Blockade of corticotropin-releasing hormone receptors reduces spontaneous waking in the rat

FANG-CHIA CHANG1 AND MARK R. OPP1,2

1Neuroscience Graduate Program and 2Department of Psychiatry and Behavioral Sciences, University of Texas Medical Branch, Galveston, Texas 77550–0431

Chang, Fang-Chia, and Mark R. Opp. Blockade of corticotropin-releasing hormone receptors reduces spontaneous waking in the rat. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R793–R802, 1998.—We have previously hypothesized that corticotropin-releasing hormone (CRH) is involved in the regulation of physiological waking. To further elucidate this role for CRH, we administered intracerebroventricularly into rats two specific CRH-receptor antagonists, α-helical CRH-(9–41) (α-hCRH) or astressin, and determined changes in electroencephalogram-defined waking and sleep. Our results indicate that both of these receptor antagonists reduce the amount of time spent awake in a dose-related manner when administered before the dark period of the light-dark cycle. However, the time courses for these effects differ between antagonists; effective doses of α-hCRH reduce waking during the first 2 h postinjection, whereas effective doses of astressin reduce waking during postinjection hours 7–12. In contrast to dark-onset administrations, the amount of waking is not altered by either CRH-receptor antagonist when administered before the light period. These results support our hypothesis that CRH contributes to the regulation of physiological waking, since interfering with the binding of CRH to its receptor reduces spontaneous waking.

METHODS

Substances

Stock solutions of α-hCRH (Peninsula Laboratories, Belmont, CA), astressin (Bachem, Torrance, CA), and CRH (Peninsula Laboratories) were prepared in pyrogen-free saline (PFS). Aliquots of these stock solutions were stored at −70°C until use, when they were then brought to an appropriate injection volume. The doses of the substances used in these experiments were as follows: for α-hCRH, 0.26, 1.3, 6.5, and 13 nmol (1, 5, 25, and 50 µg, respectively); for astressin, 0.14, 0.7, and 3.5 nmol (0.5, 2.5, and 12.5 µg, respectively); and for CRH, 0.05 and 0.1 nmol (0.25 and 0.5 µg, respectively). Animals

A total of 68 male Sprague-Dawley rats (250–300 g; Harlan, Indianapolis, IN) were used in these experiments. These animals were anesthetized (87 mg/kg ketamine and 13 mg/kg xylazine) and injected with an analgesic [butorphanol tartrate (Torbugesic)] and a broad-spectrum antibiotic [penicillin G benzathine (Bicillin LA)]. The rats were surgically implanted with EEG screw electrodes, a guide cannula directed into the lateral ventricle, and a calibrated 30-kΩ thermistor (model 44008, Omega Engineering, Stamford, CT).
Intracerebroventricular administration of astressin. Essentially the same protocol outlined above for α-hCRH was used to determine effects of astressin on spontaneous waking. Group 4 (n = 8) was administered PFS on the 1st recording day and subsequently received 0.14 nmol of astressin on the 2nd recording day. Group 5 (n = 12) received PFS on the 1st recording day and 0.7 and 3.5 nmol of astressin on the 2nd and 3rd recording days, respectively. The intracerebroventricular injections for groups 4 and 5 consisted of a 3-µl bolus administered over a 2-min period. These injections started 20 min before dark onset; recordings were initiated at dark onset and continued for 24 h.

The same protocol was used to determine the effects on waking of central administration of astressin during the light period of the light-dark cycle. Group 6 (n = 8) was administered PFS and 0.7 and 3.5 nmol of astressin (in 3 µl) 20 min before light onset on 3 consecutive days. Recordings for this group began at light onset and continued for 24 h.

Intracerebroventricular administration of CRH and a high dose of α-hCRH. Groups of rats were also used to determine the effects of CRH peptide itself on waking. Group 7 (n = 8) received 3 µl of PFS on the 1st recording day. Two days later, these animals were injected with 0.05 nmol of CRH. After another 2-day interval, 0.1 nmol of CRH was injected intracerebroventricularly into these animals. These injections of CRH were given as a 3-µl bolus beginning 20 min before dark onset, at which time 24-h recordings were initiated. Two days after the completion of the final recordings of responses to CRH, these same rats were injected with a high dose (13 nmol) of the CRH-receptor antagonist, α-hCRH. As previously mentioned, α-hCRH at doses above 6.5 nmol is a partial agonist. We elected to administer a dose of α-hCRH at twice the reported threshold dose for partial agonist actions when administered intracerebroventricularly. In this way, we could directly compare responses of rats to the agonist (CRH) with responses in the same animals to a high dose of α-hCRH exhibiting mixed agonist-antagonist actions to evaluate the degree of agonist actions of α-hCRH in our rat sleep-assay system. Intracerebroventricular administration of this high dose of α-hCRH was given before dark onset. Recordings were initiated at dark onset and continued for 24 h.

Group 8 (n = 8) was used to determine the effects of 0.05 and 0.1 nmol of CRH peptide on waking during the light period using the same protocol described previously for dark period recordings, except that injections began 20 min before and 24-h recordings started at light onset. To directly compare the effects of CRH peptide with a high dose of α-hCRH administered before light onset, group 8 was also injected intracerebroventricularly with 13 nmol of α-hCRH 2 days after the last administration of CRH.

Apparatus and Recording

Signals from the EEG electrodes and thermostors were fed into amplifiers (Colbourn Instruments, Lehigh Valley, PA, models S75–01 and S71–20, respectively). The EEG was amplified (factor of 3,000) and analog band-pass filtered between 0.1 and 40 Hz (frequency response ±3 dB; filter frequency roll off 12 dB/octave). The EEG amplifiers were calibrated before the initiation of these experiments by use of a Bio-Systems Calibrator (Colbourn Instruments). Similarly, the amplifiers used to record T Griffin were calibrated with a thermostor and water bath. Gross body movements were detected by custom-built ultrasonic detectors (Biomedical Instrumentation, University of Tennessee, Memphis, TN). These conditioned signals (EEG and T Griffin) as well as those from the movement detectors were subjected to analog-to-digital conversion with 12-bit precision at a sampling rate of 128 Hz.
(AT-MIO-64F5, National Instruments, Austin, TX). The digitized EEG waveform, Tbr samples, and integrated values for body movements were stored as binary computer files until subsequent analyses.

Postacquisition determination of vigilance state was done by visual scoring of 12-s epochs using custom software written in LabView for Windows (National Instruments) as previously described. In addition to the amount of time spent in each vigilance state, the number and duration of individual bouts of behavior were determined using criteria modified from those of Tobler and colleagues (7, 18), as previously described (36). Briefly, a wakefulness (Wake) bout is considered initiated if three consecutive 12-s epochs are scored as Wake (36-s) and terminated if three consecutive epochs are scored as slow-wave sleep (SWS). Wake bouts with a duration of >60-min are eliminated from subsequent statistical analyses because such bouts encompass parts of either two or three individual hourly time blocks and therefore skew subsequent determination of hourly bout duration. This exclusion criterion has previously been applied to studies of rat sleep-wake architecture (36, 45). A SWS bout is considered initiated if three consecutive 12-s epochs (36 s) are scored as SWS and terminated if two consecutive 12-s epochs (24 s) are scored as either Wake or rapid-eye-movement sleep (REMS). A REMS bout is initiated by two consecutive 12-s epochs (24 s) being scored as REMS and terminated if two consecutive 12-s epochs (24 s) are scored as either SWS or Wake. Finally, transitions from one state to another are determined from consecutive 12-s epochs without regard to criteria for episodes of vigilance state defined above. Therefore a transition is considered to occur if two consecutive 12-s epochs are scored dissimilarly.

Table 1. Percentage of recording time spent in vigilance states and average cortical $T_{br}$ determined from rats after ICV administration of CRH-receptor antagonists or CRH

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hours</th>
<th>L/D Cycle</th>
<th>Wake</th>
<th>SWS</th>
<th>REMS</th>
<th>$T_{br}$, °C</th>
<th>Hours</th>
<th>Wake</th>
<th>SWS</th>
<th>REMS</th>
<th>$T_{br}$, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-hCRH-(9–41)</td>
<td>1–6 D</td>
<td>68.0 ± 35</td>
<td>26.2 ± 2.7</td>
<td>5.8 ± 1.0</td>
<td>36.9 ± 0.07</td>
<td>7–12</td>
<td>70.2 ± 4.0</td>
<td>25.0 ± 3.3</td>
<td>4.8 ± 0.9</td>
<td>36.6 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>0.26 nmol</td>
<td>1–6 D</td>
<td>68.1 ± 35</td>
<td>27.6 ± 2.9</td>
<td>4.3 ± 0.8</td>
<td>36.7 ± 0.05</td>
<td>7–12</td>
<td>69.3 ± 4.0</td>
<td>26.0 ± 3.3</td>
<td>4.7 ± 0.9</td>
<td>36.4 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>1.3 nmol</td>
<td>1–6 D</td>
<td>65.7 ± 38</td>
<td>29.1 ± 3.1</td>
<td>5.2 ± 0.9</td>
<td>36.9 ± 0.06</td>
<td>7–12</td>
<td>69.2 ± 3.8</td>
<td>27.0 ± 3.3</td>
<td>3.9 ± 0.7</td>
<td>36.7 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>6.5 nmol</td>
<td>1–6 D</td>
<td>66.9 ± 25</td>
<td>28.8 ± 2.1</td>
<td>4.3 ± 0.7</td>
<td>37.2 ± 0.19</td>
<td>7–12</td>
<td>79.7 ± 2.8</td>
<td>17.3 ± 2.3</td>
<td>3.0 ± 0.5</td>
<td>36.8 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>13 nmol</td>
<td>1–6 D</td>
<td>60.8 ± 32</td>
<td>31.3 ± 2.5</td>
<td>8.0 ± 1.0</td>
<td>37.3 ± 0.09</td>
<td>7–12</td>
<td>71.9 ± 3.6</td>
<td>23.3 ± 2.9</td>
<td>4.8 ± 0.9</td>
<td>37.1 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>PFS</td>
<td>1–6 L</td>
<td>33.0 ± 25</td>
<td>59.2 ± 3.2</td>
<td>7.7 ± 0.9</td>
<td>36.7 ± 0.10</td>
<td>7–12</td>
<td>43.7 ± 2.6</td>
<td>46.0 ± 2.2</td>
<td>10.3 ± 1.0</td>
<td>36.7 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>0.14 nmol</td>
<td>1–6 L</td>
<td>37.1 ± 33</td>
<td>54.8 ± 3.0</td>
<td>8.0 ± 0.9</td>
<td>36.4 ± 0.09</td>
<td>7–12</td>
<td>49.5 ± 2.8</td>
<td>42.5 ± 2.1</td>
<td>8.0 ± 0.9</td>
<td>36.4 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>PFS</td>
<td>1–6 D</td>
<td>60.0 ± 28</td>
<td>35.5 ± 2.5</td>
<td>4.6 ± 0.7</td>
<td>37.6 ± 0.07</td>
<td>7–12</td>
<td>71.1 ± 3.2</td>
<td>24.6 ± 2.5</td>
<td>4.3 ± 0.8</td>
<td>37.6 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>0.07 nmol</td>
<td>1–6 D</td>
<td>56.0 ± 30</td>
<td>37.2 ± 2.5</td>
<td>6.8 ± 0.9</td>
<td>37.3 ± 0.07</td>
<td>7–12</td>
<td>72.5 ± 3.6</td>
<td>23.9 ± 3.0</td>
<td>3.6 ± 0.7</td>
<td>37.3 ± 0.16</td>
<td></td>
</tr>
<tr>
<td>PFS</td>
<td>1–6 D</td>
<td>57.0 ± 26</td>
<td>37.3 ± 2.1</td>
<td>5.6 ± 0.7</td>
<td>36.9 ± 0.03</td>
<td>7–12</td>
<td>72.0 ± 2.9</td>
<td>24.8 ± 2.5</td>
<td>3.2 ± 0.5</td>
<td>36.5 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>3.5 nmol</td>
<td>1–6 D</td>
<td>55.2 ± 26</td>
<td>40.7 ± 2.2</td>
<td>4.1 ± 0.6</td>
<td>36.7 ± 0.04</td>
<td>7–12</td>
<td>64.5 ± 2.5*</td>
<td>32.3 ± 2.2*</td>
<td>3.3 ± 0.6</td>
<td>36.7 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>CRH</td>
<td>1–6 D</td>
<td>60.8 ± 32</td>
<td>31.3 ± 2.5</td>
<td>8.0 ± 1.0</td>
<td>37.3 ± 0.04</td>
<td>7–12</td>
<td>71.9 ± 3.6</td>
<td>23.3 ± 2.9</td>
<td>4.8 ± 0.9</td>
<td>37.1 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>0.05 nmol</td>
<td>1–6 L</td>
<td>49.8 ± 4.3*</td>
<td>44.4 ± 3.8*</td>
<td>5.9 ± 1.0</td>
<td>36.7 ± 0.03</td>
<td>7–12</td>
<td>43.8 ± 2.2</td>
<td>44.3 ± 1.8</td>
<td>12.8 ± 1.0</td>
<td>36.4 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>0.1 nmol</td>
<td>1–6 L</td>
<td>54.4 ± 5.2*</td>
<td>40.9 ± 4.7*</td>
<td>4.7 ± 0.9*</td>
<td>36.9 ± 0.04</td>
<td>7–12</td>
<td>41.0 ± 2.5</td>
<td>47.8 ± 2.3</td>
<td>11.2 ± 0.8</td>
<td>36.7 ± 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE percentages of recording time determined from freely behaving rats after intracerebroventricular (icv) administration of indicated substances. Differences were detected by 1-way ANOVA within indicated time blocks. α-hCRH-(9–41), α-helical corticotropin-releasing hormone (CRH)-(9–41); PFS, pyrogen-free saline (vehicle); Hour, 6-h postinjection time blocks; L/D cycle, period of light-dark cycle immediately before which injections were given (D, dark period; L, light period); Wake, wakefulness; SWS, slow-wave sleep; REMS, rapid-eye-movement sleep; Tbr, brain temperature (measured at surface of frontal cortex). *Statistically significant difference between values obtained after vehicle (PFS) and those obtained after administration of CRH-receptor antagonists.

Statistical Analyses

All values are presented as means ± SE for the indicated sample sizes. One-way ANOVA for the duration of each vigilance state (SWS, REMS, and Wake) for Tbr values and for sleep architecture parameters were performed across the two 6-h time blocks comprising either the 12-h light period or the 12-h dark period. The main effect consisted of manipulation (vehicle, α-hCRH, astressin, or CRH peptide doses). If statistically significant differences were detected, post hoc comparisons were made to determine which hourly intervals during experimental conditions deviated from values obtained from the same animals during control conditions. An α-level of P < 0.05 was taken as indicating a statistically significant difference between vehicle and active substances.

RESULTS

Effects of Intracerebroventricular Administration of α-hCRH

Intracerebroventricular administration of α-hCRH before dark onset transiently reduced spontaneous waking in a dose-related manner. One-way ANOVA across 6-h time blocks did not reveal consistent alterations in waking or REMS after intracerebroventricular administration of any dose of α-hCRH (Table 1). However, the amount of time spent in SWS increased significantly after the 6.5-nmol dose of α-hCRH (Table 1). Visual inspection of the data revealed that there were consistent and reproducible reductions in waking and increases in SWS during the first 2-h postinjection period.
(Fig. 1). Therefore subsequent statistical analyses were confined to this 2-h postinjection period. The two lowest doses of α-hCRH (0.26 and 1.3 nmol) tended to reduce waking and enhance SWS during the first 2-h postinjection, although these changes did not achieve statistical significance (data not shown). The amount of time spent awake during this 2-h period after the 6.5-nmol dose was reduced from 75.1 ± 3.7% of recording time after vehicle to 58.9 ± 4.2% of recording time after α-hCRH (P < 0.01). This reduction in waking was mirrored by increases in the amount of SWS (Fig. 1). Although there was a slight increase in REMS during the first 2-h postinjection, this difference did not achieve statistical significance.

The reduction in waking during the 2-h postinjection period after 6.5 nmol of α-hCRH was primarily due to a decrease in Wake bout duration. Analyses of sleep architecture parameters across postinjection hours 1–6 did not reveal statistically significant alterations in sleep-wake architecture, although the numbers of transitions from one state of vigilance to another was increased significantly after administration of 6.5 nmol of α-hCRH, indicating that waking was fragmented (Table 2). However, analyses restricted to the 2-h postinjection period when the amount of time spent awake was reduced indicated that the duration of Wake bouts reduced from 7.1 ± 2.1 min after vehicle to 2.3 ± 0.7 min after α-hCRH (P < 0.05). The number and duration of SWS bouts were not statistically altered by this manipulation.

In contrast to intracerebroventricular administration before dark onset, the 0.26- to 6.5-nmol doses of α-hCRH when injected before light onset failed to alter any aspect of sleep-wake activity determined in these experiments during any time point (Fig. 1 for responses to 6.5-nmol dose; other data not shown). Similarly, Tbr was not altered by any of the doses of α-hCRH used in these experiments, regardless of the timing of administration (data not shown).

Responses to Intracerebroventricular Administration of Astressin

Intracerebroventricular administration of astressin also reduced spontaneous waking in a dose- and time-related manner. When injected intracerebroventricu- larly before the dark period, each dose of astressin used in this experiment tended to slightly reduce waking and increase SWS during postinjection hours 1–6; these minor alterations in behavior did not deviate
statistically from control values (Table 1). During postinjection hours 7–12, however, the amount of time spent in waking was reduced, but the amount of time spent in SWS was increased in a dose-related manner (Table 1, Fig. 2); most of these effects were confined to postinjection hours 7–10 (Fig. 2). During this 4-h period, the amount of time spent awake was reduced from 77.0 ± 3.3% of recording time after vehicle to 63.4 ± 3.1% of recording time after 3.5 nmol of astressin (P < 0.005; Fig. 2). This reduction in waking was accompanied by increases in SWS, whereas REMS was not consistently altered. During postinjection hours 1–6, there were no consistent alterations in sleep-wake architecture, although the number of transitions from one state to another were increased after the 3.5-nmol dose (Table 3). There

<table>
<thead>
<tr>
<th>Treatment</th>
<th>L/D Cycle</th>
<th>No. of Bouts</th>
<th>Bout Duration, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFS D</td>
<td>4.7 ± 0.4</td>
<td>7.1 ± 0.7</td>
<td>8.1 ± 1.9</td>
</tr>
<tr>
<td>0.26 nmol D</td>
<td>3.8 ± 0.4</td>
<td>6.4 ± 0.6</td>
<td>10.4 ± 2.3</td>
</tr>
<tr>
<td>1.3 nmol D</td>
<td>4.4 ± 0.4</td>
<td>7.8 ± 0.7</td>
<td>8.1 ± 2.1</td>
</tr>
<tr>
<td>PFS D</td>
<td>7.2 ± 0.6</td>
<td>9.4 ± 0.8</td>
<td>4.8 ± 1.4</td>
</tr>
<tr>
<td>6.5 nmol D</td>
<td>8.1 ± 0.6</td>
<td>11.5 ± 0.9</td>
<td>3.1 ± 0.7</td>
</tr>
<tr>
<td>PFS D</td>
<td>5.4 ± 0.4</td>
<td>7.8 ± 0.6</td>
<td>7.1 ± 1.9</td>
</tr>
<tr>
<td>13 nmol D</td>
<td>5.6 ± 0.4</td>
<td>8.4 ± 0.5</td>
<td>4.6 ± 1.2*</td>
</tr>
<tr>
<td>PFS L</td>
<td>6.8 ± 0.5</td>
<td>10.5 ± 0.6</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>13 nmol L</td>
<td>6.1 ± 0.5</td>
<td>10.8 ± 0.8</td>
<td>4.7 ± 1.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Differences were detected by 1-way ANOVA within indicated time blocks. *Statistically significant difference between values obtained after vehicle (PFS) and those obtained after administration of indicated nmol of α-hCRH-(9–41).
were, however, statistically significant alterations in sleep-wake architecture parameters during postinjection hours 7–12. During this 6-h time period, bout durations for Wake were reduced (Table 3). During postinjection hours 7–10, when waking was significantly reduced, the Wake bout duration decreased from 11.3 ± 2.3 to 6.7 ± 1.3 min after 0.7 nmol of astressin and 5.7 ± 1.4 after 3.5 nmol of astressin. In addition, during this same 4-h time period, the number of transitions from one state of vigilance to another increased from 30.7 ± 3.4 after administration of vehicle to 39.7 ± 6 after the 3.5-nmol doses of astressin, indicating a fragmentation of normal sleep-wake activity. As with α-hCRH, astressin when injected before light onset failed to alter any aspect of sleep-wake activity determined in these experiments (Fig. 2 for 3.5-nmol dose; other data not shown). Similarly, T Pr values were not altered by any dose of astressin regardless of time of administration (data not shown).

Intracerebroventricular Administration of CRH and High Doses of α-hCRH

Intracerebroventricular administration of CRH increased waking and reduced sleep in a dose-related manner. When administered before the dark period, both doses of CRH (0.05 and 0.1 nmol) increased waking and reduced REMS during postinjection hours 1–6 (Table 1). The corresponding reduction in SWS for this 6-h period achieved statistical significance after the 0.1-nmol dose only. Most of the effects of CRH were apparent for 2–3 h postinjection (Fig. 3). Intracerebroventricular administration of these doses of CRH before the light period resulted in a pattern of alterations in sleep-wake activity that was generally similar to that observed when injections were given prior to dark onset (Table 1), except the duration of the effects was longer (Fig. 3). Although both doses of CRH increased waking and reduced SWS and REMS, the effects of the 0.05-nmol dose were primarily limited to the first 2 h postinjection, whereas these changes lasted 3 h after the 0.1-nmol dose (Fig. 3).

The groups of rats that received CRH also received a high dose (13 nmol) of the CRH-receptor antagonist α-hCRH. No statistically significant departures from controls were revealed after analyses of 6-h time blocks when 13 nmol of α-hCRH was administered before dark onset (Table 1). However, visual inspection of the data revealed a consistent and reproducible reduction in waking concurrent with reductions in SWS and REMS during the 2nd postinjection hour after this dose of α-hCRH (Fig. 3). When confined to hourly time blocks, statistical analyses indicated a significant departure from control values during this time point. In marked contrast, however, intracerebroventricular administration of this dose of α-hCRH before light onset increased waking and reduced SWS during the 1st postinjection hour (Fig. 3). During postinjection hour 1 after α-hCRH, waking was increased from 28.5 ± 9.3 min after vehicle to 67.7 ± 12.2% of recording time after vehicle to 67.7 ± 10.1% of recording time after α-hCRH. This increase in waking was at the expense of SWS; REMS was not affected (Fig. 3). During the 1st postinjection hour after the low dose (0.05 nmol) of CRH, waking increased from 28.5 ± 3.0% after vehicle to 81.4 ± 12.2%, a value statistically indistinguishable from the 67.7 ± 10.1% observed after 13 nmol of α-hCRH during this same time period in the same animals (Fig. 3); i.e., the increase in waking induced by 13 nmol of the CRH antagonist α-hCRH and a low dose (0.05 nmol) CRH were identical. The 1-h increase in Wake after this dose of α-hCRH during the light period was due to an increase in Wake bout duration, which increased from 3.6 ± 0.4 min after vehicle to 27.5 ± 9.3 min after α-hCRH.

**DISCUSSION**

There is ample indirect evidence to suggest that CRH may be involved in the regulation of physiological waking in addition to its role as a mediator of responses to stressors (reviewed in Ref. 35). We now extend these observations and provide direct evidence that CRH may be involved in the regulation of waking by reporting that two separate and specific CRH-receptor antagonists reduce waking in a dose- and time-dependent manner in freely behaving animals.

α-hCRH is a selective and competitive CRH-receptor antagonist that was initially characterized for its abil-
ity to inhibit CRH-induced ACTH secretion in vitro (43). α-hCRH binds to CRH receptors with ~10 times less potency than does CRH itself (5). Although it is not very potent, α-hCRH has been widely used to elucidate mechanisms whereby CRH contributes to responses to stressors and is more effective when administered into the CNS than when administered systemically (17). For example, α-hCRH administered intracerebroventricularly into rats inhibits CRH- or ether stress-induced ACTH secretion (43), blocks CRH-induced changes in locomotor activity and conflict test responding (3), reverses the stress-induced inhibition of exploratory behavior (24), reverses the “anxiogenic” responses to ethanol withdrawal (1), and reduces measures of “emotionality” in socially defeated animals (25). These and many other similar studies in which α-hCRH has been used as a CRH antagonist provide much of the evidence that indicates a pivotal role for CRH as a mediator of responses to stressors.

Our present study extends the aforementioned observations of the involvement of CRH in responses to stressors to include behavior of normal, nonstressed rats freely behaving in their home cages. Rats that are well habituated to handling and injection procedures respond to α-hCRH with a transient reduction in the amount of physiological waking. The reduction in waking induced by 6.5 nmol (25 µg) of α-hCRH lasts only 2 h. This relatively short effect on sleep-wake behavior is similar in duration to that reported in other studies using α-hCRH and may be due to a relatively short half-life. For example, intravenous administration of α-hCRH into adrenalectomized rats reduces plasma ACTH concentrations for 2 h (43). The ability of α-hCRH to reduce waking is dose related; low doses [0.26 and 1.3 nmol (1 and 5 µg, respectively) in our study] tend to reduce waking in the 2nd postinjection hour, whereas a statistically significant reduction in waking in the first 2-h postinjection period is apparent after administration of 3.5 nmol (25 µg). This effective dose of α-hCRH in our rat sleep assay of freely behaving animals is generally similar to doses reported to be effective in blocking or attenuating behavioral responses to stressors. However, in some models of stressor-induced alterations in behavior, α-hCRH may effectively attenuate responses at doses in the range of 1–5 µg (e.g., see Ref. 24, 25). That α-hCRH is effective at lower doses in interfering with behavioral responses to stressors is probably due to the fact that CRH concentrations are elevated well above basal values in response to stressors.

Our observation that spontaneous waking in the rat is reduced when α-hCRH is administered intracerebroventricu-
arily into freely behaving rats is in contrast to that recently reported by Gonzalez and Valatx (20a).
These authors report that a single 100-µg (~26-nmol) dose of α-hCRH administered before dark onset does not alter spontaneous waking in the rat, although it is effective in blocking alterations in REMS induced by immobilization stress. These authors conclude that CRH is not involved in the regulation or modulation of spontaneous waking. This conclusion is problematic, however, because α-hCRH is widely reported to exhibit partial agonist activity (1, 10, 24, 25, 32, 41, 42, 47), particularly at doses of >25 µg. The partial agonist effect of high doses of α-hCRH is clearly observed in the results obtained in our experiments when 13 nmol (50 µg) of α-hCRH is administered before the light period, the time when endogenous concentrations of CRH in the rat are at their lowest. In the 1st postinjection hour, waking increases after this dose of the antagonist by an amount statistically indistinguishable from the increase in waking that occurs during this same time period after administration of a low dose (0.05 nmol) of CRH itself. As such, high doses of the CRH antagonist α-hCRH may be functionally equivalent to low doses of CRH. It is therefore not surprising that when 100 µg of α-hCRH is given before the dark period of the light-dark cycle, when endogenous concentrations of CRH in the rat are at their greatest, there is no detectable alteration in CRH-mediated behavior, since, in effect, a functional low dose of CRH has been added to a system in which endogenous CRH is already high. When in our studies this 13-nmol (50-µg) dose of α-hCRH was administered before the dark period, some antagonist actions were apparent. However, the magnitude of these alterations more closely resembled the middle dose (1.3 nmol or 5 µg) than the highest effective dose (6.5 nmol or 25 µg). The fact that α-hCRH possesses partial agonist actions is one of several factors that has hampered the use of this antagonist in basic and preclinical research. As such, the development of specific and potent CRH antagonists devoid of partial agonist properties continues to be a priority, and several new CRH antagonists have recently been developed and characterized that meet this particular criterion (22, 31, 32, 42).

One of the members of this new generation of CRH-receptor antagonists is astressin. Astressin is ~100 times more potent than α-hCRH at inhibiting ACTH secretion in vitro and is extremely effective in blocking ACTH release in stressed or adrenalectomized rats in vivo (22). Furthermore, astressin exhibits little if any intrinsic agonist activity. Astressin is effective in blocking responses to a variety of stressors. For example, astressin blocks CRH- and alcohol-induced increases in ACTH (42) and stress-induced alterations in gastric and colonic motor function (31) with ~30 times more potency than does α-hCRH. We extend these observations of biological actions of astressin to include a reduction of spontaneous waking in freely behaving rats.

To date, three CRH-receptor subtypes that differ in anatomical distribution and pharmacological profile have been described (reviewed in Refs. 6, 11); they are termed CRH-R1, CRH-R2α, and CRH-R2β. In pituitary, CRH-R1 mRNA is expressed in both the anterior and intermediate lobes (11, 40, 46). In contrast, the expression of CRH-R2 mRNA in the anterior pituitary is either undetectable (46) or only detected in scattered cells (11). Therefore CRH-R1 appears to be the major receptor isoform regulating pituitary ACTH secretion. Within the rat brain, the distribution of hybridization signals for CRH-R1 mRNA is widespread, including neo-, olfactory, and hippocampal cortices, cerebellum, septum, amygdala, and brainstem sensory relay structures, with only low levels of expression detected in thalamic and hypothalamic nuclei (11, 40, 46). In contrast, the expression of CRH-R2 mRNA within rat brain is generally confined to subcortical structures, in the lateral septal nucleus, ventromedial hypothalamic nucleus, olfactory bulb, amygdala, and the choroid plexus (11, 46). CRH-R2β is the predominant CRH-R2 isoform in neuronal systems, whereas CRH-R2α is localized in nonneuronal tissues of the CNS (e.g., choroid plexus and cerebral arteries; Ref. 11) but is primarily expressed in the periphery in heart, skeletal muscle, lung, kidney, and intestine (11, 46). Of relevance to our experiments, α-hCRH is a more effective antagonist of CRH-R2β than CRH-R1 (11). As such, α-hCRH is more effective in blocking brain CRH receptors than pituitary CRH receptors (42), which may explain why in most models α-hCRH is more effective when administered centrally than peripherally (e.g., Ref. 17). Astressin on the other hand, effectively blocks both CRH-R1 and CRH-R2 in brain and pituitary (42). These differences in CRH-receptor distributions and affinities for α-hCRH and astressin may account for the different time courses of alterations in waking previously described. We hypothesize that, in our rat sleep-assay system, the relatively quick reduction in waking after intracerebroventricular administration of α-hCRH is due to central actions mediated by subcortical CRH-R2β. In contrast, the behavioral effects of astressin in our system may be primarily mediated by the HPA axis by blocking CRH-R2 in the paraventricular nucleus of the hypothalamus and/or the CRH-R1 in the anterior pituitary. Such potential sites or mechanisms of action may explain the relatively long delay from the administration of astressin until behavior is altered. Experiments to further elucidate the respective roles for these CRH-receptor subtypes in the modulation of waking are currently in progress.

The circadian rhythmicity of HPA axis activity in the rat is particularly striking, with peak activity occurring during the dark period of the light-dark cycle (e.g., Refs. 19, 21). Changes in CRH in hypothalamus parallel those in the periphery and generally reflect the status of the HPA axis (48). Thus endogenous CRH concentrations are low during the light (rest) period and high during the dark (active) period in rats. As such, neither of the CRH-receptor antagonists used in these experiments affected sleep-wake behavior during the light period, with the exception of the partial agonist actions described above for high doses of α-hCRH. Our observations that CRH antagonists are ineffective in altering spontaneous behavior of rats during the light period...
are not surprising and, in fact, are expected on the basis that low endogenous CRH concentrations indicate a system with relatively "little" to antagonize. Previously published observations that α-hCRH (at low doses) does not affect "nonstressed" animals are probably due to this circadian rhythmicity, since most studies using rats are conducted during the light (rest) period. In contrast to light period administration, if CRH antagonists are administered during the dark (active) period when CRH concentrations are high, they are effective in altering spontaneous behavior (this study). The circadian modulation of responses to CRH antagonists is also observed when CRH itself is administered. Intracerebroventricular administration of CRH increases waking (e.g., Refs. 15, 37) regardless of timing of administration (this study); however, the magnitude and duration of the response are time dependent. Administration of CRH during the light period, when endogenous CRH is normally low, results in an increase in waking of a larger magnitude than when the same dose is administered during the dark period, when endogenous CRH concentrations are already high. Similarly, when animals are subjected to stressors during the light period, the normally low concentrations of CRH are greatly elevated in response to the stressor, and CRH-receptor antagonists are effective in blocking or attenuating responses mediated by this CRH surge. As with administration of CRH itself, the magnitude of responses of rats to stressors may not be as great during the dark (active) period as those observed to the same manipulation conducted during the light (rest) period, since the activity of the system is already high.

In conclusion, we have shown that central administration of two specific CRH-receptor antagonists reduces, in a time- and dose-related manner, spontaneous waking in freely behaving rats. The time courses of responses to these antagonists differ, an effect that may be due to tissue-specific distribution patterns for CRH-receptor subtypes. Although additional experiments must be conducted before definitive conclusions can be made concerning the involvement of specific CRH-receptor subtypes in these responses, our results provide direct evidence that CRH may be involved in the regulation or modulation of sleep-wake activity because interfering with the binding of CRH to its receptors reduces waking.

Perspectives

Animals respond to stressors with a variety of complex behavioral, physiological, and autonomic processes that act in concert to eliminate the stressor. There has been extensive effort to determine mechanisms that mediate responses to stressors, and in fact most studies focusing on CRH are fundamentally "stress" studies. As a result, there is now a large body of knowledge indicating a major role for CRH in responses to stressors. We have previously suggested, for example, that the involvement of CRH in negative-feedback mechanisms for immune activation is a critical feature in the complex alterations in sleep that occur throughout the course of an acute infection.

Indeed, CRH through multiple pathways is able to modulate behavioral responses to immune active substances. Although determining mechanisms responsible for changes in behavior during patholgy is important, it is equally important to understand the regulation of normal behavior in the absence of overt stressors. In our opinion, this is one aspect of research concerning CRH that is generally lacking. How will we be able to fully understand the role of CRH in psychiatric illness, immune challenge, or responses to "psychological stressors" if we do not know what CRH does in brain in the absence of these conditions? Studies such as this current one are important because they form the basis for understanding more completely the basic neurobiology of CRH. With the development of new tools, e.g., more selective receptor antagonists, we will be able to explore in greater detail the role of CRH in normal behavior.

The technical assistance of Kristi Overgaard and William Dalmeida is gratefully acknowledged.

This work was supported in part by National Institute of Mental Health Grant MH-52275.

Address for reprints: M. R. Opp, Dept. of Psychiatry and Behavioral Sciences, University of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0431.

Received 19 March 1998; accepted in final form 14 May 1998.

REFERENCES

R802 CRH ANTAGONISTS REDUCE SPONTANEOUS WAKING


25. Rainnie, D. G., B. J. Farnhout, and P. Shinnick-Gallagher. Differential actions of corticotropin releasing factor on basolat-


32. Yokoe, T., T. Audhya, C. Brown, B. Hutchinson, J. Pas-