Brain-derived neurotrophic factor enhances spontaneous sleep in rats and rabbits

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Kushikata, Tetsuya, Jidong Fang, and James M. Krueger. Brain-derived neurotrophic factor enhances spontaneous sleep in rats and rabbits. Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1334–R1338, 1999.—Various growth factors are involved in sleep regulation. Brain-derived neurotrophic factor (BDNF) belongs to the neurotrophin family; it and its receptors are found in normal brain. Furthermore, cerebral cortical levels of BDNF mRNA have a diurnal variation and increase after sleep deprivation. Therefore, we investigated whether BDNF would promote sleep. Twenty-four male Sprague-Dawley rats (320–380 g) and 25 male New Zealand White rabbits (4.5–5.5 kg) were surgically implanted with electroencephalographic (EEG) electrodes, a brain thermistor, and a lateral intracerebroventricular cannula. The animals were injected intracerebroventricularly with pyrogen-free saline and, on a separate day, one of the following doses of BDNF: 25 or 250 ng in rats; 10, 50, or 250 ng in rabbits. The EEG, brain temperature, and motor activity were recorded for 23 h after the intracerebroventricular injections. BDNF increased time spent in non-rapid eye movement sleep (NREMS) in rats and rabbits and REMS in rabbits. Current results provide further evidence that various growth factors are involved in sleep regulation.

neurotrophin-2; rapid eye movement sleep; growth factor; brain temperature; slow-wave sleep

THE HYPOTHESIS that sleep serves a synaptic function began with Moruzzi (26) and has been adopted by many others (5, 6, 17, 20, 22). Related hypotheses of brain organization as it applies to sleep suggest that sleep begins as a local event within small groups of highly interconnected neurons (1, 17, 20, 22) [neuronal groups as defined by Edelman (8)]. The notion that sleep is dependent on prior duration of wakefulness was modified in these theories to posit that sleep is dependent on prior neuronal use. It is thought that sleep, at the neuronal group level, is induced by growth factors that are produced locally in response to neuronal use. Those growth factors, in turn, alter synapses and thus input-output relationships of the neuronal group within which they are produced. Sleep function (growth factor-induced synaptic change) is thus posited to be inseparable from sleep mechanisms (growth factor-induced altered circuit dynamics of neuronal groups) (20, 22). There are of course many other ideas concerning sleep function (reviewed in Ref. 13); however, the notion that growth factors are central to both sleep mechanism and sleep function led to the current experiments.

A variety of growth factors are implicated in sleep regulation; the list includes endocrines, such as growth hormone (GH), GH-releasing hormone (GHRH), prolactin (PRL), and insulin, as well as autocrines or exocrines, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), acidic fibroblast growth factor (aFGF), and neurotrophin 1 (NT-1). All of these substances, if injected, have the capacity to enhance non-rapid eye movement sleep (NREMS) (insulin, IL-1β, TNF-α, aFGF), rapid eye movement sleep (REMS) (GH, PRL), or both (GHRH, NT-1) (27). Inhibition of GH, IL-1, TNF, or GHRH inhibits spontaneous sleep and, in the case of GHRH, IL-1, and TNF, sleep rebound after sleep deprivation (reviewed in Ref. 21). These and many additional data strongly suggest that these growth factors are involved in physiological sleep regulation (3, 21). Brain-derived neurotrophic factor (BDNF; also called neurotrophin-2) is another growth factor whose production by neurons is stimulated by neuronal use (36) and has the ability to promote the growth and survival of neurons in the central nervous system. BDNF and its receptor, a specific tyrosine kinase receptor (trkB), are widely distributed in the brain (7); both BDNF mRNA and trkB mRNA have diurnal rhythms in brain (4). Furthermore, BDNF mRNA levels in the cortex increase after sleep deprivation (32). We thus thought it likely that BDNF might promote sleep; we report here that injection of BDNF induces enhanced sleep in rats and rabbits.

MATERIALS AND METHODS

Recombinant human BDNF was purchased from Sigma (St. Louis, MO). BDNF bioactivity, its ability to support survival and stimulate neurite outgrowth of cultured embryonic chick dorsal root ganglia, was determined by the manufacturer. It contained 250 µg BSA per 5 µg. Substances were dissolved in pyrogen-free isotonic NaCl (PFS; Abbott, North Chicago, IL). Injection volumes were 4 µl for rats and 25 µl for rabbits.

Animals. Twenty-four male Sprague-Dawley rats (320–380 g) and 25 male New Zealand White rabbits (4.5–5.5 kg) were surgically implanted with electroencephalographic (EEG) electrodes, a brain thermistor, a lateral intracerebroventricular cannula, and electromyographic (EMG) electrodes (only in rats); ketamine-xylazine (35 and 5 mg/kg) anesthesia was used as previously described (16, 18). In rats, the patency and free drainage of the guide cannula were verified by injecting intracerebroventricularly 40 ng of angiotensin II (Sigma) in 4 µl of PFS. If the cannula placement was correct, angiotensin II elicited a drinking response (35). 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 (Hot Pack 352600, Philadelphia, PA). Rats were habituated to the recording procedure for at least 3 days; during this period, the rats were connected to recording cables and injected with...
PFS daily at the same time that the experimental treatments were to be done. Rabbits were habituated to the recording chamber for at least 1 day. The animals were kept on a 12:12-h light-dark cycle (lights on at 0800 for rats or 0600 for rabbits) at 21 ± 1°C (for rabbits) and 22 ± 1°C (for rats) ambient temperature. Water and food were available ad libitum throughout the experiment. A flexible tether connected the electrode and thermistor leads to an electronic swivel. The animals were allowed relatively unrestricted movement inside the recording cages. EEG, brain temperature (Tbr), motor activity (only in rabbits, detected by an ultrasonic sensor; Biomedical Instrumentation, Univ. of Tennessee), and EMG (only in rats) were recorded. The EEG was filtered below 0.1 and above 35 Hz. The amplified signals were then calculated. In addition, the number of NREMS and REMS episodes, mean episode lengths, and mean length of sleep cycle (REMS-REMS interval) were determined using a computer program with the criterion that each episode lasted ≥ 30 s.

Experimental protocol. A total of 24 rats and 25 rabbits were used in these experiments. In rats, each animal received an injection of 4 µl PFS intracerebroventricularly on a separate day to obtain control values. The same animals were then injected intracerebroventricularly with one of three doses of BDNF: 10 (n = 6), 50 (n = 6), or 250 ng (n = 7). The injections took place at dark onset. In rabbits, each animal received 25 µl PFS intracerebroventricularly as control, in the same manner as rats. On the next day, the animals were injected intracerebroventricularly with one of two doses BDNF: 25 (n = 7) or 250 ng (n = 8). The injections took place between 0845 and 0920. In addition, five rabbits were injected intracerebroventricularly with 250 ng BDNF at dark onset. Furthermore, five rats and five rabbits were injected intracerebroventricularly with 12.5 µg of BSA (Sigma) as an additional control, because high doses of BSA (140 mg) injected intraperitoneally induced increases in sleep and Tbr (28).

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

RESULTS

In rabbits, control injections of BSA had no statistically significant effects on any of the parameters measured in this study. The typical diurnal variations in sleep and Tbr parameters persisted in BSA- and physiological saline-injected controls. Intracerebroventricular administration of the lowest dose of BDNF tested

Table 1. Effects of BDNF on spontaneous sleep in rats and rabbits

<table>
<thead>
<tr>
<th>Substance</th>
<th>Injection Time</th>
<th>n</th>
<th>NREMS, min</th>
<th>REMS, min</th>
<th>SWA, %control</th>
<th>Tbr, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF, 10 ng</td>
<td>6:00 PM</td>
<td>6</td>
<td>553.4 ± 3.7</td>
<td>145.8 ± 3.7</td>
<td>100.0 ± 0</td>
<td>36.2 ± 0.1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6:00 PM</td>
<td>6</td>
<td>582.4 ± 19.1</td>
<td>121.3 ± 10.1</td>
<td>95.3 ± 2.8</td>
<td>36.4 ± 0.2</td>
</tr>
<tr>
<td>BDNF, 50 ng</td>
<td>6:00 PM</td>
<td>6</td>
<td>550.1 ± 21.2</td>
<td>135.6 ± 8.9</td>
<td>100.0 ± 0</td>
<td>36.2 ± 0.3</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6:00 PM</td>
<td>6</td>
<td>601.2 ± 25.6*</td>
<td>143.9 ± 5.6</td>
<td>92.1 ± 7.4</td>
<td>36.2 ± 0.3</td>
</tr>
<tr>
<td>BDNF, 250 ng</td>
<td>6:00 PM</td>
<td>6</td>
<td>557.8 ± 16.4</td>
<td>146.3 ± 8.6</td>
<td>100.0 ± 0</td>
<td>36.1 ± 0.2</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6:00 PM</td>
<td>7</td>
<td>638.6 ± 16.6*</td>
<td>147.9 ± 10.3</td>
<td>99.3 ± 4.3</td>
<td>35.9 ± 0.2</td>
</tr>
<tr>
<td>BSA, 12,500 ng</td>
<td>6:00 PM</td>
<td>5</td>
<td>544.6 ± 10.6</td>
<td>130.0 ± 7.0</td>
<td>100.0 ± 0</td>
<td>36.3 ± 0.1</td>
</tr>
<tr>
<td>Vehicle</td>
<td>6:00 PM</td>
<td>5</td>
<td>542.3 ± 22.7</td>
<td>138.5 ± 9.0</td>
<td>93.1 ± 4.1</td>
<td>35.8 ± 0.3</td>
</tr>
<tr>
<td>Rabbit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BDNF, 25 ng</td>
<td>9:00 AM</td>
<td>7</td>
<td>507.1 ± 18.1</td>
<td>50.3 ± 4.4</td>
<td>100.0 ± 0</td>
<td>38.0 ± 0.4</td>
</tr>
<tr>
<td>Vehicle</td>
<td>9:00 AM</td>
<td>7</td>
<td>511.6 ± 14.6</td>
<td>49.2 ± 1.9</td>
<td>99.3 ± 3.5</td>
<td>38.1 ± 0.4</td>
</tr>
<tr>
<td>BDNF, 250 ng</td>
<td>9:00 AM</td>
<td>8</td>
<td>513.7 ± 24.8</td>
<td>47.6 ± 5.2</td>
<td>100.0 ± 0</td>
<td>38.3 ± 0.2</td>
</tr>
<tr>
<td>Vehicle</td>
<td>9:00 AM</td>
<td>8</td>
<td>553.9 ± 25.8*</td>
<td>56.8 ± 5.4*</td>
<td>101.6 ± 4.0</td>
<td>38.3 ± 0.2</td>
</tr>
<tr>
<td>BDNF, 250 ng</td>
<td>6:00 PM</td>
<td>5</td>
<td>508.4 ± 22.4</td>
<td>44.0 ± 3.5</td>
<td>100.0 ± 0</td>
<td>38.3 ± 0.2</td>
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<tr>
<td>Vehicle</td>
<td>6:00 PM</td>
<td>5</td>
<td>566.1 ± 26.2*</td>
<td>63.8 ± 2.9*</td>
<td>91.1 ± 2.7*</td>
<td>38.3 ± 0.3</td>
</tr>
<tr>
<td>BSA, 12,500 ng</td>
<td>9:00 AM</td>
<td>5</td>
<td>512.7 ± 20.1</td>
<td>45.3 ± 8.2</td>
<td>100.0 ± 0</td>
<td>38.1 ± 0.7</td>
</tr>
<tr>
<td>Vehicle</td>
<td>9:00 AM</td>
<td>5</td>
<td>520.3 ± 7.9</td>
<td>50.1 ± 7.9</td>
<td>91.7 ± 7.3</td>
<td>38.3 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of animals. Rapid eye movement sleep (REMS) and non-REMS (NREMS) are measured in minutes occupied by sleep during 23-h postinjection period. SWA, slow-wave activity; Tbr, brain temperature; BDNF, brain-derived neurotrophic factor. *P < 0.05 vs. corresponding vehicle treatment.
(25 ng) also had no effect on NREMS, REMS, SWA, or Tbr (Table 1). In contrast, intracerebroventricular administration of BDNF (250 ng) during the light period (LP) increased the amount of time spent in NREMS (Table 1, Fig. 1) [ANOVA treatment effect; F(1, 7) = 29.92, P < 0.01] with an interaction of treatment and time [ANOVA treatment effect; F(1, 7) = 2.32, P < 0.05]. Further intracerebroventricular administration of BDNF (250 ng) at dark onset had a greater effect on the amount of time spent in NREMS; after dark onset injections rabbits spent ~1 h extra in NREMS over the recording period, whereas after daytime injection there were ~40 min extra in NREMS [ANOVA treatment effect; F(1, 4) = 21.54, P < 0.01]. REMS was also increased after intracerebroventricular administration of BDNF (250 ng) during either LP [ANOVA treatment effect; F(1, 7) = 13.15, P < 0.01] or at dark onset [ANOVA treatment effect; F(1, 4) = 22.66, P < 0.01] (Fig. 1, Table 1). The increases in REMS after dark onset injections resulted from an increase of the number of REMS episodes [1.7 ± 0.1 control vs. 2.2 ± 0.1 experiment; ANOVA treatment effect; F(1, 4) = 49.19, P < 0.01]. Sleep cycle length (REMS-REMS intervals) decreased [37.0 min ± 2.4 control vs. 27.0 ± 1.3 experiment; ANOVA treatment effect; F(1, 4) = 138.89, P < 0.01] with an interaction of treatment and time [ANOVA treatment effect; F(1, 4) = 2.47, P < 0.05]. EEG SWA decreased after 250 ng BDNF given at dark onset (Table 1) [ANOVA treatment effect; F(1, 4) = 14.68; P < 0.05]. BDNF failed to affect Tbr after any dose (Table 1).

In rats, BSA and physiological saline also failed to alter the normal diurnal variations of sleep in this species. The lowest dose of BDNF tested in rats (10 ng) failed to affect any of the parameters measured in this study. In contrast, administration of the moderate or highest dose of BDNF increased the amount of time spent in NREMS [ANOVA treatment effect; F(1, 5) = 8.41, P < 0.05 for 50 ng; F(1, 6) = 40.34, P < 0.01 for 250 ng] (Fig. 2, Table 1). For example, after the 250-ng dose rats spent ~1.3 h in NREMS over the 23-h recording period. About one-half of this increase took place in the initial 12-h postinjection dark period, and the other half took place during the subsequent 12-h light period. In rats, unlike in rabbits, BDNF failed to significantly affect REMS (Fig. 2, Table 1). In rats, the BDNF-induced increases in NREMS resulted from small increases in both the number of NREMS episodes and their duration, but neither individual effect reached significance. In rats, BDNF failed to affect sleep cycle length or the number or duration of REMS episodes.

Although not systematically quantified, BDNF did not induce abnormal behavior in either rats or rabbits, insofar as animals continued to cycle through sleep-
wake episodes, were easily aroused if disturbed, and failed to exhibit any gross abnormal motor behavior.

DISCUSSION

The effects of BDNF on sleep were dependent on the species and the time of administration. BDNF increased NREMS and REMS in rabbits but, in rats, increased only NREMS. The reasons for this species difference are unknown. Nevertheless, other sleep-promoting substances have similar species-specific effects on sleep. For example, prostaglandin D2 enhances sleep in rats but not in rabbits (12, 19). Furthermore, in rabbits, the effect of BDNF administration at dark onset on NREMS seemed to be greater than that observed after administration of BDNF during LP. Previous differences in sleep responses after administration of sleep-promoting or sleep-inhibiting substances at different times of the day in both rats and rabbits were described. For example, administration of 10.0 ng IL-1β at dark onset enhances NREMS in rats, whereas the same dose of IL-1β suppresses NREMS if given during LP (29). These differences likely resulted from the interaction of the circadian and homeostatic processes regulating sleep (reviewed in Refs. 2, 3).

BDNF failed to affect Tbr. There is a rather extensive literature describing the multiple links between thermoregulation and sleep (reviewed in Ref. 23). For example, there is a regulated decrease in Tbr during the entry into NREMS (39), and an acute mild increase in ambient temperature is a well-characterized somnogen (37). Nevertheless, under a variety of circumstances, thermoregulation can be separated, in part, from sleep regulation (23). Some substances enhance both NREMS and Tbr (e.g., TNF and IL-1β), but the pyrogenic actions of IL-1β can be pharmacologically blocked without affecting sleep responses (24); the effect of IL-1β on sleep and temperature is also differentially affected depending on where in the brain it is injected (38). Furthermore, the somnogenic actions of IL-1β, but not its pyrogenic actions, are blocked by central administration of nitric oxide synthase inhibitors (16). Other substances increase Tbr and inhibit sleep (e.g., corticotropin-releasing hormone). Collectively, such considerations clearly indicate separate yet linked mechanisms for sleep and body temperature.

EEG SWA decreased during NREMS after BDNF administration at dark onset in rabbits. Although this effect was significant, its biological importance is questioned because control injections of BSA induced similar decreases, though these were not significant. In many circumstances, EEG SWA is thought to be indicative of NREMS intensity. For example, after sleep deprivation, supranormal EEG slow waves characterize NREMS (31) and are associated with higher arousal thresholds (30). Nevertheless, there is an extensive literature describing the separation of EEG SWA from state regulation. For example, intraperitoneal injections of IL-1β or TNF-α can induce increases in NREMS and decreases in EEG SWA in rats (11) and mice (9), whereas intracerebroventricular injections of IL-1 or TNF usually enhance both (14, 29). Systemic injection of atropine induces EEG synchronization regardless of state (34). Removal of basal forebrain cholinergic neurons reduces EEG SWA but has little effect on the amount of NREMS (15). Regardless of such considerations, it does not appear that BDNF has a major role in regulation of EEG SWA.

Results presented here clearly indicate that BDNF has the capacity to enhance NREMS in both rats and rabbits. These results, coupled with the previous findings that BDNF mRNA increases in the cortex during sleep deprivation (32), suggest that BDNF may have a role in sleep regulation. Such a role for BDNF is also consistent with theoretical considerations suggesting that growth factors are part of sleep mechanisms and that their actions on synapses are part of sleep function (20, 22). Thus that BDNF is produced by GABAergic neurons in an activity-dependent manner (25) and that BDNF promotes synaptic plasticity (10) and affects cortical reorganization after nerve injury or after cutting or stimulation of facial whiskers (33) are consistent with the notion that sleep serves a synaptic function. Regardless of such consideration, current results provide further evidence that sleep is, in part, regulated by growth factors.

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REFERENCES


