Pituitary adenylate cyclase-activating peptide affects homeostatic sleep regulation in healthy young men

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Submitted 31 March 2006; accepted in final form 31 October 2006

Murck H, Steiger A, Friebœs RM, Antonijevic IA. Pituitary adenylate cyclase-activating peptide affects homeostatic sleep regulation in healthy young men. Am J Physiol Endocrinol Metab 292: E853–E857, 2007. First published November 22, 2006; doi:10.1152/ajpendo.00152.2006.—Pituitary adenylate cyclase-activating peptide (PACAP) is involved in autonomous regulation, including timekeeping, by its action on the suprachiasmatic nucleus and on neuroendocrine secretion, energy metabolism, and transmitter release. In particular, the interactions between PACAP and the glutamatergic system are well recognized. We compared the effect of intravenously administered PACAP to that of placebo in eight healthy male subjects. PACAP in a concentration of 4 \times 12.5 \mu g was administered in a pulsatile fashion hourly between 2200 and 0100. Sleep EEG was recorded from 2300 to 1000, which was also the time when subjects were allowed to sleep. Blood samples were taken every 20 min between 2200 and 0700 for the determination of cortisol, GH, and prolactin. PACAP administration led to no changes in the macro-sleep structure as assessed according to standard criteria. Spectral analysis revealed a significant reduction in the \theta-frequency range in the first 4-h interval and of the spindle frequency range in the second 4-h interval of the registration period. This was accompanied by an increase in the time constant of the physiological \beta-power decline in the course of the night, i.e., a less pronounced dynamic of the reduction of \beta-power with time. This was accompanied by a trend (P < 0.1) toward decreased prolactin secretion in the first 4-h period of the night. No other changes in endocrine secretion were observed. We concluded that PACAP leads to a reduction of the dynamics of homeostatic sleep regulation and prolactin secretion. Both effects are the opposite of those seen after sleep deprivation but similar to the changes after napping, i.e., a reduced sleep propensity. This implies that PACAP might be involved in homeostatic sleep regulation.

cortisol; growth hormone; prolactin

Both VIP and PACAP promote the effect of glutamate within the brain; in particular, N-methyl-D-aspartate (NMDA) evokes Ca²⁺ release and the following cascade of PKC activation and the expression of brain-derived neurotrophic factor (16). In the time frame of hours, both peptides lead to an increase in astrocytic glycogen synthesis (16), which has been proposed to be one of the functions of sleep (6).

Both VIP and PACAP induce changes in sleep parameters in experimental animals. Local administration of PACAP at the onset of darkness into the pontine reticular formation of rats leads to a promotion of rapid-eye-movement (REM) sleep (1) that is similar to the action of VIP when administered intracerebroventricularly (22, 24; see Ref. 29 for review). For VIP, the anatomical area responsible for this effect is less clear because microinjection of VIP into the amygdala also leads to an increase in REM sleep (26). Furthermore, both peptides stimulate melatonin secretion in vitro (27), which might mediate an indirect effect on sleep structure. We (19) have shown recently that repetitive administration of VIP affects the temporal structure of sleep regulation, since it decelerates non-REM-REM cycles in healthy young men and leads to a phase advance of the sleep structure and of neuroendocrine secretion. PACAP has also been demonstrated (4) to cross the blood-brain barrier and probably reaches intracerebral concentrations that are sufficient to modulate astrocyte function. We now want to assess whether intravenous PACAP administration also influences the temporal structure of sleep in humans.

METHODS

Subjects

This study was approved by the Ethics Committee for Human Experiments at the Max Planck Institute of Psychiatry. Written, informed consent was obtained from eight healthy young men (age range 22–30 yr) of normal height and weight. They underwent extensive psychiatric, physical, and laboratory (hematology, virology, clinical chemistry, endocrinology, EEG, and electrocardiographic) examinations. Individuals with a personal or family history of psychiatric disorders or a recent stressful life event were excluded, as were shift workers and persons who had recently made a transmeridian flight. Other exclusion factors were abuse of drugs, nicotine (>2 cigarettes per day), alcohol, and caffeine.

Peptide

PACAP-35 was obtained from Clinalfa (Läufelfingen, Switzerland).

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Study Design

Each subject participated in two sleep-endocrine sessions, each consisting of two successive nights (adaptation night followed by the recording night) separated by 1 wk. The sleep EEG was recorded from 2300 to 1000. PACAP (4 × 12.5 µg equivalent to a total of 11 nmol) or saline was injected at 2200, 2300, 2400, and 0100 through an indwelling intravenous catheter connected to plastic tubing that ran through a soundproof lock into the adjacent room. The dose was chosen to be of a similar dose range as VIP in our previous experiment (19) but also to be tolerated by the subjects. This led to the decision of a lower range than for VIP. This allowed drug administration and repeated blood sampling in the adjacent laboratory without disturbing the subject’s sleep. Blood samples were collected every 30 min between 2000 and 2200 and every 20 min between 2200 and 1000. Specimens collected before 2200 served to control for stress effects of a lower range than for VIP. This allowed drug administration and repeated blood sampling in the adjacent laboratory without disturbing the subject’s sleep. Blood samples were collected every 30 min after cannulation (1930), whereas those collected between 2200 and 1000 were included in the time course analysis. The electrodes were fixed between 2100 and 2200. After 2200 the subjects stayed in bed, aside from restroom visits. The subjects were not allowed to sleep until the lights were turned off at 2300, when the polysomnographic registration also started. They were allowed to sleep as long as they required but were finally awakened at 1000 when necessary.

Hormone Measurements

For the determination of hormone measurements, we used commercial radioimmunoassays. The detection limit of the GH assay (Advantage; Nichols Institute, San Juan Capistrano, CA) was 3 ng/ml. The intra-assay coefficients of variation were <12% for GH concentrations between 30 and 800 ng/ml. The interassay coefficients of variation at the same GH concentration range were between 6 and 11%. For cortisol (RIA Kit J125; ICN Biomedicals, Carson, CA), the detection limit was 0.3 ng/ml plasma; intra- and interassay coefficients of variation for 20 and 40 ng/ml levels were <7%. Immunoradiometric assays without extraction procedures (Nichols Institute) were used for prolactin measurements. For prolactin, the minimum detectable amount was 0.1 ng/ml plasma; the coefficients of variation at 2 ng/ml plasma were <8%. In general, the hormone analyses for both treatment conditions were performed with the same assay to avoid interassay variability for the comparison.

From the hormone concentrations, the values for the area under the curve were calculated using the trapezoid rule. For comparison with our other data, we decided focus on two intervals of the night, i.e., the first (0–240 min) and the second 4-h periods after start of the registration (240–480 min).

Sleep-EEG Analysis

Due to technical problems, sleep-EEG analysis could be obtained in only seven subjects. Polysomnographic sleep recordings consisted of two EEGs (C3-A2, C4-A1; time constant 0.3 s, low-pass filtering 70 Hz), vertical and horizontal electrooculograms, an electromyogram, and an electrocardiogram. Sleep recordings again followed our standard procedure (14). Sleep EEGs were rated visually according to standard criteria (23) by an experienced rater who was blind to the study protocol. Sleep-EEG parameters used for the analysis were the following: sleep onset latency [sleep onset defined as the interval between lights off and the first epoch of 30 s containing stages 2, 3, 4, or REM sleep (min)]; total sleep time (min); sleep period time; time spent in each of the following sleep stages during time in bed (min): intermittent wakefulness, stage 2, stage 3, stage 4, slow-wave sleep (SWS; non-REM stages 3 and 4), and REM; and REM latency [interval from sleep onset until the first epoch-containing stage REM (min)]. For the spectral analysis, EEG signals were additionally sampled by an 8-bit analog to digital converter at a sampling rate of 100 Hz and stored on disk for further spectral analysis using the single frequency bins (frequency resolution 0.38 Hz; see Ref. 19). Spectral analysis was done for non-REM sleep stages (stages 2–4) only. For the standard criteria, we determined the parameters for the whole period (2300–1000) as well as for the first and second 4-h period, in accordance with the hormone measures and to allow comparison with our other studies.

Statistical Analysis

Our primary interest was to examine the changes in the time course of sleep regulation. The changes in the remaining sleep and endocrine parameters were examined in an exploratory way to allow the comparison with our earlier studies and are therefore regarded as secondary variables. Because of the exploratory nature of the study, we did not use a Bonferroni correction. Statistical analysis was performed using SPSS version 13 for Windows. Because the data were not normally distributed, we performed a Wilcoxon rank test for the single parameters of interest. This was done for the first and second 4-h periods of the registration (2300–0300 and 0300–0700) as in our earlier related studies (18, 20).

Our primary attempt was to use an analysis of the data cycle by cycle for as many non-REM-REM cycles as available for all subjects. The sleep cycle duration was our intended primary outcome variable. However, there was great variability of the sleep cycle duration of the first sleep cycle (see RESULTS), which made this approach questionable and unreliable. Therefore, we had to choose an alternative, but closely-related, approach toward the primary intention of the experiment. We chose a more global approach. It is known (7) that, during sleep, the δ-power declines in a fashion that can mathematically be described by an exponential function. In detail, we calculated the δ-power amplitude (0.5–4.5 Hz) for the non-REM sleep epochs (stages 2–4). The other sleep stages were not included in the fitting process. For better homogeneity, we did this for the first 8 h of the registration period, first for comparability and second to avoid artifacts by different length of the period for the fit. The two process models used a formulation for the δ-power (DP) over time as DP(t) = Amp × exp (−t/τ), where Amp is a factor for the δ-power amplitude over the night, t is the actual time, and τ the time constant of the decline. In accordance with our hypothesis, the primary variable was chosen to be τ. This will be given in the unit of minutes. All other variables are secondary outcome variables.

All values are expressed as means ± SD when not otherwise stated. A significance level of P < 0.05 is considered to be significant, and data with P < 0.1 are presented and considered as trends.

**RESULTS**

**Sleep EEG**

Conventional sleep-EEG analysis. Following PACAP administration, no significant changes were observed for either the total registration period (Table 1) or the first and second 4-h

### Table 1. Comparison of conventional sleep-EEG parameters for the total registration period (2300–1000)

<table>
<thead>
<tr>
<th>Sleep Parameter</th>
<th>Placebo</th>
<th>PACAP</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPT</td>
<td>557.8±57.6</td>
<td>549.0±75.5</td>
<td>NS</td>
</tr>
<tr>
<td>Sleep onset latency</td>
<td>31.2±24.4</td>
<td>29.6±28.7</td>
<td>NS</td>
</tr>
<tr>
<td>No. of awakenings</td>
<td>19.1±7.8</td>
<td>23.9±6.7</td>
<td>NS</td>
</tr>
<tr>
<td>REM latency</td>
<td>94.5±26.4</td>
<td>123.0±113.6</td>
<td>NS</td>
</tr>
<tr>
<td>Intermittent wake</td>
<td>42.4±27.3</td>
<td>65.1±70.6</td>
<td>NS</td>
</tr>
<tr>
<td>Stage 2</td>
<td>292.4±41.2</td>
<td>295.7±88.7</td>
<td>NS</td>
</tr>
<tr>
<td>Stage 3</td>
<td>36.9±10.7</td>
<td>28.2±14.2</td>
<td>NS (P &lt; 0.1)</td>
</tr>
<tr>
<td>Stage 4</td>
<td>9.8±11.4</td>
<td>4.4±7.2</td>
<td>NS</td>
</tr>
<tr>
<td>SWS</td>
<td>46.6±20.2</td>
<td>32.6±16.7</td>
<td>NS</td>
</tr>
<tr>
<td>REM time</td>
<td>122.6±20.9</td>
<td>104.9±36.9</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SD and in min, except for no. of awakenings. PACAP, pituitary adenylate cyclase-activating peptide; SPT, sleep period time; SWS, slow-wave sleep; REM, rapid eye movement; NS, not significant.
intervals of the registration period (data not shown). For the total registration period there was a trend for a reduction in stage 3 sleep (Table 1). Our original aim to perform a cycle-wise analysis could not be conducted reliably, because PACAP caused a sleep-onset REM in one subject (REM latency 1.5 min) and a prolonged duration of wakefulness before the first REM period of 221 min duration, which led to REM latency of 293 min in another subject.

It is important to note that the time course of SWS is altered in a way that suggests a reduced accumulation of SWS during the first few hours of the night (Fig. 1). The dynamics of REM sleep and wakefulness are not changed. The apparent increase in cumulative wakefulness can be attributed to the one patient who was awake for an extended period of time (see above).

**Spectral analysis.** We performed spectral analysis for non-REM sleep separately for the first (2200–0300) and the second (0300–0700) 4-h intervals of the registration period. PACAP induced a significant decrease in EEG power in several frequency bins ranging from the δ- to the α-frequency (2.2–9.5 Hz), whereas in the second half of the night the EEG power bins in a range including the lower spindle frequency range (10.5–13.5 Hz) were significantly reduced (Fig. 2).

![Fig. 1. Cumulative sum of sleep stages averaged for the 7 subjects. Rapid-eye-movement (REM) sleep and wakefulness are not changed between the conditions. The apparently higher amount of wakefulness can be attributed to 1 subject (see text). For slow-wave sleep (SWS) there is a qualitative change in the dynamic that suggests a lower sleep propensity.](image)

To describe the dynamics of the sleep process, we used the simple model of an exponential decline of δ-power with time and calculated the time constant for this. Because there is no change in the dynamics of REM sleep, this procedure seems to be justified. The time constant τ was significantly increased with the administration of PACAP. It increased from 339 ± 75 to 558 ± 326 min (means ± SD, P < 0.05). Figure 3 shows one representative example. This reduction of the dynamic of δ-power, as expressed by the increase in τ, is also reflected in the cumulative δ-power with time (Fig. 4). As with SWS, there is a reduced δ-power accumulation in the early part of the registration period, whereas in the later intervals the cumulative SWS runs in parallel in the PACAP and in the placebo group. This is again suggestive for a reduced SWS propensity after PACAP administration.

**Hormone secretion.** Cortisol and GH secretion were not affected throughout the 8-h registration period (Fig. 5). However, prolactin secretion was significantly reduced during the first 4-h registration period between 2300 and 0300.

**Further Observations**

PACAP administration led, in all subjects, to an increase in heart rate (±120 beats/min) and a flush. In two of the subjects the flush was still visible the next morning after the end of the registration period at 1000.

**DISCUSSION**

We studied the effect of intravenous administration of 4 × 12.5 μg PACAP in healthy young subjects on the sleep EEG and nocturnal hormone secretion compared with placebo. Our main finding is that PACAP leads to changes in the dynamics of the sleep structure with an increased time constant of the δ-power, a reduced activity in the δ- to α-frequency range between 2300 and 0300, and a reduction in the spindle frequency range between 0300 and 0700. This was accompanied...
by a reduced prolactin secretion in the first 4 h of the registration period.

As described in the introduction, PACAP shares large structural similarities with VIP. Both peptides affect brain metabolism by way of increasing glutamate-mediated glycogene synthesis in astrocytes (16). Because the increase in the glycogen content of astrocytes has been suggested to be related to the homeostatic function of sleep (6), it might be suggested that both peptides affect the homeostatic sleep regulation. Furthermore, the timing of sleep might be affected by a direct influence of both peptides on the suprachiasmatic nucleus, the hypothalamic clock (13). We found pronounced change in the time pattern of sleep after the administration of VIP (19), in particular a slowing of the ultradian rhythm.

Our findings share a pattern with earlier observations, which rather point to an effect on the homeostatic control of sleep. The pattern of a reduced slow-wave activity at the beginning of the night and of a reduced activity in the spindle frequency range is similar to those seen after napping, i.e., a situation with reduced sleep propensity (30), and is in contrast to the changes observed after sleep deprivation in healthy young subjects (12, 18). Accordingly, after sleep deprivation we observed a reduced time constant $\tau$, i.e., an increased dynamic of the $\delta$-power reduction (unpublished data). Furthermore, the similarities also include the pattern of endocrine changes, i.e., the unchanged GH and cortisol secretion, but the changed prolactin secretion, which is increased during recovery sleep after total sleep deprivation (18), is reduced here. In rats, PACAP leads to an increase in REM sleep but not slow-wave sleep (11). This is of particular interest in the context of the effect of...
different forms of sleep deprivation in rats, where in contrast to the situation in humans an REM rebound occurs regularly, which might even suppress the recovery of slow-wave activity (10). This might also be related to the difference of the effect of PACAP in humans compared with rats (see introduction).

A relationship exists between the present findings and the changes after administration of the NMDA antagonistic magnesium-salt (Mg2+) administration, which mirror each other. After Mg2+ administration we observed an increase in slow-wave activity in the first half of the night and of sleep-EEG activity in the low-spindle frequency activity in the second half of the night (14, 21), in contrast to the changes observed here. In fact, PACAP leads to a sensitization of glutamatergic neurotransmission, for example, in the hippocampus (15, 25), whereas Mg2+ blocks hippocampal NMDA receptors (5). Similarly, sleep deprivation leads to a reduced NMDA receptor activation (8, 17). From this mosaic of effects we suggest that PACAP affects homeostatic sleep regulation by its ability to sensitize NMDA receptor-mediated neurotransmission, which is opposite to the effect of sleep deprivation, or wakefulness in general, which leads rather to a desensitization of NMDA receptor function. This might translate into modifications of sleep-related processes like glycogene synthesis.

In conclusion, PACAP modifies the dynamic of the sleep structure and sleep-related neuroendocrine changes. PACAP increases the time constant of the δ-power decline, i.e., it leads to a less dynamic δ-power change overnight and reduces prolactin concentration in the first half of the night. It can therefore be suggested that mechanisms activated by PACAP are closely involved in sleep-homeostatic processes.

ACKNOWLEDGMENTS

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