Antagonism of corticotropin-releasing hormone alters serotonergic-induced changes in brain temperature, but not sleep, of rats

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1Institute of Human Physiology II, and 2Giuseppe Moruzzi Centre for Experimental Sleep Research, University of Milan Medical School, Milan, Italy; and 3Department of Anesthesiology, 4Neuroscience Graduate Program, and 5Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, Michigan

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Imeri, Luca, Susanna Bianchi, and Mark R. Opp. Antagonism of corticotropin-releasing hormone alters serotonergic-induced changes in brain temperature, but not sleep, of rats. Am J Physiol Regul Integr Comp Physiol 289: R1116–R1123, 2005. First published June 30, 2005; doi:10.1152/ajpregu.00074.2005.—Serotonin is involved in many physiological processes, including the regulation of sleep and body temperature. Administration into rats of low doses (25, 50 mg/kg) of the 5-HT precursor L-5-hydroxytryptophan (5-HTP) at the beginning of the dark period of the 12:12-h light-dark cycle initially increases wakefulness. Higher doses (75, 100 mg/kg) increase nonrapid eye movement (NREM) sleep. The initial enhancement of wakefulness after low-dose 5-HTP administration may be a direct action of 5-HT in brain or due to 5-HT-induced activation of other arousal-promoting systems. One candidate arousal-promoting system is corticotropin-releasing hormone (CRH) and the hypothalamic-pituitary-adrenal axis. Serotonergic activation by 5-HTP at the beginning of the dark period also induces hypothermia. Because sleep and body temperature are influenced by circadian factors, one aim of this study was to determine responses to 5-HTP when administered at a different circadian time, the beginning of the light period. Results obtained show that all doses of 5-HTP (25–100 mg/kg) administered at light onset initially increase wakefulness; NREM sleep increases only after a long delay, during the subsequent dark period. Serotonergic activation by 5-HTP at light onset induces hypothermia, the time course of which is biphasic after higher doses (75, 100 mg/kg). Intracerebroventricular pretreatment with the CRH receptor antagonist α-helical CRH does not alter the impact of 5-HTP on sleep-wake behavior but potentiates the hypothermic response to 50 mg/kg 5-HTP. These data suggest that serotonergic activation by peripheral administration of 5-HTP may modulate sleep-wake behavior by mechanisms in addition to direct actions in brain and that circadian systems are important determinants of the impact of serotonergic activation on sleep and body temperature.

thermoregulation; hypothermia; hypothalamic-pituitary-adrenal axis; corticotropin-releasing factor; 5-hydroxytryptamine

EXEMPLARY EXPERIMENTAL DATA and clinical observations indicate that brain serotonin (5-HT) plays a pivotal role in the regulation of many physiological processes and complex behaviors (23), including sleep (25, 35) and thermoregulation (29, 30). It is now thought that 5-HT, release of which is maximal during wakefulness, promotes wakefulness per se by actions on 5-HT2 receptors and subsequently triggers sleep via induction of sleep-promoting systems (25, 26). Recent data directly support this dual role for 5-HT in regulation of the arousal state. We showed that increasing serotonergic activity at the beginning of the dark period by intraperitoneal injection of the 5-HT precursoir L-5-hydroxytryptophan (5-HTP) results in dose-related and biphasic changes in sleep-wake behavior of rats; low doses initially enhance wakefulness, which is later followed by increased nonrapid eye movement (NREM) sleep (20). Under those experimental conditions, serotonergic activation at dark onset induces hypothermia, the duration of which encompasses periods of both increased wakefulness and enhanced NREM sleep.

The contribution of 5-HT to the regulation of arousal state and thermoregulation involves multiple systems. Brain 5-HT acts directly on neural systems implicated in regulation of arousal state, such as neurons in the pontine tegmentum (laterodorsal and pedunculopontine nuclei), neurons in the ventrolateral preoptic and suprachiasmatic nuclei (8, 24, 35, 40), as well as on hypothalamic thermoregulatory neurons (29). In addition to direct actions on neural systems that regulate arousal state and thermoregulation, effects of 5-HT on these processes may be mediated by indirect actions on other systems. For example, serotonergic axons synapse on corticotropin-releasing hormone (CRH)-immunoreactive neurons in the paraventricular nucleus of the hypothalamus (28); increasing synaptic 5-HT stimulates the release of CRH (3, 13). Central administration of CRH increases wakefulness (12, 32, 33). Elevated CRH in the paraventricular nucleus of the hypothalamus increases pituitary ACTH. ACTH, in turn, stimulates the secretion of adrenal glucocorticoids. Each of these components of the hypothalamic-pituitary-adrenal (HPA) axis is capable of modulating arousal state and thermoregulation (reviewed in Refs. 31 and 41). In addition to actions of central 5-HT on CRH-immunoreactive neurons in the hypothalamus, 5-HT also increases HPA axis activity by direct actions on the anterior pituitary and the adrenal cortex. As such, 5-HT impacts the HPA axis by both central and peripheral mechanisms.

Rats are nocturnal, and the dark period is, for these animals, the active phase when they are awake the most and their body temperatures are highest. Conversely, the light period is the rest phase for rats, during which time they sleep the most and body temperatures are at their lowest. Because sleep-wake behavior and thermoregulation are strongly influenced by circadian factors, the impact of serotonergic activation on arousal state and body temperature may differ depending on the timing of activation. We hypothesized that if serotonergic activation occurs at the beginning of the light period, the initial increase in wakefulness of rats would be proportionally greater than that occurring when 5-HTP is administered before the beginning of...
the dark period. Furthermore, we hypothesized that because body temperatures of rats are lower during the light period than during the dark period, the hypothermic response to serotonergic activation at the beginning of the light period would be proportionally less than that following serotonergic activation at the beginning of the dark period. To test these hypotheses, rats were injected intraperitoneally at light onset with 5-HTP, sleep-wake behavior was determined, and brain cortical temperature (Tcort) was recorded. Finally, in an initial attempt to elucidate the mechanisms responsible for the wake-promoting effects of serotonergic activation, we hypothesized that if elevated 5-HT in brain stimulates CRH in the hypothalamus, which would induce wakefulness, then antagonizing the central actions of this peptide would reduce the extent to which wakefulness increases after 5-HTP administration. To test this hypothesis, rats were pretreated intracerebroventricularly at light onset with a CRH receptor antagonist and then injected intraperitoneally with 5-HTP. We now report that 5-HTP administration at light onset initially increases wakefulness of rats and induces hypothermia. Central antagonism of CRH receptors does not affect 5-HTP-induced increases in wakefulness but does alter the magnitude of the hypothermic response.

MATERIALS AND METHODS

Parallel studies were conducted in two laboratories. Experiment 1 (see Experimental Protocols. Experiment 1: effects of 5-HTP administration on sleep-wake behavior and Tcort) was conducted at the University of Milan (by L. Imeri and S. Bianchi) to facilitate comparison with data derived from previous studies of the impact of 5-HTP on sleep-wake behavior of rats. These studies were conducted on the same strain of rats and under the same conditions previously reported (20). Experiment 2 (see Experimental Protocols. Experiment 2: effects of central CRH receptor antagonism on 5-HTP-induced changes in sleep-wake behavior and Tcort), in which the effects of intracerebroventricular administration of a specific CRH receptor antagonist on 5-HTP-induced alterations in brain temperature and sleep were determined, was conducted at the University of Michigan (by M. R. Opp); previous studies using this antagonist have been conducted by this laboratory under the same conditions using the same rat strain and vendor (5, 6).

Substances

5-HTP was purchased from Sigma-Aldrich (St. Louis, MO), dissolved in isotonic pyrogen-free saline (PFS; Abbott Laboratories, North Chicago, IL) before each administration and injected intraperitoneally. The CRH receptor antagonist α-helical CRH (αhCRH) was purchased from Peninsula Laboratories (Belmont, CA) and prepared in PFS. Aliquots of these stock solutions were stored at −70°C until use, when they were brought to the appropriate injection volume.

Animals

Male Sprague-Dawley rats (225–300 g at time of surgery; Harlan, Indianapolis, IN and Charles River, Calco, Italy) were used in these experiments. All procedures performed in these studies were approved by the University of Michigan Animal Care and Use Committee in accordance with the United States Department of Agriculture Animal Welfare Act and the United States Public Health Service Policy on Humane Care and Use of Laboratory Animals. Procedures also conformed to European Union (European Economic Community Council Directive 86/609, OJ L 358.1; 12 December 1987) and Italian (Decreto Legge n.116, Gazette Ufficiale suppl. 40, 18 February 1992) laws and policies.

Animals were anesthetized (either ketamine/xylazine, 87/13 mg/kg, respectively, or isoflurane) and injected with an analgesic (butorphanol tartrate) and a broad spectrum antibiotic (penicillin G benzathine). Stainless-steel screws placed over the frontal, parietal, and occipital brain cortices served as EEG electrodes. A calibrated 30-kohm thermometer (model 44008; Omega Engineering, Stamford, CT) was implanted over the parietal cortex to monitor Tcort. Body movements were evaluated either by recording electromyographic activity (EMG) with Teflon-coated silver wires inserted in the neck muscles (experiment 1) or through custom-built ultrasonic detectors (Biomedical Instrumentation, University of Tennessee, Memphis, TN; experiment 2). Insulated leads were routed from the EEG electrodes (and EMG electrodes, when used) and the thermometer to a Teflon pedestal (Plastics One, Roanoke, VA) that was cemented in place with dental acrylic (Isocryl; Lang Dental Supply, Wheeling, IL). This pedestal was used to connect the animals to the recording apparatus via a flexible tether and electronic swivel. The incision was treated topically with polysporin (polymyxin B sulfate-bacitracin zinc), and the animals were placed under heat lamps and monitored until recovery from anesthesia. The animals were allowed 7 days of recovery from the surgical procedures. On the second postsurgical day, the rats were connected to the recording apparatus (see Apparatus and Recording) via a flexible tether that allowed relatively unrestricted movement within the cage. For the next 5 days the animals were habituated by daily handling that coincided with scheduled experimental protocols. Rats were housed individually in environmentally controlled chambers, maintained between 22 and 23°C on a 12:12-h light-dark cycle. Food and water were available ad libitum. Rats were fed a standard rat maintenance diet (Harlan, Madison, WI, or Mucedola, Settimo Milanese, Italy), containing 18.5–25.03% crude proteins, and a tryptophan to other amino acids ratio of 0.23–0.30%.

Apparatus and Recording

Signals from the EEG electrodes, the EMG electrodes when used, and the thermistor were fed into amplifiers (Colbourn Instruments, Lehigh Valley, PA, or Grass, Quincy, MA) in an adjacent room. The EEG was amplified (factor of 3,000) and analog bandpass-filtered between 0.1 and 40 Hz (frequency response: ±3 dB; filter frequency rolloff, 12 dB/octave). Conditioned signals were digitized with 12- or 16-bit precision at a sampling rate of 128 Hz (model AT-MIO-64FS or PCI-3033E; National Instruments, Austin, TX) and stored as binary computer files until subsequent analyses. Closed-circuit television cameras connected to video monitors allowed observation of animal behavior.

Postacquisition determination of the vigilance state was done by visual scoring of 12-s epochs using custom software (ICELUS, by M. R. Opp) written in LabView for Windows (National Instruments). Animal behavior was classified as wakefulness, NREM sleep, or REM sleep on the basis of characteristic changes in the EEG, brain temperature, and muscle tone/activity detected by the EMG or ultrasonic detectors, respectively. The same criteria were applied and the same software used in both laboratories to determine arousal state. Analyses of sleep-wake behavior of rats under basal conditions in each laboratory revealed no significant differences in the duration of time spent in each arousal state.

Experimental Protocols

Experiment 1: effects of 5-HTP administration on sleep-wake behavior and Tcort. Rats were injected intraperitoneally with both vehicle (PFS) and at least two doses of 5-HTP. As such, each animal served as its own control. Four doses of 5-HTP were tested: 25 (n = 7), 50 (n = 7), 75 (n = 6), and 100 mg/kg (n = 5). These are the same doses we used in our previous study (20) to determine the impact on sleep of rats of 5-HTP injected at dark onset. Experimental manipulations were randomly scheduled with an interval of at least 3 days between injections, and no animal received more than one vehicle and
three doses of 5-HTP. All injections were given 15 min before light onset. Recordings began at light onset and continued for 23 h.

Experiment 2: effects of central CRH receptor antagonism on 5-HTP-induced changes in sleep-wake behavior and Tcort. A double injection protocol was used in which injections were given both intracerebroventricularly and intraperitoneally. This protocol consisted of three conditions. The rats (n = 8) first received control injections consisting of vehicle intracerebroventricularly plus vehicle intraperitoneally. The second manipulation consisted of vehicle icv + 50 mg/kg 5-HTP ip. Finally, animals were injected with 5 μg (6.5 nmol) ohCRH icv + 50 mg/kg 5-HTP ip. This dose of ohCRH was chosen because when injected intracerebroventricularly into rats at the beginning of the light period it does not alter sleep-wake behavior and its effective duration of action when given at dark onset is 2 h (5). The 50 mg/kg dose of 5-HTP was selected because results of experiment 1 (see results) indicated it alters sleep-wake behavior of rats for about 2 h. All intracerebroventricular injections were initiated 45 min before light onset, and the intraperitoneal injections were given 30 min later. All recordings began at light onset and continued for 24 h. The order of the manipulations varied among animals, recordings were not made on consecutive days, and a minimum of 3 days elapsed between injections.

Statistical Analyses

All values are presented as means ± SE for the indicated time blocks.

Experiment 1: effects on sleep-wake behavior and Tcort of 5-HTP administration. The duration of each vigilance state (NREM sleep, REM sleep, wakefulness), and Tcort were performed within time blocks. Rats were injected intraperitoneally with either 5-HTP or vehicle (pyrogen-free saline). NREM, nonrapid eye movement; REM, rapid eye movement. *P ≤ 0.05 was taken to indicate which condition(s) contributed to the effect. An α-level of P ≤ 0.05 was indicated a statistically significant difference between conditions.

RESULTS

Experiment 1: Effects of 5-HTP Administration at Light Onset on Sleep-Wake Behavior and Tcort

Intraperitoneal administration of 5-HTP at light onset altered sleep-wake behavior and Tcort of rats. The two lowest doses of 5-HTP used in this study (25 and 50 mg/kg) induced a transient hypothermic response (Table 1), the majority of which occurred during the first 2 h postinjection (Fig. 1). During this time, the maximal Tcort reduction relative to control values was 0.9 ± 0.2 and 1.3 ± 0.5°C after administration of 25 and 50 mg/kg 5-HTP, respectively. After 75 mg/kg 5-HTP, there was an initial reduction in Tcort similar in magnitude and duration to that observed after the 25 mg/kg dose. However, this initial hypothermic response was followed by a second reduction in Tcort that occurred about 6 h postinjection, during which Tcort was modestly reduced by 0.8 ± 0.2°C (Fig. 1). Changes in Tcort after the 75 mg/kg 5-HTP did not differ as indicating a statistically significant difference between values obtained after administration of vehicle and 5-HTP.

Table 1. The impact of intraperitoneal administration of l-5-hydroxytryptophan (5-HTP) at light onset on the amount of time rats spend in vigilant states and on cortical brain temperature

<table>
<thead>
<tr>
<th>Postinjection Time Block</th>
<th>Manipulation</th>
<th>Wakefulness</th>
<th>NREM Sleep</th>
<th>REM Sleep</th>
<th>Brain Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hours 1–4, light period</strong></td>
<td><strong>Vehicle, 25 mg/kg 5-HTP</strong></td>
<td>41.5±3.9</td>
<td>51.4±3.4</td>
<td>7.1±1.2</td>
<td>35.2±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 50 mg/kg 5-HTP</strong></td>
<td>52.5±5.2</td>
<td>45.5±5.0</td>
<td>2.0±0.7*</td>
<td>34.8±0.1*</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 75 mg/kg 5-HTP</strong></td>
<td>43.4±3.4</td>
<td>51.1±3.1</td>
<td>5.5±0.8</td>
<td>35.3±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 100 mg/kg 5-HTP</strong></td>
<td>66.0±5.4*</td>
<td>33.4±5.3*</td>
<td>0.6±0.3*</td>
<td>34.7±0.1*</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 125 mg/kg 5-HTP</strong></td>
<td>38.5±4.3</td>
<td>53.3±3.6</td>
<td>8.2±1.4</td>
<td>35.2±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 150 mg/kg 5-HTP</strong></td>
<td>76.1±5.2*</td>
<td>23.5±5.1*</td>
<td>0.5±0.2*</td>
<td>35.0±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 200 mg/kg 5-HTP</strong></td>
<td>42.7±4.8</td>
<td>50.3±4.2</td>
<td>7.0±1.5</td>
<td>35.2±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 250 mg/kg 5-HTP</strong></td>
<td>86.0±4.6*</td>
<td>14.0±4.6*</td>
<td>0.0±0.0*</td>
<td>34.5±0.1*</td>
</tr>
<tr>
<td><strong>Hours 5–12, light period</strong></td>
<td><strong>Vehicle, 25 mg/kg 5-HTP</strong></td>
<td>35.8±2.7</td>
<td>51.4±2.1</td>
<td>12.8±0.9</td>
<td>35.2±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 50 mg/kg 5-HTP</strong></td>
<td>37.7±2.4</td>
<td>49.5±1.9</td>
<td>12.9±1.0</td>
<td>35.2±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 75 mg/kg 5-HTP</strong></td>
<td>31.5±2.2</td>
<td>54.2±1.8</td>
<td>14.3±0.8</td>
<td>35.2±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 100 mg/kg 5-HTP</strong></td>
<td>31.7±2.0</td>
<td>56.3±1.6</td>
<td>11.9±1.0</td>
<td>35.0±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 125 mg/kg 5-HTP</strong></td>
<td>35.2±2.8</td>
<td>51.9±2.2</td>
<td>13.0±0.9</td>
<td>35.2±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 150 mg/kg 5-HTP</strong></td>
<td>33.9±2.4</td>
<td>54.2±2.2</td>
<td>11.8±1.1</td>
<td>35.1±0.1</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 200 mg/kg 5-HTP</strong></td>
<td>33.5±2.9</td>
<td>51.8±2.4</td>
<td>12.9±0.9</td>
<td>35.2±0.1</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 250 mg/kg 5-HTP</strong></td>
<td>38.8±3.7</td>
<td>53.9±3.3</td>
<td>7.3±1.2*</td>
<td>33.8±0.3*</td>
</tr>
<tr>
<td><strong>Hours 13–23, dark period</strong></td>
<td><strong>Vehicle, 25 mg/kg 5-HTP</strong></td>
<td>67.1±2.3</td>
<td>25.1±1.7</td>
<td>7.8±0.8</td>
<td>36.0±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 50 mg/kg 5-HTP</strong></td>
<td>64.4±2.6</td>
<td>26.4±1.8</td>
<td>9.2±0.9</td>
<td>35.0±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 75 mg/kg 5-HTP</strong></td>
<td>70.3±2.1</td>
<td>22.8±1.5</td>
<td>6.9±0.7</td>
<td>35.9±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 100 mg/kg 5-HTP</strong></td>
<td>59.7±2.3*</td>
<td>29.5±1.6*</td>
<td>10.8±1.0*</td>
<td>35.7±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 125 mg/kg 5-HTP</strong></td>
<td>69.9±2.6</td>
<td>22.9±1.9</td>
<td>7.2±0.9</td>
<td>35.9±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 150 mg/kg 5-HTP</strong></td>
<td>61.4±3.3*</td>
<td>28.7±2.4†</td>
<td>9.2±1.0†</td>
<td>35.6±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 200 mg/kg 5-HTP</strong></td>
<td>66.8±2.6</td>
<td>25.0±1.8</td>
<td>8.1±0.9</td>
<td>35.9±0.0</td>
</tr>
<tr>
<td></td>
<td><strong>Vehicle, 250 mg/kg 5-HTP</strong></td>
<td>35.6±2.7*</td>
<td>33.9±1.9*</td>
<td>10.5±2.1</td>
<td>35.5±0.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE across the indicated time blocks. Rats were injected intraperitoneally with either 5-HTP or vehicle (pyrogen-free saline). NREM, nonrapid eye movement; REM, rapid eye movement. *P ≤ 0.05 vs. values obtained after vehicle injection; †P = 0.056; ‡P = 0.052.

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statistically from those obtained after vehicle administration because of the biphasic time course during which temperatures were recorded both above and below values obtained during control recordings. This biphasic hypothermic pattern was profoundly evident after administration of the 100 mg/kg dose of 5-HTP (Fig. 1). Rats injected intraperitoneally with this dose of 5-HTP were hypothermic for essentially the entire recording period (Table 1), although the nadir of this hypothermia was evident 8 h postinjection and amounted to \(2.2^\circ C\) (Fig. 1).

5-HTP administration at the beginning of the light period initially increased wakefulness and reduced NREM sleep (Table 1, Fig. 1). During postinjection hours 1–4, there were no statistical differences between values for NREM sleep obtained after injection of 25 mg/kg 5-HTP and vehicle (Table 1). However, restricting data analyses to the first postinjection hour revealed that 25 mg/kg of 5-HTP transiently increased wakefulness and reduced NREM sleep (Fig. 1). After 50 mg/kg of 5-HTP, wakefulness increased and NREM sleep was reduced during the first 4 h postinjection (Table 1). The majority of these changes occurred during the first 2 h postinjection (Fig. 1). The duration of the 5-HTP-induced increase in wakefulness and reduction in NREM sleep after 75 mg/kg 5-HTP was 4 h (Table 1, Fig. 1). The highest dose of 5-HTP used (100 mg/kg) increased wakefulness for 8 h (Fig. 1). The 100 mg/kg dose of 5-HTP reduced NREM sleep during postinjection hours 1–4 (Table 1, Fig. 1). REM sleep was reduced during the first 4 h postinjection after each of the doses of 5-HTP used in this study (Table 1). The reduction in REM sleep persisted for 12 h postinjection after the 100 mg/kg dose of 5-HTP (Table 1, Fig. 1).

During the subsequent dark period (postinjection hours 13–23) wakefulness was reduced after the 50–100 mg/kg doses of 5-HTP (Table 1, Fig. 1). NREM sleep increased during this time after 50 and 100 mg/kg 5-HTP, and there was a tendency for increased NREM sleep after the 75 mg/kg dose, which did not quite achieve statistical significance (Table 1). There was an increase in REM sleep after administration of 50 mg/kg 5-HTP, and a tendency for increased REM sleep after 75 mg/kg 5-HTP (Table 1).

Experiment 2: Effects of CRH Receptor Antagonism on Changes Induced by 5-HTP Administration on Sleep-Wake Behavior and Tcort

Intraperitoneal administration of 50 mg/kg 5-HTP into this group of rats induced hypothermia. During the first 4 h postin-
jection, mean Tcort dropped from 37.0 ± 0.1°C after control manipulations (vehicle icv + vehicle ip) to 36.2 ± 0.1°C after 5-HTP (vehicle icv + 5-HTP ip). The peak effect was observed 2 h postinjection, when Tcort was reduced by 1.0 ± 0.3°C (Fig. 2). This hypothermic response was about the same duration and magnitude as that observed in rats injected with this dose of 5-HTP in experiment 1 (Fig. 1). When rats were pretreated intracerebroventricularly with 5 μg αhCRH, the hypothermic response to 5-HTP was exacerbated; Tcort reached a nadir of 33.7 ± 0.6°C 2 h postinjection (Fig. 2), a reduction of 3.3 ± 0.6°C relative to corresponding control values. Relative to values obtained after control manipulations, intraperitoneal administration of 5-HTP increased wakefulness and inhibited both NREM and REM sleep, an effect that lasted 4 h (Fig. 2). Intracerebroventricular pretreatment of animals with αhCRH did not alter changes in sleep-wake behavior of rats induced by peripheral administration of 5-HTP (Fig. 2).

**DISCUSSION**

There are four major findings of this study: 1) 5-HTP administration into rats at the beginning of the light period initially increases wakefulness and induces hypothermia, 2) NREM sleep increases 12 h after injection of 5-HTP, 3) intracerebroventricular administration of the CRH receptor antagonist αhCRH does not alter 5-HTP-induced wakefulness, and 4) the hypothermic response to peripheral administration of 5-HTP is exacerbated when αhCRH is administered intracerebroventricularly. Each dose of 5-HTP used in this study increases wakefulness for 1 to 4 h, an effect not altered by central antagonism of CRH receptors. In contrast to the monophasic hypothermia after all doses of 5-HTP administered at dark onset (20), the hypothermic response to 75 and 100 mg/kg 5-HTP given at light onset (this study) is biphasic, with a clear second nadir of temperature apparent 7–8 h after
injections. The hypothermic response to 50 mg/kg 5-HTP is exacerbated when animals are pretreated intracerebroventricularly with the CRH receptor antagonist αhCRH.

Our previous results (20) indicate that 5-HTP administration into rats at dark onset induces dose-dependent changes in sleep-wake behavior. When given at dark onset, 25 and 50 mg/kg 5-HTP increase wakefulness and reduce NREM sleep, whereas higher doses (75 and 100 mg/kg) increase NREM sleep and reduce wakefulness. These responses to 5-HTP administration at dark onset are apparent during the initial 12 h postinjection. Because low doses of 5-HTP (25, 50 mg/kg) administered at dark onset promote wakefulness, whereas high doses (75 and 100 mg/kg) promote NREM sleep (20), our previous results suggest that the impact on sleep-wake behavior of serotonergic activation at dark onset by peripheral administration of 5-HTP depends on the degree to which the system is activated. Our previously obtained data also support the hypothesis that 5-HT promotes wakefulness per se, but triggers subsequent sleep through the induction of the synthesis and/or release of as yet to be identified sleep-inducing factor(s) (25, 26). Results of the present study indicate that serotonergic activation by the same doses of 5-HTP administered at light onset initially enhances wakefulness regardless of dose; NREM sleep increases only after a delay of 12 h. During the light period immediately following 5-HTP administration, changes in sleep-wake behavior of rats are limited to increases in wakefulness and reductions in NREM and REM sleep. As such, under the conditions of this study, increases in NREM sleep induced by 5-HTP occur only during the subsequent dark period. Increases in NREM sleep some 12 h after 5-HTP administration may be a rebound effect, similar to that observed after sleep deprivation; prolonged wakefulness is followed by increases in sleep. However, observations suggest the increase in NREM sleep during the subsequent dark period is not likely a sleep rebound. For example, rats injected with 5-HTP at light onset do not lose all sleep, i.e., they are not totally sleep deprived. After the first postinjection hour, rats injected with 5-HTP obtain approximately half the NREM sleep they do after injection of vehicle. In addition, the impact of short-term sleep deprivation of rats at light onset (1 to 2 h) is modest at best, with minimal impact on subsequent sleep-wake behavior. More prolonged periods of total sleep deprivation at light onset (4 to 8 h) induce increases in NREM sleep apparent during the remainder of the light period and during the subsequent dark period.

An alternate hypothesis for observations that increases in NREM sleep following administration of 5-HTP are limited to the dark period is that such responses represent a fundamental property of the serotonergic system. Increases in NREM sleep during the subsequent dark period following light onset administration (this study) are similar in magnitude and duration to the increases in NREM sleep that follows administration of the same doses of 5-HTP at dark onset. Therefore, collective results of this study and our previous study (20) suggest that 5-HTP increases NREM of rats only during the dark period, regardless of whether injections are given at the beginning of the dark period or at the beginning of the previous light period. Although additional experiments are necessary to determine the precise mechanisms involved, these time-dependent differences in responses to 5-HTP underscore the importance of the interactions between circadian and sleep-regulatory mechanisms.

The second aim of this study was to begin to determine if CRH in the brain provides an interface between activation of the serotonergic system and subsequent alterations in sleep-wake behavior. Wakefulness increases after each dose of 5-HTP administered at light onset in this study, an effect lasting from 1 to 4 h. When administered at dark onset, only the lowest doses (25 and 50 mg/kg) of 5-HTP promote wakefulness during the initial postinjection period (20), and the duration of this effect is limited to 1 h. Present observations are consistent with the hypothesis that 5-HT is an arousal-promoting neurotransmitter; activation of arousal-promoting systems of rats during the light period when they are less active and sleep the most is expected to have a greater impact on wakefulness than when activation of such systems occurs during the dark period.

Results of this study suggest that hypothalamic CRH release stimulated by increased 5-HT in brain (3, 13) may not mediate the initial wakefulness that follows peripheral 5-HTP administration. Although additional experiments are required to definitively rule out hypothalamic CRH as a mediator of 5-HTP-induced wakefulness, the finding that intracerebroventricular pretreatment with the CRH receptor antagonist αhCRH does not reduce 5-HTP-induced wakefulness under the conditions of this study suggests there are other mechanisms and mediators of this response. Receptor-mediated actions of 5-HT within the CNS on neuronal systems implicated in the regulation of sleep are well documented (e.g., Refs. 8, 24, 35, 40). Peripheral administration of 5-HTP increases 5-HT synthesis in the CNS (e.g., Ref. 10) but also increases synthesis and release of 5-HT in the periphery. Among the many systems impacted and actions of 5-HT in the periphery, 5-HT acts directly on the pituitary and adrenal glands of the HPA axis. Because αhCRH was administered centrally (icv), peripheral (pituitary, adrenal cortical) CRH receptors were not targeted, nor were 5-HT receptors on the pituitary and/or adrenal cortex blocked. By acting directly on the pituitary, 5-HT induces the synthesis and release of proopiomelanocortin (POMC)-derived peptides such as ACTH and αMSH (18). ACTH and α-melanocyte stimulating hormone (αMSH) both induce wakefulness (7, 33). Similarly, 5-HT acts on the adrenal gland (9) to induce the secretion of glucocorticoids, which may also increase wakefulness (reviewed in Ref. 41). As such, effects of 5-HT on the pituitary and adrenal components of the HPA axis may still increase wakefulness after peripheral 5-HTP administration, even if actions of hypothalamic CRH are antagonized.

In addition to peripheral effects on the HPA axis, nonspecific effects in the CNS may contribute to the wakefulness-promoting actions of 5-HTP. Aromatic L-amino acid decarboxylase is the enzyme that decarboxylates 5-HTP to 5-HT. This enzyme also is present in catecholaminergic neurons. High concentrations of 5-HTP may be taken up by catecholaminergic neurons, which may convert it to 5-HT (14). As such, synthesis and release of 5-HT from nonserotonergic neurons may mimic effects of activation of serotonergic neurons. In addition, dopaminergic neurons may release dopamine in response to 5-HT, which may also contribute to arousal. However, peripheral administration of 5-HTP as a tool to increase global 5-HT in brain is frequently used (reviewed in Ref. 1).
and the doses of 5-HTP administered in this study constitute approximately half the daily dietary tryptophan intake (2).

The reduction in Tco2 induced by serotonergic activation in this and our previous study (20), suggests that 5-HT may play a role in lowering body and brain temperature. 5-HT stimulates release of POMC-derived peptides from the pituitary, including αMSH (4, 15). Because the cryogenic effects of αMSH are well established (reviewed in Ref. 33), it is possible that the hypothemic response to 5-HTP observed in this present study results from 5-HTP-induced increases of this pituitary peptide. However, the role 5-HT plays in thermoregulation is complex. For example, central 5-HT pathways may facilitate thermogenesis by regulating brown adipose tissue activity (39). In addition, a balance between hypothalamic 5-HT and noradrenaline may regulate body temperature, with 5-HT elevating body temperature (reviewed in Ref. 29). In agreement with the thermogenic role of 5-HT for 5-HT, cortical brain temperature and hypothalamic serotonergic activity correlate positively in freely behaving rats under physiological conditions (19). Moreover, brain 5-HT depletion by p-chlorophenylalanine decreases body and brain temperature (22, 27) and flattens the circadian temperature rhythm (27). Finally, both activation of presynaptic inhibitory 5-HT1A and 5-HT1B receptors (17, 42) and blockade of postsynaptic 5-HT2 receptors (11, 21) induce hypothermic responses.

Results of this study suggest yet another mechanism by which 5-HT may contribute to the regulation of body temperature. When central CRH is antagonized, the hypothemic response to 5-HTP is exacerbated. The magnitude of the reduction in body temperature after 50 mg/kg of 5-HTP is more than three times as great when rats are pretreated intracerebroventricularly with αCRH than when they are not. This synergistic interaction between the serotonergic system and central CRH has, to our knowledge, not been previously reported. Elevated CRH induces thermogenesis (34, 37, 38) through actions on brown adipose tissue (39). Because 5-HT increases CRH in brain (reviewed in Ref. 18), our data suggest that normally the hypothemic response to elevated 5-HT may be opposed by CRH-induced thermogenesis. As such, the hypothemic response of animals to which 5-HTP has been administered peripherally may be limited in magnitude by the thermogenic actions of CRH. If the balance of these opposing physiological processes is altered by antagonizing central CRH-induced thermogenesis, as by CRH receptor antagonism, the hypothemic response to 5-HTP is exacerbated, perhaps mediated by pituitary αMSH.

In conclusion, 5-HTP administered intraperitoneally into rats at light onset alters sleep-wake behavior and cortical brain temperature; initially, wakefulness is promoted and sleep is reduced with increases in NREM sleep not apparent until 12 h postinjection. Results of this present study provide additional evidence supporting the hypothesis that 5-HT is a wake-promoting neurotransmitter. Finally, differences in responses to 5-HTP depending on timing of administration (wakefulness promoting at light onset, NREM sleep promoting at dark onset) underscore the importance of interactions between sleep regulatory and circadian mechanisms. Additional experiments are necessary to fully elucidate potential interactions between the serotonergic system, CRH, and/or the HPA axis as they pertain to the modulation of sleep-wake behavior and temperature regulation.

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