Full Paper

The Effect of Baclofen on Alterations in the Sleep Patterns Induced by Different Stressors in Rats

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Abstract. We have previously reported that sleep patterns are significantly affected by both physical and psychological stress induced by a communication box; however, the mechanism by which stress alters sleep patterns was not established. In the present study, we investigated the role of γ-aminobutyric acid (GABA), acting through the GABA B receptor, on stress-induced changes in sleep patterns. Our results show that physical stress increased the total wakefulness time by increasing sleep latency and inhibiting both rapid eye movement (REM) and non-rapid eye movement (NREM) sleep during a 6 h sleep-recording period. The GABA B agonist baclofen (20 pmol/2 μl) attenuated the effects of physical stress on sleep latency, total wakefulness, and NREM sleep, but not total REM sleep. In contrast, psychological stress enhanced total REM sleep and shortened REM sleep latency without altering other sleep patterns. The effect of psychological stress on total REM sleep was also reversed by baclofen. These results suggest that GABA via GABA B receptors may play a role in the regulation of specific sleep patterns by both physical and psychological stress.

Keywords: stress, rapid eye movement (REM) sleep, electroencephalogram (EEG), microinjection

Introduction

In past decades, abundant evidence has shown that stress greatly influences sleep in mammals, including humans (1–5). However, the mechanisms underlying how stress affects sleep is less well understood. Recently, we and other groups have reported that physical (foot shock) and psychological stress can be simultaneously induced in two groups of rats using a communication box (6–9). Psychological stress is generated by exposure to emotional responses, for example, visual, olfactory, and auditory stimuli arising from the adjacent foot shock (physical stress)-stressed animals without any direct physical stress (10, 11). Therefore, the effects of psychological stress and physical stress can be compared using the communication box.

Many neurotransmitters regulate sleep patterns, including neurotransmitters and hormones activated by stress such as dopamine (2), acetylcholine (12), and corticosterone (2, 13). The γ-aminobutyric acid (GABA) B receptor is widely distributed and plays an important role in regulating central nervous system function (14). The GABA B agonist baclofen has been shown to inhibit the release of neurotransmitters and stress hormones (15, 16), including, serotonin (17, 18), dopamine (19, 20), prolactin (21), acetylcholine (22), corticosterone (23), and corticotropin-releasing hormone (CRH) (24) in rats. Furthermore, GABA levels are decreased during stress, for example, in the nucleus accumbens, cortex, and brainstem of rats exposed to acute forced swimming stress (25) and in the hippocampus of rats undergoing chronic mild stress (26). GABA concentrations in the basolateral amygdala (BLA) of mice are also reduced by classic psychological stress: a conditioned fear stimulus (27). Interestingly, the GABA B agonist baclofen, but not the GABA A agonist muscimol, attenuated handling stress-induced increases in extracellular dopamine (28). However, the role of GABA B-receptor activation in stress-induced changes in sleep patterns is not known. Therefore, in this present study we examine whether baclofen normalizes the changes in sleep patterns caused by physical and psychological stress.
Materials and Methods

Animals

Male Wistar rats at 8–10 weeks of ages were obtained from Charles River (Yokohama). All animals were housed 2 rats to a cage in cages 42-cm-long × 26-cm-wide × 15-cm-high. The animal room was maintained at 22 ± 1°C under a 12-h/12-h light/dark cycle with lights on from 7:00 am. Food and water were available ad libitum. The animal experiments were performed in compliance with the Guidelines for Animal Experimentation and with the approval of the Committee of Animal Experimentation, Ehime University School of Medicine. Every effort was made to minimize the number of animals used and their suffering.

Surgery

The animals were anesthetized by the injection of sodium pentobarbital (Nembutal, 50 mg/kg i.p.) and electrodes for the recording of an electroencephalogram (EEG), electrooculogram (EOG), and electromyelogram (EMG) were implanted. The electrodes for EEG recording, consisting of a twisted pair of stainless steel wires (tip diameter, 0.2 mm) insulated except for the last 0.5 mm of the tips, were stereotactically implanted (SR-5; Narishige, Tokyo) in the frontal cortex and dorsal hippocampus (A: 4.3 mm, L: 2.5 mm, V: 2.5 mm) according to the stereotactic apparatus (29). The EEG from the cortex and hippocampus was recorded against a ground electrode placed over the frontal bone. The EMG was recorded from the neck with the same stainless steel electrodes. The EOG was recorded with a silver ball electrode (0.2 mm in diameter), which was placed in the orbit of the eye. Each electrode was connected to the pins of a small socket, which was fixed to the skull with dental cement together with two screws driven into the skull. In addition, stainless steel cannulae were implanted into the intracerebroventricular space (i.c.v., A: 0.8 mm, L: 1.4 mm, V: 3.0 mm) for microinjection of drug. At least 7 days were allowed for recovery from the surgery.

Experimental protocols

Psychological stress was employed using a communication box according to the previously described method (30). This box (90 cm × 90 cm × 90 cm) was equipped with a floor grid composed of stainless steel rods (0.5-cm diameter) placed 1.3-cm-apart. The box consisted of 9 small compartments (30 cm × 30 cm) divided by transparent plastic walls. In the current study, 2 compartments were used. Plastic plates were placed on the grid floors of 5 compartments to prevent the rats from receiving electric shocks. An electric foot shock generator (MSG-001; Toyo Sangyo Ltd., Toyama), combined with a timer box (MTB-001, Toyo Sangyo Ltd.) was used to produce a scrambled electric foot shock (2 mA) through the floor grid lasting for 10 s at intervals of 60 s for 1 h.

The rats placed directly on the electric grid floors were used as the physical stress group, while the rats placed in the compartments with plastic plates on the grid floor were used as the psychological-stress group. They could see the rats receiving foot shock via three sides of the transparent acrylic panels and hear the sounds and smells. These rats were exposed to various emotional stimuli from the rats in the compartments with the electric grid floors. These rats were only exposed to psychological stress without foot shock stress.

In the present study, the rats were then divided into 6 groups after recovery from surgery. Seven rats were used in each of the groups, which included a control group (saline, 2 μl); baclofen group (baclofen, 20 pmol/2 μl); physical-stress group with microinjection of saline (physical + saline, 2 μl); physical-stress group with microinjection of baclofen (physical + baclofen, 20 pmol/2 μl); psychological stress group with microinjection of saline (psychological + saline, 2 μl); psychological stress group with microinjection of baclofen (psychological + baclofen, 20 pmol/2 μl). Baclofen was purchased from Sigma-Aldrich (St. Louis, MO, USA) and was dissolved in 0.9% saline solution. Saline and baclofen were infused into the i.c.v. space in a volume of 2 μl; the dose of baclofen was determined by the preliminary experiments. The injection cannulae were connected to polyethylene tubing that in turn was connected to 5.0-μl Hamilton syringes. All of the microinjections were performed immediately after 1 h of psychological stress, followed by sleep recording for 6 h. Each animal was used only once and at the end of each experiment, crystal violet was injected in order to verify the injection sites after sleep recording.

The EEG, EMG, and EOG were recorded with an electroencephalograph (Model EEG 5113; Nihon Kohden, Tokyo) in the freely moving rat. The rat was moved into the recording plastic cage (30 cm × 18 cm × 24 cm), which was placed in a soundproof and electrically shielded box (100 cm × 100 cm × 100 cm) immediately after psychological stress. All electrophysiological recordings were started simultaneously when the rat was put into the recording plastic cage and continued. The formal recording was performed for 6 h after two 6-h adaptation periods of sleep recording. The signals were amplified and filtered (EEG, 0.5–30 Hz; EMG, 16–128 Hz; EOG, 0.1–30 Hz) simultaneously and stored on a computer hard disk for offline analyses. The sleep states were automatically classified by 10-s epochs as wakefulness, non-rapid eye movement.
(NREM) sleep, and REM sleep by OPS023 software (Nihon Kohden), according to the previously described criteria (31). The following parameters were used: sleep latency (from wakefulness to the onset of a consecutive 120 s sleep), REM sleep latency (from the onset of the consecutive 120 s sleep to first onset of REM sleep), total REM sleep, total NREM sleep, and total wakefulness time.

Statistical analyses

All values are presented as the mean ± S.E.M (n = 7). The significance of the data was analyzed using one-way ANOVA with Tukey’s test. P values less than 0.05 were considered significant.

Results

Figure 1 shows a representative polygraph recording of wakefulness, REM sleep, and NREM sleep by EEG, EMG, and EOG in a rat. Each state was characterized as follows: The wakefulness state was characterized by a low voltage EEG and a high amplitude EMG and EOG. The REM sleep state showed a low voltage EEG and a higher voltage EOG, which are similar with those of a wakefulness state, but the EMG was also low amplitude; in contrast, the NREM sleep state showed a high voltage EEG and a low amplitude EMG and EOG.

Figure 2 shows the effects of baclofen on sleep latency in the control, physical-stress, and psychological-stress group. Sleep latency was prolonged by physical stress (P<0.01) compared to the saline group. However, sleep latency was not influenced by psychological stress or baclofen alone.

Figure 3 represents the effects of baclofen on REM sleep latency in the control, physical-stress, and psychological-stress group. REM sleep latency was significantly inhibited in the psychological-stress group in comparison to that of the saline group (P<0.05). In

![Fig. 2. The effects of baclofen on sleep latency in the control, physical-stress, and psychological-stress group. The data represent the means ± S.E.M. (n = 7). ##P<0.01, vs. saline.](image)

![Fig. 3. The effects of baclofen on REM sleep latency in the control, physical-stress, and psychological-stress group. The data represent the mean ± S.E.M. (n = 7). #P<0.05, ##P<0.01, vs. saline group.](image)
Contrast, physical stress did not affect REM sleep latency, but REM sleep latency was significantly increased in the physical-stress group with injection of baclofen (physical + baclofen, P<0.01).

Figure 4 shows the effects of baclofen on the total REM sleep in the control, physical-stress, and psychological-stress group. Total REM sleep was inhibited in the physical-stress group (physical + saline, P<0.01), and increased by psychological stress in comparison to the control group (P<0.05). Moreover, the total REM sleep was significantly enhanced in the psychological stress group with baclofen (psychological + baclofen) in comparison to the saline group.

The effect of baclofen on the total NREM sleep in all groups is presented in Fig. 5. The total NREM sleep was significantly reduced by physical stress compared to the saline group (P<0.01). However total NREM sleep was not influenced in the other groups.

Figure 6 shows the effects of baclofen on the total wakefulness time in the control, physical stress, and psychological-stress group. The total wakefulness time significantly increased by physical stress but not by psychological stress. Furthermore, there is no significant difference in the wakefulness time between the physical stress with baclofen group and the saline group.

Discussion

The results of the present study show that physical stress inhibited both REM sleep and NREM sleep and increased wakefulness. These results are consistent with our reported findings (8) and other previous findings (32, 33). We also confirmed that psychological stress enhanced total REM sleep without any change of total NREM sleep or total wakefulness (8, 9). Changes in sleep patterns are thought to be regulated by multiple neurotransmitters, including acetylcholine (12), dopamine (2), and other stress-induced hormones and transmitters (12, 34, 35). GABA_B receptors are distributed throughout the brain (36); and baclofen, a GABA_B agonist, inhibits the release of many of these stress-regulated neurotransmitters and hormones, including acetylcholine (22), serotonin (17, 18), dopamine (19, 20), prolactin (21), corticosterone (23), and CRH (24) in rats. Furthermore, the levels of GABA in the central nervous system (CNS) are reduced by several kinds of stress, including acute forced swimming stress in rats (25) and conditioned fear stimulus stress in mice (27). Interestingly, the GABA_B agonist baclofen, but not the GABA_A agonist muscimol suppressed increases in extracellular dopamine by acute handling-stress (28). Therefore, in this present study we determined whether baclofen could normalize the changes in the sleep patterns caused by physical and psychological stress. Baclofen significantly enhanced total NREM sleep, but not total REM sleep in the physical-stress group. Furthermore, baclofen also significantly attenuated physical stress–induced increase in total wakefulness as well as increasing NREM sleep. These results are consistent with studies showing that microinjection of
baclofen into the pedunculopontine tegmentum (PPT) increased slow wave sleep and reduced wakefulness in rats (37, 38). Therefore, some of the effects of baclofen we report here may be due to inhibition of cholinergic neurons by baclofen in the PPT (37). However, in the present study microinjection of baclofen into the i.c.v. space should cause a general effect throughout the brain in comparison to a local microinjection into the PPT. This may explain why the present dose of baclofen was much lower in comparison to that used by Ulloor et al. (37).

Another noteworthy observation was that the effect of the same dose of baclofen on the sleep patterns was much greater in the physical and psychological-stress group in comparison to the control group. This discrepancy may be due to stress-activated neurotransmitters and stress hormones, which result in a greater sensitivity to baclofen in the stress group. In addition, we and others have previously reported that corticosterone, an index of hypothalamic-pituitary adrenal (HPA) activity (39–44), is significantly increased following physical stress (2, 7, 45). As baclofen inhibits the release of corticosterone (23) and CRH (24), it is possible that baclofen regulates sleep by inhibiting the HPA axis in the physical-stress group. Interestingly we found that baclofen did not change total REM sleep following physical stress while Ulloor et al. found that baclofen reduced REM sleep in the normal rats (37). These results suggest that REM and NREM sleep may be controlled by different neurotransmitters or stress hormones and that the changes in REM sleep following physical stress are not directly regulated by GABA_B receptors.

Total REM sleep was significantly elevated in the psychological-stress group. Previously we have found that the increase of total REM sleep induced by psychological stress was dose-dependently inhibited by atropine (9). This finding suggests that cholinergic neurons are important for the enhancement of total REM sleep induced by psychological stress. In the present study, total REM sleep was significantly inhibited by a microinjection of baclofen in the psychological-stress group; however, the total NREM sleep and wakefulness were not influenced. The same dose of baclofen was applied in the control group, physical-stress group, and psychological-stress group in the present experiment. However the observed results were completely different. One possible explanation is the difference in the nature of the physical and psychological stresses. Recently, evidence has shown that the release of acetylcholine was increased in response to psychological stress, including sensory stress (46), auditory stimulation (12), and fear-conditioned stress (47). Furthermore, local microinjection of baclofen into the PPT, where most cholinergic neurons are located in the brain, significantly reduced the total REM sleep through inhibition of cholinergic activity (37). Therefore, the reduction of REM sleep could reflect inhibition of cholinergic activity by baclofen in the psychological-stress group. Alternatively, the difference in sensitivity of REM sleep to baclofen between physical and psychological stress could be due to the intensity of the stress response. It is well known that the size of the stress response is much greater following physical stress than following psychological stress. For example, we have previously shown that the corticosterone concentration was not significantly increased after psychological stress (8). This increased stress response could induce a greater release of neurotransmitters in the CNS in the physical-stress group compared to the psychological-stress group. Therefore, it is possible that the dose of baclofen we used is unable to affect REM sleep as it is insufficient to overcome the larger stress response and concomitant increase in neurotransmitter release in the physical-stress group. This would suggest that changes in sleep patterns are not related to a stress-activated HPA axis in the psychological-stress group. In addition, it is possible that the increase in total REM sleep induced by psychological stress is related to sleep rebound. However, several studies have shown that sleep patterns are not be modified by sleep deprivation or mild stress for 1 or 2 h (48, 49). Therefore, total REM sleep induced by psychological stress cannot simply be attributed to sleep rebound.

In the present study, we did not quantitatively determine changes in GABA_B-receptor activity between the two types of stress groups. However, our results are consistent with the hypothesis that stress inhibits the release of GABA leading to a reduced tone on GABA_B receptors as baclofen normalized NREM sleep patterns in the physical-stress group as well as REM sleep patterns in the psychological-stress group. Interestingly, GABA release has been shown to be decreased in some models of depression such as learned helplessness (50) and forced swimming stress (25). Furthermore, GABA_B receptors are down-regulated in learned helplessness stress, an effect prevented by antidepressants (51). These preclinical studies and our own findings are consistent with the reduced GABA levels in depressed patients (52, 53). This relationship between stress, GABA_B-receptor activity, and depression needs to be studied further in the future.

In summary, our results show that both physical and psychological stress significantly altered sleep patterns. In the physical-stress group, total REM sleep and NREM sleep were both inhibited, with the reduction of total NREM sleep, but not REM sleep, being reversed by
microinjection of baclofen. These results suggest that the reduction of total NREM sleep may be related to reduction in GABA$_B$-receptor function. In contrast, the total REM sleep was greatly enhanced by psychological stress without any alteration of total NREM sleep, and the enhancement of total REM sleep was completely inhibited by baclofen. Taken together with previous experiments, we hypothesize that this effect may be due to inhibition of a population of cholinergic neurons activated by psychological stress. Therefore, the present study extends our previous findings and clarifies the relationship between GABA, sleep, and stress type, which may be useful in specific clinic applications in the future.

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