Neuroscience 209 (2012) 108–118

SALUBRINAL, AN ENDOPLASMIC RETICULUM STRESS BLOCKER, MODULATES SLEEP HOMEOSTASIS AND ACTIVATION OF SLEEP- AND WAKE-REGULATORY NEURONS

M. METHIPPARA,[a] B. MITRANI,[a] F. X. SCHRADER,[b] R. SZYMUSIAK[b,c] AND D. MCGINTY[a,b]*

[a]*Department of Psychology, UCLA, 405 Hilgard, Los Angeles, CA 90095, USA*

[b]*Veterans Administration Greater Los Angeles Healthcare System, 16111 Plummer Street, North Hills, CA 91343, USA*

[c]*Department of Medicine, UCLA, 405 Hilgard, Los Angeles, CA 90095, USA*

Abstract—Endoplasmic reticulum (ER) stress has been associated with the regulation of sleep and wake. We have previously shown that i.c.v. administration of a specific ER stress modulator, Salubrinal (SALUB), which inhibits global protein translation by blocking the dephosphorylation of eukaryotic initiation factor 2α (p-eIF2α), increased non-rapid eye movement (NREM) sleep. Here we report on the relationship between ER stress response and sleep homeostasis by measuring the amount and intensity of homeostatic recovery sleep in response to the i.c.v. administration of SALUB in adult freely behaving rats. We have also tested the hypothesis that SALUB induces sleep by activating sleep-promoting neurons and inhibiting wake-promoting neurons in the basal forebrain (BF) and hypothalamus by quantifying the effects of SALUB treatment on c-Fos expression in those neuronal groups. The present study found that i.c.v. administration of SALUB significantly modified the homeostatic sleep response. SALUB administered during sleep deprivation increased sleep intensity, indicated by slow-wave activity (SWA), during recovery sleep, whereas its administration during recovery sleep increased the amount of recovery sleep. We also found that SALUB induced c-Fos activation of GABAergic neurons in the sleep-promoting rostral median preoptic nucleus while simultaneously reducing c-Fos activation of wake-promoting lateral hypothalamic orexin-expressing neurons and magnocellular BF cholinergic neurons. The current findings suggest that ER stress pathway plays a role in the homeostatic control of NREM sleep in response to sleep deprivation and provides a mechanistic explanation for the sleep modulation by molecules signaling the need for brain protein synthesis. Published by Elsevier Ltd on behalf of IBRO.

Key words: Salubrinal, sleep homeostasis, Fos expression, preoptic GABAergic, perifornical orexinergic, basal forebrain cholinergic neurons.

The endoplasmic reticulum (ER) has evolved an adaptive response, the unfolded protein response (UPR), to cope with the accumulation of unfolded or misfolded proteins in the ER lumen, a condition termed ER stress (Zhang and Kaufman, 2004). Involvement of the sleep–wake cycle in the UPR was hypothesized on the basis of sleep deprivation (SD) experiments in rodents and flies. Sleep deprivation increased the markers of ER stress and the UPR in mouse and rat brains (Terao et al., 2003, 2006; Naidoo et al., 2005, 2008). As a component of the UPR, translation of mRNAs is repressed by phosphorylation of eukaryotic initiation factor 2α (eIF2α). We showed that 96-h sleep fragmentation increased phosphorylation of eIF2α in rat basal forebrain (BF) cholinergic neurons (Methippara et al., 2008). In *Drosophila*, expression of the immunoglobulin binding protein (BiP), an early marker of the ER stress response, was increased by waking (Shaw et al., 2000) and sleep deprivation (Naidoo et al., 2007). Moreover, flies with the mutant heat shock protein HSP 83, a cytosolic chaperone playing a role in protein folding, died from sleep deprivation, whereas activation of heat shock proteins rescued the flies from the lethal effects of sleep deprivation (Shaw et al., 2002).

Recently, we found that i.c.v. administration of a specific ER stress modulator, Salubrinal (SALUB), increased non-rapid eye movement (NREM) sleep and strongly induced the expression of p-eIF2α in rat BF neurons (Methippara et al., 2009). By preventing dephosphorylation of p-eIF2α, SALUB blocks global protein translation. We hypothesized that SALUB promoted NREM sleep because (1) sleep normally facilitates brain protein synthesis (Shapiro and Girdwood, 1981; Ramm and Smith, 1990), (2) sleep is regulated, in part, by pathways controlling brain protein synthesis (Cirelli, 2009), and (3) blocking brain protein synthesis increases sleep need (Methippara et al., 2008, 2009). We further hypothesize that molecules of the UPR pathway, which signal the accumulation of unfolded proteins, directly modulate sleep- and wake-promoting mechanisms. Here we test the hypothesis that SALUB induces sleep by activating sleep-promoting neurons and inhibiting wake-promoting neurons in BF and hypothalamus. We tested this hypothesis by measuring effects of

*Correspondence to: D. McGinty, VAGLAHS, 16111 Plummer Street, North Hills, CA 91343, USA. Tel: +1-818-891-7711 x7579; fax: +1-818-895-9575.
E-mail address: dmcginty@ucla.edu (D. McGinty).

Abbreviations: ACSF, artificial cerebrospinal fluid; ANG II, angiotensin II; ATF4, activating transcription factor 4; BF, basal forebrain; BiP, binding protein; ChAT, choline acetyltransferase; CREB, cyclic AMP response element-binding protein; DMSO, dimethyl sulfoxide; EEG, electroencephalogram; EMG, electromyogram; ER, endoplasmic reticulum; FFT, fast Fourier transform; GAD, glutamic acid decarboxylase; mBF, magnocellular BF; MnPO, median preoptic nucleus; NREM or NR, non-rapid eye movement; Orx, orexin; PBS, phosphate-buffered saline; p-eIF2α, eukaryotic initiation factor 2α, phosphorylated; PF/LH, perifornical/lateral hypothalamus; REM or R, rapid eye movement; SALUB, salubrinal; SD, sleep deprivation; SWA, slow-wave activity; TBS, tris-buffer saline; UPR, unfolded protein response; VLPO, ventro-lateral preoptic; W, wake.

0306-4522/12 $36.00 Published by Elsevier Ltd on behalf of IBRO.
doi:10.1016/j.neuroscience.2012.02.016

Table 1. Experimental groups, type of treatment, treatment time and duration, and the end points measured

Experiment	Group	Treatment	Time of treatment	*n*	Parameters measured
1	1S-SD	6 h SALUB+SD; 18 h ACSF+recovery	ZT0–ZT6; ZT6–ZT24	7	Sleep
	1V-SD	6 h vehicle+SD; 18 h CSF+recovery	ZT0–ZT6; ZT6–ZT24		
	2S-RS	6 h SD; 6 h SALUB+recovery; 12 h CSF+recovery	ZT0–ZT6; ZT6–ZT12; ZT12–ZT24	8	
	2V-RS	6 h SD; 6 h vehicle+recovery; 12 h CSF+recovery	ZT0–ZT6; ZT6–ZT12; ZT12–ZT24		
2	3	3 h SALUB	ZT12–ZT15	10	Sleep and Fos expression
	4	3 h vehicle	ZT12–ZT15	6	
Total				31	

Each rat in experiment 1 ($n=7$) and ($n=8$) received two infusions: SALUB followed by ACSF on one day and vehicle followed by ACSF on another day. Rats in groups 1S-SD and 1V-SD received infusions of SALUB and vehicle, respectively, concurrent with sleep deprivation; in group 2S-RS and 2V-RS, SALUB and vehicle were respectively administered for 6 h immediately following 6-h sleep deprivation. In group 3 ($n=10$) and group 4 ($n=6$), each rat received only one treatment: 3 h of infusion with either SALUB or vehicle.

SALUB treatment on c-Fos expression in established sleep-promoting and wake-promoting neuronal groups, preoptic GABAergic neurons and magnocellular BF (mBF) cholinergic, and perifornical/lateral hypothalamic (PF/LH) orexinergic neurons.

A critical feature of sleep physiology is sleep homeostasis, as shown by the occurrence of recovery sleep after sleep deprivation. The involvement of ER stress response in sleep homeostasis was demonstrated in BiP mutant flies; recovery sleep after sleep deprivation was increased in flies overexpressing BiP and decreased in flies expressing a dominant negative of BiP (Naidoo et al., 2007). Here we report on the relationship between ER stress response and sleep homeostasis by quantifying the amount and intensity of homeostatic recovery sleep in response to the i.c.v. administration of SALUB in adult freely behaving rats. Specifically, we tested the hypotheses that (1) enhancing the UPR by administering SALUB during extended waking would further drive sleep pressure higher and thereby would increase the amount and intensity of recovery sleep and (2) enhancing the UPR by SALUB infusion during the recovery sleep from extended waking would prolong recovery sleep.

EXPERIMENTAL PROCEDURES

All experiments were conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and with the approval of IACUC at Veterans Affairs of Greater Los Angeles Healthcare System. Thirty-one adult male Sprague–Dawley rats (Harlan, IN, USA), weighing 250–275 g at the beginning of the experiments, were housed individually in Plexiglas cages under 12-h light/dark illumination (0700 lights ON:1900 lights OFF), constant temperature (23 °C), with *ad libitum* supply of food and water. Every effort was made to minimize the number of animals used and their suffering. Rats were randomly assigned to four groups in two experiments. Rats were administered SALUB or vehicle, i.c.v., in a counterbalanced order. Exp 1 examined the effects of SALUB on the homeostatic properties of sleep–wake. SALUB or vehicle was administered (a) during 6 h of SD followed by 18 h of recovery sleep with continuous artificial cerebrospinal fluid (ACSF) perfusion or (b) during the first 6 h of recovery sleep immediately after 6 h of SD, followed by an additional 12 h of recovery sleep with ACSF perfusion. In all groups, SD was initiated at ZT0, when sleep amounts are normally high. Exp 2 examined the effects on sleep-related Fos expression in selected hypothalamic cell groups with continuous infusion of SALUB or vehicle for 3 h during spontaneous sleep, beginning at ZT12, when sleep amounts are normally low (Table 1).

Surgery

Rats were anesthetized i.p. with a mixture of ketamine HCl (80 mg/kg) and xylazine (8 mg/kg) and surgically prepared for polygraphic sleep–wake recording under aseptic conditions as previously described (Methippara et al., 2009). Briefly, gold-plated stainless steel machine screws with soldered leads were threaded over the parietal and frontal cortices for electroencephalogram (EEG) recording, and Teflon-coated stranded stainless steel wires with bared ends were hooked into the dorsal neck muscles for electromyogram (EMG) recording. A screw electrode threaded onto the nasal bone was used as the ground. All electrodes were soldered to a standard connector. A guide cannula assembly consisting of 23-G thin-walled stainless steel tubing, which was shorter by 1–1.5 mm than an injector cannula, was stereotaxically implanted unilaterally over the lateral ventricle (coordinates AP=−0.8 mm, L=1.4 mm, and H=3.6 mm from bregma [Paxinos and Watson, 1998]) and fixed in place with dental acrylic. A removable obdurator stylet was inserted through the guide cannula and sealed to keep it patent. The plug with the soldered electrodes and the guide cannula was affixed on the skull with dental cement. Rats were housed individually in Plexiglas cages for 5–7 days of recovery from surgery. Postoperative care consisted of administration of the analgesic Buprenex (0.02 mg/Kg, im; 2×/d) for 2 days and application of a topical antibiotic around the incision (2×/d) for 3 days.

Adaptation

For Exp 1, rats were adapted for 2 h daily for 3 days to the recording enclosure and counter-weighted recording cable. On the 4th day, rats were placed on a treadmill without the treadmill movement; the next 4 days the treadmill was activated 3 h daily in the dark phase, with the treadmill running at the intervals required