An ether stressor increases REM sleep in rats: possible role of prolactin

B. BODOSI, F. OBÁL JR., J. GARDI, J. KOMLÓDI, J. FANG, AND J. M. KRUEGER. An ether stressor increases REM sleep in rats: possible role of prolactin. Am J Physiol Regulatory Integrative Comp Physiol 279: R1590–R1598, 2000.—Sleep alterations after a 1-min exposure to ether vapor were studied in rats to determine if this stressor increases rapid eye-movement (REM) sleep as does an immobilization stressor. Ether exposure before light onset or dark onset was followed by significant increases in REM sleep starting ~3–4 h later and lasting for several hours. Non-REM (NREM) sleep and electroencephalographic slow-wave activity during NREM sleep were not altered. Exposure to ether vapor elicited prolactin (Prl) secretion. REM sleep was not promoted after ether exposure in hypophysectomized rats. If the hypophysectomy was partial and the rats secreted Prl after ether exposure, then increases in REM sleep were observed. Intracerebroventricular administration of an antiserum to Prl decreased spontaneous REM sleep and inhibited ether exposure-induced REM sleep. The results indicate that a brief exposure to ether vapor is followed by increases in REM sleep if the Prl response associated with stress is unimpaired. This suggests that Prl, which is a previously documented REM sleep-promoting hormone, may contribute to the stimulation of REM sleep after ether exposure.

Address for reprint requests and other correspondence: J. M. Krueger, 205 Wegner Hall, Dept. of Veterinary and Comparative Anatomy, Pharmacology and Physiology, Washington State Univ., Pullman, Washington 99164-6520 (E-mail: krueger@vetmed.wsu.edu).

Received 8 March 2000; accepted in final form 3 July 2000

METHODS

Animals and surgery. Male Sprague-Dawley rats (300–550 g) were used. The surgeries were carried out under ketamine-xylazine (87 and 13 mg/kg, respectively) anesthesia. Stainless steel jewelry screws for electroencephalograph (EEG) recording were implanted over the frontal and parietal cortices and over the cerebellum on the right side. A thermistor placed over the left parietal cortex was used to record brain cortical temperature (Tcrt). The chronic intracerebroventricu-
ular cannula was stereotaxically inserted into the left lateral ventricle [−0.80 mm from the bregma, 1.4 mm from midline, and −3.4 mm from the top of the skull (29)]. The injection cannula protruded by 0.5 mm from the guide cannula. The location of the cannula was determined by the gravity method (sudden drop in pressure) during implantation. The function of the cannula and the drainage of the ventricular system were tested by the drinking response to intracerebroventricularly injected angiotensin (100 ng) before the recording, and the rats that failed to respond were left out of the experiments. Finally, Trypan blue was injected into the cannula, and the ventricles were examined at the termination of the experiments; data were used from those rats only in which Trypan blue stained the entire ventricular system.

For blood sampling, rats previously used in sleep experiments were chronically implanted with a Silastic catheter in the right atrium. The catheter was introduced via the right external jugular vein, and the distal end was exteriorized on the back of the neck. The catheters were flushed daily with heparinized physiological saline (200–300 IU/day in 0.2–0.3 ml) to prevent formation of blood clots. After surgery, the rats were placed in the bottom of the jar, and it was separated from the recording cage. A cotton cloth soaked with ether was placed in a jar that did not contain ether vapor but was exposed to the empty jar on day 1 (control) and to ether vapor on day 2. Because maintenance of HYPOX rats required relatively high ambient temperature and glucose supplement, the effects of ether exposure on REM sleep were also tested in a group of intact rats (n = 4) that were kept at 29–30°C and received drinking water containing 5% glucose.

Rabbit “polyclonal antiserum for rat Prl immunohistochemistry” (anti-rPrl-IC-5, lyophilized after 1:10 dilution in phosphate buffer) was obtained from the National Institute of Diabetes, Digestive, and Kidney Diseases (NIDDK) and used for in vivo immunoneutralization. The Prl-AS is provided without preservative and is devoid of antibodies to pituitary hormones other than Prl. The 1:10 dilution of the Prl-AS was reconstituted, and three injections of 4 μl of the solution were intracerebroventricularly injected 30, 20, and 10 min before ether exposure in six rats. On the control day, the rats were placed in the empty jar, and they received intracerebroventricular injections of pyrogen-free physiological saline at the same time points as the injections of Prl-AS on the experimental day. In another group of rats (n = 5), instead of Prl-AS, normal rabbit serum in the same dilution and volume as the Prl-AS was intracerebroventricularly injected. These rats were also recorded for 2 days; a control day with intracerebroventricular injection of physiological saline + exposure to the empty jar and an experimental day with intracerebroventricular administration of normal rabbit serum + ether stress. In all the experiments with Prl-AS or normal rabbit serum, the ether exposure occurred before light onset, and the rats were recorded from during the 12-h light period.

Finally, the effects of Prl-AS were tested on spontaneous sleep without ether exposure in one group of rats (n = 11). The rats received intracerebroventricular physiological saline on the control day and Prl-AS on the experimental day; the volume, dose, and timing of the injections were the same as those used in the group with ether stress, but these rats were not exposed to an empty jar or ether vapor. Sleep-wake activity was recorded during the 12-h light period.

The effects of various treatments were as follows. Each rat was allowed 7 to 10 days of recovery after the implantation of the EEG electrodes, the thermistor, and the intracerebroventricular cannula. During this period, the rats were connected to the recording tether and habituated to the experimental conditions. In addition, the rats prepared for intracerebroventricular injections were tested with angiotensin 2 to 3 days after implantation, and they received intracerebroventricular injections of physiological saline (4 μl) daily for 4 days before the onset of recording. The rats with an intracerebroventricular cannula were intracerebroventricularly injected with Trypan blue and killed immediately after the experiment to check the staining of the ventricular system. A group of rats that was used in sleep recording without intracerebroventricular cannula was implanted with the cardiac catheter 3 to 5 days after the termination of recording. These rats were used to determine the effects of ether exposure on plasma Prl for blood sampling 4 to 5 days postimplantation. Rats implanted with EEG electrodes and thermistors were HYPOX 7 to 10 days after surgery. Sleep in these rats was studied 7 to 8 days after hypophysectomy. Blood collection for Prl measurements occurred 3 to 4 days after sleep recording in HYPOX rats.

Recording. The rats were housed in individual Plexiglas cages. The cages were placed in recording rooms with a 12:12-h light-dark cycle and with an ambient temperature.
regulated at 26°C (29–30°C for the HYPOX rats). The rooms were sound attenuated. Food and water were continuously available. The rats were kept in conditions identical to those in the recording rooms for at least 1 mo before the operation.

The tethers were attached to commutators. The motor activity of the rats was assessed by recording potentials generated in electromagnetic transducers attached to the tethers. Cables from the commutators and electromagnetic transducers were connected to amplifiers (filtering below 0.1 and above 30 Hz) in an adjacent room. The signals were digitized (64-Hz sampling rate) and collected by a computer and stored on compact discs. For scoring, the EEG, Tcrt, and motor activity signals were restored on the computer screen. In addition, power density values were calculated by fast-Fourier transformation for consecutive 8-s epochs in the frequency range between 0.25 and 20.0 Hz for 0.25-Hz bands and integrated for 0.5-Hz bins; the spectral resolution was 0.125 Hz. The power density spectra were also displayed on the computer screen. The states of vigilance were determined over 8-s epochs by the usual criteria as NREM sleep (high-amplitude EEG slow waves, lack of body movements, and declining Tcrt on entry), REM sleep (highly regular theta activity in the EEG, general lack of body movements with occasional twitches, and a rapid rise in Tcrt at onset), and wakefulness (EEG activities similar to, but often less regular, with lower amplitude than those in REM sleep, frequent body movements, and a gradual increase in Tcrt after arousal). The percentage of the time spent in each state of vigilance of 1-h periods was determined. The 8-s Tcrt values were averaged for 1-h periods. Mean power density spectra were calculated for 8-s uninterrupted periods of artifact-free NREM sleep in each hour. The power density values for the 0.25- to 4-Hz (delta) frequency range were integrated and used as an index of EEG slow-wave activity (SWA) during NREM sleep to characterize sleep intensity in each recording hour (SWA was calculated for 2-h blocks in the dark-onset group to avoid time blocks without NREM sleep). In the light-onset group, power density values were calculated in the frequency bands between (in Hz) 0.25 and 4.0, 4.5 and 7.0, 7.5 and 12, and 12.5 and 20 for both NREM sleep and wakefulness in hour 1 of the recording of the control day (empty jar) and of the day with ether exposure (day 3) to determine if ether exposure altered EEG activity. The mean number per 12 h (frequency) and the mean duration of REM-sleep epochs were determined in both groups. Brief NREM sleep-wake sequences may intrude on REM sleep. When the time interval between two consecutive REM-sleep periods was <1 min, then these REM-sleep periods were counted as one epoch. The fragments of REM sleep were summed, but the intervening periods were not included in the calculated duration of the REM-sleep epochs. The selection of 1-min time windows for the occurrences of fragmented REM-sleep episodes was on the basis of the assumption that occurrences of REM sleep tend to peak at 8- to 12-min intervals, which are the normal duration of the sleep cycle in the rat, in both the oscillation and homeostatic models of REM-sleep regulation (3).

Determination of Prl. The rats (n = 10) fitted with a chronic catheter in the right atrium were exposed to the empty jar on the control day and to ether vapor on the experimental day. Blood samples (0.2 ml each) were withdrawn immediately before moving the rats into the jar and 5, 15, 30, and 60 min after they were returned to their recording cage. Silastic tubing was connected to the cardiac catheters, and the tubing was routed out of the cage. Thus the rats could move freely, and they were not disturbed by sampling. The dead-space volume was 0.15 ml. The lost volume was replaced by means of physiological saline.

HYPOX rats are not anticipated to respond with Prl secretion to ether exposure. Therefore, Prl was determined in a single blood sample obtained from the trunk by means of decapitation with guillotine 5 min after a second ether exposure in these rats. In this way, implantation of the chronic catheter could be avoided in the HYPOX rats.

Prl was determined in a single radioimmunoassay in duplicates. The blood was centrifuged, and the plasma was stored at −20°C until assayed. The intra-assay coefficient of variation was <7%. The immunoreagents (rat Prl-AS: anti-rPrl-S-9; iodination grade rat Prl antigen: rPrl-I-6; and rat Prl reference preparation: rPrl-RP-3) were provided by The National Hormone and Pituitary Program (NIDDK).

Statistics. The mean values of the states of vigilance, SWA, and Tcrt calculated for 2-h periods were used in the statistics. SWA is presented as deviation from baseline, but the statistical tests were also performed on the absolute values. The states of vigilance, SWA, and Tcrt during the 12-h recording periods were compared by means of two-way ANOVA for repeated measures between the recording days in a group of rats. The treatment effect and the time effect (variations across the individual hours) were the two factors of the ANOVA. In general, the F statistics are only provided for the treatment effect and for the interactions between the treatment and the time factors when statistically significant differences are noted, and significant variations in time are not discussed. Baseline values of REM sleep and NREM sleep were compared by ANOVA for independent samples among the various groups recorded in the light period. Differences in the mean duration and frequency of REM-sleep epochs were analyzed by means of one-way ANOVA for repeated measures among the 4 days of recording in the light-onset group and the dark-onset group. When several groups or more than 2 days were compared, the Student-Newman-Keuls test was used for post hoc analysis to identify which group or day differed from other groups or days. The changes in Prl after ether exposure were analyzed by means of ANOVA for repeated measures with respect to the baseline day. Plasma Prl concentrations 5 min after ether exposure were compared by means of Student’s t-test between the intact rats and the HYPOX rats. An α-level of P < 0.05 was considered to be significant in all tests.

RESULTS

Changes in sleep after ether exposure in intact rats. The rats groomed vigorously for a few minutes after exposure to ether. Then, their behavior was normal and they were mostly quiet. The time spent in the various states of vigilance in hour 1 of the recording after ether exposure did not differ from those values on any other day. Also, ether exposure did not alter the power density values in the various frequency bands either in wakefulness or NREM sleep as determined in hour 1 of recording in the light-onset group on the control day and the day with ether exposure. The changes in power densities during wakefulness after ether stress were as follows: delta band (0.5–4.0 Hz): −2.0 ± 3.2%; theta band (4.5–7.5 Hz): +2.8 ± 3.6%; alpha band (8.0–12.0 Hz): −4.2 ± 1.9%; and power densities from 12.0 to 20.0 Hz: −2.7 ± 1.6%. None of these changes were statistically significant, and in fact, the EEG looked normal.
Fig. 1. Effects of 1-min exposure to ether vapor on intact rats on the duration of the states of sleep (rapid eye-movement sleep (REMS) and non-REM sleep (NREMS)), electroencephalograph slow-wave activity (SWA, power densities in the 0.25- to 4.0-Hz range) during NREMS, and brain cortical temperature (Tcrt). Mean ± SE values calculated for 2-h blocks are presented. Light-onset group (n = 14): ether exposure was done before light onset, and the recordings were taken during the 12-h light period. Dark-onset group (n = 11): ether exposure was done before dark onset, and recordings were taken during the dark period (top: horizontal bar marks the dark period). ▪, day 1: the rats were undisturbed; ◀, day 2: 1-min exposure to a jar without ether; ●, day 3: 1-min exposure to ether vapor; and ◆, day 4: the rats were undisturbed. For SWA, percentage deviations from baseline day (day 1) and the control day (day 2: empty jar), REM sleep failed to increase in 3 of 14 rats in the 12-h light period after ether exposure. The amount of REM sleep after ether exposure was lower than on the control day (day 2) but higher on the baseline day (day 1) in one rat in the dark-onset group.

Increases in REM sleep began about 3–4 h after ether exposure (Fig. 1). In the light-onset group, maximum values of REM sleep were observed between hours 6 and 8 of the recording, and REM sleep returned to baseline by the end of the light period. In the dark-onset group, the amount of REM sleep after ether exposure approached values that were normally observed during the light period. The time × treatment interaction was statistically significant for the dark-onset group \( F(15,150) = 1.85, P < 0.05 \). Although increases in REM sleep continued until the end of the dark period, REM sleep returned to normal in the first hour of the subsequent light period (4.0 ± 1.47 and 5.29 ± 1.60% on the control day and on the ether day, respectively), and REM sleep calculated for the 11-h light period was also normal (9.7 ± 0.82 and 9.3 ± 0.69%). Increases in the time spent in REM sleep resulted from an increased number of REM-sleep epochs in both the light-onset group and the dark-onset group \( F(3,39) = 3.47, P < 0.05, \) and \( F(3,33) = 8.43, P < 0.05 \), respectively, with the ether-exposure day being different from the other recording days (Table 1). The mean duration of individual REM-sleep epochs tended to be slightly longer after ether exposure than on the other days, but these differences were not statistically significant.

Table 1. Mean frequency and duration of REM-sleep epochs during 12 h of recording

<table>
<thead>
<tr>
<th>Light-Onset Group</th>
<th>Dark-Onset Group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frequency</strong></td>
<td><strong>Duration</strong></td>
</tr>
<tr>
<td></td>
<td>n/12 h</td>
</tr>
<tr>
<td>Undisturbed</td>
<td>47.4 ± 3.16</td>
</tr>
<tr>
<td>Empty jar</td>
<td>47.4 ± 2.62</td>
</tr>
<tr>
<td>Ether exposure</td>
<td>56.6 ± 3.43*</td>
</tr>
<tr>
<td>Undisturbed</td>
<td>49.6 ± 3.29</td>
</tr>
</tbody>
</table>

Values are mean ± SE. REM, rapid eye movement. *Significantly different from the other days (ANOVA followed by Student-Newman-Keuls test, \( P < 0.05 \)).
There were no significant differences in NREM sleep among the 4 days of recording in that the ether exposure did not significantly alter NREM sleep in either group of rats (Fig. 1). Nevertheless, NREM sleep tended to decrease on day 4 (the day after ether exposure) in the light-onset group. SWA during NREM sleep differed among the 4 days in the light-onset group \((F[3,39] = 3.06, P < 0.05)\) due to significant decreases in SWA on day 4 (Student-Newman-Keuls test). No such differences in SWA were observed in the dark-onset group. Significant differences in Tcrt were not detected among the 4 days. Changes in Prl after ether exposure. Exposure to ether significantly stimulated Prl secretion \((F[1,8] = 8.99, P < 0.05)\); the effect varied with time \((F[4,32] = 3.91, P < 0.05)\). Prolactin was already increased in the sample taken 5 min after ether exposure (Fig. 2). Although Prl started to decline thereafter, it stayed above baseline for 30 to 60 min. Response to ether exposure in HYPOX rats. ANOVA was used to compare the time spent in REM sleep among the control days in all groups of rats studied in the light period, including day 2 of the light-onset group reported above. There were significant differences among the groups \((F[4,35] = 5.82, P < 0.05)\) with REM sleep being significantly less in the HYPOX rats than in any other groups (Table 2). In contrast, NREM sleep was normal in the HYPOX rats. Ether exposure failed to alter REM sleep, NREM sleep, or SWA (not shown) in the HYPOX rats (Fig. 3, Table 2). The four intact rats maintained at 29–30°C and provided with 5% glucose in drinking water had an increased amount of REM sleep on the control day (mean ± SE: 13.9 ± 1.0% recording time), but each of them responded with increases in REM sleep to ether exposure producing a group mean of 16.5 ± 1.2% amount of REM sleep.

The plasma PRL concentration was 2.6 ± 0.4 ng/ml in the HYPOX rats 5 min after ether exposure. This value was significantly less (Student’s t-test) than the concentration of PRL after ether exposure (13.7 ± 2.0 ng/ml) or on the control day (7.6 ± 0.8 ng/ml) at the same time point in the intact rats (Fig. 2). PRL concentration after ether exposure varied greatly in the three rats in which post mortem examination indicated partial hypophysectomy (8.5, 11.9, and 63.7 ng/ml), but each value was in or above the range of stimulated PRL secretion in the intact rats at the same time point. The mean amount of REM sleep and NREM sleep in the partially HYPOX rats was as low as in the HYPOX group. Unlike the HYPOX rats, however, each partially HYPOX rat responded with increases in REM sleep to ether exposure.

Table 2. Mean duration of sleep states (% recording time) during 12-h light period on control day and experimental day

<table>
<thead>
<tr>
<th></th>
<th>NREM Sleep</th>
<th>REM Sleep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Control</td>
</tr>
<tr>
<td>Intact + ether (Light-onset group)</td>
<td>14</td>
<td>49.0 ± 1.9</td>
</tr>
<tr>
<td>HYPOX + ether</td>
<td>7</td>
<td>50.9 ± 4.4</td>
</tr>
<tr>
<td>Partial HYPOX + ether</td>
<td>3</td>
<td>50.2 ± 7.3</td>
</tr>
<tr>
<td>Normal rabbit serum + ether</td>
<td>5</td>
<td>53.6 ± 4.9</td>
</tr>
<tr>
<td>PRL-AS + ether</td>
<td>6</td>
<td>54.3 ± 2.0</td>
</tr>
<tr>
<td>PRL-AS-no ether</td>
<td>9</td>
<td>54.7 ± 2.3</td>
</tr>
</tbody>
</table>

Values are mean ± SE. The names of the groups refer to the treatment on the experimental day (ether, ether exposure before light onset; HYPOX, hypophysectomized rats; PRL-AS, antiserum to prolactin). The rats were exposed to an empty jar on the control day except the PRL-AS-no ether group that was not stressed. The rats injected with PRL-AS or normal rabbit serum received pyrogen-free physiological saline on the baseline day. *Significantly different from control (treatment factor in ANOVA); †significantly different from the control day in the other groups (Student-Newman-Keuls test after ANOVA). Partial HYPOX + ether data are not included in statistical analysis because of the low sample size. NREM, non-REM.
Ether exposure after Prl-AS. Intracerebroventricular injections caused fever irrespective of the nature of the solution; physiological saline, normal rabbit serum, or Prl-AS. Figure 4 depicts Tcrt values in the groups injected with normal rabbit serum or Prl-AS with or without ether exposure. REM sleep was suppressed during the hyperthermic period, i.e., for 4–6 h, on both the control and the experimental days in each group. In contrast, NREM sleep tended to increase in these rats, although the changes did not reach the level of statistical significance (Table 2).

Exposure to ether promoted REM in the rats injected with normal rabbit serum [F(1,4) = 9.327, P < 0.05] (Fig. 4, Table 2). In contrast, ether exposure failed to stimulate REM sleep in the group injected with Prl-AS (Fig. 4, Table 2). In fact, decreases in REM sleep were observed in five of six rats, although these changes analyzed by ANOVA did not reach the level of statistical significance [F(1,5) = 2.936, P = 0.1]. REM sleep also decreased in the rats that received Prl-AS without ether exposure (Fig. 4, Table 2). The changes in REM sleep after Prl-AS with or without ether exposure were of similar magnitude, but the reduction in REM sleep in the later group was statistically significant [F(1,8) = 7.83, P < 0.05].

With respect to saline baseline, NREM sleep and SWA (not shown) were not altered after normal rabbit serum or Prl-AS with or without ether exposure (Table 2).

**DISCUSSION**

A brief ether exposure promotes REM sleep. This finding is consistent with reports on the effects of another stressor, acute immobilization, on REM sleep in rats in that the increases in REM sleep result from an increase of the frequency of REM-sleep epochs (4, 6, 13), and the changes in REM sleep occur mostly within 12 h after exposure to the stressor (6). Furthermore, like in the experiments with immobilization (6, 30), a few rats failed to respond with increases in REM sleep to ether vapor in our studies. In the current experiments, ether exposure selectively stimulated REM sleep; NREM sleep was not altered. Similar findings are often observed after immobilization (13, 30). Nevertheless, possible long-term changes in NREM sleep cannot be excluded as indicated by the decreases in NREM-sleep intensity 1 day after the exposure to ether. The explanation and the biological significance of this finding is currently not clear. Finally, current findings indicate that ether exposure could also be used to stimulate REM sleep when applied just before the light period without significant sleep loss during the exposure to the stressor. It is noted that manipulation of rats before light onset also means that the rats were exposed to a brief light exposure when they were moved into the jar or when intracerebroventricular injections were administered. These treatments, however, did not differ between the control and the experimental days, and therefore the changes in REM sleep after ether exposure were not due to a phase advance of the diurnal rhythm of REM sleep.

Our idea that Prl might be involved in the mediation of the stressor-induced enhancements in REM sleep is on the basis of several observations as follows. Increases in REM sleep after ether exposure develop slowly, over a period of several hours, and culminate long after the acute stressor is over. This time course suggests an action that involves several metabolic steps rather than a direct neurotransmitter-mediated effect. A similar time course is observed for the increases in REM sleep induced by systemic administration of Prl (27, 33). Further in our experiments, Prl secretion in response to ether exposure was verified (11, 17, 23). Prl is transported into the cerebrospinal fluid by a receptor-mediated mechanism residing in the choroid plexus (38). The expression of the Prl transporter is stimulated by Prl itself (18) resulting in enhanced intracerebral Prl concentrations when systemic Prl is increased. Stress-associated rises in Prl secretion, e.g., Prl release induced by a 30-min immobilization, are effective in promoting the expression of the
Prl transporter in the rat (10). Hypophysectomy and the administration of Prl-AS were two approaches used to estimate the possible contribution of Prl to promotion of REM sleep after exposure to ether. Although caution is required in the interpretation of the results of the individual experiments, collectively, the findings support an involvement of Prl in the REM-sleep alterations.

Spontaneous REM sleep was significantly reduced, whereas NREM sleep was normal in the HYPOX rats. The literature on sleep alterations in HYPOX rats varies. In a previous experiment in our laboratories, HYPOX rats exhibited decreased NREM sleep and normal REM sleep; the hypophysectomy was verified by means of undetectable growth hormone (GH) in systemic blood (24). HYPOX rats, however, were also reported to have decreased or normal REM sleep and decreased, normal, or increased NREM sleep (37, 41, 42). The time elapsed between hypophysectomy and recording might contribute to the divergent findings. The rats studied in our previous experiments (24) were HYPOX 1 to 2 mo before the sleep recording. Some metabolic alterations might be compensated, whereas others might result in chronic deficiency during such a long period. Valatx et al. (37) demonstrate that the amount of REM sleep is practically normalized 30 days after surgery in HYPOX rats if these rats are recorded at 30°C instead of normal room temperature. Our rats were recorded in a relatively early stage after the removal of the pituitary because the low baseline Prl-like activity in the acutely HYPOX rats rises up to 50% of normal levels after several weeks (22). Small nests of Prl-producing cells are believed to be the source of this Prl; these cells remain in the sella turcica after any method of hypophysectomy (40). Low concentrations of Prl were, in fact, detected in our HYPOX rats after ether exposure, but these Prl levels were only one-third of the baseline Prl measured in intact rats without ether stress. Exposure to ether vapor did not promote REM sleep in the HYPOX rats. The absence of REM-sleep responses in the HYPOX rats was not due to the glucose supplement and relatively high ambient temperature for both the partially HYPOX rats and the group of intact rats maintained at the same conditions as the HYPOX rats exhibited increases in REM sleep after ether exposure. Stimulation of REM sleep occurred in the intact rats despite an already enhanced REM sleep on the control day at 29–30°C, the ambient temperature at which REM sleep normally peaks in rats (36). Our finding indicates that the presence of the pituitary is important for the effects on REM sleep, but it does not necessarily imply that Prl is involved. The bulk of the pituitary was also removed in the partially HYPOX rats. The low-baseline amount of REM sleep indicated that these rats had the same fundamental deficiencies as the HYPOX rats. Nevertheless, the capacity of the remaining cells to release Prl on stress was obviously enough to produce high plasma Prl concentrations in the partially HYPOX rats. A decreased activation of the ACTH-corticosterone axis may promote Prl secretion in the partially HYPOX rats for corticosterone attenuates Prl release in intact rats (11). Not only Prl secretion, but also REM sleep was enhanced in response to ether exposure in the partially HYPOX rats. These findings suggest that a rise in plasma Prl might be necessary for the stimulation of REM sleep after exposure to ether.

The immunoneutralization of Prl inhibited the increases in REM sleep after ether exposure. There are, however, two complicating factors that have to be considered in the interpretation of the results. First, Prl-AS decreased spontaneous REM sleep in the absence of exposure to ether vapor. Thus low-REM sleep after ether exposure might result from an inhibition of spontaneous REM sleep. Second, the intracerebroventricular injections of a serum may alter REM sleep independently of Prl, e.g., via fever.

The decreases in spontaneous REM sleep after Prl-AS in rats without ether exposure correspond to previous observations after intrahypothalamic injection of Prl-AS (34), and they may result from the removal of the REM sleep-promoting activity of intraneuronal Prl (31). The major aim of Prl-AS injections in the current experiment was the inhibition of Prl entering the brain from the systemic circulation but, of course, immunoneutralization of intraneuronal Prl could not be avoided. A very high dose of Prl-AS was therefore injected; in fact, the dose of Prl-AS in our studies was large enough to suppress systemic Prl after leakage into the pituitary circulation (21). The decreases in REM sleep were of the same magnitude in the rats injected with Prl-AS with and without exposure to ether vapor. Thus inhibition of ether-stress-associated promotion of REM sleep is not attributed to the low spontaneous REM sleep in the rats injected with Prl-AS.

The intracerebroventricular injections caused fever, NREM sleep tended to increase, and REM sleep tended to decrease in these rats. The large dose of Prl-AS required large volumes of intracerebroventricular injections that can induce sleep alterations in the rat (5). In addition, because the injection cannula was 0.5 mm longer than the implanted guide cannula, microlesions by the tip of the cannula might also cause fever. Increases in NREM sleep and suppression of REM sleep are characteristic responses to cytokine release (16). Fever was also observed after physiological saline administration on the baseline days, and it did not interfere with the promotion of REM sleep after ether exposure in the rats injected with normal rabbit serum. Thus the inhibition of promotion of REM sleep after ether exposure is attributed to the neutralization of Prl-like immunoreactivity and not to some nonspecific actions of the Prl-AS.

The similarities in the sleep responses to ether exposure and acute immobilization and the fact that immobilization stress is associated with Prl releases (e.g., Ref. 1) open up the possibility that Prl may also contribute to REM-sleep promotion after immobilization in the rat. Currently, different substances are implicated in the mechanism of stimulation of REM sleep by immobilization. Corticotropin-like intermedi-
ate lobe peptide (CLIP) injected into the dorsal raphe nuclei promotes REM sleep (9). Bonnet et al. (4) report that CLIP-like activity increases in the dorsal raphe nuclei in rats subjected to immobilization. The authors suggest that CLIPergic neurons projecting from the arcuate nucleus to the raphe are activated during stress and may mediate the increases in REM sleep. González and Valatx (13) showed that corticotropin-releasing hormone (CRH) has a key role in the immobilization-induced enhancements in REM sleep because intracerebroventricular administration of a CRH antagonist blocks the sleep response. These authors proposed that CRH acts on REM sleep via the locus ceruleus. The importance of the various REM sleep-promoting mechanisms may vary with the type of stressor, and it is possible that these mechanisms are related. For example, CRH is involved in stress-associated Prl secretion because inhibition of CRH suppresses the Prl response to stressors (1).

Perspectives

It is not known how Prl stimulates REM sleep. Our speculation is that the site of the action is in the brain stem because Prl continues to enhance REM sleep-like activity in cats with mesencephalic transection (15). Expression of Prl receptors is well-documented in the forebrain (e.g., Refs. 2, 32), but Prl receptors are also reported inpons and medulla (35) and the ventral tegmental area of the mesencephalon (32). The long latency of the REM-sleep response to Prl suggests that the sleep effects are mediated by some metabolic actions. Stimulation of pyruvate dehydrogenase by Prl in cholinergic neurons is a likely candidate. Increased pyruvate dehydrogenase activity may result in enhancements in REM sleep (14) due to the increased availability of acetylcholine in the brain stem neurons involved in the genesis of REM sleep. Prl, in fact, stimulates pyruvate dehydrogenase in some peripheral tissues (8, 39), and preliminary findings in our laboratory indicate that it may also do so in the brain stem. Regardless of these possibilities, current results are consistent with the hypothesis that increases in REM sleep after ether exposure are mediated in part by Prl. The mechanism outlined above is a permissive action by Prl on REM sleep, and in that case CLIP could provide the trigger that eventually results in increases in REM sleep after a stressor.

The authors thank Dr. G. B. Makara (Institute of Experimental Medicine, Budapest, Hungary) for the help in hypophysectomy and S. Tóth for the technical assistance.

This work was supported by ETT-627/1996–04 and OTKA-T0-30456 to F. Obal Jr. and by the National Institutes of Health Grant NS-27250 to J. M. Krueger.

Permanent address of J. Gardi: Endocrine Unit, Albert Szent-Györgyi Medical University, 6720 Szeged, Hungary.

REFERENCES


36. Szymbusiak R and Satinoff E. Maximal REM sleep time defines a narrower thermoneutral zone than does minimal metabolic rate. Physiol Behav 26: 687–690, 1981.


