Glial cell line-derived neurotrophic factor promotes sleep in rats and rabbits

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Kushikata, Tetsuya, Takeshi Kubota, Jidong Fang, and James M. Krueger. Glial cell line-derived neurotrophic factor promotes sleep in rats and rabbits. Am J Physiol Regulatory Integrative Comp Physiol 280: R1001–R1006, 2001.—Various growth factors (e.g., growth hormone-releasing hormone, acidic fibroblast growth factor, nerve growth factor, brain-derived neurotrophic factor, and interleukin-1) are implicated in sleep regulation. It is hypothesized that neuronal activity enhances the production of such growth factors, and therefore, they in turn form part of the sleep regulatory mechanism. Glial cell line-derived neurotrophic factor (GDNF) promotes development, differentiation, maintenance, and regeneration of neurons, and its production is induced by well-characterized sleep regulatory substances such as interleukin-1 and tumor necrosis factor. Therefore, we investigated whether GDNF would promote sleep. Twenty-six male Sprague-Dawley rats and 30 male New Zealand White rabbits were surgically implanted with electroencephalogram (EEG) and electromyogram (EMG; rats only) electrodes, a brain thermistor, and a lateral intracerebroventricular cannula. The animals were injected intracerebroventricularly with pyrogen-free saline and on a separate day only) electrodes, a brain thermistor, and a lateral intracerebroventricular cannula. The animals were injected intracerebroventricularly with pyrogen-free saline and on a separate day with one of the following doses of GDNF: 5, 50, and 500 ng in rabbits and 50 and 500 ng in rats. The EEG, brain temperature, EMG (in rats), and motor activity (in rabbits) were recorded for 23 h after the intracerebroventricular injection. GDNF (500-ng dose) increased the time spent in nonrapid eye movement sleep in both rats and rabbits. Rapid eye movement sleep was not affected by the lower doses of GDNF but was inhibited in rabbits after the high dose. EEG slow-wave activity was not affected by GDNF. The current results provide further evidence that various growth factors are involved in sleep regulation.

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ment was correct, ANG II elicited a drinking response (31). Only rats with a positive drinking response were used. After a 1– to 2-wk recovery period, the animals were placed in sleep-recording chambers (model 352600; Hot Pack, Philadelphia, PA). The rats were habituated to the recording procedure for 3 days; during this period, the rats were connected to recording cables and injected daily with PFS at the same time as the experimental treatments were to be done. The rabbits were habituated to the recording chamber for 1 day. The animals were kept on a 12:12-h light-dark cycle (lights on at 0800 with the rats or at 0600 with the rabbits) at 21 ± 1°C ambient temperature. Water and food were available ad libitum throughout the experiment. A flexible tether connected the electrodes and thermistor leads to an electronic swivel. The animals were allowed relatively unrestricted movement inside the recording cages. EEG, brain temperature (Tbr), motor activity detected by an ultrasonic sensor movement inside the recording cages. EEG, brain temperature (Tbr), motor activity detected by an ultrasonic sensor

Results

Rats given heat-treated GDFN or physiological saline continued to exhibit the normal diurnal variations of sleep and Tbr that are characteristic of this species. The lower dose of GDFN tested in rats (50 ng) at dark onset (2000) also failed to affect any of the parameters measured in this study. Administration of the higher dose of GDFN (500 ng) at dark onset increased the amount of time spent in NREM sleep recorded period was normalized to 100%. The relative changes in EEG power density values from the baseline were then calculated. In addition, a computer program was used to determine the number of NREMS and REMS episodes and their mean episode lengths, with the criterion that each episode lasted at least 30 s.

Experimental protocol. Twenty-six rats and 30 rabbits were used in these experiments. Each rat received an injection of 4 μL PFS intracerebroventricularly to obtain control values. On a separate day, the same animals were then injected intracerebroventricularly with one of two doses of GDFN as follows: group 1, 50 ng (n = 6); group 2, 500 ng (n = 7). The injections took place on the dark onset (2000). In addition, seven rats were injected intracerebroventricularly with 500 ng GDNF at light onset (0800). Each rabbit received 25 μL PFS intracerebroventricularly in the same manner as rats. On the next day, the animals were injected intracerebroventricularly with one of three doses of GDFN as follows: group 1, 5 ng (n = 6); group 2, 50 ng (n = 8); group 3, 500 ng (n = 8). The injections took place between 0850 and 0925. As an additional control to exclude the effects of contaminants such as endotoxin, six rats and eight rabbits were injected intracerebroventricularly with 500 ng of heat-treated (90°C for 30 min) GDNF.

All statistical analyses were performed with two-way ANOVA for repeated measures across the entire recording period. A significance level of P < 0.05 was accepted.

RESULTS

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increases in NREMS resulted from an increase in the number of NREMS episodes \(327.1 \pm 8.6\) control vs. \(388.7 \pm 12.1\) during experiment, ANOVA; treatment effect; \(F(1,6) = 6.04; P < 0.05\) with an interaction of treatment and time; \(F(7,42) = 5.45; P < 0.01\). In contrast, the higher dose of GDNF at light onset (0800) failed to enhance NREMS, although it slightly decreased the number of NREMS episodes \(372.4 \pm 10.7\) control vs. \(347.3 \pm 5.8\) during experiment, ANOVA; treatment effect; \(F(1,6) = 13.26; P < 0.05\). In rats, GDNF failed to affect REMS (Tables 1 and 2) and also failed to affect EEG SWA after any dose. The higher dose of GDNF at dark onset slightly increased \(T_{br}\) [ANOVA; treatment effect; \(F(1,6) = 7.61; P < 0.05\)].

Heat-treated GDNF or physiological saline failed to alter the normal diurnal variations of sleep in rabbits (Fig. 2).

Intracerebroventricular administration of the lowest (5 ng) or middle (50 ng) dose of GDNF tested also had no effect on NREMS, REMS, SWA, or \(T_{br}\). However, intracerebroventricular administration of the highest dose of GDNF (500 ng) increased the amount of time spent in NREMS by \(~1\) h over the 23-h recording period [Fig. 2 and Table 1; ANOVA; treatment effect; \(F(1,7) = 11.84; P < 0.05\); with an interaction of treatment and time; \(F(7,49) = 2.94; P < 0.05\)]. In contrast, REMS was decreased after intracerebroventricular administration of the highest dose of GDNF [ANOVA; treatment effect; \(F(1,7) = 13.15; P < 0.01\); Table 2]. The decreases in REMS resulted from a decrease in the number of REMS episodes \(65.1 \pm 6.3\) control vs. \(57.4 \pm 6.0\) during experiment, ANOVA; treatment effect; \(F(1,7) = 7.36; P < 0.05\). The middle dose of GDNF also decreased the number of REMS

### Table 1. GDNF enhances spontaneous sleep in rats and rabbits

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Variables</th>
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<tr>
<td>Species</td>
<td>NREMS, min</td>
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<tr>
<td>Rat</td>
<td>GDNF</td>
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Values are means ± SE; \(n\), no. of animals. GDNF, glial cell line-derived neurotrophic factor; NREMS, nonrapid eye movement sleep; REMS, rapid eye movement sleep; SWA, slow-wave activity; \(T_{br}\), brain temperature. *Significant difference, \(P < 0.05\).
episodes \[67.8 \pm 5.2 \text{ control vs. } 59.8 \pm 5.3 \text{ during experiment, ANOVA; treatment effect; } \text{F}(1,7) = 30.37; \ P < 0.01\]. GDNF failed to affect SWA or T_{br} after any dose in rabbits (Fig. 2 and Table 1).

Although not systematically quantified, GDNF did not induce abnormal behavior in either rats or rabbits in the sense that animals continued to cycle through sleep-wake episodes, they were easily aroused if disturbed, and they exhibited no gross abnormal motor behavior.

**DISCUSSION**

The current results clearly indicate that GDNF promotes sleep in rats and rabbits. The effects of GDNF on sleep depended on the time of administration in rats. Previously, differences in sleep responses in rats were reported after administration of sleep-promoting or sleep-inhibiting substances at different times of the day. For example, administration of 10.0 ng IL-1\(\beta\) at dark onset enhances NREMS in rats, whereas the same dose of IL-1\(\beta\) suppresses NREMS if given during the light period (26). These differences likely resulted from the interaction of the circadian and homeostatic processes regulating sleep.

GDNF failed to enhance EEG delta wave activity during NREMS. In contrast, several other somnogenic growth factors, such as IL-1\(\beta\), TNF-\(\alpha\), GHRH, and aFGF, enhance EEG SWA at doses that also enhance duration of NREMS. The mechanisms responsible for EEG SWA remain unknown. In many circumstances, EEG SWA is thought to be indicative of NREMS intensity. For example, after sleep deprivation, “supranormal” EEG slow waves characterize NREMS (28) and are associated with higher arousal thresholds (27). Nevertheless, there is an extensive literature describing the separation of EEG SWA from state regulation.

For instance, intraperitoneal injections of IL-1\(\beta\) or TNF-\(\alpha\) induce increases in NREMS and decreases in EEG SWA in rats (7) and mice (4), whereas intracerebroventricular injections of IL-1 or TNF enhance both of these parameters (8, 26). Electrolytic lesions of the hypothalamus result in long-term reduction of EEG SWA, whereas NREMS moves toward normal duration after ~8 days (32). Furthermore, rats restricted to a daytime diet shift their diurnal rhythm of NREMS, becoming daytime active, but do not shift the diurnal rhythm of EEG SWA (30). Regardless of such considerations, it does not appear that GDNF has a major role in the regulation of EEG SWA, although it has the capacity to enhance NREMS, thereby suggesting that the mechanisms responsible for EEG SWA are different, in part, from those involved in sleep regulation.

The significance of the GDNF-induced inhibition of REMS in rabbits after the high dose is unknown. The mechanisms responsible for REMS regulation are, in part, independent from those responsible for NREMS (reviewed in Ref. 14), and it is possible to differentially stimulate or inhibit NREMS or REMS (e.g., see Table 1). Nevertheless, one possible mechanism of GDNF inhibition of REMS could be via dopaminergic neurons. GDNF is dopaminotrophic (2, 23), and dopaminergic neurons are implicated in sleep regulation (35). For instance, dopamine autoreceptor antagonists inhibit REMS (24). Furthermore, GFR\(\alpha\)-1 receptors are in many areas of brain, including the cholinergic system (22), which is also implicated in REMS regulation (reviewed in Ref. 33). Regardless, the effects of GDNF on REMS were small and only occurred in one species after the highest dose; thus, it remains to be determined whether this effect has biological significance.

The biological significance of the enhanced T_{br} in rats after GDNF is also questionable since rabbits, a
species often considered more sensitive to pyrogens, failed to display fever after GDNF. In rats, GDNF induced very small increases in Tbr, and it is possible that the hyperthermia affected sleep responses. Regardless, there is a rather extensive literature describing the multiple links between thermoregulation and sleep. For example, there is a regulated decrease in Tbr during the entry into NREMS, and an acute mild increase in ambient temperature is a well-characterized somnogen. Nevertheless, under a variety of circumstances, thermoregulation can be separated from sleep regulation (reviewed in Ref. 16). Some substances enhance both NREMS and Tbr (e.g., TNF and IL-1β), yet the pyrogenic actions of IL-1β can be pharmacologically blocked without affecting sleep responses (17). In contrast, the NREMS-promoting actions of IL-1β, but not its pyrogenic actions, are blocked by central administration of nitric oxide synthase inhibitors (9). Furthermore, some substances (e.g., IL-6) are pyrogenic but not somnogenic (25). Collectively, such considerations clearly indicate separate, yet linked, mechanisms for sleep and body temperature.

Microbial products such as endotoxin are common contaminants of manufactured peptides (21). Endotoxin induces production of a variety of somnogenic cytokines, and it is, itself, somnogenic. We thus tested heat-inactivated GDNF as a control, since endotoxin and other pyrogenic bacterial cell wall products are heat stable. Because the heat-inactivated GDNF had no effect on the sleep parameters studied, it seems unlikely that results obtained were due to contaminating bacterial cell wall products.

Perspectives

That GDNF promotes NREMS provides further evidence for the involvement of the brain cytokine network in sleep regulation. Previously, we hypothesized that cytokines and other somnogenic growth factors are important components of sleep mechanisms and are indicative of, and play a role in, sleep function (reviewed in Ref. 12). Thus we proposed that neuronal activation stimulates production of these sleep regulatory growth factors, e.g., GDNF mRNA levels are enhanced by neuron excitation (29). These growth factors alter membrane potentials of nearby neurons and thereby change their firing patterns. Thus synapses that were not activated by the initial train of events are secondarily activated with a time delay due to the production and diffusion times of upregulated growth factors (modeled in Ref. 13). As a consequence, the input-output activity patterns of neuronal groups are changed and thereby divorce environmental input from output; when this occurs, we hypothesize that the neuronal groups are in a state of sleep. Kavanau (10) also reached the conclusion that neuronal groups alter between states, although he used different reasoning. Finally, these growth factors also alter expression of genes involved in synaptic function. As a consequence, the efficacy of transmission at affected synapses is changed for longer periods of time because of structural changes. Thus sleep function (growth factor-induced synaptic sculpturing) is inseparable from sleep mechanisms (growth factor-induced altered circuit dynamics of neuronal groups; reviewed in Ref. 15).

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