of AD in the orexinergic LH and its influences on sleep–wakefulness, we designed two experiments in freely behaving rats: First, we bilaterally microinjected 1,3-dipropyl-8-phenylxanthine (DPX) (1.5 pmol and 15 pmol), a selective A1R antagonist into the LH during the light cycle and examined its effect on spontaneous sleep–wakefulness. Second, we performed 6 h of sleep deprivation. Thirty minutes before the animals were allowed to enter recovery sleep, 15 pmol of DPX was bilaterally microinjected into the LH and its effects on recovery sleep were monitored.

Microinjection of DPX into the orexinergic LH produced a significant increase in wakefulness with a concomitant reduction in sleep, both during spontaneous bouts of sleep–wakefulness and during recovery sleep. Local administration of DPX into the LH produced a significant increase in the latency to non-REM sleep during recovery sleep. However, total slow wave (delta) activity during non-REM sleep phase of recovery sleep remained unaffected after DPX treatment.

This is the first study that implicates endogenous adenosine to have a functional role in controlling orexinergic tone and influencing the homeostatic regulation of sleep–wakefulness. **© 2008 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: orexin, perifornical-lateral hypothalamus, sleep, adenosine, A1 receptor.

The orexinergic zone of the perifornical lateral hypothalamus (LH) contains several neuronal subtypes including the orexin-containing and the melanin concentrating hormone containing neurons. However, there is strong and consistent evidence implicating only the orexin neurons in the regulation of wakefulness (Selbach and Haas, 2006; Datta and Maclean, 2007). While deficiency of orexinergic neurotransmission resulted in a reduced wakefulness and narcolepsy (Lin et al., 1999; Chen et al., 2006; Gerashchenko et al., 2001; Chemelli et al., 1999; Thakkar et al., 1999; Thannickal et al., 2000; Mignot, 2004), increased orexinergic tone exerts wake-promoting effects (Bourgin et al., 2000; Thakkar et al., 2001; Methippara et al., 2000). The orexin neurons exhibited the wake-on/rapid eye movement (REM) –Off discharge pattern (W>non-REM>REM) (Alam et al., 2002; Lee et al., 2005; Mileykovskiy et al., 2005).

Several lines of evidence implicate adenosine (AD) to be the homeostatic regulator of sleep–wakefulness (Basheer et al., 2004). It has been suggested that during wakefulness, when neural metabolism is highest, AD, a by-product of neuronal (ATP) metabolism, gradually builds up and mediates the homeostatic need for sleep (Strecker et al., 2000).

We have previously shown that the orexin neurons express adenosine A1 receptor (A1R) (Thakkar et al., 2002). An *in vitro* study recently reported that AD inhibits orexin neurons via A1R (Liu and Gao, 2006). To further evaluate the role of AD in the orexinergic LH and its influences on sleep–wakefulness, we examined the effects of a selective AD A1R antagonist DPX locally administered into the LH on spontaneous bouts of sleep–wakefulness and on recovery sleep following 6 h of sleep deprivation in freely behaving rats.

EXPERIMENTAL PROCEDURES

Experimental design

Our first set of experiments monitored the effects of A1R antagonist on spontaneous sleep–wakefulness. AD is implicated in the homeostatic regulation of sleep. Since recovery sleep following sleep deprivation is an essential paradigm to study the homeostatic mechanisms regulating sleep–wakefulness, our second set of experiments monitored the effects of A1R antagonist on recovery sleep following sleep deprivation.

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Abbreviations: AD, adenosine; A1R, adenosine A1 receptor; DPX, 1,3-dipropyl-8-phenylxanthine, a selective adenosine A1 receptor antagonist; LH, orexinergic zone of the perifornical lateral hypothalamus; REM, rapid eye movement.

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Table 1. Protocol for experiment 1

Day	Microinjections		
	Batch 1 (N=3)	Batch 2 (N=3)	Batch 3 (N=3)
1	Saline (0.9%)	DPX (1.5 pmol)	DPX (15 pmol)
2	DPX (1.5 pmol)	DPX (15 pmol)	Saline (0.9%)
3	DPX (15 pmol)	Saline (0.9%)	DPX (1.5 pmol)
4	Saline (0.9%)	Saline (0.9%)	Saline (0.9%)

All microinjections were performed 6 h before the beginning of the dark cycle.

Animals

Adult male Sprague–Dawley rats were housed under constant temperature, with *ad libitum* access to food and water, and with 12-h light/dark (light (02:00 h to 14:00 h) and dark (14:00 h to 02:00 h)) cycle at least 10 days before surgery. All surgeries were conducted before 14:00 h (before the beginning of the dark period). Every effort was made to minimize animal suffering and to reduce the number of animals used. All animals were treated in accordance with the American Association for Accreditation of Laboratory Animal Care's policy on care and use of laboratory animals. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Animal Research Committee of the Harry S. Truman Memorial Veterans Hospital.

Surgery

Under sterile condition and using standard surgical protocol (for details see Thakkar et al., 2001), the animals (N=9) were implanted with electrodes for recording electroencephalogram and electromyogram for determination of behavioral state. Intracerebral guide cannulas (22 G, PlasticsOne, Roanoke, VA, USA) were also implanted bilaterally at a 90° angle above the target site in the orexinergic LH (Chen et al., 2006). The target coordinates for the tip of the injector canulae were: AP −3.3, ML ±1.5, DV −8.5 [relative to bregma (Paxinos and Watson, 1998)].

Experiments were conducted in a sound-attenuated chamber with the same light conditions (light on=02:00–14:00 h) with food and water available *ad libitum*. After 3 days of post-operative recovery, the rats were tethered to a lightweight recording cable and habituated to the recording setup for at least 7 days before the experiment was begun and remain tethered until the end of the experiment except during microinjections.

1,3-Dipropyl-8-phenylxanthine (DPX)

DPX, a selective A1R antagonist (Daly et al., 1985) was purchased from Tocris Biosciences, Ellisville, MO, USA, and dissolved in ethanol to make a stock solution of 500 μM. Subsequent dilutions were made in saline. Two concentrations, 1.5 and 15 pmol (300 nL of 5 and 50 μM) were used in this study.

Acquisition of sleep–wakefulness data

Sleep–wakefulness signals were amplified by EEG machine (Model 15, Astromed Inc., West Warwick, RI, USA) digitized and acquired (128 Hz/channel) by Icelus data acquisition software (build 071503, authored by Mark Opp, University of Michigan, Ann Arbor, MI, USA) and stored in Dell Desktop computer.

Experiment 1: Effect of DPX administration on spontaneous sleep–wakefulness

The experimental protocol is as described in Table 1. All microinjections were performed in the light period between 09:30 and 10:00 h (6 h before dark onset). The microinjection protocol: The rat was disconnected from the recording cable and gently swaddled in a towel. The injector cannula was gently inserted into the ipsilateral guide cannula after removing the stylus. After waiting for approximately 1 min, DPX (or saline) was gently injected. The injector cannula was kept in place for 1 min before retracting. The same protocol was repeated on the contralateral side after 1 min. The rat was reconnected and the behavioral state monitoring was continued for 6 h.

Experiment 2: Effect of DPX administration on recovery sleep

The second experiment was begun 1 week after the completion of the first experiment. The experimental protocol is described in Table 2. Rats were sleep deprived during the last 6 h of the light cycle (08:00–14:00 h). Sleep deprivation was conducted by gentle handling (Thakkar et al., 2003b). Bilateral microinjections of either 0.9% saline or 15 pmol of DPX (300 nL of 50 μM) was performed 30 min (between 13:30 and 14:00 h) before the animals were allowed to enter recovery sleep and recovery sleep was monitored for 3 h.

Localization of the injection site

Six injections were performed in each animal. On completion of the experiment, the animals were killed under deep phenobarbital anesthesia and perfused with 0.9% saline followed by perfusion of 10% formalin. The brains were isolated, blocked and processed for immunohistochemistry. Orexin-A immunohistochemistry was used to illustrate the locations of orexin neurons relative to the drug injector cannula track (Chen et al., 2006).

Analysis of sleep–wakefulness

The data analysis was performed by Icelus data analysis software (build 111904, authored by Mark Opp, University of Michigan) using a Dell Desktop computer. The sleep–wakefulness was visually scored in 10 s epochs as (1) Wake, which included both active and quiet waking; (2) non-REM sleep; (3) REM sleep.

To emphasize the homeostatic component of sleep regulation (Borbely, 1982), we measured: 1) latency to non-REM and REM sleep and 2) total slow wave activity (total delta activity) during 3 h of recovery sleep following 6 h of sleep deprivation.

Table 2. Protocol for experiment 2

Day	Treatment	Microinjections		
		Batch 1 (N=3)	Batch 2 (N=3)	Batch 3 (N=3)
1	Sleep deprivation	Saline	Saline	DPX (15 pmol)
2		No treatment		
3	Sleep deprivation	DPX (15 pmol)	DPX (15 pmol)	Saline

Sleep deprivation was performed for 6 h ending at the onset of dark period. The onset of recovery sleep coincided with the onset of dark period. All microinjections were performed 30 min before the onset of recovery sleep.

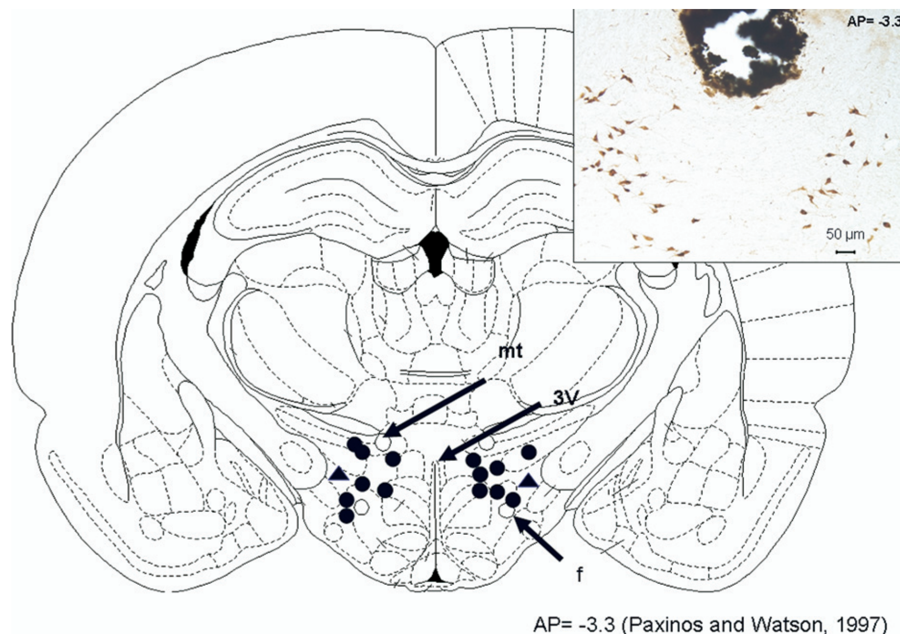

Fig. 1. Schematic representation of the anatomical location of the histologically identified injection sites. Triangles represent the "missed" sites in one animal that received DPX injection outside the orexinergic zone. The missed sites were posterior (AP −4.2) to the target site. All other sites (black circles) were located between AP −3.1 and −3.6 and are all mapped onto this one coronal brainstem section (−3.3, adapted from Paxinos and Watson, 1998). Microinjections were performed bilaterally in each animal. Abbreviations: f=fornix; mt=mammillothalamic tract; 3V=third ventricle. For identification of unlabeled structures see Paxinos and Watson (1998). Inset: a representative photomicrograph depicting the localization of the injection site in the midst of orexin neurons in the orexinergic zone of the LH. Scale bar=50 μm.

Latency to non-REM sleep was defined as the amount of time between the injection and the first non-interrupted 60 s bout of non-REM sleep. Latency to REM sleep was defined the amount of time between the injection and the first non-interrupted 30 s bout of REM sleep. Total slow wave (delta) activity was defined as total power in the delta band (1–4.0 Hz) in non-REM sleep during 3 h of recovery sleep (Wurts and Edgar, 2000).

Statistical analysis

The effect of DPX on the spontaneous sleep–wakefulness was analyzed by repeated measure ANOVA followed by Bonferroni's post hoc test (EZAnalyze Ver 3.0. http://www.ezanalyze.com) whereas paired *t*-test was used to evaluate the effect of DPX on behavioral states during recovery sleep.

RESULTS

Out of a total of nine rats, one rat was discarded due to poor quality of electrographic signals. Another animal was a "mishit" or the injection sites were not localized in the orexinergic LH and therefore not included in the data analysis.

Histology

The localization of the injection tips for each of the animals is shown in a coronal schematic, Fig. 1 (N=7, adapted from Paxinos and Watson, 1998). All injection sites (N=7) were in the orexinergic LH between AP levels −3.1 and −3.6. The "missed" injection sites (shown as triangle in Fig. 1A) were posterior (AP=−4.2) and not localized in the orexinergic LH. A representative photomicrograph depicting the injection site in the orexinergic LH is shown in Fig. 1 (inset).

Experiment 1: Effect of DPX administration on spontaneous sleep–wakefulness

As there was no significant difference in any behavioral state after two saline treatments, behavioral state data after two saline treatments was pooled together. Microinjection of DPX into the orexinergic LH produced a significant change in the behavioral state during the first 3 h (1–3

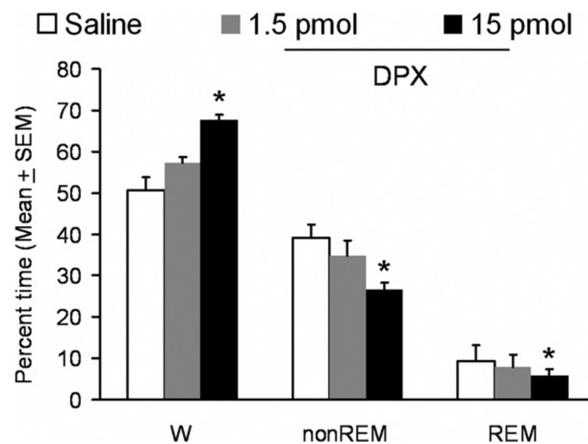

Fig. 2. Bilateral local microinjections of a selective A1R antagonist, DPX into the orexin-rich perifornical hypothalamus increased the amount of time spent in wakefulness and decreased the amount of time spent in both non-REM and REM phases of sleep during 3 h of spontaneous bouts of sleep–wakefulness during the light period compared with saline treatment. Repeated measures ANOVA followed by Bonferroni's post hoc test revealed that the maximum effect was produced by the highest (15 pmol/300 nL) dose of DPX. * $P<0.05$; level of significance (see text for details).

h) after treatment. There was no change in any behavioral state during the next 3 h (4–6 h) after treatment (data not shown).

As illustrated in Fig. 2A, wakefulness was significantly increased during the first 3 h after DPX administration [F=11.99; DF=20; P<0.001, one-way RM ANOVA] as compared with wakefulness following saline injections. Post hoc test (Bonferroni's) revealed that only the 15 pmol dose of DPX increased wakefulness (t=4.6; P<0.05) as compared with saline treatment.

There was a significant reduction in non-REM [F=6.91; df=20; P<0.01, one-way RM ANOVA] and REM sleep [F=3.896; df=20; P<0.05, one-way RM ANOVA] following DPX treatment. Bonferroni's test revealed a significant reduction in non-REM (t=4.4; P<0.05) and REM sleep (t=4.2; P<0.05) with the 15 pmol dose as compared with non-REM sleep following saline treatment (Fig. 2).

Experiment 2: Effect of DPX administration on recovery sleep following 6 h of sleep deprivation

As illustrated in Fig. 3A, DPX treatment produced a significant increase [t=5.74, P<0.01; paired t-test] in wakefulness with a concomitant reductions in non-REM [t=5.42, P<0.01, paired t-test] and REM phases [t=3.16, P<0.05] during 3 h of recovery sleep.

In order to understand the onset and the progression of the drug effects on recovery sleep, we conducted fine grain analysis of behavioral state during recovery sleep in smaller time bins of 30, 90 and 180 min of recovery sleep. While there was no change in any behavioral state during first 30 min post-DPX injection, significant increases in wakefulness (P<0.05) with a concomitant decreases in non-REM (P<0.05) were observed 90 and 180 min post-DPX injections (Fig. 3B and

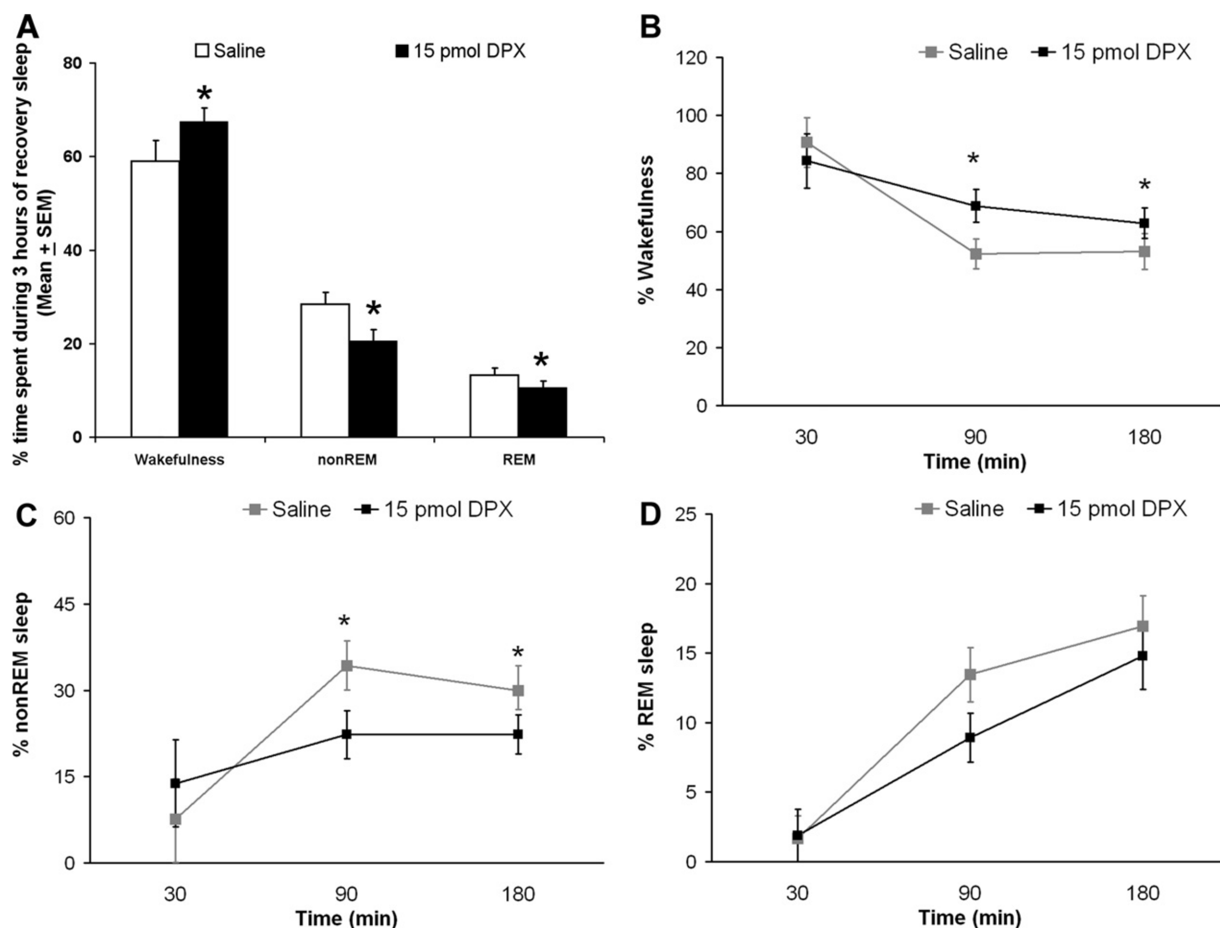

Fig. 3. (A) Bilateral local microinjections of a 15 pmol/300 nL of A1 receptor antagonist, DPX into the orexin-rich perifornical hypothalamus produced a significant decrease in both non-REM and REM phases of sleep with a concomitant increase in wakefulness during 3 h recovery sleep. In order to understand the onset and the progression of the effect of DPX on recovery sleep and since handling of the animals for microinjection is likely to produce arousal especially during first 30 min, we analyzed the behavioral state data in smaller time bins. Excluding the first 30 min post-injection, bilateral local microinjections of a 15 pmol/300 nL of A1 receptor antagonist, DPX into the orexin-rich perifornical hypothalamus produced a significant increase in wakefulness (B) with a concomitant decrease in non-REM sleep (C) during 90 and 180 min of recovery sleep. REM sleep did not show any significant change when the data were analyzed in smaller time bins (D). We used the sleep deprivation–recovery sleep paradigm because recovery sleep following sleep deprivation is a direct measure of the homeostatic regulation of sleep. Sleep deprivation was performed by gentle handling. The animals were treated with DPX (15 pmol/300 nL) 30 min before they were allowed to enter recovery sleep. * P<0.05; level of significance (see text for details).

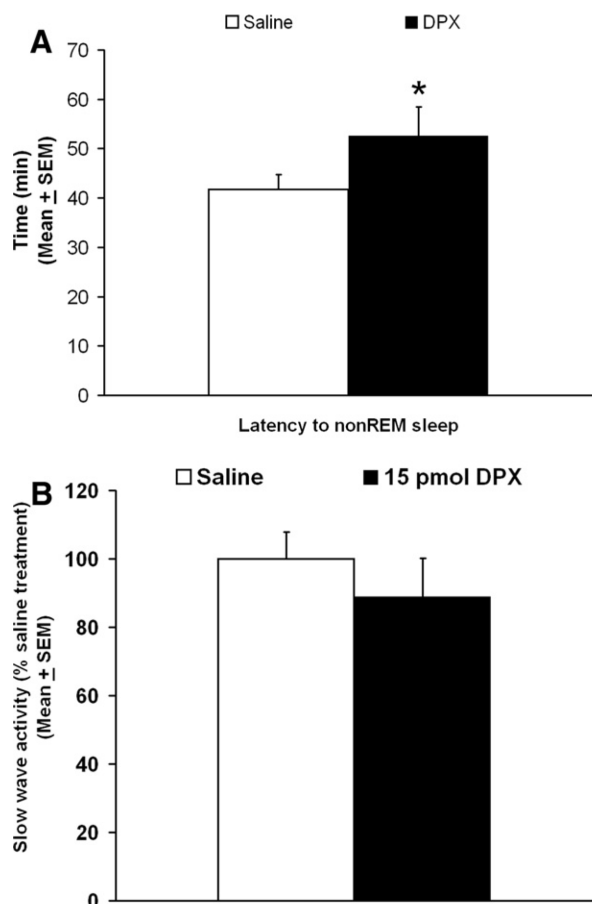

Fig. 4. (A) Bilateral local microinjections of a 15 pmol/300 nL of A1 receptor antagonist, DPX into the orexin-rich perifornical hypothalamus produced a significant increase in the latency to the non-REM phase of recovery sleep. Latency to non-REM sleep is an indicator of sleep propensity. Increased latency to the non-REM sleep phase of recovery sleep suggests that the DPX-treated animals were less sleepy as compared with saline treated animals. (B) Total slow wave (delta activity) during recovery sleep remained unaffected following bilateral local microinjections of a 15 pmol/300 nL of A1 receptor antagonist, DPX into the orexin-rich perifornical hypothalamus. * $P<0.05$; level of significance (see text for details).

3C). REM sleep did not show any significant change (Fig. 3D).

There was a significant increase [$t=2.49$, $P<0.05$, paired *t*-test, Fig. 4A] in latency to non-REM sleep following DPX treatment as compared with after saline treatment. Latency to REM sleep was unaffected (data not shown). In contrast, a non-significant decrease in total slow wave activity [$t=0.791$, $P=0.46$, paired *t*-test, Fig. 4B] was observed during 3 h of recovery sleep.

DISCUSSION

The results of our study suggest that blockade of A1R in the orexinergic LH, by local microinjection of a selective A1R antagonist DPX, produced a significant increase in wakefulness with a concomitant reduction in sleep during spontaneous bouts of sleep–wakefulness and during recovery sleep. To our knowledge, this report is the first to implicate the interaction of AD and orexins in the homeostatic regulation of sleep.

As described above, we had one "mishit" animal with the injection site outside the orexinergic zone. There was no effect of DPX (both doses, data not shown) on any behavioral state during spontaneous sleep–wakefulness or during recovery sleep (DPX dose=15 pmol) suggesting that the DPX effects were site specific.

We found an increase in wakefulness with concomitant reductions in non-REM and REM sleep during spontaneous sleep–wakefulness following local infusion of a selective A1R antagonist into the LH. This results are not surprising because: 1) orexin neurons are wake-active and reduction in orexinergic tone reduced wakefulness (McCarley, 2007). 2) AD is a somnogen, implicated to mediate sleep via A1 receptor (Radulovacki et al., 1984; Alam et al., 1999, 2006; Blanco-Centurion et al., 2006; Thakkar et al., 2003a,b; Thakkar and Mallick, 1996; Portas et al., 1997). 3) Orexin neurons are reported to express A1R (Thakkar et al., 2002). 4) There is *in vitro* evidence implicating adenosinergic inhibition of orexin neurons via A1R (Liu and Gao, 2006) and 5) A recent *in vivo* study reported that activation of AIR by local application of A1R agonist, N^6-cyclopentyl-AD into the lateral hypothalamic promotes sleep (Kumar et al., 2006).

Since recovery sleep is a direct measure of sleep homeostasis, we studied the effects of blockade of A1R on recovery sleep. To build up maximum sleep pressure and allow its optimal measurement, we performed 6 h of sleep deprivation during the light period, when the animals normally sleep. Our sleep deprivation ended coincident with lights off, the time when the animals normally wake up, and thus allow a measure of post-deprivation sleep without any "ceiling effect" (Thakkar et al., 2003b). Handling of the animals for microinjection may cause arousal, especially during the first 30 min, that could delay the onset of sleep (Pollock and Mistlberger, 2003). Thus, in addition to cumulative 3 h analysis of behavioral state during recovery sleep, we also analyzed behavioral state in smaller time bins of 30, 90 and 180 min.

Our results suggest that blockade of A1R increased wakefulness, reduced recovery sleep, and increased latency to non-REM sleep. Sleep latency is a robust measure of sleep propensity. Thus, increased latency to non-REM sleep suggested that the animals treated with DPX were less sleepy as compared with animals treated with saline. Total slow wave delta activity is another marker of the homeostatic sleep drive. However, although there was a decrease in total slow wave (delta) activity following DPX treatment, the effect was not significant. These findings are consistent with a prior report that systemic injection of caffeine (a non-specific AD receptor antagonist) reduced recovery sleep but did not change compensatory slow-wave activity during recovery sleep (Wurts and Edgar, 2000).

While there was a significant decrease in REM sleep during the cumulative 3 h of recovery sleep, no change in REM sleep was observed when the data were analyzed in smaller time bins, suggesting a milder effect.

Our study suggests that AD acting via A1R in the orexin rich LH may have a key role in the regulation of sleep–wakefulness by reducing the activity of the orexin neurons in the LH, in addition to its actions in other brain areas and further reinforces the role of AD as a homeostatic regulator of sleep.

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REFERENCES

Alam MN, Gong H, Alam T, Jaganath R, McGinty D, Szymusiak R (2002) Sleep-waking discharge patterns of neurons recorded in the rat perifornical lateral hypothalamic area. J Physiol 538:619–631.

Alam MN, Szymusiak R, Gong H, King J, McGinty D (1999) Adenosinergic modulation of rat basal forebrain neurons during sleep and waking: neuronal recording with microdialysis. J Physiol 521 (Pt 3):679–690.

Alam MN, Szymusiak R, McGinty D (2006) Adenosinergic regulation of sleep: multiple sites of action in the brain. Sleep 29:1384–1385.

Basheer R, Strecker RE, Thakkar MM, McCarley RW (2004) Adenosine and sleep-wake regulation. Prog Neurobiol 73:379–396.

Blanco-Centurion C, Xu M, Murillo-Rodriguez E, Gerashchenko D, Shiromani AM, Salin-Pascual RJ, Hof PR, Shiromani PJ (2006) Adenosine and sleep homeostasis in the basal forebrain. J Neurosci 26:8092–8100.

Borbely AA (1982) A two process model of sleep regulation. Hum Neurobiol 1:195–204.

Bourgin P, Huitron-Resendiz S, Spier AD, Fabre V, Morte B, Criado JR, Sutcliffe JG, Henriksen SJ, de Lecea L (2000) Hypocretin-1 modulates rapid eye movement sleep through activation of locus coeruleus neurons. J Neurosci 20:7760–7765.

Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, Richardson JA, Williams SC, Xiong Y, Kisanuki Y, Fitch TE, Nakazato M, Hammer RE, Saper CB, Yanagisawa M (1999) Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell 98:437–451.

Chen L, Thakkar MM, Winston S, Bolortuya Y, Basheer R, McCarley RW (2006) REM sleep changes in rats induced by siRNA-mediated orexin knockdown. Eur J Neurosci 24:2039–2048.

Daly JW, Padgett W, Shamim MT, Butts-Lamb P, Waters J (1985) 1,3-Dialkyl-8-(p-sulfophenyl)xanthines: potent water-soluble antagonists for A1- and A2-adenosine receptors. J Med Chem 28:487–492.

Datta S, Maclean RR (2007) Neurobiological mechanisms for the regulation of mammalian sleep-wake behavior: Reinterpretation of historical evidence and inclusion of contemporary cellular and molecular evidence. Neurosci Biobehav Rev 31:775–824.

Gerashchenko D, Kohls MD, Greco M, Waleh NS, Salin-Pascual R, Kilduff TS, Lappi DA, Shiromani PJ (2001) Hypocretin-2-saporin lesions of the lateral hypothalamus produce narcoleptic-like sleep behavior in the rat. J Neurosci 21:7273–7283.

Kumar S, Rai S, Szymusiak R, McGinty D, Alam N (2006) Effects of adenosine A_1 receptor agonist into the perifornical lateral hypothalamic area on sleep. Program No. 458.12.2006 Neuroscience Meeting Planner, Atlanta, GA: Society for Neuroscience [online].

Lee MG, Hassani OK, Jones BE (2005) Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. J Neurosci 25:6716–6720.

Lin L, Faraco J, Li R, Kadotani H, Rogers W, Lin X, Qiu X, de Jong PJ, Nishino S, Mignot E (1999) The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. Cell 98:365–376.

Liu ZW, Gao XB (2006) Adenosine inhibits activity of hypocretin/orexin neurons via A1 receptor in the lateral hypothalamus: a possible sleep-promoting effect. J Neurophysiol.

McCarley RW (2007) Neurobiology of REM and NREM sleep. Sleep Med 8:302–330.

Methippara MM, Alam MN, Szymusiak R, McGinty D (2000) Effects of lateral preoptic area application of orexin-A on sleep- wakefulness. Neuroreport 11:3423–3426.

Mignot E (2004) Sleep, sleep disorders and hypocretin (orexin). Sleep Med 5 (Suppl 1):S2–S8.

Mileykovskiy BY, Kiyashchenko LI, Siegel JM (2005) Behavioral correlates of activity in identified hypocretin/orexin neurons. Neuron 46:787–798.

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates. Boston: Academic Press.

Pollock MS, Mistlberger RE (2003) Rapid eye movement sleep induction by microinjection of the GABA-A antagonist bicuculline into the dorsal subcoeruleus area of the rat. Brain Res 962:68–77.

Portas CM, Thakkar M, Rainnie DG, Greene RW, McCarley RW (1997) Role of adenosine in behavioral state modulation: a microdialysis study in the freely moving cat. Neuroscience 79:225–235.

Radulovacki M, Virus RM, Djuricic-Nedelson M, Green RD (1984) Adenosine analogs and sleep in rats. J Pharmacol Exp Ther 228:268–274.

Selbach O, Haas HL (2006) Hypocretins: the timing of sleep and waking. Chronobiol Int 23:63–70.

Strecker RE, Morairty S, Thakkar MM, Porkka-Heiskanen T, Basheer R, Dauphin LJ, Rainnie DG, Portas CM, Greene RW, McCarley RW (2000) Adenosinergic modulation of basal forebrain and preoptic/anterior hypothalamic neuronal activity in the control of behavioral state. Behav Brain Res 115:183–204.

Thakkar M, Mallick BN (1996) Effect of rapid eye movement sleep deprivation on 5′-nucleotidase activity in the rat brain. Neurosci Lett 206:177–180.

Thakkar MM, Delgiacco RA, Strecker RE, McCarley RW (2003a) Adenosinergic inhibition of basal forebrain wakefulness-active neurons: a simultaneous unit recording and microdialysis study in freely behaving cats. Neuroscience 122:1107–1113.

Thakkar MM, Winston S, McCarley RW (2003b) A1 receptor and adenosinergic homeostatic regulation of sleep-wakefulness: effects of antisense to the A1 receptor in the cholinergic basal forebrain. J Neurosci 23:4278–4287.

Thakkar MM, Ramesh V, Cape EG, Winston S, Strecker RE, McCarley RW (1999) REM sleep enhancement and behavioral cataplexy following orexin (hypocretin)-II receptor antisense perfusion in the pontine reticular formation. Sleep Res Online 2:112–120.

Thakkar MM, Ramesh V, Strecker RE, McCarley RW (2001) Microdialysis perfusion of orexin-A in the basal forebrain increases wakefulness in freely behaving rats. Arch Ital Biol 139:313–328.

Thakkar MM, Winston S, McCarley RW (2002) Orexin neurons of the hypothalamus express adenosine A1 receptors. Brain Res 944:190–194.

Thannickal TC, Moore RY, Nienhuis R, Ramanathan L, Gulyani S, Aldrich M, Cornford M, Siegel JM (2000) Reduced number of hypocretin neurons in human narcolepsy. Neuron 27:469–474.

Wurts SW, Edgar DM (2000) Caffeine during sleep deprivation: sleep tendency and dynamics of recovery sleep in rats. Pharmacol Biochem Behav 65:155–162.

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