Reduced Glutathione Regulates Sleep in Unrestrained Rats by Producing Oxidized Glutathione

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The present study was conducted to examine whether reduced glutathione (GSH), a naturally occurring tripeptide in the brain, exert somnogenic activity in freely behaving rats, since its dimer oxidized glutathione (GSSG) is a potent endogenous sleep-promoting substance. Nocturnal 10-h intracerebroventricular (icv) infusion of 50 nmol GSH increased amount of non-rapid-eye-movement (nonREM) sleep during the 12-h dark period (maximally 21 % above the baseline). Dose-response relations exhibited a bell shape at 4 different doses in a range 10-100 nmol. However, inhibition of GSH biosynthesis by diurnal 10-h icv infusion of a GSH peroxidase inhibitor, N-ethylmaleimide (NEM), at 4 different doses in a range 0.01-10 mol, dose-dependently brought about suppression of both nonREM and REM sleep. These results indicate that GSSG biosynthesized from GSH is actually responsible for the GSH-induced enhancement of sleep. (Sleep and Hypnosis 2000;1:26-30)

Key words: glutathione, GSH peroxidase inhibitor, intracerebroventricular infusion, N-ethylmaleimide, neuronal detoxification, nonREM sleep, sleep-promoting substance

INTRODUCTION

We identified oxidized glutathione (GSSG), a hexapeptide, as an active component of the Sleep-Promoting Substance (SPS) which was extracted from brainstems of sleep-deprived rats (1). Nocturnal intracerebroventricular (icv) infusion of GSSG induces an enhancement of both rapid-eye-movement (REM) sleep and nonREM sleep in rats (2). Further, it is reported that icv administration of GSSG increases sleep in rabbits (3) and mice (4). In addition, systemic administration of GSSG reduces rectal temperature in rats sleep-deprived by forced locomotion and shortens latencies to sleep in their recovery sleep (5).

GSSG is the dimer of reduced glutathione (GSH), a tripeptide, which is easily converted into GSSG by GSH peroxidase (6). GSH commonly exists in the mammalian brain (7-9). Then, a question arises as to whether GSH can exert somnogenic activity. If GSH be somnogenic, further questions arise as to what dose-response relation exists between GSSG and GSH, and which form of glutathione, GSSG or GSH or both, be responsible for sleep modulation. The present study deals with experimental approaches to such questions and shows that GSSG but not GSH is the actual sleep modulator.

MATERIALS AND METHODS

Male rats of the Sprague-Dawley strain raised in our closed colony were used. They were kept on a 12-h light and 12-h dark schedule (lights on: 08.00

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to 20.00 h) in a constant air-conditioned environment of 25 – 1 ßC and 60 – 6 % relative humidity with free access to rat chow and water. At the age of 60-70 days, rats weighing 300-350 g were anesthetized with pentobarbital sodium (50 mg/kg intraperitoneally), fixed on a stereotaxic apparatus and implanted with a stainless-steel cannula in the third cerebral ventricle for continuous icv infusion, three cortical gold-plated screw electrodes for recording electroencephalogram (EEG) and two nuchal stainless-steel electrodes for recording electromyogram (EMG). The electrodes and cannula were chronically fixed over the skull by a dental acrylic resin. The surgical techniques are described elsewhere (10). After the operation each animal received a total 40,000 U of penicillin G potassium subcutaneously and locally. Then they were individually housed in a special experimental cage which continuously enabled icv infusion and monitoring of EEG and EMG along with locomotor and drinking activities.

The experimental cages were placed in a soundproof, electromagnetically shielded room with the same environmental conditions as described above. Lead wires of the electrodes were connected with a polygraph (Nihon-Kohden EEG-4317). The external end of the cannula was connected via a thin polyethylene tubing (PE10) to an infusion pump (Central Kagaku Boh-eki, CKI-100), which continuously flowed out a sterile physiological saline at a rate of 10 l/h. A cannular feed-through slip ring, inserted in between the lead wires along with the infusion tubing, and fixed above the experimental cage, guaranteed free movements to the animals. Polygraphic recordings of EEG and EMG were done under continuous icv infusion of saline.

After more than one week of recovery from surgery and acclimatization to icv infusion, a 4-day sleep assay was conducted under continuous recordings of EEG and EMG along with continuous icv infusion: day 1 as baseline starting at the onset of the light period, day 2 as experiment when the saline infusion was replaced by an infusion of either four doses (10, 25, 50 and 100 nmol) of GSH (Sigma Chemical) dissolved in 100 l of saline for a 10-h period starting 1 h before the dark onset or four doses (0.01, 0.1, 1 and 10 mol) of N-ethylmaleimide (NEM) (Sigma Chemical), a GSH peroxidase inhibitor, dissolved in 100 l of saline for a 10-h period starting 1 h before the light onset, and days 3 and 4 as recovery when saline was icv infused as before. On the basis of polygraphic records of EEG and EMG, the vigilance state was classified into nonREM sleep, REM sleep and wakefulness at 12-s intervals according to our routinized criteria (11), fed into a computer and further analyzed statistically.

RESULTS

Sleep-waking pattern in rats under continuous icv saline infusion

As previously reported (12), the rats kept under continuous icv infusion of saline exhibited a light-dark cycle-entrained circadian rhythm of sleep and wakefulness that clearly manifested a night-active pattern of the rat.

Sleep-waking pattern in rats under nocturnal icv infusion of GSH

Table 1 summarizes the effect of 4 doses of GSH on sleep parameters.

Table 1. Changes in sleep parameters (mean – S.E.M., n=6) before, during and after l00-h nocturnal icv infusion of 50 nmol reduced glutathione on day 2 in male rats

		Day 1 (Baseline)	Day 2 (Experiment)	Day 3 (Recovery 1)	Day 4 (Recovery 2)
Light period					
NonREM sleep	Total time (min)	486.0 – 14.9	472.7 – 14.3	456.0 – 6.0	451.0 – 11.5
	Episode number	88.2 – 9.2	86.2 – 8.4	89.0 – 6.3	93.2 – 5.5
	Episode duration (min)	5.9 – 0.9	5.7 – 0.4	5.3 – 0.4	5.0 – 0.4
REM sleep	Total time (min)	79.5 – 5.7	77.6 – 2.1	85.7 – 3.5	85.9 – 4.5
	Episode number	36.5 – 1.7	33.5 – 1.8	38.7 – 1.3	39.0 – 2.5
	Episode duration (min)	2.2 – 0.1	2.4 – 0.2	2.2 – 0.1	2.2 – 0.2
Dark period					
NonREM sleep	Total time (min)	239.1 – 6.7[$]	280.2 – 10.8[$,#]	215.0 – 9.9[$]	231.7 – 11.1[$]
	Episode number	72.0 – 6.7	71.0 – 5.7	61.3 – 5.8	60.8 – 5.6
	Episode duration (min)	3.5 – 0.3	4.1 – 0.4	3.6 – 0.3	3.9 – 0.2
REM sleep	Total time (min)	26.3 – 3.9	31.3 – 4.8	19.7 – 4.2	26.8 – 3.9
	Episode number	20.5 – 1.6	22.2 – 1.6	14.8 – 3.8	20.0 – 3.4
	Episode duration (min)	1.3 – 0.1	1.4 – 0.2	1.4 – 0.1	1.4 – 0.1

[$] P< 0.005, ANOVA, $F_{3,20} = 9.57$.

[#] P< 0.01, significantly different from the corresponding baseline value (Student's t-test).

The 10-h nocturnal icv infusion of GSH induced an enhancement of sleep at the expense of wakefulness during the 12-h dark period. The total amount of nocturnal nonREM sleep was significantly enhanced by administration of 25 and 50 nmol/10 h. The latter dose was most effective and brought about an increment of 21 % above the baseline value. The lower and higher doses exerted less somnogenic activity, showing a definite bell-shaped dose-response relation (Figure 1, left). Although the administration of GSH induced an increase in the nocturnal amount of REM sleep by 13-21 %, the change was statistically insignificant due to large individual varieties (Figure 1, right).

Figure 1. Dose-response relation of the effects of nocturnally icv infused GSH on the amount of nonREM sleep and REM sleep during the 12-h dark period in otherwise saline-infused rats. Each value was expressed as percent change from the corresponding baseline. Vertical lines on each column stand for S.E.M. *P<0.05, **P<0.01 (Student's t-test). For 10 nmol, n=5. For 25 and 100 nmol, n=7. For 50 nmol, n=6

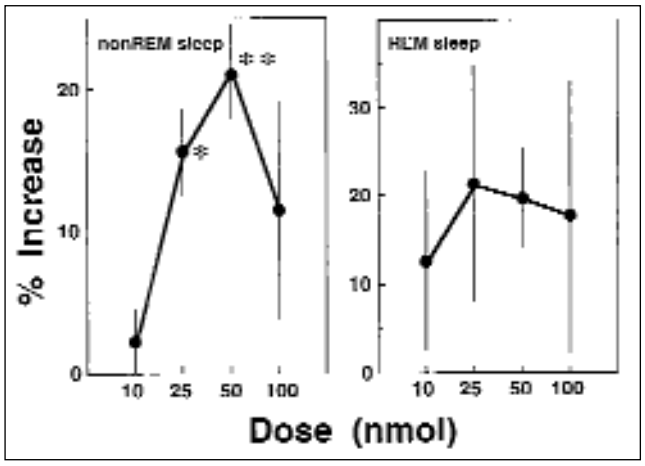
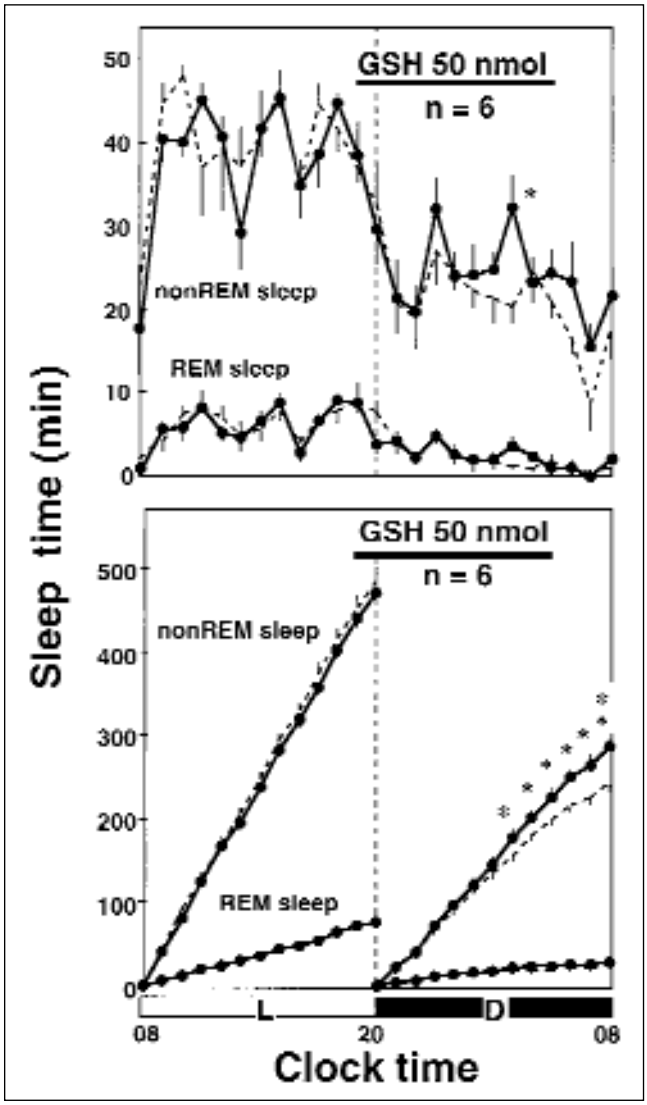


Figure 2 illustrates time-course changes in hourly amounts of sleep modulated by 50 nmol GSH. The enhancement of nonREM sleep appeared rather slowly, exhibiting statistically significant difference from the baseline of cumulative value 7 h after the initiation of GSH infusion. In the subsequent two recovery days, the time-course pattern of hourly amounts of sleep was largely equal to that of the baseline day. The sleep parameters during the light and dark periods of the recovery days showed no difference from the baseline values as shown in Table 1.

Sleep-waking pattern in rats under diurnal icv infusion of NEM

Table 2 summarizes the effect of 4 doses of NEM on the amounts of sleep. The 10-h diurnal icv infusion of NEM dose-dependently suppressed both nonREM sleep and REM sleep during the infusion

Figure 2. Effects of 10-h icv infusion of 50 nmol of GSH between 19.00 to 05.00 h (indicated by a solid bar) on the time-course changes in the amounts of nonREM sleep and REM sleep in otherwise saline-infused rats. Top: hourly amounts. Bottom: cumulative values for the light (L) and dark (D) period. Broken lines indicate baseline values on the previous day. Vertical lines on each hourly value stand for S.E.M. *P<0.05, **P<0.01 (Student's t-test).



period, i.e. the light period. In the 10 mol NEM group, the magnitude of sleep suppression was so striking that the decrements of nonREM sleep and REM sleep were 239.0 min (-57%) and 62.2 min (-89%), respectively, as compared to the previous baseline day. The diurnal suppression of sleep was followed by a significant rebound of nocturnal sleep. The increments of nonREM sleep and REM sleep were as much as 86.4 min (+33%) and 68.0 min (+259%), respectively, as compared to the previous baseline night. Figure 3 illustrates time-course changes in hourly amounts of sleep modulated by 10 mol NEM. The sleep parameters during the light and dark periods of the recovery days showed no significant difference from the baseline values.

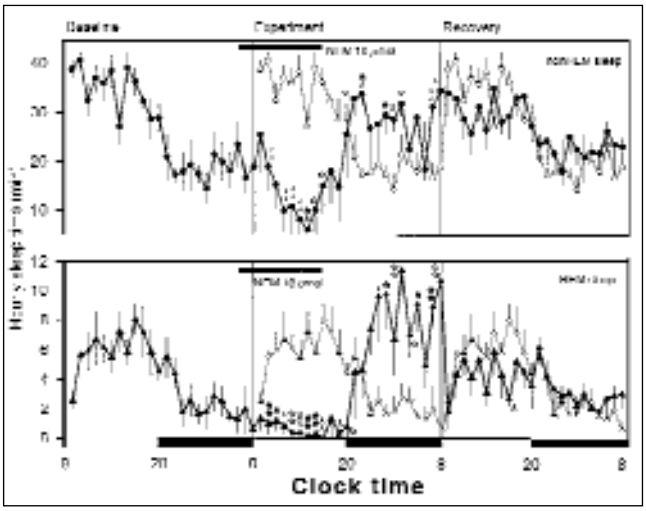
Table 2. Effect of intracerebroventricular infusion of N-ethylmaleimide (NEM) on the amounts of sleep (mean – S.E.M.) in male rats

		Light period		Dark period	
Groups	n	nonREM sleep** (min)	REM sleep** (min)	nonREM sleep* (min)	REM sleep** (min)
Baseline	22	417.8 – 6.4	70.0 – 2.5	260.7 – 9.6	26.3 – 2.7
NEM (mol)					
0.01	5	473.9 – 6.3	66.0 – 7.3	278.8 – 17.2	31.3 – 3.5
0.1	5	423.8 + 21.2	67.3 – 6.7	282.2 – 17.7	29.0 – 7.0
1	6	361.4 – 13.0[a]	52.7 – 6.1[b]	288.6 – 15.7	47.8 – 7.7[b]
10	6	178.8 – 53.5[c]	7.8 – 3.1[c]	347.1 – 19.5[c]	94.3 – 10.5[c]

**P < 0.001, *P < 0.01, one-way ANOVA, $F_{5,39} = 24.31$ for nonREM sleep and $F_{5,39} = 28.95$ for REM sleep during the light period; $F_{5,39} = 4.59$ for nonREM sleep and $F_{5,39} = 22.88$ for REM sleep during the dark period.

[a] significantly different from 0.01 and 0.1 mol groups (P < 0.05 for all combinations, Duncan's MRT).
[b] significantly different from baseline (P < 0.01), 0.01 mol (P < 0.05), 0.1 mol (P < 0.05) and 10 mol (P < 0.01) groups (Duncan's MRT).
[c] significantly different from each of the other dose groups (P < 0.01 for all combinations, Duncan's MRT).

Figure 3. Effects of 10-h icv infusion of 10 mol of N-ethylmaleimide (NEM) between 07.00 to 17.00 h (indicated by a solid bar) on the time-course changes in the hourly amounts of nonREM sleep and REM sleep in otherwise saline-infused rats. *P<0.05, **P<0.01 (Student's t-test).



DISCUSSION

In the present study we demonstrated for the first time that GSH, a naturally occurring tripeptide in the brain, exhibited sleep-enhancing effects, when it was icv infused during a 10-h nocturnal period in unrestrained rats. The dose-response relation was bell-shaped for the nonREM sleep-enhancing activity in the dosage range from 10 to 100 nmol, in which the maximal effect was induced by 50 nmol. The somnogenic effects induced by this dose of GSH was comparable to those induced by 25 nmol of GSSG (2). Since GSH is structurally a half part of GSSG, it is reasonable that the two-fold dose was required for the somnogenicity of GSH equivalent to that of GSSG.

It is well known that both forms of glutathione, GSH and GSSG, are detectable in the cerebral cortex, cerebellum, brainstem and cerebrospinal fluid in mammals (7). These peptides are localized in glial cells along with neuronal axons and terminals (9). In cultured cells, glutathione is present in high concentrations in astrocytes (13) and biosynthesized in astrocytes (14). Concentrations of GSH are always higher than those of GSSG (7,9). Since GSH is easily converted into GSSG by GSH peroxidase and GSH also promoted sleep as reported above, it became of special interest to analyze which form of glutathione could be responsible for the promotion of sleep.

Our experiments on the effects of NEM on sleep clearly demonstrates that the inhibition of biosynthesis of GSSG from GSH strongly suppressed both nonREM sleep and REM sleep during the administration period. These reactions appear to be physiological because the NEM-induced loss of sleep was immediately compensated by a rebound during the subsequent dark period which was then followed by a normal circadian sleep-waking rhythm.

This fact suggests that GSSG but not GSH is responsible for the modulation of sleep, and that the peroxidation process, i.e. conversion from GSH to GSSG, is inevitably required for sleep promotion. Thus, it is concluded that GSSG biosynthesized from GSH is actually responsible for the GSH-induced enhancement of sleep.

Since GSH serves as an important detoxicant in biological organisms during the peroxidation process which coincides with sleep promotion, we proposed that glutathione contributes the detoxification of neurons at the cellular level in synchrony with sleep in the behavior level (2,15,16). The present study can positively substantiate our speculation.

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