Neuroscience 153 (2008) 551–555

RAPID REPORT

EFFECT OF MICRODIALYSIS PERFUSION OF 4,5,6,7-TETRAHYDROISOXAZOLO-[5,4-C]PYRIDINE-3-OL IN THE PERIFORNICAL HYPOTHALAMUS ON SLEEP–WAKEFULNESS: ROLE OF δ-SUBUNIT CONTAINING EXTRASYNAPTIC $GABA_A$ RECEPTORS

M. M. THAKKAR,[a]* S. WINSTON[b] AND R. W. McCARLEY[b]

[a]*Harry Truman Memorial Veteran's Hospital and Department of Neurology, University of Missouri, 800 Hospital Drive, Columbia, MO 65201, USA*

[b]*Boston VA Healthcare System, and Department of Psychiatry, Harvard Medical School, Brockton, MA 02301, USA*

Abstract—Gaboxadol or 4,5,6,7-tetrahydroisoxazolo-[5,4-c] pyridine-3-ol (THIP) is a selective agonist for the δ-subunit containing extrasynaptic $GABA_A$ receptors that will soon enter the U.S. market as a sleep aid [Winsky-Sommerer R, Vyazovskiy VV, Homanics GE, Tobler I (2007) The EEG effects of THIP (gaboxadol) on sleep and waking are mediated by the GABA(A)delta-subunit-containing receptors. Eur J Neurosci 25:1893–1899]. Numerous studies have shown that systemic administration of THIP reduces wakefulness and increases sleep both in humans and rats [Lancel M, Langebartels A (2000) Gamma-aminobutyric acid(A) (GABA(A)) agonist 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol persistently increases sleep maintenance and intensity during chronic administration to rats. J Pharmacol Exp Ther 293:1084–1090; Walsh JK, Deacon S, Dijk DJ, Lundahl J (2007) The selective extrasynaptic GABAA agonist, gaboxadol, improves traditional hypnotic efficacy measures and enhances slow wave activity in a model of transient insomnia. Sleep 30:593–602]. However, it is yet unclear where in the brain THIP acts to promote sleep. Since the perifornical lateral hypothalamus (PFH) contains orexin neurons and orexin neurons are critical for maintenance of arousal [McCarley RW (2007) Neurobiology of rapid eye movement (REM) and NREM sleep. Sleep Med 8:302–330], we hypothesized that THIP may act on PFH neurons to promote sleep. To test our hypothesis, we used reverse microdialysis to perfuse THIP unilaterally into the PFH and studied its effects on sleep–wakefulness during the light period in freely behaving rats.

Microdialysis perfusion of THIP (100 μM) into the PFH produced a significant reduction in wakefulness with a concomitant increase in non-rapid eye movement or slow wave sleep as compared with artificial cerebrospinal fluid perfusion. REM sleep was unaffected.

This is the first study implicating the δ-subunit containing extrasynaptic $GABA_A$ receptors in PFH in control of sleep–wakefulness in freely behaving rats. Published by Elsevier Ltd on behalf of IBRO.

*Corresponding author. Tel: +1-573-814-6000x53697.
E-mail address: thakkarmm@health.missouri.edu (M. M. Thakkar).

Abbreviations: ACSF, artificial cerebrospinal fluid; non-REM, non-rapid eye movement or slow wave sleep; PFH, perifornical lateral hypothalamus; REM, rapid eye movement; THIP, 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol.

0306-4522/08$32.00+0.00 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2008.02.053

Key words: orexin/hypocretin, perifornical hypothalamus, THIP, reverse microdialysis.

The GABA system is closely linked with the regulation of sleep–wakefulness. Thus, it is not surprising that pharmacological landscape for treatment of various sleep disorders including insomnia have been dominated by agents that activate $GABA_A$ receptors (Wafford and Ebert, 2006). Classical synaptic GABA transmission results in phasic inhibition that is mainly mediated by γ2 subunits containing postsynaptic $GABA_A$ receptors (Rudolph and Mohler, 2006; Ebert et al., 2006; Olsen et al., 2007). In contrast, tonic inhibition is mainly mediated by δ subunit containing "extrasynaptic" $GABA_A$ receptors (Olsen et al., 2007). These "extrasynaptic" $GABA_A$ receptors have a higher affinity for GABA and slower rates of desensitization and deactivation than do the classical synaptic receptors.

The $GABA_A$ agonist 4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol (THIP) selectively activates extrasynaptic $GABA_A$ receptors (Sommerer et al., 2007). Systemic administration of THIP induces sleep in rats and humans (Faulhaber et al., 1997; Lancel, 1997). However it is yet unknown where in the brain does THIP act to induce sleep.

There is strong evidence indicating that the perifornical lateral hypothalamus (PFH) is critical for wakefulness. Although the PFH contains several cell types, including the orexin/hypocretin and the melanin concentrating hormone containing neurons, there is compelling and consistent evidence implicating the orexin neurons in the control of wakefulness (McCarley, 2007). For example, local administration of orexin in various brain regions produced wakefulness (Bourgin et al., 2000; Thakkar et al., 2001; Xi et al., 2001; Methippara et al., 2000). In contrast, a deficiency or reduction of orexinergic neurotransmission resulted in a reduction in wakefulness and cataplexy like episodes in rodents (Lin et al., 1999; Chen et al., 2006; Gerashchenko et al., 2001; Chemelli et al., 1999; Thakkar et al., 1999) and narcolepsy in humans (Thannickal et al., 2000; Mignot, 2004). Single unit recording studies suggest that the orexin neurons exhibited the Wake-On/rapid eye movement (REM)–Off discharge pattern (W>non-rapid eye movement or slow wave sleep (non-REM)<REM) (Alam et al., 2002; Lee et al., 2005; Mileykovskiy et al., 2005).

The δ-subunit-containing $GABA_A$ receptors are present in the PFH (Pirker et al., 2000) and the role of PFH $GABA_A$ receptors in sleep induction has been previously shown (Alam et al., 2004). However, it is yet unclear whether extrasynaptic $GABA_A$ receptors in the PFH have any role in control of sleep–wakefulness. To evaluate the role of δ-subunit containing extrasynaptic $GABA_A$ receptors in the PFH and its influences on sleep–wakefulness, we examined the effects of THIP locally administered into the PFH on spontaneous bouts of sleep–wakefulness in freely behaving, naturally sleeping rats.

EXPERIMENTAL PROCEDURES

Animals and surgery

Adult male Sprague–Dawley rats were housed under constant temperature, with *ad libitum* access to food and water, and with 12-h light/dark cycle (07:00 h to 19:00 h; 19:00 h to 07:00 h) at least 10 days before surgery.

Under sterile conditions and using the standard surgical protocol (for details see Thakkar et al., 2001, in press), the animals were implanted with electrodes for recording electroencephalogram and electromyogram for determination of behavioral state. Intracerebral guide cannulas (CMA/Microdialysis, Acton, MA, USA; for lateral insertion of the microdialysis probes) were implanted at a 90° angle above the target site in the orexinergic zone of the PFH. The target coordinates (Paxinos and Watson, 1998) for the tip of the microdialysis probe were: AP −3.3, ML ±1.5, DV −8.5, relative to bregma and skull surface at bregma. 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 Boston VA Healthcare System.

THIP-hydrochloride

THIP, a selective agonist for δ-subunit- containing extrasynaptic $GABA_A$ receptors (Winsky-Sommerer et al., 2007) was purchased from Tocris Biosciences, Ellisville, MO, USA and dissolved in artificial cerebrospinal fluid (ACSF=NaCl 147 mM, KCl 3 mM, $CaCl_2$ 1.2 mM, $MgCl_2$ 1.0 mM, pH 7.2) to make a stock solution of 1 mM. Three concentrations (1, 10 and 100 μM) were used in this study to provide an effective concentration of 0.1, 1 and 10 μM at the probe tip (Thakkar et al. 1998).

Post-operative recovery, habituation and sleep–wakefulness recordings of sleep–wakefulness

Experiments were conducted in sound-attenuated chambers with food and water available *ad libitum* and lights on from 07:00 to 19:00 h; 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. The animals remained tethered until the end of the experiment except during probe insertion.

Unilateral microdialysis perfusion of THIP coupled with sleep-W recordings in freely behaving rats

A unilateral microdialysis probe was implanted. The following protocol for probe insertion was used: The rat was disconnected from the recording cable and gently swaddled in a towel. After removing the stylus, a microdialysis probe (CMA 11, 1 mm membrane length, 0.24 mm O.D; CMA/Microdialysis) was gently inserted into the guide cannula. The flow was checked and the rat was reconnected. After allowing 12 h for recovery from probe-insertion and for equilibrium at the probe tip, the experiment was begun. The experimental protocol is described in Table 1. ACSF and/or THIP were perfused at 2 μl/min. Most previous studies have performed systemic administration of THIP during the light (inactive) period (Lancel and Faulhaber, 1996; Vyazovskiy et al., 2005; Winsky-Sommerer et al., 2007) and found subsequent effects on sleep. Since a key purpose of this study was to define brain region where THIP might its sleep inducing effects in rats; in this initial study, we decided to perform microdialysis perfusion of THIP during the light period.

Table 1. Experimental protocol

ACSF perfusion	Day 1	ACSF perfusion begins at 09:30–15:30 h with two syringe changes to match syringe changes on THIP perfusion days
THIP (1 μM) perfusion	Day 2	ACSF perfusion: 09:30–11:00; THIP perfusion (1 μM): 11:00–14:00 h ACSF: 14:00–15:30 h:
ACSF perfusion	Day 3	Same as described for day 1
THIP (10 μM) perfusion	Day 4	Same as described for day 2 except 10 μM of THIP will be used
ACSF perfusion	Day 5	Same as described for day 1
THIP (100 μM) perfusion	Day 6	Same as described for day 2 except 100 μM of THIP will be used
ACSF perfusion	Day 7	Same as described for day 1

Localization of the injection site

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 orexin-A immunohistochemistry (Chen et al., 2006) to localize the injection site in the PFH.

Analysis of behavioral states

Behavioral state data were acquired and digitized using the Harmonie software (Stellate Systems, Montreal, Canada and sleep–wakefulness was visually scored in 10 s epochs as (1) Wakefulness (which included both active and quiet wakefulness), determined by the presence of low amplitude, high frequency desynchronized EEG with the concomitant presence of active muscle tone; (2) non-REM sleep; determined by the presence of low frequency, high amplitude, synchronized EEG with low EMG tone; and (3) REM sleep, determined by complete absence of muscle tone along with desynchronized EEG (Thakkar et al., 2003). The effect of THIP on the sleep–wakefulness was analyzed by repeated measure ANOVA followed by Bonferroni's test (EZAnalyze Ver. 3.0., Boston, MA, USA, http://www.ezanalyze.com).

RESULTS

Only those animals with microdialysis probe tips ($N=7$) in the PFH as revealed by orexin-A immunohistochemistry were included in data analysis. A representative photomicrograph illustrating the perfusion site in the midst of orexin neurons is shown in Fig. 1.

The behavioral state data during two ACSF perfusions were comparable. Therefore behavioral state data during ACSF perfusion were pooled together. As described in

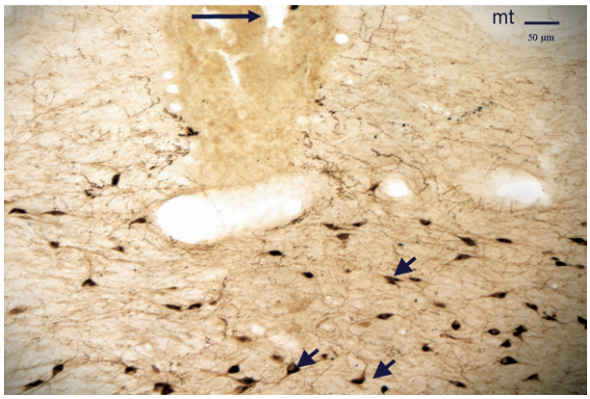

Fig. 1. A representative photomicrograph illustrating the localization of the microdialysis probe tip (black arrow) in the midst of orexin neurons (black arrowheads) in the orexinergic PFH is shown. All the probe tips (N=7) were localized within the orexinergic PFH. Abbreviation: mt, mammillothalamic tract. Scale bar=50 μm.

Table 2 and illustrated in Fig. 2, there was significant decrease in wakefulness ([F=3.25; df=27; P<0.05, N=7, one-way RM ANOVA] with a concomitant increase in non-REM sleep [F=3.54; df=27; P<0.05, N=7, one-way RM ANOVA] during 3 h of unilateral THIP perfusion into the PFH as compared with during ACSF perfusion. Post hoc Bonferroni tests revealed that the 100 μM dose of THIP significantly reduced wakefulness (mean t=3.9; P<0.05) and increased non-REM sleep (t=4.1; P<0.05). Although REM sleep was decreased during THIP perfusion, the effect did not reach significance [F=0.71; df=27; P=0.559, N=7, one-way RM ANOVA] (see Table 2 and Fig. 2). The changes in the behavioral states returned to baseline during 90 min of post-THIP ACSF perfusion (data not shown).

DISCUSSION

Local unilateral administration of THIP, a selective agonist for δ-subunit containing extrasynaptic $GABA_A$ receptors in the PFH produced a significant increase in non-REM sleep with a concomitant reduction in wakefulness during the light period in freely behaving rats. To our knowledge, this report is the first implicating the δ-subunit containing extrasynaptic $GABA_A$ receptors in the PFH in the regulation of sleep–wakefulness in freely behaving rats.

The use of a microdialysis probe to apply drugs locally in specific regions of the brain provides precise control over the concentration and duration of the drug administration and offers several advantages over other techniques including the ability to deliver low and constant concentrations of drugs without disturbing the animal (Thakkar et al., 1998). In addition, the drug delivered through microdialysis probe does not diffuse more than 1 mm from the probe membrane (Hocht et al., 2007; Westerink and De Vries, 2001). One limitations associated with reverse microdialysis is that the brain concentration of the delivered drug needs to be estimated from *in vitro* probe experiments. Previous studies done in our laboratory suggest that ~10% of the drug diffuses out of the probe (Portas et al., 1996). Thus, perfusion of 100 μM of THIP will deliver 10 μM concentration of THIP outside the probe.

Table 2. Percent time (mean±S.E.M., N=7) spent in during 3 h of THIP perfusion

	ACSF	THIP		
		1 μM	10 μM	100 μM
Wakefulness	53.51±2.9	47.21±3.2	46.20±2.8	42.87±5.4
non-REM	41.83±2.5	47.54±3.1	48.21±2.6	52.69±4.9
REM	4.84±0.7	5.43±1.2	5.78±0.7	4.63±1.1

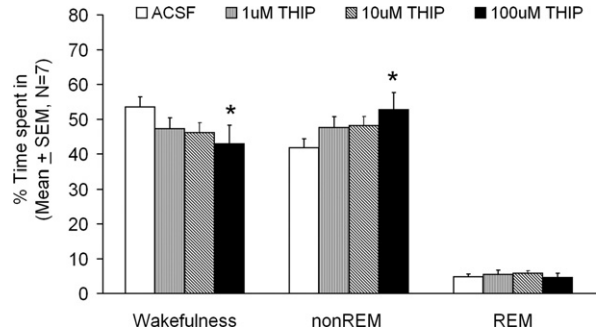

Fig. 2. Unilateral microdialysis perfusion (3 h) of THIP (also known as gaboxadol), a selective agonist for δ-subunit containing extrasynaptic GABAA, into the orexin-rich perifornical hypothalamus increased the amount of time (mean±S.E.M.; N=7) spent in non-REM and decreased the amount of time spent in wakefulness during light period compared with ACSF perfusion. REM sleep was not affected. One-way repeated measures ANOVA followed by Bonferroni's post hoc test revealed that the maximum effect was produced by the highest (100 μM; effective concentration at the probe tip=10 μM) dose of THIP. * P<0.05; level of significance (see text for details).

Numerous studies have shown that THIP selectively activates the δ-subunit containing extrasynaptic $GABA_A$ receptors in the brain (Krogsgaard-Larsen et al., 2004; Winsky-Sommerer et al., 2007; Wafford and Ebert, 2006) and systemic administration of THIP, in rats and humans, increases non-REM sleep and reduces wakefulness without affecting REM sleep (Lancel and Faulhaber, 1996; Lancel and Langebartels, 2000). Although, *in vitro* studies conducted in mice suggest that the ventrobasal thalamus may be critical for THIP-induced sleep promotion (Belelli et al., 2005; Jia et al., 2005), recent *in vivo* studies have shown that systemic THIP administration does not promote sleep in mice (Vyazovskiy et al., 2005; Winsky-Sommerer et al., 2007).

Our study suggests that unilateral administration of 100 μM THIP into the PFH increased non-REM sleep and reduced wakefulness as compared with ACSF perfusion. This effect may be due to THIP-induced inhibition of orexin neurons because Alam et al. (2004) have shown that orexinergic neurons are under GABAergic control during sleep although, THIP-induced inhibition of other no-orexinergic neurons cannot be ruled out.

Unilateral perfusion of THIP in the PFH did not produce any significant effect on REM sleep, most likely because the critical REM sleep promoting neurons are in the brainstem (Datta, 2007).

CONCLUSION

In conclusion, while further studies especially bilateral infusion of THIP in the PFH and other wakefulness centers (Datta and Maclean, 2007) and monitoring the effects of THIP during the dark period are necessary; our initial study suggests that unilateral perfusion of THIP, a selective extrasynaptic $GABA_A$ receptor agonist, into the orexinergic PFH increased non-REM sleep and reduced wakefulness during the light period in freely behaving, naturally sleeping rats. This is the first study to implicate extrasynaptic $GABA_A$ receptors in the orexinergic PFH in the control of sleep–wakefulness and the first to localize the effects of THIP to a specific brain region in freely behaving rats.

Acknowledgments—We thank Kristen Winston and Erika Mello for their help with data acquisition and analysis, John Franco for animal care. This research was supported by the Department of Veterans Affairs Medical Research Service Award (R.W.M.), NIH-R37 MH039683 (R.W.M.), NIH-R03 NS059831-01A1 (M.M.T.) and the NARSAD award (M.M.T.).

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, Kumar S, Bashir T, Suntsova N, Methippara MM, Szymusiak R, McGinty D (2004) GABA-mediated control of hypocretin- but not melanin-concentrating hormone-immunoreactive neurones during sleep in rats. J Physiol 563:569–582.

Belelli D, Peden DR, Rosahl TW, Wafford KA, Lambert JJ (2005) Extrasynaptic GABAA receptors of thalamocortical neurons: a molecular target for hypnotics. J Neurosci 25:11513–11520.

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.

Datta S (2007) Activation of pedunculopontine tegmental PKA prevents GABAB receptor activation-mediated rapid eye movement sleep suppression in the freely moving rat. J Neurophysiol 97: 3841–3850.

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.

Ebert B, Wafford KA, Deacon S (2006) Treating insomnia: Current and investigational pharmacological approaches. Pharmacol Ther 112: 612–629.

Faulhaber J, Steiger A, Lancel M (1997) The GABAA agonist THIP produces slow wave sleep and reduces spindling activity in NREM sleep in humans. Psychopharmacology (Berl) 130:285–291.

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.

Hocht C, Opezzo JA, Taira CA (2007) Applicability of reverse microdialysis in pharmacological and toxicological studies. J Pharmacol Toxicol Methods 55:3–15.

Jia F, Pignataro L, Schofield CM, Yue M, Harrison NL, Goldstein PA (2005) An extrasynaptic GABAA receptor mediates tonic inhibition in thalamic VB neurons. J Neurophysiol 94:4491–4501.

Krogsgaard-Larsen P, Frolund B, Liljefors T, Ebert B (2004) GABA(A) agonists and partial agonists: THIP (gaboxadol) as a non-opioid analgesic and a novel type of hypnotic. Biochem Pharmacol 68: 1573–1580.

Lancel M (1997) The GABA(A) agonist THIP increases non-REM sleep and enhances non-REM sleep-specific delta activity in the rat during the dark period. Sleep 20:1099–1104.

Lancel M, Faulhaber J (1996) The GABAA agonist THIP (gaboxadol) increases non-REM sleep and enhances delta activity in the rat. Neuroreport 7:2241–2245.

Lancel M, Langebartels A (2000) Gamma-aminobutyric acid(A) (GABA(A)) agonist 4,5,6,7-tetrahydroisoxazolo[4,5-c]pyridin-3-ol persistently increases sleep maintenance and intensity during chronic administration to rats. J Pharmacol Exp Ther 293:1084–1090.

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.

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.

Olsen RW, Hanchar HJ, Meera P, Wallner M (2007) GABAA receptor subtypes: the "one glass of wine" receptors. Alcohol 41:201–209.

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

Pirker S, Schwarzer C, Wieselthaler A, Sieghart W, Sperk G (2000) GABA(A) receptors: immunocytochemical distribution of 13 subunits in the adult rat brain. Neuroscience 101:815–850.

Portas CM, Thakkar M, Rainnie D, McCarley RW (1996) Microdialysis perfusion of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) in the dorsal raphe nucleus decreases serotonin release and increases rapid eye movement sleep in the freely moving cat. J Neurosci 16:2820–2828.

Rudolph U, Mohler H (2006) GABA-based therapeutic approaches: GABAA receptor subtype functions. Curr Opin Pharmacol 6:18–23.

Thakkar MM, Engemann S, Walsh K, Sahota P (2008) Microinjections of adenosine antagonist into the orexinergic lateral hypothalamus: Effects on sleep-wakefulness. Neuroscience, in press.

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, Strecker RE, McCarley RW (1998) Behavioral state control through differential serotonergic inhibition in the mesopontine cholinergic nuclei: a simultaneous unit recording and microdialysis study. J Neurosci 18:5490–5497.

Thakkar MM, Winston S, McCarley RW (2003) 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.

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.

Vyazovskiy VV, Kopp C, Bosch G, Tobler I (2005) The GABAA receptor agonist THIP alters the EEG in waking and sleep of mice. Neuropharmacology 48:617–626.

Wafford KA, Ebert B (2006) Gaboxadol: a new awakening in sleep. Curr Opin Pharmacol 6:30–36.

Walsh JK, Deacon S, Dijk DJ, Lundahl J (2007) The selective extrasynaptic GABAA agonist, gaboxadol, improves traditional hypnotic efficacy measures and enhances slow wave activity in a model of transient insomnia. Sleep 30:593–602.

Westerink BH, De Vries JB (2001) A method to evaluate the diffusion rate of drugs from a microdialysis probe through brain tissue. J Neurosci Methods 109:53–58.

Winsky-Sommerer R, Vyazovskiy VV, Homanics GE, Tobler I (2007) The EEG effects of THIP (gaboxadol) on sleep and waking are mediated by the GABA(A)delta-subunit-containing receptors. Eur J Neurosci 25:1893–1899.

Xi MC, Morales FR, Chase MH (2001) Effects on sleep and wakefulness of the injection of hypocretin-1 (orexin-A) into the laterodorsal tegmental nucleus of the cat. Brain Res 901:259–264.

(Accepted 20 February 2008)
(Available online 6 March 2008)